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About the Conference

International Conference on Therapeutics and Pharmacy Practice - **“Exploring and Advancing Healthcare through Novel Strategies in Pharmacy Practice”** organized by Nirmala College of Pharmacy, Mangalagiri in collaboration with Findlay University, USA & Pharmacon Society, Hyderabad held on 17th & 18th December, 2019. The Conference main objectives are to cultivate an international forum for the ever widening scope of Pharmacy Practice research, to Create a forum for the dissemination of quality, international Pharmacy Practice research for all stake holders, to increase Pharmacy Practice contributions to global health through the provision of greater access to the latest high quality international Pharmacy Practice and to develop a policy statement on current and future Pharmacy Practice research.

In the two day deliberations of Resource Persons from India and Abroad highlighted about Life threatening drug interactions and adverse drug reactions in critical care; Artificially intelligent, comprehensive, clinical history taking tool; Pathophysiology and Pharmacology as a basis for Pharmacotherapy; Advance therapies in Heart failure; Higher education and Career options of Pharma professionals in USA; Professional Community Pharmacy services in India. The above discussions facilitated to the delegates to aware about role of Clinical Pharmacist, advances happening in healthcare profession and novel strategies in Pharmacy Practice.

Many researchers are contributed their extensive research work to publish articles in our partnered reputed journal an International Journal of Life Science and Pharma Research. On behalf of Organizing Committee, International advisory committee and Peer Review Committee; we would like to thank all the authors for their efforts for making our International Conference a grand success. We would like to extend our sincere thanks to the Editorial team of International Journal of Life Science and Pharma Research for publishing our conference proceeding in a form of Special Issue.

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BIOANALYTICAL ESTIMATION OF GLICLAZIDE IN RABBIT PLASMA BY RP-HPLC: METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT

An isocratic simple, precise, selective and rapid assay was developed and validated for the determination of gliclazide in rabbit plasma using the RP-HPLC method. The assay was performed using Agilent HPLC system, isocratic pump (1200 series) and Hyper chrome ODS-BP (5 μ m, 4.6 mm x 250 mm) analytical column with 0.1% formic acid: acetonitrile (70:30, v/v) as the mobile phase at a flow rate of 1.0 ml/min and UV detection done at 228 nm. The method was found to be specific for gliclazide with no interfering peaks at the retention time of 4.2 $\pm$ 0.3 min. The developed method was validated within the concentration range of 20-800 ng/ml with a correlation coefficient of 0.9999. The validation parameters, system suitability, selectivity, linearity, accuracy, precision, recovery, ruggedness, robustness, LOD, LOQ and drug stability (both at ambient temperature and freeze-thaw conditions) were found to be within the limits as per standard guidelines. The method is simple and rapid and was fully validated.

KEYWORDS: gliclazide, rabbit plasma, RP-HPLC, development, validation

1. INTRODUCTION

Gliclazide, a second-generation sulphonylurea is used in the treatment of type II diabetes¹⁻³. Different methods for the determination of gliclazide in biological fluids like rat⁴⁻⁵, rabbit⁶⁻⁷ and human plasma⁸⁻¹¹ using RP-HPLC has been reported in the literature. UV-Visible¹²⁻¹⁴ and HPLC¹⁵ methods were reported in the literature for its estimation alone and combination in dosage forms¹⁶⁻¹⁷. There is no finding on simple and complete analysis and validation for gliclazide estimation in rabbit plasma. Hence, a simple, sensitive, rapid, precise, accurate and robust RP-HPLC method was developed and validated for gliclazide estimation in rabbit plasma as per US-FDA guidelines¹⁸.

2. MATERIALS AND METHODS

2.1. Materials

Gliclazide was obtained as a gift sample from Dr. Reddy's Labs, Hyderabad. HPLC grade solvents methanol, acetonitrile and formic acid were purchased from Merck Co. whereas remaining chemicals and solvents were of analytical grade.

2.2. Instrumentation and chromatographic conditions

The Agilent HPLC system (Agilent 1200 liquid chromatography system, Agilent Technologies Inc., USA.) equipped with isocratic pump (1200 series) and hyper chrome ODS-BP (5 μ m, 4.6 mm x 250 mm) analytical column was used for the study. A mixture of 0.1% formic acid: acetonitrile (70:30, v/v) was used as mobile phase at a flow rate of 1.0 ml/min. The mobile phase was filtered through 0.45 μ m membrane filter and degassed by ultrasonication. Duration of 10 min was taken as total chromatographic run time per sample. The drug was monitored at 228 nm using a variable wavelength detector (VWD). The data analysis and

quantitation were performed with Agilent Chemstation software (Agilent Technologies). The retention time obtained was 4.2 ± 0.3 min.

2.3. Preparation of stock solution and working solutions

Gliclazide stock solution was prepared by dissolving 10 mg of gliclazide in methanol and making the volume up to 10 ml in a volumetric flask, resulting in a solution containing 1 mg/ml. The stock solution was diluted with the mobile phase to get working solutions having concentrations of 20, 80, 200, 400 and 800 ng/ml.

2.4. Preparation of calibration and quality control standards

Absence of endogenous interferences that hinder the retention time was confirmed by testing the blank rabbit plasma before spiking; hence no internal standard was used. Blank rabbit plasma was used to prepare five calibration standards ranging over 20 to 800 ng/ml of gliclazide. They were prepared freshly by spiking the 20 μ l each of the working solutions in to 180 μ l blank rabbit plasma to get the concentrations of 20, 80, 200, 400 and 800 ng/ml respectively. The quality control (QC) samples were also prepared in a similar way by spiking the working solutions in to blank rabbit plasma to get the concentration levels of the lower limit of quantification (LLOQ) of 20 ng/ml, low quality control (LQC) of 60 ng/ml, medium quality control (MQC) of 400 ng/ml and high quality control (HQC) of 770 ng/ml. All the solutions were stored until analysis in a deep freezer.

2.5. Treatment of plasma samples

Liquid-liquid extraction method was applied to isolate drug from rabbit plasma. Polypropylene tubes were used to collect 100 μ l of plasma sample, vortexed, followed by the addition of 100 μ l of 0.5 N sodium carbonate and 2.5 ml of extraction solvent (methyl tertiary butyl ether), further vortexed for 10 min. The samples were then centrifuged for 10 min at 4,000 rpm at 20°C. The supernatant was subsequently transferred into a tube and allowed for evaporation under nitrogen at 40°C. Thus obtained dried form of residue is reconstituted using 500 μ l of mobile phase and briefly vortexed. Thus, extracted sample was then injected into HPLC for analysis.

2.6. Method validation

The current developed analytical method was validated according to US-FDA guidelines¹⁸.

2.6.1. System suitability

It was studied using 6 replicate aqueous injections of MQC (400 ng/ml) following the established chromatographic conditions.

2.6.2. Selectivity and sensitivity

Selectivity and sensitivity were evaluated to observe the interference peaks due to endogenous plasma constituents and/or any degradation peaks at the analyze retention time using 6 blank plasma samples at the level of lower limit of quantification (LLOQ).

2.6.3. Linearity (Calibration curve)

All the prepared calibration standards in the range of 20-800ng/ml were assayed in six replicates and a calibration curve was constructed between peak area and concentration. Linearity of the results was assessed by employing linear regression equation and correlation coefficient (R^2) parameters. The regression line equation $y = mx + c$ was obtained, where; y =response, m =slope, x =concentration, c =intercept.

2.6.4. Accuracy and precision (intraday and interday)

Intraday and interday studies using 4 different concentrations (quality control standards of LLOQ (20 ng/ml), LQC (60 ng/ml), MQC (400 ng/ml) and HQC (770 ng/ml)) of quality control standards in rabbit plasma at 6 replicates were performed to determine the precision. Accuracy was determined at each case at each concentration level.

2.6.5. Extraction efficiency (Recovery)

The extraction efficiencies of 3 different concentrations (LQC (60 ng/ml), MQC (400 ng/ml) and HQC (770

ng/ml)) at 6 replicates were performed to estimate the percent recovery by matching the peak areas of extracted standards and un-extracted standards.

2.6.6. Ruggedness

It is estimated by analysing the homogeneous lots by different analysts in different laboratories. The operational and environmental conditions are within the specified limits.

2.6.7. Robustness

Robustness studies were performed using a sample concentration of 80 ng/ml by allowing minor variations in flow rate, mobile phase composition and wavelength.

2.6.8. LOD and LOQ

The LOD and LOQ were established according to the following formulae:

Detection limit, $LOD = 3.3SD/slope$

Quantification limit, $LOQ = 10SD/slope$

where SD= standard deviation of the calibration curve y-intercept.

2.6.9. Stability

Stability studies were performed at room temperature and freeze-thaw (frozen at -20°C and thawed 3 times) conditions. Analyze area response was compared for stability sample and fresh stock sample using LQC and HQC levels at 6 replicates for each level.

3. RESULTS AND DISCUSSION

3.1. Conditions of chromatography

Sample preparation and chromatographic conditions were optimized by several extensive preliminary trials for suitable method development. Mobile phase composition was also optimized in order to achieve good chromatographic peak shape and resolution. Best results were obtained when 0.1% formic acid: acetonitrile (70:30, v/v) was used as mobile phase at a flow rate of 1.0 ml/min. The UV detector for analyte measurement at its retention time of 4.2 ± 0.3 min was programmed at 228 nm. For treatment of plasma samples, liquid-liquid extraction method was adopted which gave sharp and symmetric peaks.

3.2. Method validation

3.2.1. System suitability

The calculated results are shown in **Table 1**. It was found that the obtained % CV is within the acceptable limits of not more than (NMT) 5%, hence the established chromatographic conditions were suitable for the estimation of gliclazide in rabbit plasma.

Table 1: System suitability data for RP-HPLC method

Injection Number	Peak area
1	97071286
2	96986954
3	97163859
4	98125146
5	97046581
6	96875362
n	6
Mean	97211531.33
SD(±)	457665.07
% CV (% RSD)	0.47

3.2.2. Selectivity and sensitivity

Typical chromatograms obtained from blank rabbit plasma and the drug loaded plasma are shown in Figures 1 and 2, which indicated that no peaks of endogenous components appeared at the same retention time of drug, showing no interferences. Hence, no internal standard was used in the study.

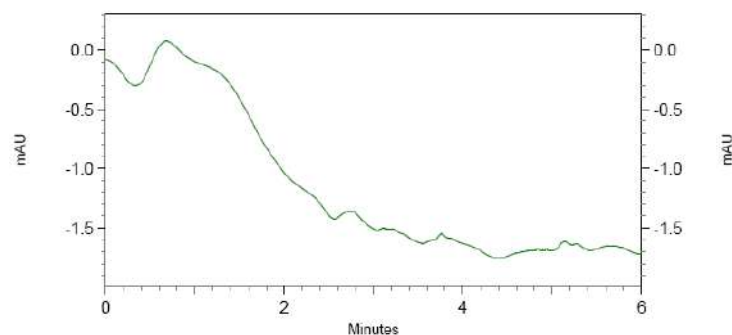


Fig. 1: Chromatogram of blank rabbit plasma sample

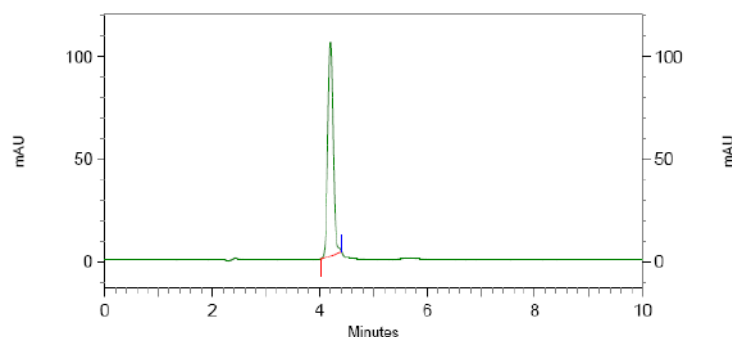


Fig. 2: Chromatogram of drug loaded rabbit plasma (gliclazide quality control standard of 80 ng/ml) at 228 nm by RP-HPLC

3.2.3. Linearity (Calibration curve)

Calibration curve was constructed by five-point analysis to determine the linearity over a concentration range of 20 - 800 ng/ml. The linear regression equation was found to be $y = 232634x + 4472000$, with a correlation coefficient (R^2) value of 0.9999 showing good linearity. The results are shown in Table 2 and calibration curve is presented in Fig.3.

Table 2: Details of calibration curve (gliclazide in rabbit plasma)

S.No.	Spiked plasma conc. (ng/ml)	Peak area	Conc. measured (Mean, ng/ml)	SD, n=6	%CV	Accuracy (%)
1	20	8966318.00	19.32	18733.6	0.21	96.60
2	80	22718500.67	78.43	112168.6	0.49	98.04
3	200	51945654.00	204.07	24309.9	0.05	102.04
4	400	97072130.00	398.05	12600.8	0.01	99.51
5	800	190610588.33	800.13	68623.6	0.04	100.02

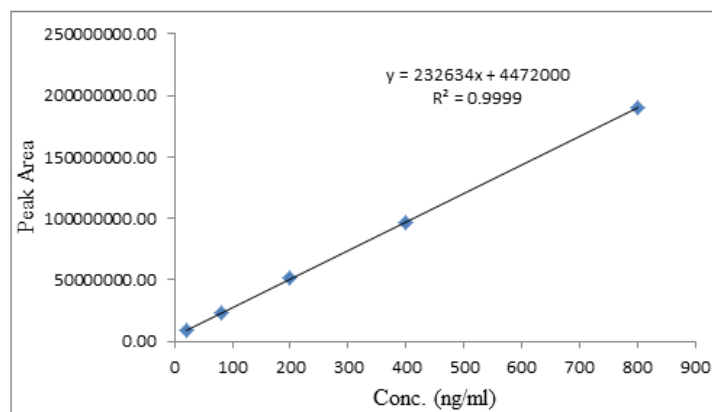


Fig. 3: Calibration curve of gliclazide in spiked rabbit plasma

3.2.4. Accuracy and precision (intraday and interday)

The obtained results as shown in Table 3, demonstrated good accuracy and precision for the back calculated concentrations over the selected concentration range with %CV within the limits of $\pm 15\%$ (whereas $\pm 20\%$ for LLOQ).

Table 3: Accuracy and precision data

Spiked plasma conc. (ng/ml)	Intraday (Within run) n=6			Interday (Between run) n=6		
	Conc. measured (mean, ng/ml, Mean $\pm$ SD)	%CV	Accuracy (%)	Conc. measured (mean, ng/ml, Mean $\pm$ SD)	%CV	Accuracy (%)
LLOQ (20)	18.81 $\pm$ 0.37	1.94	94.05	19.44 $\pm$ 0.78	4.04	97.18
LQC (60)	59.62 $\pm$ 1.10	1.84	99.37	59.80 $\pm$ 1.62	2.7	99.67
MQC (400)	395.51 $\pm$ 6.89	1.74	98.88	395.04 $\pm$ 3.67	0.93	98.76
HQC (770)	754.47 $\pm$ 7.99	1.06	97.98	771.32 $\pm$ 13.44	1.74	96.42

3.2.5. Extraction efficiency (Recovery)

The results for percentage of drug recovery are shown in Table 4. The mean % recoveries of gliclazide ranged from 96.09% to 99.79% indicating the closeness to true values with accuracy.

Table 4: Recovery data

S.No.	Spiked conc. (ng/ml)	Measured conc. (ng/ml)	Recovery	%CV
1	16 (80%)	15.97	99.79	0.26
2	20 (100%)	19.22	96.09	1.44
3	24 (120%)	23.8	99.17	0.98

3.2.6. Ruggedness

The results were found to be within the acceptance limits of %CV less than 2%. The results are shown in Table 5.

Table 5: Ruggedness data

S.No.	Accuracy (%)		
	Set-I	Set-II	Set-III
1	99.89	99.45	99.4
2	98.77	99.2	99.7
3	98.43	99.67	99.88
4	99.81	99.54	99.6
5	98.2	98.98	98.2
6	96.6	98.2	99.56
Average	98.62	99.17	99.39

SD, n=6	1.21	0.54	0.60
%CV	1.23	0.54	0.61
Overall Average	99.06		
Overall %CV	0.79		

Where, set-I represent the variability due to HPLC system, set-II represents the variability due to HPLC column and set-III represents the variability due to analyst

3.2.7. Robustness

The results were found to be within the acceptance limit of %CV less than 2%. The results are shown in Table 6.

Table 6: Robustness data

S.No.	Parameter	Condition	Mean	SD, n=6	%CV	Accuracy (%)
1	Standard	Standard conditions	79.38	0.93	1.18	99.23
2	Mobile phase composition (±5%)	0.1% formic acid: acetonitrile, 65:35 (v/v)	78.09	0.49	0.63	97.61
		0.1% formic acid: acetonitrile, 75:25 (v/v)	78.46	0.79	1.00	98.08
3	Wavelength (±2 nm)	226	77.82	1.33	1.72	97.28
		230	78.64	0.61	0.78	98.30
4	Flow rate (ml) (±0.2 ml)	1.2	80.88	0.71	0.88	101.10
		0.8	79.51	0.72	0.90	99.39

3.2.8. LOD and LOQ

The LOD and LOQ of gliclazide were found to be 0.67 mg/ml and 2.03 ng/ml respectively.

3.2.9. Stability

The results shown in Table 7 indicated that the gliclazide drug is stable in rabbit plasma for at least 24 hours at room temperature and at frozen conditions.

Table 7: Stability data

Spiked plasma conc. (ng/ml)	Bench top stability		Freeze and thaw stability	
	24 h		(3 cycles, 48 h)	
	Conc. measured, (n=6; ng/ml; Mean±SD)	%CV	Conc. measured, (n=6; ng/ml; Mean±SD)	%CV
		(n=6)		(n=6)
60	59.7±0.46	0.77	57.90±0.99	1.7
770	771.96±6.02	0.78	751.71±9.20	1.22

4. CONCLUSION

In the present bioanalytical study, a simple, accurate, and precise RP-HPLC method was developed for the estimation of gliclazide in rabbit plasma as per US FDA guidelines. The developed method was fully validated, and the results were found to be within the acceptable limits. The simplicity involved in the developed technique and the corresponding high sensitivity project this as an attractive technique for the quantification of gliclazide in rabbit plasma. Even it is an easily adaptable method for the routine quality control analysis.

5. ACKNOWLEDGEMENT

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COMPARING THE PRESCRIBING PRACTICES IN MANAGEMENT OF CHRONIC ALCOHOLIC LIVER DISEASE IN DEPARTMENTS OF GENERAL MEDICINE, GASTROENTEROLOGY & HEPATOLOGY

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ABSTRACT

Patients of alcoholic liver diseases (ALD) attended the General Medicine, Gastroenterology & Hepatology with varieties of chief complaints like jaundice, fatigue, digestive issues, and tenderness in the right upper abdomen. The use of diuretics, antibiotics, vitamin supplements, proton pump inhibitors, anti hypertensive, laxatives, nutritional supplements are very common for the treatment of ALD. The research was done to analyze the prescription pattern of drugs used in alcoholic liver disease patient in KGH, Vishakhapatnam. A prospective observational; unicentric study was conducted in the medicine and Gastroenterology & Hepatology department of KGH from December 2017 to May 2018. Patients who were attending to medicine department were enrolled in the study as per selection criteria. The information on alcoholic liver disease prescriptions was recorded and pattern of drugs used for it was analyzed.

KEYWORDS: *ALD (Alcoholic Liver Disease), WHO (World Health Organization), Gastroenterology, NLEM (National List of Essential Medicines).*

INTRODUCTION

Alcohol is a depressant, which means it slows the function of the central nervous system. Alcohol reduces communication between the brain and body. Slurred speech is one of the first sign if the person had taken more amount of alcohol.¹ Dizziness², fatigue³, nausea⁴, headache⁵, stomach aches⁶, vomitings⁷, hangovers⁸, poor memory⁹, weakened hand eye coordination¹⁰ are the other side effects. Alcoholic liver disease is a condition that includes fatty liver, alcoholic hepatitis and chronic hepatitis. Signs and symptoms of liver disease¹¹ include: Skin and eyes that appear yellowish (jaundice), Abdominal pain and swelling, Swelling in the legs and ankles, Itchy skin, Dark urine color, Pale stool color or bloody or tar-colored stool, Chronic fatigue, nausea or vomiting. Tertiary Problems¹² that alcoholic beverages bring are alcoholic liver disease, heart disease and cancer. The use of alcoholic beverages has been an integral part of many cultures for thousands of years. There are very few studies which describe the prescribing patterns of drugs in Alcoholic Liver Disease¹³. The present article is an attempt to gain insight into prescribing patterns of drugs in various complications of ALD including specific therapies at General Medicine, Gastroenterology and Hepatology department in a tertiary care teaching hospital. In present study, the prescribing patterns of drugs used in ALD drugs were analyzed by WHO prescribing core indicators. The main purpose of the study is to identify the prescribing patterns of drugs used in various complications of alcoholic liver disease and the prescribing patterns of alcoholic liver disease drugs would be analyzed by using WHO prescribing core indicators.

MATERIALS AND METHODS

A hospital based prospective observational study was conducted in the Department of General Medicine, Gastroenterology and Hepatology of King George Hospital, Vishakhapatnam, India. This study was carried out for a period of six month during which we have collected 102 prescriptions after obtaining the consent from the patient.

Study design: A hospital based prospective and observational study.

- **Study subjects:** Study carried out on 102 subjects diagnosed with ALD attended General Medicine, Gastroenterology and Hepatology department.

Study Criteria

Inclusion Criteria

- 1) Patients of any age, either sex who will be admitted to the emergency ward in the department of General Medicine and also Gastro enteric and hepatic ward in KGH.
- 2) Patients those diagnosed with ALD followed by the Portal Hypertension, as cites, Jaundice, Alcoholic Hepatitis, and Anemia etc.
- 3) Subjects who shall accept and give informed consent.
- 4) Patients who are clinically stable and could answer the questions and those willing to contribute, with or without the help of family within 72 hours of admission.

Exclusion criteria

- 1) General medicine department out patients.
- 2) Special population includes pregnancy and psychiatry.
- 3) Patient below 18 years.

Table 1: Prescribing Indicators.

1	Total number of prescriptions	102
2	Total number of drugs prescribed	979
3	Average number of drugs per prescription	9.59
4	Percentage of drugs prescribed by generic name	66.08%
5	Percentage of prescriptions with an injection prescribed	97.05%
6	Percentage of prescriptions with an antibiotic prescribed	77.45%
7	Percentage of drugs prescribed from NLEM-2015	74.23%

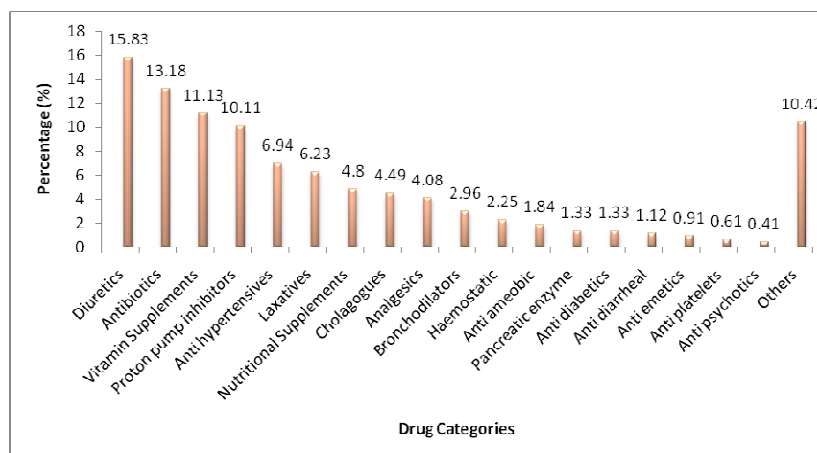


Figure 1: Distribution of prescribed drugs based on their category.

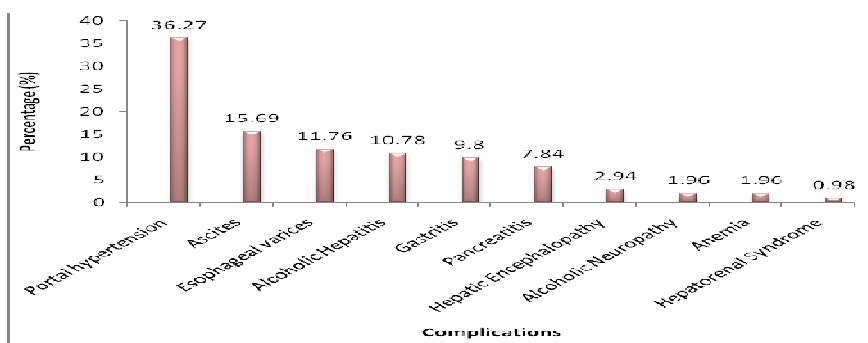


Figure 2: Complications Associated with ALD.

RESULTS AND DISCUSSION

The objective of this work is to identify the prescribing patterns of drugs in various complications of alcoholic liver disease including specific therapies and it was reported in literature that the prescribing patterns of alcoholic liver disease drugs would be analyzed by using WHO prescribing core indicators. The objective of this work is to identify the prescribing patterns of drugs in various complications of alcoholic liver disease including specific therapies and it was reported in literature that the prescribing patterns of alcoholic liver disease drugs would be analyzed by using WHO prescribing core indicators. The research project was carried out as per the protocol laid out, subjects were approached and the research project and its objectives are explained to them. A total of 102 populations were included in study. Their Informed consent was taken. Patients coming to King George Hospital were taken in to the study under the direction of the doctor. Patient details are written in the profile till he discharged with discharge summary. The drug classification was done & the data collected was analyzed for WHO recommended prescribing indicators. At the end of the study, results were analyzed and interpreted using statistical methods. Table no: 1 gives prescribing indicators. Figure: 1 indicates the complications associated with ALD. Figure 2 indicates the distribution of prescribed drugs based on their category. The percentage of drugs prescribed by generic name, percentage of encounters with an injection, percentage of encounters with antibiotics and percentage of drugs prescribed from National list of Essential Medicine (NLEM) were 66.08%, 97.05%, 77.45% and 74.23% respectively. Moreover all WHO prescribing indicators showed a deviation from the standard prescribing indicators values. Therefore, these factors should be monitored very closely in order to ensure proper prescribing in the hospitals.

CONCLUSION

In this unicentric study, we found that ceftriaxone, pantoprazole, furosemide, spironolactone, vitamin B-complex, lactulose, ursodeoxycholic acid etc., are the commonly used medications in the gastroenterology, hepatology & general medicine wards in KGH. Ceftriaxone and Pantoprazole remains most widely used drugs in the treatment of alcoholic liver disease, more or less in all the patients attending in KGH. If study would be done on more number of patients, it may draw best results on prescribing patterns of alcoholic liver disease. Our study highlights that there is enough scope for improving the prescribing pattern among the health care practitioners and reducing the use of antibiotics to avoid antibiotic resistance. The proper development of the formulary in-house will have a major impact on drug prescribers and healthcare professionals.

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SP-03

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACT OF ACALYPHA INDICA IN RATS

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ABSTRACT

Acalypha is the fourth largest genus of the Euphorbiaceae family. It has various traditional uses, a cure for stomach ache, dyspepsia, venom antidote, rheumatism and dermatitis. The presence of chemical constituents like phenolic compounds, steroids, flavonoids, tannins and saponins are proved to be of therapeutic significance from previous studies. To investigate the anti-inflammatory activity of chloroform and hydro alcoholic leaf extract of *Acalypha indica* in carrageenan induced inflammation in rat paw. The chloroform, hydro alcoholic leaf extracts were administered at respective concentrations in carrageenan induced inflammation in rat paw at 125mg/kg and 250mg/kg body weight of rats. The results of the study were expressed as mean $\pm$ S.E and the data was analyzed by using one way analysis (ANOVA). Values with $P < 0.05$ were considered as significant. Among them hydro alcoholic leaf extract of *Acalypha indica* showed a significant result at specified dose ($p < 0.001$) of 250mg/kg against the standard drug Indomethacin in concentration of 25mg/kg body weight. The results obtained were promising and paves a way to further studies for developing a formulation. Due to wider scope of its pharmacological uses further studies are to be carried out to isolate the constituents and in designing new drug entities.

KEYWORDS: *Acalypha indica*, Hydro alcoholic, Indomethacin, Prednisolone.

INTRODUCTION

Acalypha indica (Euphorbiaceae Family) (English: Indian Acalypha, Indian Mercury, Indian Copperleaf, Indian Nettle, Three-seeded Mercury) is an erect, often simple-stemmed annual herb distributed widely throughout the tropics and is believed to originate from India, Indochina and Ethiopia.¹ Its potent pharmacological activities enhance its use in traditional medicine such as an anti-inflammation, anti-bacterial, anti-cancer, anti-diabetes, anti-hyperlipidemic, diuretic, and anti-helminthic herbal plant. It is known to be a good cure for respiratory problems, rheumatoid arthritis, scabies, treatment of insect bites, and it also promotes wound healing. The decoction made from the whole plant is used for treatment of bronchitis, epilepsy, emmenagogue, mouth ulcers, and as an expectorant to treat asthma and pneumonia.² The leaf decoction is dropped into the ears to treat earache and infections. The paste made from powdered leaves is applied for skin infections. The Indian people have the most documented records of plant utilization for their traditional medicines (Martin, 1995; Savithramma et al., 2007). Chemical analysis of *Acalypha indica* shows that the plant contains phenolic compounds, steroids, flavonoids, saponins and tannins.³ It contains an alkaloid, acalyphine along with cyanogenic glycoside, inositol, resin, triatomine and volatile oils. *Acalypha indica* is listed in the Pharmacopoeia of India as an expectorant to treat asthma and pneumonia.⁴ It was formerly listed in the British Pharmacopoeia. The plant has also been eaten as a vegetable in Africa and India, but care needs when eating it since it contains several alkaloids as well as hydrocyanic acid. Due to its wide range of therapeutic effects and as per previous results we choose this plant for anti-inflammatory activity.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *Acalypha indica* was collected from the local area of Mangalagiri in the month of November. The whole plant was authenticated by Dr.P.Satyanarayana Raju, Plant Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh. The leaves were

collected and dried in shade for 7 days. Then dried leaves were grounded coarsely to powder. Further it was used to prepare the required extracts by using solvents from less polar to more polar. (i.e chloroform, ethanolic, hydro alcoholic).

Preparation of Plant Extracts

An equal quantity of leafy powder was taken and a solvent mixtures of (chloroform-water in 3:1 ratio), ethanolic and (ethanol-water in 3:1 ratio) were added and sonicated for 30 min to enhance the solubility of powder in the solvent mixtures. Afterward the powdered solution was subjected to soxhlet extraction for 5 days at a temperature below 40⁰c. The obtained chloroform, ethanolic and hydro alcoholic extracts were concentrated by simple distillation.

Preparation of test samples

Test samples were prepared by using the extracts, which were suspended in saline solution containing 1% Tween 80 such that each ml of suspension contained 125mg and 250mg of the extract. Tween 80 was used as it is the commonly used suspending agent in earlier reported studies.⁵

Preparation of standard sample

Standard sample was prepared by suspending the drug 10mg/kg Indomethacin and Prednisolone in saline solution containing 1% Tween 80.

Experimental Animals

Albino rats of Wistar strain of either sex (150-200gm) were obtained from Mahaveer enterprises; Hyderabad for the study. Gender of the rats does not make a difference for inflammatory activity as reported in many earlier studies.^{5,6} The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 5°C), humidity (55 ± 5%) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum accordingly as per the instructions given by Institutional Animal Ethical Committee. (CPCSEA)⁷.

Drugs & Chemicals

Albino rats were procured from Mahaveer enterprizes, Hyderabad, Chloroform, ethanol, saline, Carrageenan, Tween-80 were purchased from Lobe chemicals. All the reagents are of laboratory grade.

Acute toxicity studies

The chloroform, hydro alcoholic extracts of *Acalyph aindica* were studied for acute oral toxicity according to the guideline No: 423 set by Organization of Economic Cooperation and Development (OECD).^[8] Two doses of 200mg/kg (p.o) and 500mg/kg (p.o) were tested on three groups containing five animals in each group for 14 days. The animals were monitored for mortality and general behaviour. None of the doses were observed of toxicity.

Experimental procedure

The acute anti-inflammatory activity was evaluated by carrageenan – induced rat paw edema as earlier described by Turner et al.⁹ Animals were divided into six groups of six animals each. Acute inflammation was produced by injecting 0.1 ml of 1% carrageenan into sub-plantar surface of rat hind paw. Group-I control group received 0.1ml of 1% tween 80. Group-II, III, IV received (125mg/kg & 250mg) of chloroform, ethanolic and hydro alcoholic leaf extract by oral route. Group-V received the standard Indomethacin 10mg/kg by oral route. Group-VI received the standard Prednisolone 10mg/kg by oral route. Doses were given orally by using oral catheter. After thirty minutes of administration of extract, saline or standard, 0.1ml of 1% w/v carrageenan was injected to induce edema in right hind paw of rat. Paw volume was measured by Vernier calipers. The readings were recorded at intervals of 1hr, 2hr, 3hr, 4hr and 5hr. Then the paw swelling of the extract treated groups were compared with that of standard groups.

STATISTICAL ANALYSIS

The results of the study were expressed as mean ± S.E. data. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dennett's Multiple Comparison Test and p values implied significance (p<0.05).¹⁰

RESULTS

Table-1: Phytochemical constituents of *Acalypha aindica* leaf

S.No	Type of constituent	Chloroform extract	Ethanol extract	Hydro-alcoholic extract
1	Carbohydrates	+	+	+
2	Steroids	+	+	+
3	Proteins	-	-	-
4	Amino acids	-	-	-
5	Glycosides	+	+	+
6	Flavonoids	-	+	+
7	Tannins	+	+	+
8	Alkaloids	+	+	+

Table 2: Anti-inflammatory effect of chloroform extract of *Acalypha indica* L In carrageenan-induced rat paw inflammation

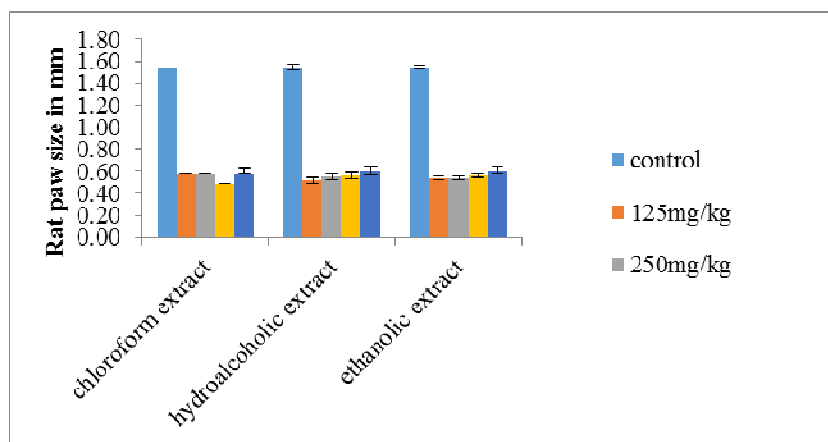
Treatment	Dose (mg/kg body weight)	Increase in paw volume			
		1 h	2 h	3 h	4 h
Control(saline)	---	1.15 ±0.29	1.52±0.29	1.71±0.29	1.8±0.29
Hydro alcoholic extract	125	0.5±0.03	0.6±0.03	0.5±0.03	0.5±0.03
Hydro alcoholic extract	250	0.6±0.05	0.6±0.05	0.6±0.05	0.5±0.05
Indomethacin	10	0.55±0.05	0.6±0.05	0.6±0.05	0.5±0.05
Prednisolone	10	0.65±0.04	0.6±0.04	0.6±0.04	0.55±0.04

Table 3: Anti-inflammatory effect of ethanol extract of *Acalypha indica* L In carrageenan-induced rat paw inflammation

Treatment	Dose (mg/kg body weight)	Increase in paw volume			
		1 h	2 h	3 h	4 h
Control(saline)	---	1.15 ±0.29	1.52±0.29	1.71±0.29	1.8±0.29
Chloroform extract	125	0.55±0.065	0.65±0.065	0.6±0.065	0.5±0.065
Chloroform extract	250	0.55±0.065	0.65±0.065	0.6±0.065	0.5±0.065
Indomethacin	10	0.45±0.048	0.5±0.048	0.55±0.048	0.45±0.048
Prednisolone	10	0.6±0.05	0.6±0.05	0.6±0.05	0.5±0.05

Table 4: Anti-inflammatory effect of hydro alcoholic extract of *Acalypha indica* L In carrageenan-induced rat paw inflammation

Treatment	Dose (mg/kg body weight)	Increase in paw volume			
		1 h	2 h	3 h	4 h
Control(saline)	---	1.15 ±0.29	1.52±0.29	1.71±0.29	1.8±0.29
Ethanol extract	125	0.60±0.57	0.60±0.57	0.52±0.57	0.55±0.57
Ethanol extract	250	0.6±0.06	0.6±0.06	0.5±0.06	0.5±0.06
Indomethacin	10	0.45±0.048	0.5±0.048	0.55±0.048	0.45±0.048
Prednisolone	10	0.65±0.06	0.6±0.06	0.6±0.06	0.5±0.06



Graph-1: Values were given as mean $\pm$ S.D. for six rats in each group. Values are statistically significant at $**p < 0.001$ as compared with standard group

DISCUSSION

All the three extracts chloroform, ethanolic and Hydro alcoholic extract at a dose of 250mg showed significant anti-inflammatory activity than the standard drug Indomethacin in carrageenan induced edema in right hind paw of rat ($p < 0.01$). Hydro alcoholic extract at a dose of 250mg showed more significant effect than the standard drug Indomethacin. Chloroform extract of 125mg & 250mg showed significant activity then the standard drug Prednisolone. Ethanolic extract at 125mg & 250mg showed a significant effect than the standard drug Indomethacin than prednisolone. It is well known that carrageenan induced paw edema model is commonly used as an experimental model for evaluating anti inflammatory activity of natural products¹¹ and is believed to be biphasic, of which the first phase is mediated by the release of histamines and 5-hydroxy tryptamine in the early stage followed by kinin release and then prostaglandin in the later stage¹². Prostaglandins are chemicals that the body produces and which can cause the fever and pain that are associated with inflammation. It works by inhibiting the production of prostaglandins, endogenous signalling molecules known to cause these symptoms. It does this by inhibiting cyclooxygenase, an enzyme that catalyzes the production of prostaglandins.^{13,14} In this study, an attempt was made to evaluate the anti-inflammatory activity of *Acalypha indica*. This study reveals that chloroform and hydro alcoholic extract showed good anti-inflammatory activity, which may be attributed due to the combined activity of the phytochemical constituents like alkaloids, steroids, flavonoids present in *Acalypha indica*. These experimental results have established an evidence for the folklore pharmacological activity of *Acalypha indica*.

CONCLUSION

This study proves that all the three extracts of *Acalypha indica* showed good anti inflammatory activity than the standard drugs paving a path to develop a formulation and then evaluate it.

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HEPATOPROTECTIVE ACTIVITY OF *CELASTRUS EMARGINATA* [W] LEAVES METHANOLIC FRACTION OF ETHANOLIC EXTRACT ON CCl₄ INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

It is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury. Prolonged administration of CCl₄ can lead to cirrhosis and hepatic carcinoma. However, the protection of *Celastrus emarginata* [W] Leaves Methanolic fraction of Ethanolic extract against hepatic injury on CCl₄ Induced rat, yet remains unclear. The research aimed to investigate the mitigation effect of MF-EE-CE against CCl₄ Induced hepatotoxicity in experimental animals. The rat were administered CCl₄ (1.5 ml/kg i.p.) as a positive control to compare MF-EE-CE. The hepatic function was assessed in liver tissue of control rat. Levels of serum hepatic enzymes (SGOT, SGPT) as well as Total protein and Albumin levels were significantly increased in CCl₄ induced rats. Co-treatment with MF-EE-CE (100 mg/kg and 200 mg/kg p.o) significantly decreased the serum levels of liver function biomarkers. Furthermore, MF-EE-CE treatment showed a significant reduction of lipid peroxidation and increase of enzyme and glutathione concentrations. Histopathological analysis was parallel to these biochemical findings. The results clearly demonstrated that MF-EE-CE is effective for ameliorating hepatic cytotoxicity arising from CCl₄.

KEYWORDS: *Celastrus emarginata*, hepatoprotective, Serum hepatic enzymes, CCl₄

INTRODUCTION

CCl₄ is a well known hepatotoxic industrial solvent.^{1,2} CCl₄ is commonly used for free radical induced liver injury.³⁻⁵ Liver is not the only target organ of CCl₄ but it also affects several organs of the body such as lungs, hearts, testes, kidneys, and brain.⁶ It was reported from the investigation carried out on animal models of acute CCl₄ induced liver damage, It is now generally accepted that CCl₄ toxicity results from bioactivation of CCl₄ into trichloromethyl free radical by cytochrome P450 system in liver microsomes and causes lipid peroxidation of membranes that leads to the liver.^{4,7} However, the cellular antioxidant action is reinforced by the presence of dietary antioxidants.^{8, 9} Antioxidants and anti-inflammatory agents play a critical role against CCl₄ intoxication by scavenging active oxygen and free radicals and neutralizing lipid peroxides.¹⁰ Mounting evidence suggests that natural agents with antioxidant potential use as dietary supplements perform their protective and therapeutic potential against all of the liver diseases such as fatty liver.¹¹ Based on the above information, antioxidants are proposed to be valid in precaution against some poisoning substances including CCl₄. In fact, a previous report has investigated the protection activities of several naturally-occurring antioxidants against CCl₄ induced acute hepatotoxicity.¹² *Celastrus emarginata* Willd. (CE), Taxonomically *Celastrus paniculatus* belongs to the family Celastraceae is commonly known as Malkangani, Kangani, Jyotishmati, Sphutabandhani, Svarnalota, Black oil tree, Intellect tree, Climbing-staff plant is an important medicinal large, woody, a climbing shrub, distributed almost all over India up to an altitude of 1800-2000 meter is known for its ability to improve memory¹³. Ayurveda, the ancient Indian traditional system of medicine has used this plant seed for prevention and treatment of various diseases¹⁴. The plant has used for prevention and treatment of various diseases as cough, asthma, headache, leprosy, paralysis, leucoderma, rheumatism, and gout. The leaf sap is a good antidote for opium poisoning and leaves are emmenagogue. The bark is reported abortifacient activity¹⁵. CE Seed oil has been reported to improve memory and it is one of the components of the formulation recommended for memory enhancing and mental disorders 'Mentat Syrup'. The methanolic extract exhibits free-radical-scavenging properties and antioxidant

effects in human non-immortalized fibroblasts. The bark is used as a brain tonic and depurative. The seeds are acrid, bitter, thermogenic, emollient, stimulant, intellect promoting, digestive, laxative, emetic, expectorant, appetizer, aphrodisiac, cardiogenic, anti-inflammatory, diuretic, diaphoretic, febrifuge and tonic, abdominal disorders, pruritus, skin diseases, cephalalgia, arthralgia, cardiac debility, nephropathy, amenorrhoea, dysmenorrhoea. The seed oil is bitter, thermogenic and intellect promoting and is useful in abdominal disorders, beriberi and sores¹⁶. The seed of this plant has been found to the antioxidant properties¹⁷. The seeds also experimentally showed sedative action, as an antirheumatic agent, alleviation of intestinal spasms, analgesic, anti inflammatory and anti-diarrhoeal activities^{18,19,20}. Appetizer, emetic, soporific, aphrodisiac, powerful brain and liver tonic²³. Seeds used externally on foul, indolent ulcers and scabies; useful both as an external and internal remedy in rheumatism, gout, paralysis, leprosy Used against pruritis, skin diseases, leucoderma, cephalalgia, arthralgia, asthma, cardiac debility, inflammation, strangury, neuropathy, amenorrhoea, dysmenorrhoea, epilepsy Used to treat beriberi (vitamin B1) deficiency disease^{21,22}; powerful agent against malaria; useful in treating anxiety, "brain fog", etc.; The seeds were also reported as an anxiolytic, sedative, and anticonvulsant properties. The powdered root and root bark considered to be useful in the treatment of malaria and cancerous.²³ Despite frequent and regular use, there is no reported work to examine its effect on damage caused by CCl₄. The study was thus intended to bridge the gap in our continued efforts to establish the effects on diseases and medications (CE) on CCl₄-induced liver damage. However, few comprehensive studies related to this activity are available until now. Therefore, the aim of this study was to complement the protective role of MF-EE-CE against CCl₄-induced hepatocellular injury in the animal system in terms of the fact that the supplementation of some antioxidants is popularly effective in counteracting the toxicity of CCl₄.²⁴

MATERIALS AND METHODS

Procurement of plant materials

The Leaves of *C. emarginata* were collected from Nanded of Marathwada region in Maharashtra during January, 2012, cleaned and dried at room temperature in shade, away from direct sunlight. Identification of the plant was done by Dr. Vishal R. Marathe, Department of Botany, Science College, Nanded, authenticated plant by comparing morphological features and a sample voucher specimen of plant is deposited for future reference.

Drying and size reduction of plant

The leaves of CE were separated from other parts, cleaned to evacuate the adhered foreign material and were washed under tap water, air dried, homogenized to powder.

Procurement of chemicals

All chemicals and reagents used for testing were analytical grade obtained from Ranbaxy Fine Chemicals Ltd., New Delhi and Loba Chemie, Mumbai, India.

Preparation of extracts

450 g of powdered leaves of the plant was completely extracted with ethanol using soxhlet apparatus. The ethanol extract (EE) concentrated in vacuum yielded solid mass (6.25%; w/w). The ethanol extract (25 g) was adsorbed on to the 250 g of silica gel (60-120 mesh) and fractionated using Pet. ether (PEF-EE-CE), Chloroform (CHF-EE-CE), Ethyl acetate (EAF-EE-CE) and Methanol (MF-EE-CE). The recovered fractions were finally evaporated to give yields of 12.08%, 5.04%, 6.02% and 51.1% of solid respectively. The thin layer chromatographic (TLC) studies of EE revealed the presence of flavonoids, Tannins, Alkaloids, steroids and Glycosides; the MF-EE-CE showed the trace quantities of flavonoids; the fractions, MF-EE-CE selected at dose levels of 100, 200 mg/kg for in vivo studies. The results were compared with a standard hepatoprotective drug silymarin (10 mg/kg in vivo). All the test substances were suspended in vehicle CMC (1% v/v).

Animal

Wistar albino rats weighing between 170-250g of either sex, maintained under standard husbandry conditions (Temp 20 ± 2°C, relative humidity 54 ± 10% and 12 h in light dark cycle) were used for all studies. Animals were allowed to take tap water and standard laboratory feed. The experiments were

performed after the approval of experimental protocols by the institutional animal ethics committee. Groups consisted of 6 rats each unless otherwise noted.

Hepatoprotective activity in vivo Toxicity studies

Acute toxicity studies were performed for MF-EE-CE according to the acute toxic classic method²⁵ described by OECD. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight and providing only water. The rats were divided into two groups of 3 animals each. The groups of rats were administered orally with appropriate extracts of *C. emarginata* at a dose of 100 and 200 mg/kg the animals were observed continuously after dosing during first 30 min, periodically for first 24 h with special attention given during first 4 h and daily thereafter, for a total of 14 days. At this dose level as there was no mortality seen, the procedure was repeated with further dose (2000 mg/kg) using fresh animals.

Carbon tetrachloride-induced hepatotoxicity

Rats were divided into 8 groups of 6 each, control, and carbon tetrachloride, silymarin, and test groups. The rats of control the group received three doses of CMC (1% v/v, p.o.) at 12 h intervals (0 h, 12 h and 24 h). The rats of carbon tetrachloride (CCl₄) group received three doses of vehicle at 12 h intervals and a single dose of CCl₄ (1.5 ml/kg *i.p.*) diluted in liquid paraffin (1:1) 30 min after administration of the first dose of vehicle. The animals in silymarin group received three doses of silymarin (10 mg/kg) at 0 h, 12 h and 24 h. CCl₄ (1.5 ml/kg *i.p.*) was administered 30 min after the first dose of silymarin while the test groups were given first the dose of extract in acacia mucilage at 0 h which was followed by a dose of carbon tetrachloride (1.5 ml/kg *i.p.*) after 30 min, while at 12 h, and 24 h the second and third dose of respective extracts (100 and 200 mg/kg/ *p.o.*). After 36 h of administration of carbon tetrachloride, blood was collected and serum was separated and used for the determination of biochemical parameters²⁶.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifuging at 2500 rpm at 300°C for 15 min. To determine the functional state of the liver and biochemical parameters the serum was used. Based on the reference method of International Federation of Clinical Chemistry Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated,²⁷ in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidised to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malate dehydrogenase is used to reduce oxaloacetate to malate whereas for SGPT the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase.²⁸ Serum bilirubin (SB) by Jendrassik and Grof method²⁹ which involves the reaction of bilirubin with diazotized sulphanilic acid to form an azocompound, the color of which is measured at 546 nm. Total protein (TP) was estimated by Biuret method³⁰ where proteins produce a violet colour complex with copper ions in an alkali solution. The absorbance of the colour complex is directly proportional to the protein in the sample, while the albumin (ALB) was estimated by BCG³¹ involving formation of blue-green complex with bromocresol green at slightly acidic pH which is photometrically measured. All the estimations were carried out using standard kits on auto analyser of Merck make (300 TX, E.Merck-Micro Labs, Mumbai).

Histopathological studies

Animals from control and treated groups were used for this purpose. The animals were sacrificed and abdomen was cut and opens to remove liver. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 hour, then embedded in paraffin using conventional methods³² and cut into 5 µm thick sections and stained using haematoxylineosin dye and finally mounted in diphenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

STATISTICAL ANALYSIS

The mean values ± SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analyzed separately and one-way analysis of variance³³ was carried out and the individual comparisons of the group mean values were done using Dennet's test.³⁴

RESULTS AND DISCUSSION

Acute toxicity studies

The MF-EE-CE did not cause any mortality up to 2000 mg/kg and were considered as safe. When normal hepatocytes were treated with the extracts under test, there are no alterations in the values of % viable cells and TP content as compared to the control at the dose level up to 1500 µg/ml indicating that the extracts were not toxic to the cells. The histological examination of the liver sections reveals that the normal liver architecture was distributed by hepatotoxin intoxication. In the liver sections of the rats treated with MF-EE-CE and intoxicated with CCl₄ the normal cellular architecture was retained as that of silymarin, thereby confirming the protective effect of the extract. A significant increase in serum SGOT, SGPT, SB, Total protein and Albumin levels (Graph 1, 2,3,4 &5) was observed in animals treated with CCl₄ (1.5 ml/kg i.p.) as compared to normal pretreatment with MF-EE-CE (100 mg/kg and 200 mg/kg p.o) for 7 days decreases the above parameters significantly as compared to CCl₄ treated group. Silymarin pretreatment produced significant decreases in the above parameter when compared to CCl₄. Histopathological examination of liver section of normal rats showed normal lobular architecture is seen central vein and portal tract seen. Central vein in the rbc's in lumen (Figure 1) where as CCl₄ treated group showed various degree of hydropic degeneration of hepatocytes seen. Fatty change was also noted. Portal tract shows abundant mono nuclear cells infiltration. Architecture of normal liver maintained (Figure 2). Silymarin treated group significantly normalized (Figure 3). Administration of MF-EE-CE at higher doses (200 mg/kg p.o.) significantly normalized these defects in the histological architecture of the liver no fatty change was seen sinusoids are congested focally mild hydropic change noted (Figure 4). In accordance with these results, it may be hypothesized that flavonoids, which are present in MF-EE-CE, could be considered responsible for the hepatoprotective activity. In conclusion this study underlines the therapeutic potential of *C. emarginata*. Further work is in progress in an attempt to isolate and estimate the flavonoids of bio active extract.

CONCLUSION

The finding of the study suggests that the extract contains phenolic compounds. It is therefore concluded that the leaves extract of *C. emarginata* has promising hepatoprotective potentials.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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PRNIO SOMAL GEL THERAPY

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ABSTRACT

Proniosomes are one of the novel prove secular drug system which are dry formulations coated with carrier such as non-ionic surfactants. Proniosomes are formulated in such a manner that they can overcome the drawbacks of niosomes such as physical instabilities, fusion and aggregation. Proniosomes can be administered by various routes like oral, intravenous, buccal, topical, transdermal etc. in which Proniosomes are formulated as gels for topical drug delivery. Proniosomal gels are translucent gels and liquid lamellar crystals of vesicular bilayers which can be formed by the addition of small quantity of gelling agent or water to the dry Proniosomes (mixture of non-ionic surfactant, lecithin and cholesterol). Proniosomal gels offer better resistance towards stress caused by skin flexion; mucocilliary movement and better percutaneous absorption due to non-ionic surfactants are used. Because of their high stability, ease of application and better percutaneous absorption they are widely used for various category of drugs such as antifungals, NSAIDS, antihypertensive etc. As Proniosomal gels offer good attention towards the topical drug delivery, present review focuses on its preparation methods, applications and recent developments.

KEYWORDS: *Coacervation phase separation; Non-ionic surfactants; Proniosomal gel; Topical drug delivery.*

INTRODUCTION

Proniosomes are¹ the prove secular drug delivery systems which are defined as the dry formulations coated with carriers such as non-ionic surfactants. Proniosomes can be converted into niosomes upon hydrating with hot water right before use. As niosomes are associated with various drawbacks such as physical instabilities like fusion, aggregation of particles and leakage of the drug these are formulated into Proniosomes. Proniosomes can deliver both the hydrophilic and hydrophobic drugs. The principal advantage of Proniosomes is that amount of carrier required for maintaining the surfactant ratio can be easily adjusted. Proniosomal gels are the very recent prove secular drug delivery systems which offer the drug delivery through topical or transdermal route in a versatile manner. Proniosomal gels are a mixture of non-ionic surfactant, lecithin and cholesterol¹.

Formulation of proniosomal gels²

The essential components of proniosomal gels are as follows

Non-ionic surfactants

The non-ionic surfactants used in the formulation of proniosomal gels are selected based on the HLB value of the surfactant as the entrapment efficiency of drug is affected by:

- The HLB value: The HLB value between 4-8 will usually give the vesicles with high compatibility.

Phase transition temperature

Spans 40 & 60 produce the vesicles with high temperature efficiency and the leakage of drug from the vesicles is also reduced due to high transition temperature. The geometry of the vesicles can be predicted by the critical packaging parameter of the surfactant which must be between 0.5-1 for spherical vesicles.

Lecithin

In the formulation of proniosomal gels lecithin acts as a permeation enhancer and it enhances the % entrapment of the drug due to high phase transition temperature. Based on the penetration capability soy lecithin acts as a good permeation enhancer.

Cholesterol

Cholesterol is one of the important components in the formulation of Proniosomes which impart stability and permeability to the vesicles. The entrapment efficiency of the drug depends on the concentration of the cholesterol used. On the further increasing the concentration of cholesterol the disruption of the bilayer takes place and the entrapped drug leaks out.

Aqueous phase

The most widely used aqueous phases in the formulation of Proniosomes includes hot water, 0.1% glycerol and phosphate buffer of pH7 etc. The pH of the aqueous phase plays an important role in the entrapment of the drug.

Solvent

The selection of the suitable solvent plays an important role in formulating the Proniosomes as they affect the vesicles size and drug permeation rate of the drug. The size of the vesicles depends on the type of solvent used and the order is as follows:

Drug selection criteria

The drugs with the following criteria are suitable for the formulation of

Proniosomal gels;

Low aqueous solubility, High dosage frequency, Low therapeutic index and drugs with more adverse effects².

Methods of preparation of proniosomal gel³

- a) Coacervation phase method.
- b) Slow spray coating method.
- c) Slurry method.

Coacervation phase separation

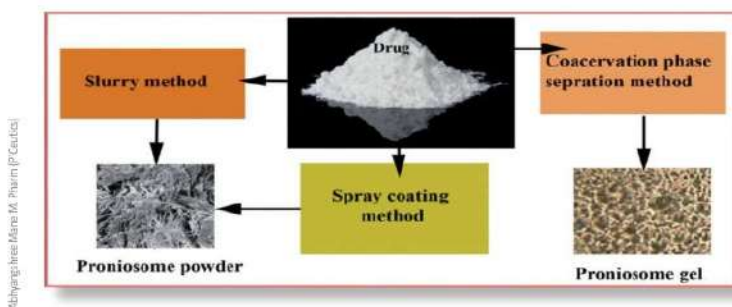
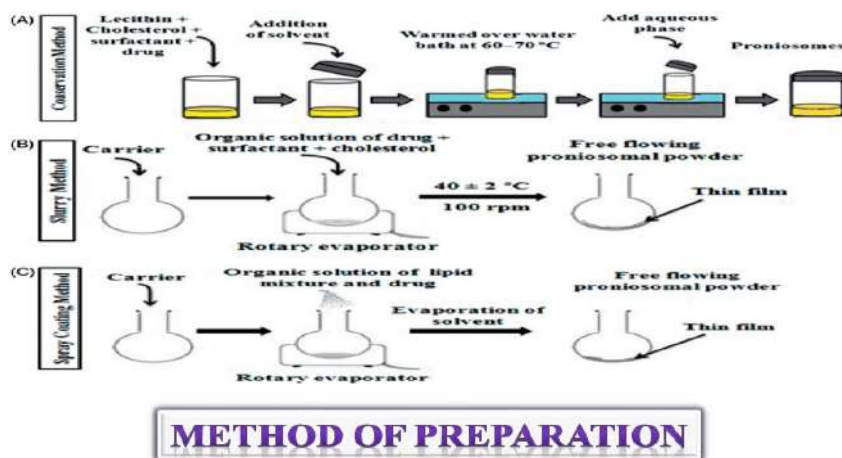
Proniosomal formulae were prepared by a method reported by Alsarra et al. with slight modification, using different types of non-ionic surfactants, lecithin, and cholesterol. Appropriate amounts of proniosomal components mixed together with the drug were mixed with 2.5ml of absolute ethanol in a clean and dry, wide-mouthed glass tube. After mixing all the ingredients, the open end of the glass tube covered with lid to prevent loss of solvent from it and warmed in a water bath at $65 \pm 3^\circ\text{C}$ for 5 min, until the surfactants were dissolved completely. Then, 1.6ml of pH of 7.4 phosphate buffer was added, and warming on the water bath for ~2 min till a clear solution was observed. The mixture was allowed to cool at room temperature until the dispersion was converted to proniosomal gel.

Slurry method

Carrier material to a 250-ml flask and the entire volume of surfactant solution was added to the flask to form slurry. If the surfactant solution volume is less, then additional organic solvent can get slurry. The flask was attached to a rotator evaporator was applied until the free-flowing. The flask was removed from the evaporator and kept under vacuum overnight. The Proniosomes powder was stored in sealed containers at 4°C . The time required to produce Proniosomes is independent of the ratio of surfactant solution to be carrier material and appears to be scalable.³

Slow spray-coating method

This method involves preparation of Proniosomes by spraying surfactant in an organic solvent onto carrier material and then evaporating the solvent. Since the carrier is soluble in the organic solvent, it repeats the process until the desired has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar when the carrier dissolves. The resulting noisome is very similar to those produced by conventional methods and the size distribution is more uniform. It is suggested that this formulation would provide hydrolysis for hydrophobic drugs formulation³.



Mohammad A., Abdul A., Ibrahim M., 2016, Technology overview & drug delivery application of proniosomes, Pharma Dev Technol., 1-10. 14

EVALUATION⁴

Scanning electron microscopy

Proniosome powder is affixed to double sided carbon tape, positioned on an aluminium stub, and excess powder is removed. The stub is stored under vacuum overnight. The samples are sputter-coated with Au/pd under an argon atmosphere at 180 mA for 1 min. Electron micrographs are obtained using a field emission SEM operating at 1 or 2 kV.

Transmission Electron Microscopy

The monography of hydrated niosome dispersion is determined using transmission electron microscopy. A drop of niosome dispersion is diluted 10-fold using deionised water. A drop of diluted niosome dispersion is applied to a carbon coated 300 mesh copper grid and is left for 1 min to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion is removed by absorbing the drop with the corner of a piece of filter paper. After twice rinsing the grid a drop of 2% aqueous solution of uranyl acetate for 1sec. The remaining solution is removed by absorbing the liquid with the tip of piece of filter paper and the sample is air dried. The sample is observed with a JEOL 100 CX transmission electron microscope at 80kv.

Angle of repose

The angle of repose of dry Proniosome powder is measured by a funnel method. In this method, the funnel is fixed at a position so that the 13mm outlet orifice of the funnel is 10cm above a level black surface. The powder is poured through the funnel to form a cone on the surface, and the angle of repose is then calculated by measuring the height of the cone and the diameter of its base.

Particle Size and Particle Size Distribution

A small aliquot (100 microliter) of niosome dispersion is dispersed in 50ml of water and is measured immediately with a Sald-1100 Laser Diffraction particle size analyser. The particle size range is set to 0.1-45 micrometre and the refractive index range is set to 0.2-1.7 micrometre. The particle size distribution of niosome is calculated internally.

Release of Drug from Proniosome

In vitro release is determined using dialysis tube. One end is sealed with cellophane membrane. Measured

amount of niosome are placed in the dialysis tube. Dialysis tube is placed into a beaker containing simulated fluid at 370. Beaker is placed on magnetic stirrer and is shaken at 100rpm at temperature of 370. Aliquots are withdrawn from receptor compartment at certain time intervals. The drug is withdrawn samples is estimated by the suitable analytical method⁽⁴⁾.

CONCLUSION

Proniosomes provide a promising drug delivery with great potential towards physical and chemical stability, ease of preparation and economical viability. They had attained a great attention for the delivery of drugs through topical and transdermal routes because of their nontoxicity, penetration enhancement due to surfactants. As proniosomes are in the form of vesicles and the components like non-ionic surfactants are used in the formulation of proniosomes itself acts as permeation enhancers and penetrate readily into the skin. Proniosomes in the dry form makes the possibility of unit dosing by converting them into beads, tablets, capsules and gels. Thus they provide an open platform to explore their suitability for drugs with various drawbacks to provide an effective and intended therapy.

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BIOSENSORS AND ITS MEDICAL APPLICATIONS

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ABSTRACT

The early disease diagnosis is key for patient survival and successful prognosis of the disease. In recent years the demand has grown in the field of medical diagnostics for simple and disposable devices that can also demonstrate fast response times, are user-friendly, cost-efficient and are suitable for mass production. Several types of biosensors have been known that have been successfully employed in the fields of environment, biomedical and food industries to detect and remove contaminants both living and non-living entities. Amperometric, Optical, Surface Plasmon Resonance, enzymatic, DNA, Phage and Bacterial biosensors are the sensors that are being employed today. These biosensors can also detect the broadband spectrum of biological analytes and have shown greater responses and success in medical laboratories, food bioanalysis, microbial detection etc. A sensor contains a specific binding site that could respond to a specific sort of an analyte in the medium. An analyte or triggers a chemical interaction at the site which initiates the conversion of information into an electrical signal. Biosensors have been widely used in different medical devices due to their ease of use and end point applications. Biosensors can be used for accurate and precise detection of tumours, pathogens, elevated blood glucose levels in diabetic patients and other toxins etc. Biosensors are environment friendly and efficiently detect pollution in air and presence of pathogens, heavy metals etc.

KEYWORDS: *Transducers, Bio analyze, Biosensors, Phase sensor, Surface Plasmon Resonance, Amperometric sensor.*

INTRODUCTION

A biosensor is a compact, reusable, user friendly portable device that provides accurate and quantifiable biologically active analyte information. The term Biosensor was first coined by Cammann in the year 1977¹. A Biosensor makes use of a specific biological recognition element such as a substrate that reacts with the enzyme present within the device in fixed proportion to develop a corresponding electrical signal. The electrical signal generated is proportionate to the amount of substrate present in the biological sample and is interpreted by the Transducer and the sensor element present in the device to calculate the concentration of the said biological sample in the biological environment.² The basic design of a biosensor is shown in Fig.1. The biosensor basically consists of 3 essential components: Highly sensitive biological response component, the transducer, and the signal sensor processors.

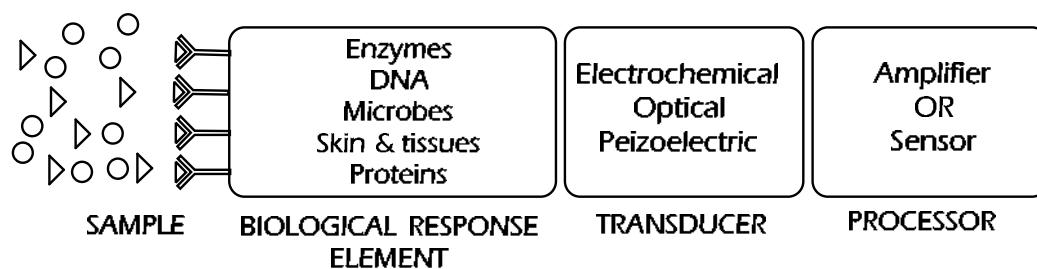


Figure 1: Basic Design of a Biosensor³

The research on biosensors is being pursued as one of the hottest research areas in the current era and has a promising future. The research in the area started almost half a decade back where the first Medical Biosensor was designed. In the year 1962, Prof. Clark of New York Academy of Sciences designed an enzyme based electrode for oxygen sensing during surgical procedures of the cardiovascular system. This development proved to be stepping stone for the development of subsequent biosensors where, interdisciplinary experts from areas such as Medicinal Chemistry, Physics, Biochemistry, Microbiology, Pharmacology, Botany, Zoology, Engineering and Medicine came together and shared their expertise in designing Biosensors. At present biosensors are widely used in the medical field for the quantification and detection of various disorders by analyzing the patient's biological samples. Disorders such as Cancer, Diabetes, Genetic deficiency, Gout, arthritis etc. are detected using biosensors. Among the Various types of biosensors enzyme based, tissue or skin based, immune sensors, DNA detecting biosensors, enzyme based biosensors are widely used. Blood Glucose sensors are now one of the common household devices for monitoring blood glucose levels in Diabetic patients. These biosensors are highly sensitive and reduce the sampling and report generation time apart from being user friendly and portable. The biosensors are very user friendly and it does not require a skilled person to handle the same. With the advent of improved technologies biosensors could be miniaturised and would become a common house hold device and an essential tool from the field of medicine and technology.

COMPONENTS OF BIOSENSORS⁴

Analyze

The substance of interest that needs detection, For instance, glucose is an 'analyte' in a biosensor designed to detect glucose.⁵

Bio receptor

A molecule that specially recognises the analyte is known as a bio receptor. Enzymes, cells, apt meters, deoxyribonucleic acid (DNA) and antibodies are some examples of bio receptors. The process of signal generation (in the form of light, heat, pH, charge or mass change, etc.) upon interaction of the bio receptor with the analyte termed bio-recognition.^{6,7}

Transducer

The transducer is an element that converts one form of energy into another. In a biosensor, the role of the transducer is to convert the bio-recognition event into a measurable signal. This process of energy conversion is known as signalization. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte-bio receptor interactions.^{8,9}

Electronics

This is the part of a biosensor process the transducer signal and prepares it for display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analog into the digital form. The processed signals are then quantified by the display unit of the biosensor.⁹

Display

The display consists of a user interpretation system such as the liquid crystal display of a computer or a direct printer that generates numbers or curves understandable by the user. This part often consists of a combination of hardware and software that generates results of the biosensor in a user friendly manner. The output signal on the display can be numeric, graphic, tubular or an image, depending on the requirements of the end user.¹⁰

BASICS OF BIOSENSING

Both biosensors and biomedical sensors fall into two general categories: Physical and chemical, Physical parameters of biomedical importance include pressure, volume, flow, electrical potential and temperature, of which pressure, temperature and flow are generally the most clinically significant and lend themselves to the use of *in vivo* sensors. The species can vary in size from the hydronium ion to a live pathogen; when the analyte is complex, an interaction with another biological entity may be required to recognize it. In general, it is necessary to distinguish this chemical species from a number of similar interferents, which can be technologically challenging, but this is an area in which bio sensors excel.¹

Biosensors for point of care testing^{12,13}

One of the most important applications of biosensors is the point of care testing. Point of care testing is the practice of performing a diagnostic or prognostic test near the patient to provide rapid results, meaning that the test itself has to be quick and easily performed without the use of expensive or complicated instrumentation. It also means that samples do not require the attention of any skilled technician as there is no need for the laboratory analysis as well as no wait time for the collection and analysis of the results. The person who is conducting the test initiates the test and receives results on the spot thus saving time. The PCOTs are feasible in various environments; in general, these are practitioner's surgery, hospital clinic, hospital ward, intensive care unit, or even patient's home. Accurate and early diagnoses play a crucial role in identifying the actual cause and nature of any disease. Millions die each year in a populated country like India, China due to infectious diseases like malaria, AIDS and tuberculosis. These developing countries are still struggling for access to treatment options available in developed countries. PCOT and its ability to generate immediate results in non-laboratory settings support more patient-centered approaches for health-care delivery and is a prospect in the field of early detection.

GLUCOSE SENSORS

Initially the Biosensor technology was focussed on monitoring blood glucose levels, due to a large number of diabetic patients. Major advances have been made since the first commercial biosensors leading to many simple self-tests that give patients the ability to control their blood sugar. Blood glucose monitoring reduces the risk of developing complications with diabetes and allows diabetics to see if the insulin and other medications they are taking are working. It gives diabetics an idea as how to exercise and food affects their blood sugar and may prevent hypoglycaemia or hyperglycemia.¹⁴

Biosensors and its applications in cancer

In most of the cases of cancer the analyte to be measured in question is a tumor biomarker. Therefore, by measuring the levels of certain proteins that are highly expressed by tumor cells biosensors can accurately detect the presence of a tumor. The best part of these biosensors is that they can detect multiple biomarkers and hence can address the detection of multiple types of tumors. The use of biosensor would not only reduce time in the detection of cancer but also reduce the cost of treatment by the administration of specific drugs for the diagnosed tumor. A biosensor is made up of three components: a recognition element, a signal transducer, and a signal processor that relays and displays the results. The molecular recognition component detects a 'signal' from the environments in the form of an analyte, and the transducer then converts the biological signal to an electrical output.¹⁵

Bio sensing Elements

Bio sensing elements are a set of biological entity, those that are capable of carrying out specific group reactions or can bind with particular group of compounds to yield a detectable signal that is read and transformed by the transducers. Commonly used Bio sensing elements are of two types namely catalytic type and affinity type. The catalytic sensors include enzymes, microbes, organelles, cells, or tissues. The affinity type sensors are antibodies, receptors and nucleic acids.¹⁶

Interferometric reflectance imaging sensor

The Interferometric reflectance imaging sensor is based on the principle of optical interference and consists of a silicon-silicon oxide substrate, standard optics, and low-powered coherent LEDs. Interferometric reflectance imaging sensors has been adapted to perform various functions. First IRIS integrated a fluorescence imaging capability into the Interferometric imaging instrument as a potential way to address fluorescence protein microarray variability. Briefly the variation in fluorescence microarrays mainly derives from inconsistent protein immobilization, on surfaces and may cause misdiagnoses in allergy microarrays. To correct from any variation in protein immobilization data acquired in the fluorescence modality is then normalised by the data acquired in the label-free modality. IRIS has also been adapted to perform single nanoparticle counting by simply switching the low magnification objective used for label-free biomass quantification to a higher objective magnification.¹⁷

DNA Biosensors

In the future, DNA will find use as a versatile material from which scientists can craft biosensors. DNA biosensors can theoretically be used for medical diagnostics, forensic science, agriculture, or even

environmental clean-up efforts. No external efforts are needed for DNA-based sensing devices. This is a significant advantage. DNA biosensors are complicated mini-machines consisting of special elements, micro-lasers and a single generator. At the heart of DNA, biosensor function is the fact that two strands of DNA stick to each other by virtue of attractive chemical forces. On such a sensor, only exact fit—that is, two strands that match up at every nucleotide position gives rise to a fluorescent signal that is then transmitted to a signal generator.¹⁸

Cardiac biosensors¹⁹

Cardiovascular disease is not a single disease but is a group of disorders that affect heart and blood vessels. Cardiovascular disease includes atherosclerosis condition that develops when a plaque build-up in the walls of the arteries. This plaque narrows the arteries and makes it difficult for blood to flow through and causes a heart attack or stroke. With regard to predicting cardiovascular disease risk, one of the active research areas recently is the use of diagnostic and prognostic biomarkers that can be identified in blood. On the basis of diagnostic and prognostic standpoint, CVD biomarkers can be categorized into pathogenetic and therapeutic types. There are several characteristics that an ideal cardiac biomarker should exhibit. They include High clinical sensitivity and specificity, Quick release of biomarker in the blood enabling early diagnosis, Capability to remain elevated for longer time in the blood, ability to be assayed quantitatively. It is difficult to select a specific marker for the diagnosis of the CVD. Therefore a range of biomarkers can potentially be analyzed simultaneously for the accurate disease diagnosis.

CONCLUSION

Treatment for a particular disease relies heavily on the early detection of the disease; where Biosensors play a major role. The use of new and upcoming biological response elements and various sensory transducer elements have improved the sensitivity of sensitivity and accuracy of the Biosensors. Biosensors play a major role in medical diagnostics. Biosensors are still plagued by the ever-present problem of biocompatibility since various types of analytes are being used for diagnosis *in-vivo*. The future of biosensors looks bright considering the fact that more technologically advanced biosensors are under development that would eliminate the biocompatibility issues associated with a variety of analyte samples.

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ROBOTICS IN PHARMACY

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ABSTRACT

Robotics is the branch of technology that deals with the design, manufacturing field, operation and applications of the robot. The first ABB robot, it was equipped in 1974 in the automatic field. Traditional robots are further segmented into articulated, scara, delta/parallel, cartesian and dual arm. Articulated robots hold majority market share owing to their capability to check the packed products at the manufacturing plant. Scara has a dominant market share in this market during the forecast period because the machines in use are relatively cheaper than ones in use in other categories. North America solely accounted for nearly 34% in primary pharmaceutical packaging vertical espousing exponential growth, with robots as the primary helping arm as per association for packaging and processing technologies. The Current state of the industry is that a tablet counter has become a standard in more than 30,000 sites in 35 countries. By the automation in pharmacy, will reduce filling errors lead into efficiencies in dispensary throughput and turnaround times and avoid contamination by storing each different drug separately. Robots increases workflow and energy; it can create greater benefits. The very real possibility of cross contamination during automated pill counting is becoming an increasing safety concern. Manufacturers of the semi automated and fully automated, (or) robotic, counting technology used in many retail and most hospital pharmacy say they consider cross contamination a major issue. It's so important, in fact that system design is used as a marketing tool in a highly competitive field. Parata RDS. System is fully automated, with a special unique feature. It is the only system that automatically caps a vial, which enhances from cross contamination. One of the biggest benefits of these robots are faster service and also can operate 24 hours a day 7 days a week without a dip inaccuracy. Robots could speed up drug development. Productivity is increased because of skills and energy of robots.

KEYWORDS: *Robotics, ABB, Dispensing in pharmacy, Industrial robots, Drug development*

INTRODUCTION

Pharmacy automation involves the mechanical process of handling and distributing medication. Any pharmacy task may be involved, including counting small objects (tablets, capsules), measuring and mixing powders and liquids for compounding, tracking and updating customer information, medical history, drug interaction, and inventory management. Robots perform tasks at a rate beyond human capability. These robots' function in probably difficult settings in closeness to biological dangers, the risk of radioactive contamination and toxic chemotherapy compounds. By the robotics in pharmacy will reduce filling errors, fill more prescriptions in less time, and increase patient safety and staff productivity. TCGRX is a leading supplier of pharmacy automation equipment for the long-term care, hospital, retail, nutraceutical, outpatient and central fill – pharmacy market; TCGRX offers range from simple packing technology to enter price level and inventory management.

HISTORY

Before 1970's medication was a time-consuming process for pharmacist and their staff. With increased workloads pharmacists' staff was working long hours in less time to focus on the patient care. The new technology replaces manual methods in many industries. In 1980's and 1990's the development of high-speed machines for counting and storing occurred. These units were designed to be fast and simple to operative. From 1990's through 2012 new pharmacy automation products come to market, shift from entry level product to the next level of pharmacy automation.

A natural progression from the electronic tablet counters introduced in the 1970's optical light beam technology developed for dispensing tablets in 1980's. As they are of the robotics in the pharmaceutical

industry increases rapidly, it's important to consider the benefits of the latest technologies for manufactures, researchers and scientists.

ADVANTAGES

- Pharmacy automation systems increased ability improve the patient care and increase the productivity, they can be a major time saver for the pharmacy. Dispensing robots offer faster service, customers do not have to wait as long to receive medication and the machine can fill a much faster than human can.
- Robots can increase pharmacy work flow and energy; it can create greater benefit for you and your staff. It can distribute resources to the other tasks, you can decrease the time spent on tedious tasks and increase the time spent in duties human must perform.
- The pharmacist had to spend a long time manually counting, filling, capping and labeling patient prescriptions. Now dispensing robots can perform this endless task, pharmacy automation helps the pharmacist to spend more time with patients and offer better care.
- Automation reduces dispensing errors leading to efficiencies in dispensary through put and turnaround times, enabling of re-engineering of pharmacy services, which might include developing a ward-based medicines management service and distributing the clinical pharmacy service.
- Dispensing robots offer high safety, pills that look alike may be easily confused or miscounted by pharmacy staff, the automated machines keep the pills and medications in a sterile environment that prevents the occurrence of medication contamination.
- Robotic pharmacy can increase your business's profits; it can create new earnings streams for your pharmacy. It can implement your pharmacy to add an additional location where you might never be able to start a full-service pharmacy, updating your payment process to include mobile pay or chip card that can help you a younger analytical.
- Pharmacy automation system helps you avoid errors and contaminations, robotics systems store each different drug in separate cells to avoid cross-contamination.
- Speed: customers do not have to wait as long to receive medications one of the biggest benefits of these machines are faster service.
- Customer services: By the allotment of robots to fill prescriptions, there by pharmacist may be free to talk to patients about their medications, administration, immunization and interactions. So, we can increase the patient life span.
- Security: We should lock or keep the medicines in automated dispensing pills that can be tracked by the machine's software only. All are not trained in how to use, open and restock the machines so robotics plays an important role in security also.
- Variety: There are different kinds of automated dispensing machines are present either older machine or newer machines. Older machines are not reliable with newer robots or machines. Newer robots perform
- Failure: To reduce human errors in pharmacy and pharmaceutical industries robots play an important role. There are perfect and very quick in actions.
- Software problems: To avoid errors in future installing software updates hooking the medicines to the pharmacy must be done.
- Capacity: The robot can store up to 35000 medicines and dispense around 12 prescriptions in less than 1 min. It will significantly reduce a person waiting time
- Benefit of design: Robots well suited for pick and place and assembly work in pharmaceutical environment.
- Reduces waste: Pharmacy automation not only get help to pharmacist, nurses and patients connected more efficiently; it will eliminate medication waste in filling labeling prescription.
- Safety: Robots protects the pharmaceutical products and health of employees and patient. And they mix toxic elements or chemicals safely. Robots work very cleanly and in the sealed arm construction to avoid contamination.
- Energy: A robot can perform a 96-man hour's project in 10 hours. With more consistency and higher quality results.
- Reliability: Robots minimize accidents and wasted materials.
- Return on investment (ROI): By the robots there is quick turn around and with the increase in quality, speed and benefits.
- Accuracy: Robotic systems are more accurate and consistent when compared with human beings. Robots weigh the ingredients accurately and prepare the dosage forms without contamination.

- Cost: Pay back for the purchase of robotic equipment in the pharmaceutical industry. Robots available in less price also.
- Can work continuously in any environment: Other advantages in the laboratory as that robots are impervious to many environments that would not be safe for humans. A robot can operate 24 hours a day, 7 days a week without dip in accuracy.

DISADVANTAGES

- Dangers and fears: Although current robots are not believed to have developed to the stage where they arrange any danger to society fear and disturb or worry about robots have been repeatedly suggest in a wide range of books and films. The principle theme is the robot's intelligence and ability to act could better than humans that they could develop a duty and motivation to take over or destroy the human race.
- Expertness: Employees will require training in programming and interacting with the new robotic equipment. This normally takes time and financial output.
- Safety: Robots protect workers from some risks but in the mean while create other safety problems. These dangers must be taken into consideration.
- The technology is available but regulation will place the growth of robotic pharmacies more than the technology.
- Although robotic pharmacies can reduce human errors it is important to point out. The fact that machines also commit the errors. Besides cost robotic pharmacies still have a lack of solutions for some type of products such as blood derivatives, flammable and cytotoxic drugs. So, some robotic pharmacies still need conventional static shelving, and considering make robotic pharmacist an unattractive option for many health services.
- Robotic pharmacy technologies are expensive without a doubt. Therefore, the loses of this technology would be staff that would suffer reduction to balance this price of the technology however there will be more losers to the technology after it is paid for. Robotic pharmacy can enhance precision and accuracy but robotics is not trained to explain medication interactions. Robotics can cause harm in the case of fault if the pharmacy heavily depends on automation, the system breakdown could less work productivity.
- Mistakes: Unfortunately, automated pharmacy dispensing machines can still make mistakes and if the wrong drug is loaded into the machine. Many customers can receive wrong medication before the problems are identified.

APPLICATIONS

- Automation dispensing robots never get bored, never get distracted and make far fewer mistakes than their human counterparts. And in this era of ever shrinking prescription margins, dispensing robots free up pharmacists and technicians for more profitable clinical services that require human judgment.
- Reduced costs and wastes: Without pharmacy automation systems, a pharmacist who draws 3 grams from a 10-gram vial has to discard what's remaining because it cannot be used again. An automated system is sterile and able to draw from the same vial twice. This results in reduced waste and substantial long-term savings
- Enhanced safety: By pharmacy automation system, errors and contamination very unlikely.
- Safety and quality: Reduction in dispensing errors. Staff released from dispensary for direct patient care supporting new clinical pharmacy model.
- Financial: Reduced stock holding. Improved stock rotation and reduction in expired stock wastage.
- Process efficiencies: Faster dispensing process to reduce patient waiting times. Out of hour's activity. On call remote working. Improved space utilization.
- Robotic dispensing machines safeguard the patient by helping to eliminate the risk of human error. And because these systems are now capable of meeting the demands of high daily prescription volume, pharmacies benefit greatly as well. Most robotic systems take advantage of numerous safeguard to ensure the right medicine gets to the right patient in the correct dosage every time. By using barcodes, advanced workflow systems, and reporting capabilities, pharmacies can make sure the whole process flows seamlessly from order entry to point of sale. This also allows technicians to minor safety standards for each prescription filled. These systems, if properly configured, also help to minimize the threat from contamination that can result air borne pharmaceutical dust, which some

machines can create. They also save workers from difficult environment and or repeated tasks. Using robots also removes the possible human contamination

- Specially made to meet project requirements. Robots and systems can be built to meet project requirements. Productivity Is increased because of the skill and energy of robots, which often perform at increased speeds and produce less fragment the slim, compact design of robots is beneficial when space is limited as well.
- Robots can meet the demand for observe with strict sanitation regulations. Clean room grade robots are available and are built specifically for clean room use and are designed to meet clean room requirements. These robots must minimize particular generation to maintain clean room classifications, typically, so class 5 or 6. Clean ability, including minimizing fracture and protect the robot is resistant to cleaning and sterilizing agent is also a requirement.
- Robotic dispensing machines are large storage containers that are computer controlled using a touch screen interface. They work much the same way vending machines do, dispensing medications to customers and staff according to their prescriptions. Koop's creates innovative design for any application and for afferent industries, including the pharmaceutical industry. The dispensing process will be paper free on the robot will store prescription as soon as a doctor document in electronically.

DIFFERENT TYPES OF PHARMACY AUTOMATION

1. **Counting scale:** This is a very simple counting machine. Scales are small to fit in any pharmacy set up and most are supplied with barcode scanners. They will take the vial, by placing it on the scale and pressing the corresponding button. This prevents the scale from counting the vial as part of the medication weight. Then, they will enter the proscription quantity and begin to fill the count will appear in the screen as the vial is filled.
2. **Tablet counting tool:** iphar machine offers an extremely accelerate automatic pill capsule and tablet counting machine of counting almost all shapes of the tablet, pill capsule, hard and soft capsule filling to bottle. Our counting machine will fulfill all your needs of tablets and capsule counting and filling. It is very accuracy and very efficiency in the pharmacy packing market.

Some of the more advanced models such as the

KL 1 PLUS: KL 1 plus Come with scanners and information screen. This allows us to scan our label and then scan the bottle to verify that we are distributing the right medication.

EYE CON: Eye con is a larger model, equipped with a tray, upward camera, and information screen. To use this model, a fill tech will pour pills onto the tray. The upward camera views these pills and tablets.

3. **Cabinet dispenser:** An automated dispensing cabinet (ADC) is a computerized drug storage device (or) cabinet for hospitals and many other healthcare settings. ADCs allow medications to be stored and dispensed near the point of care while controlling and tracking drug distribution. They are also called unit based cabinets (UBCs), automated dispensing devices (ADDs), automated distribution cabinet's (or) automated dispensing machine's (ADMs).
4. **Dispensing robot:** Robot dispensing system can count pills, cap bottles, label the prescription vial, store hundred of medications, and access the patient information to ensure storage containers which are compute r controlled using a touch screen interface. They work much the same way vending machines do, they can dispense medications to customers and staff according to their prescriptions. A prescription will be automatically filled by the robot when we make a fill in our software. The robot will read the prescription information and select the correct medication from its storage bins.
5. **Packaging robot:** These robots are strong for elderly and correspond patients who take many medications. Pharma robots are invading the packaging line, a PMMI business intelligence study showing the robotic applications in pharma packaging are still on the rise, but some manual operations remain. "A new way to decontaminate nested pre filled container tubs", a report on how by incorporating a robot arm, a machine builder is bringing. The benefits of pulsed light to the world of pre filled tub sterilization for syringes, vials and cartridges. " Merck expands packaging capacity in Switzerland ", in which the company's Aubonne manufacturing sites uses robotized logistics and automated equipment to support the growth of its current and future portfolio of biotech medicines.

ROBOTS ACCELERATE DRUG DISCOVERY

Robot scientist capable of screening possible drugs almost completely individually could speed up drug development. The access has already described some talented leads, including an anticancer compound which also shows anti-malarial properties. A corner stone of the initiative is NiCoLA-B, a drug discovery robot at the U.K. center for lead discovery, the company's research center on the Cambridge campus. Its name is a variation of the monitor for the entire robot system Co LAB, which was developed by high Res bio solutions. The robot can test more than 300,000 compounds a day in a ballet of procedures with a central, mechanical arm as the lead dancer: the company says it is the fastest of its kind in the world. The robot uses sound waves to move droplets of potential drugs, billionths of liters at a time, from storage tubes into miniature wells on assay plates. Droplet's of cells (or) biochemical solutions are added to the wells, and the robot monitors interactions for any activity that could indicate a promising drug. It works 24 hours a day, seven days a week so scientists don't have to, though they can interact with (or) easily reconfigure it at any time though an app. Intelligent motion and load sending features ensure safety even with direct user interaction. The system can slow (or) stop if it detects resistance (or) other changes to its environment. This makes it both quicker and cheaper than standard drug screening methods. The system already has proven successes, highlighting several drug candidates that could be repurposed, for example, it showed the anti-cancer compound TNP – 470 can also attack the malaria parasite 'plasmodium vivax' by inhibiting an essential enzyme.

CONCLUSION

Incorporating robots into pharmaceutical process has many advantages. Robots can perform operations 3 or 4 times faster than humans and can robots good at producing large quantities of products efficiently. Conclusion implementing pharmacy automation technologies in an outpatient (or) community pharmacy setting may reduce medication errors, increase productivity and save costs but does not affect staff satisfaction. However, the available lite nature remains patchy and more research in this area is needed.

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MECHANISMS OF RIFAMPICIN RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Tuberculosis is caused by *Mycobacterium tuberculosis* (MTB) and is a major health problem in many developing countries due to its rapidly developing resistance against anti-tubercular drugs. In the year 2018, 10 million people felt ill with TB, and 1.5 million died from the disease. Rifampicin is the most frequently used for anti-tubercular drug. Rifampicin acts by inhibiting the DNA dependent RNA polymerase resulting in the blockage of RNA transcription. The resistance to rifampicin develops through various mechanisms such as genetic mutations, cross resistance and metabolic resistance. Majority of the rifampicin-resistant clinical isolates of MTB include mutations in the rpo B gene that codes for the specific β subunit region of the RNA polymerase enzyme. Due to the above gene mediated conformational changes in the specific β subunit region of the RNA polymerase enzyme Rifampicin develops reduced affinity towards it resulting in development of resistance. Majority of the mutations in rpo B affects both the stability and the drug binding affinity of the protein resulting in development of resistance towards Rifampicin. The cross resistance between the rifampicin and rifabutin is also observed, and some mutations in the specific codons are associated with the low level resistance to rifampicin, however, they are susceptible to Rifabutin and Rifalazil. The metabolomics resistance approach explains about the importance of metabolomics markers that attributes rifampicin resistance due to instability in MTB mRNA, induced by the mutation in the rpo B gene. As a result of the rpoB mutation the total depletion the acotinic acid which is responsible for the functioning of the ribosome negatively influences the stability and transcription of mRNA. The current review is focused on getting a better insight into the mechanisms of drug resistance of Rifampicin and would help in the designing of new and improved drugs, the selection of improved drug targets and identification of new drug targets.

KEYWORDS: *MTB-mycobacterium tuberculosis, rifampicin, isoniazid, resistance, mechanism, DNA dependent RNA polymerase.*

INTRODUCTION

Tuberculosis is caused by *Mycobacterium tuberculosis* (bacteria). Despite being a controllable, preventable and curable disease, TB still remains a major cause of morbidity and mortality in several developing countries. Worldwide, around 10million people fall ill with tuberculosis each year. In 2018, 10 million people felt ill with TB, and 1.5 million died from the disease¹. The global TB reports2019 provides a up to date analysis of TB epidemic and progress at global, scale². Outbreaks of extensively drug-resistant tuberculosis has also been an increasing in threat regions around the world³. The drugs available for the treatment are categorized into first-line drugs including isoniazide (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol (EMB), and streptomycin (STR) and second line drugs including fluoroquinolones, thioamides, cycloserine and aminoglycosides. Rifampicin belongs to the rifamycin group and it is a bacteriocidal antibiotic drug introduced in 1972 as a antitubercular drug. Rifampicin in combination with isoniazide forms the backbone of chemotherapy short-course. And it is interesting to note that mono resistance to isoniazid is very common but mono resistance to rifampicin is very rare. Rifampicin acts by inhibiting bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase, thus blocking the RNA transcription^{4,5} but does not inhibit the mammalian enzyme. The recent increase in multidrug resistance (MDR-TB) and extensively drug resistance (XDR-TB) has further worsened the situation. Drug resistance in TB is occurs through 2 main mechanisms: (i) primary or transmitted drug resistance, occurs when strains are transmitted to a new host, and (ii) secondary or acquired drug resistance,

GENETIC MUTATIONS

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S450L: it is one of the most frequently occurring RFP-resistant mutations²⁶. The wild type S450 forms a hydrogen bond with rifampicin, but when it is mutated to leucine, the longer side chain causes steric clashes with the drug, reducing its affinity towards the drug.

H445R and H445Y: The wildtype residue H445 forms hydrophobic interactions with D435, which are involved in forming hydrophobic interactions with RFP, while the mutated residue R445 cause steric clashes with D435.

D435Y and D435V: The wildtype residue D435 is located within 3.1Å⁰ and forms a salt bridge with R448 and R607. It also forms a weak hydrogen bond with R607 and rifampicin, and hydrophobic interactions with H445 and RFP.

RpoC

The wildtype residue I491 is involved in making hydrophobic contacts with L487, L516, P514 and F452. It also forms a carbon-pi contact with F452 and weak hydrogen bonds with the carbonyl oxygen of the residues P514 and L487. When mutated to threonine the carbon-pi and hydrophobic contacts are lost. Wildtype residue V483 is involved in making hydrophobic contacts with W484, L449, V476 and L460, and makes a carbon-pi contact with W484. When mutated to glycine, it loses all the interactions with the surrounding residues. Most of the mutations in rpo B affect both the stability and drug binding affinity of the protein. Thereby the resistance of drugs occur²⁶.

CROSS RESISTANCE

There are currently five generic brands of rifamycins marketed in various countries: those are rifamycin SV; RIF, rifapentine and rifabutin (all three are mainly utilized for the systemic treatment of mycobacterial infections) and rifaximin. One more rifamycin had been developed that is rifalazil, for various indications. Although there is clinical evidence that the relapse and acquired resistance rates in tuberculosis patients treated with RIF or rifabutin are similar,²⁶ there have been a number of reports suggesting incomplete cross-resistance between rifabutin, RIF and rifapentine. On the other hand, it is an important finding related to resistance to rifampicin has been proposed that resistance to rifampicin can be used as a surrogate marker for MDR-TB as nearly 90% of rifampicin resistant strains are also resistant to isoniazid^{25,26}.

METABOLOMIC RESISTANCE

Considering the metabolomics results, one of the most interesting observations is the total depletion of aconitic acid in both (R46&R190) the rifampicin resistance organisms. Normally aconitase in *M. tuberculosis* is known to serve a binary function: (i) the conversion of citric acid to iso-citric acid, with aconitic acid acts as an intermediate in the tricarboxylic acid (TCA) cycle during normal iron homeostasis; or (ii) binding to mRNA, stabilizing it and protecting it from degradation during low iron conditions or oxidative stress²⁵. considering that the rpo B mutation negatively influences the functioning of the ribosome and subsequently the stability and transcription of mRNA, supported by the elevated uridine detected in the R46 strain (most likely due to up-regulation of this strain's pyrimidine salvage pathway activity as a survival response)²⁶. The bacteria may be compensating for this mobilizing aconitase to bind to its mRNA in an attempt to stabilize it. Subsequently decreasing the amount of aconitase for the synthesis of aconitic acid in the TCA cycle.

CONCLUSION

The present study explains about the mechanisms involved in developing resistance towards the rifampicin. In this study the resistance is mainly develops through the 3 main mechanisms they are listed as genetic mutations, cross resistance and metabolomics resistance. In genetic mutation, the rifampicin resistance in MTB is mainly occurs due to the mutation in rpo B, the β subunit of DNA- directed RNA polymerase of MTB. The mutations in particular codons 516, 526, 531, 451 (S450L) and 445 (H445Y) are the most frequently associated mutations in the rifampicin resistance. In the next mechanism explains about the cross resistance, here the cross resistance between rifampicin and rifabutin occurs. Some mutations in the codons 516, 518, 522 and 533 had been associated with low-level resistance to rifampicin but susceptibility to rifabutin and rifalazil. Moreover, the final mechanism involved is metabolomics resistance; the metabolite markers identified indicate instability in rifampicin resistant MTB mRNA, induced by the rpoB mutation.

This results in a total depletion of aconitic acid, due to the aconitase functionality towards mRNA binding and stability, and away from energy production and growth, a subsequent increased dependency on alternative energy sources, fatty acids in particular. A number of other metabolic changes were also observed, confirming an additional survival response for maintaining/ remodeling the cell wall. The above mentioned mechanisms provide a better and updated understanding of the mechanisms of rifampicin resistance.

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FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF METAPROLOL SUCCINATE

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ABSTRACT

According to the (WHO), hypertension is the most common cardiovascular condition in the world and there are about 600 million people at risk for heart attack, stroke and cardiac failure. High BP is estimated to cause 7.1 million deaths, about 13 percent of the global fatality total. It is believed this number will grow to approximately 11million by the year 2020. Metoprolol is abetal-selective (cardioselective) adrenergic receptor blocking agent. In this investigation metoprolol succinate floating microspheres were prepared by using ethyl cellulose, and hydroxyl propyl methyl cellulose as polymers. Incorporation of pH modifier has been the usual strategy employed to enhance the dissolution of weakly basic drug from floating microspheres. Microspheres prepared using a combination of both ethyl cellulose (EC) and hydroxypropyl methylcellulose (HPMC) which shows highest release were utilize to investigate the effect of fumaric acid (FA), citric acid (CA), ascorbic acid (AA) and tartaric acid (TA) (all 5-20% w/w) incorporation on metoprolol succinate (MS) release. The Formulation of Metoprolol Succinate was prepared by using different polymer (HPMC) with different diluents (ethyl cellulose) and then evaluated. From the results it was found that the release of drug (HPMC k 100M as polymer) gave the better release than other formulations.

KEYWORDS: Metoprolol succinate, ethyl cellulose, dichloro methane, ethanol ,hydroxypropyl methyl cellulose.

INTRODUCTION

Oral administration is the most convenient mode of drug delivery and is associated with superior patient compliance as compared to other modes of drug intake. However, oral administration has only limited use for important drugs, from various pharmacological categories, that have poor oral bioavailability due to incomplete absorption and/or degradation in the gastrointestinal (GI) tract. Some of these drugs are characterized by a narrow absorption window (NAW) at the upper part of the gastrointestinal tract.

METHODOLOGY

1. Estimation of Metoprolol succinate

1.1 Determination of $\lambda_{\max}$ of Metoprolol Succinate in 0.1N HCl

Stock solution: Metoprolol succinate in 0.1N HCl (100 mg in 100 ml). Scanning: From the stock solution 10 $\mu\text{g/ml}$ solutions of metoprolol succinate is prepared in 0.1N HCl and scanned between 200-400 nm. The absorption maxima of 221 nm was selected and used for further studies.

1.2 Standard calibration curve of Metoprolol succinate in 0.1N HCl

The calibration curve data of Metoprolol succinate in simulated gastric fluid pH 1.2 at 275nm, the standard calibration curve with a regression value of 0.993, slope of 0.0292 and intercept of 0.0097 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25 $\mu\text{g/ml}$.

1.3 Preparation of Metoprolol succinate loaded floating microspheres

The floating microspheres loaded with Metoprolol succinate were prepared by emulsion-solvent evaporation/solvent diffusion method using different polymers as follows:

1.3.1 Using EC polymer

The drug and ethyl cellulose were mixed in dichloromethane at various ratio and ethanol (10%) was added as plasticizer. The slurry was slowly introduced in to the solution of Sodium lauryl sulfate (0.25%, 250ml). The

resultant solution was stirred at 500 rpm for 1 hrs. This formed microsphere was filtered and dried at room temperature.

1.3.2 Using HPMC and EC polymers

Drug, HPMC and EC were dissolved in a mixture of ethanol and dichloromethane at room temperature. This was poured into 250 ml water containing 0.025% SLS maintained at 30-40°C and subsequently stirred for 45 min to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried overnight at room temperature.

Table 1: Formulations of metoprolol succinate loaded floating microspheres prepared using ethyl cellulose

Formulation code	Dispersed phase			Ethanol	Continuous phase
	Drug (mg)	EC (g)	Dichloromethane (ml)		
E ₁	250	0.50	15	10	0.25% SLS aqueous solution
E ₂	250	1	15	10	
E ₃	250	1.5	15	10	

Table 2: Formulations of Metoprolol Succinate loaded floating microspheres prepared using EC and HPMC

Formulation code	Dispersed phase				Continuous phase
	Drug (mg)	HPMC (g)	EC (g)	Ethanol : Dichloro methane(ml)	
HE ₁	250	0.50	1.0	1:2	250 ml water containing 0.025%w/v SLS
HE ₂	250	0.50	2.0	1:2	
HE ₃	250	0.50	3.0	1:2	

2. CHARACTERIZATION OF FLOATING MICROSPHERES

2.1. Fourier-transformation infrared (FTIR) spectroscopy

Fourier Transformation Infrared spectra of pure drug, polymer and drug loaded floating microspheres were obtained in KBr pellets at moderate scanning speed between 4000-200 cm⁻¹ in a Perkin-Elmer Fourier Transformation Infrared spectroscope.

2.2 Evaluation of floating microspheres

2.2.1 Yield of microspheres

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components, which were used for the preparation of the microspheres.

2.2.2 Particle size

The size was measured using an optical microscope under regular polarized light, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

2.2.3 Drug entrapment efficiency

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl and small quantity of methanol repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution with 0.1N HCl spectrophotometrically (UV-1700, Shimadzu, Japan) at 262 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by using the following formula:

2.2.4 Micrometric properties

The microspheres were characterized by their micrometric properties
Such as Angle of repose, tapped density, Compressibility index, and True density.

- (a) **Angle of repose:** The angle of repose θ of the microspheres, which measures the resistance to particle flow was calculated as:

$$\tan \theta = 2H/D$$

where $2H/D$ is the surface area of the free-standing height of the microsphere heap that is formed after making the microspheres flow from the glass funnel.

- (b) **Tapped density and compressibility index:** The tapping method was used to calculate tapped densities and compressibility index using tapped density:

$$\frac{\text{Mass of microspheres}}{\text{Tapped density}} = \text{Volume of microspheres after tapping}$$

$$\% \text{ compressibility index} = [1 - V/V_0] \times 100$$

Here, V and V_0 are the volumes of the sample after and before the standard tapping respectively.

- (c) **True density:** Density of hollow microspheres is determined by immersing the microspheres in 0.25% SLS solution in a metal mesh basket. The microspheres that are sunk after this process are used for density measurement as carried out by the displacement method.

RESULTS AND DISCUSSIONS

Table 3: Standard Calibration Curve of metaprolol succinate in 0.1N HCL

Concentration ($\mu\text{g/ml}$)	Absorbance
0	0
5	0.16
10	0.3
15	0.454
20	0.605
25	0.739

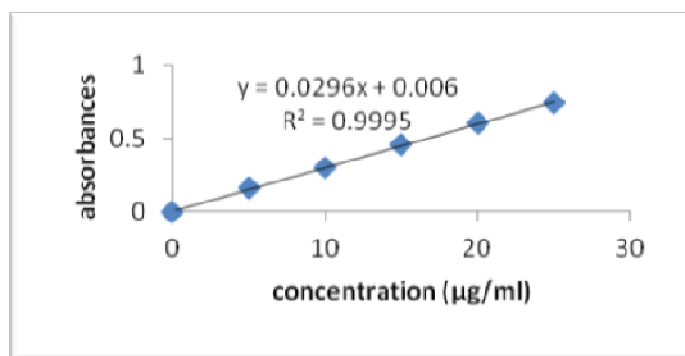


Figure 1. Standard Calibration Curve of metaprolol succinate in 0.1N HCL ($\lambda_{\text{max}}=275\text{nm}$)



Figure 2. photograph of metoprolol succinate Loaded floating microspheres of ethyl cellulose



Figure 3. Photograph of metoprolol succinate loaded floating microspheres Of EC-HPMC

DISCUSSION

Floating drug delivery system have a bulk density less than gastric fluids and thus it remains buoyant in the stomach without affecting gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from stomach. This results in an increased gastric residence time and a better control of the fluctuation in plasma drug concentration. Single unit formulations (floating tablet) are associated with problems such as sticking together or being obstructed in the gastrointestinal tract, which may have a potential danger of producing irritation.

CONCLUSION

The study conducted on "Development of metoprolol succinate loaded floating microspheres" reveals following conclusion:

- Metoprolol succinate loaded floating microspheres can be prepared using ethyl cellulose, HPMC-EC, porous polymers by solvent evaporation/ diffusion method. .
- The percent yield of all floating microspheres formulation was found to be in range of 67.48% to 94.39% suggesting that, the methods used for encapsulation was effective. The percent yield was significantly ($p < 0.05$) increases as the amount of polymer was increased in each preparation method.
- The mean particle size of floating microspheres was in the range of 128.23-389.72 μm depending upon the type and ratio of polymer used. The particle size increased significantly ($p < 0.05$) as the amount of polymer increased. The drug entrapment efficiency was in the range of 65.74 to 88.66 % suggested that optimized parameters were used in the method of preparations. The amount of polymer has significant effect on percent entrapment.
- The flow properties of all the prepared microspheres were good as indicated by low angle of repose ($\phi < 40^\circ$) and low compressibility index ($I < 25$). The good flow properties suggested that the microspheres produced were non-aggregated. The tapped density and true density was also less confirming porous nature of polymers.
- From the result it was observed that drug: polymer ratio influence the particle size, *in vitro* buoyancy as well as drug release pattern of floating microsphere. Hence, the multiple-unit floating systems of metoprolol succinate are expected to provide clinician with a new choice of safe and more bioavailable

formulation in the management of bacterial infections. The study reveals satisfactory results with a further scope of pharmacokinetic and pharmacodynamic evaluation.

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Conflict of interest

There is no conflict of interest.

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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF NEOLAMARCKIA CADAMBA FRUIT EXTRACT

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ABSTRACT

Neolamarckia cadamba has strong Anti-oxidant, Anti-fungal, Anti-helminthic, Anti-bacterial properties it plays a vital role in migrating mental disorders like depression. To evaluate invivo anti depressant activity of ethanolic extract of *Neolamarckia cadamba* fruit in swiss albino rats. For this *N.cadamba* was chopped, shade dried, pulverized with the help of electric mixer and were extracted by a continuous hot percolation method by using soxhalation apparatus. And for invivo anthelmintic activity, ethanolic and aqueous extract were prepared by soxhalation apparatus with dried powder of *N. cadamba*. The extract was subjected to phytochemical screening followed by acute oral toxicity studies in rats. In vitro experiments were done to determine anti oxidant properties *N. cadamba* extracts, the anti depressant activities of aqueous and ethanolic extract of *N.cadamba* were evaluated in rat models of depressions. Animals were divided into four groups (6 animals per group). Group 1 is served as a control group, Group 2 served as a standard drug (fluoxetine 4 mg/ml) respectively. Group 3 was given ethanolic fruit extract of *N.cadamba* orally at low dose of 40 mg/kg respectively. Group 4 were given fruit extracting of *N. cadamba* orally at high dose 80 mg/kg respectively. Following 14 days dilute dosing, all animals were tested using behavioural of depression on 15th day by using locomotor activity test, pole climbing apparatus, and tail suspension tests. And for invivo anthelmintic activity, ethanolic and aqueous extract of *Neolamarckia cadamba* were given to 9 groups of *pheretima posthuma* earthworms with different concentrations. In Locomotar activity, the low dose ethanolic extract gives the efficient therapeutic activity when compared to high dose. In Pole climbing test and tail suspension test, high dose of ethanolic fruit extract of *N.cadamba* shows efficient activity. In Anthelmintic activity, ethanolic extract of *N.cadamba* shows better therapeutic activity than the aqueous.

KEYWORDS: *Anti-depressant activity, Cadamba fruit, Neurological disorder, Locomotive activity, pole climbing test, tail suspension test.*

INTRODUCTION¹

N.cadamba is one of the most valuable plants in traditional system of the medicine from ancient time. It is a large tree with a broad crown and straight cylindrical bole. It is quick growing with broad spreading branches and grows rapidly in the first 6-8 years the trunk has a diameter of 100-160 cms but typically less than that. Leaves are 13-32 cms in long, flowering usually begins when the tree is 4-5 years old. *Cadamba* flowers are sweetly fragrant, red-orange in colour occurring in dense, globular heads of approximately 5.5 cms (2.2 in diameter). The fruit of *N.cadamba* occurs in small, fleshy capsules packed closely together to form a fleshy yellow-orange infructescence containing approximately 8000 seeds. On maturing, the fruit splits a part releasing the seeds which are dispersed by wind or rain and pericarp becoming creamy yellow on exposure and is not clearly differentiated from the heart wood.

DEPRESSION

Depression is a clearly neurological disorder characterized by disturbances in sleep and appetite as well as deficit in cognition and energy. The term depression can itself be a misleading everyone life will experience alteration in mood. Depression can be potentially life threatening condition that has an effect of millions of people across the globe. The distinctive symptoms exhibit as a trade form that include low or depressed mood "anhedonia" (reduced ability to experience natural rewards) and low energy or fatigue. The patients become chronic and after 5-10 years of potential follow up about 7-12% of them respectively are still depressed. Mood disorders are the second primary cause for disability and leading cause of years lived with

disability in all the age groups in the world .The mood becomes elevated and irritable this may be a symptom of "Mania". The term mania is used to describe severe cases frequently associated with psychiatric symptoms. Hypo mania describes less severe form of the disorder. In clinical practice this distinction often becomes blurred with hypomania being seen as patients develop or recover from mania.

METHODS

1. LOCOMOTOR ACTIVITY

The locomotor activity was measured by using Actophotometer. Six lights and six photocells were placed in the outer periphery of the bottom in such a way that a single rat can block only one beam. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was measured for a period of 10 min. technically its principle is that, a photocell is activated when the rays of light falling on the photocells are cutoff by animals crossing the beam of light. As the photocell activated, a count is recorded. The photocells are connected to an electronic automatic counting device which counts the number of "cut off"

4. POLE CLIMBING TEST

Cook's pole climbing apparatus ⁽⁵⁾ use to study cognitive function, mainly a response to conditioned stimuli during learning & its retention. The apparatus has an experimental chamber (25*25*25 cm) with the floor grid in a soundproof enclosure. Scrambled shock (6mA) is delivered to the grid floor of the chamber composed of stainless steel rods. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber. The study rat was placed in the chamber and allowed it for 45 seconds. conditioned stimulus (CS) i.e buzzer signal was turned on and unconditioned stimulus (US).ie. ...,electric shock delivered through grid floor for 45 sec. animal learned to associate the buzzer¹² with the impending foot shock by climbing the pole after buzzer signal .Avoidance response was defined as climbing reaction time <10 sec only.

3. TAIL SUSPENSION TEST (TST)

In tail suspension test (4), the animals were hanging by the tail on a plastic string 50 cm above the surface with the help of adhesive tape, placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during the test. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period, Mice were considered to be immobile only when they hang passively and were completely motionless. The test was conducted in a dim lighted room and each rat was used only once in the test.

Statistical analysis

All results were expressed as mean $\pm$ standard error of mean (S.E.M). Data was analyzed using one way ANOVA and two way repeated measures followed by Turkey's multiple comparisons and student's unpaired t test using graph pad prism statistical software $P < 0.001$ was considered as statistically extremely significant.

Table 1. Effect of Neolamarckiacadamba on immobility time in the tail suspension test using rats

S.NO	TREATMENT	IMMOBILITY TIME (in sec)
1	Control	46 $\pm$ 1.22
2	Standard (fluoxetine) 4 mg/kg	50 $\pm$ 1.28
3	Low dose 40 mg/kg	48 $\pm$ 1.29
4	High dose 80 mg/kg	80 $\pm$ 1.24*

* indicates the level of significance value $P < 0.05$. The high dose of *N. cadamba* shows the better significant value than the standard.

Table 2. Effect of *Neolamarckiacadamba* on pole climbing test using rats

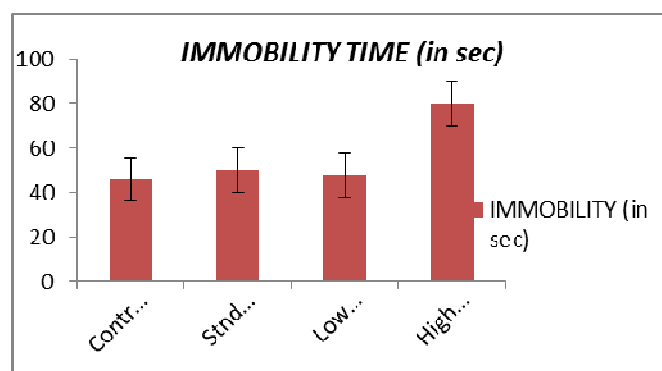
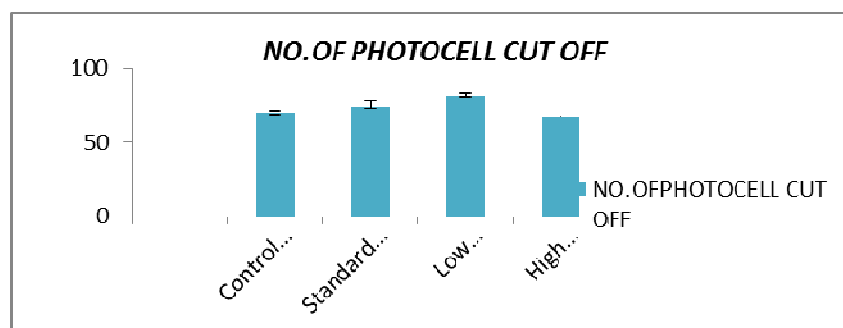
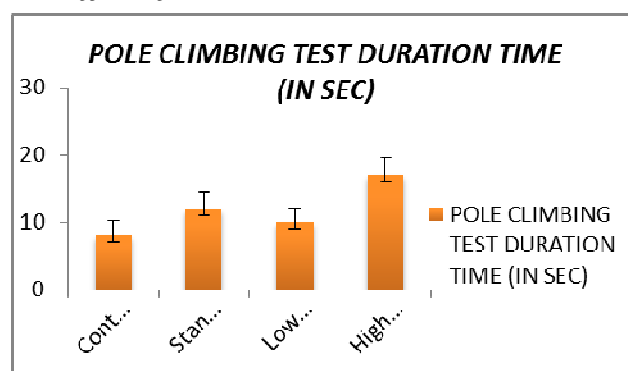
S.NO	TREATMENT	POLE CLIMBING TEST DURATION TIME (in sec)
1	Control	8 ±2.32
2	Standard (fluoxetine) 4 mg/kg	12 ±2.38
3	Low dose 40 mg/kg	10 ±1.99
4	High dose 80 mg/kg	17 ±2.48*

* indicates the level of significance value $P < 0.05$. The high dose of *N. cadamba* shows the better significant value than the standard.

Table 3. Effect of *Neolamarckiacadamba* on Locomotor activity test using rats

S.NO	TREATMENT	NO.OF PHOTOCCELL CUT OFF
1	Control	70 ±2.32
2	Standard (fluoxetine) 4 mg/kg	74 ±1.38
3	Low dose 40 mg/kg	81 ±3.39*
4	High dose 80 mg/kg	68 ±2.41

* indicates the level of significance value $P < 0.05$. The low dose of *N. cadamba* shows the better significant value than the standard.

Figure 1. Effect of *Neolamarckiacadamba* on Tail suspension test (TST)**Figure 2. Effect of *Neolamarckiacadamba* on Pole climbing test****Figure 3. Effect of *Neolamarckiacadamba* on Locomotor activity**

DISCUSSION

In the present study antidepressant effect of *Neolamarckiacadamba* fruit extract have been studied. The Ethanolic extract⁸ for this plant showed that it has antidepressant activity. Extract was found to be safe no mortality was observed following treatment with doses as high as 80 mg/kg. The increase in locomotor activity indicates a stimulant effect and *Neolamarckiacadamba* has shown stimulant effect in actophotometer. This prompted use to evaluate it further, using paradigms of depression models. Based on these requirements, we selected 3 behavioral despair models namely TST, Cook's pole climbing and Locomotor activity in rats. In the TST, rats are suspended by their tails for a defined period of time and their immobility was assessed. Acute administration of most antidepressants decreases immobility time in TST. From the above results, the Low dose (40mg/kg) gives efficient therapeutic activity when compared to the High dose (80mg/kg) of *Neolamarckiacadamba* Ethanolic fruit extract.

CONCLUSION

It is concluded that, *Neolamarckiacadamba* Ethanolic fruit extract was reported to possess, Anti depressant property; *Neolamarckiacadamba* may be an effective and acceptable alternative for the treatment of anxiety and depression conditions. Overall results of the present investigation demonstrated that *N. cadamba* extract could be the better alternative for maintaining the anxiety and depression conditions. These Studies lead to the conclusion that the herbal extract of *N. cadamba* fruit extract is found to be potent and safe. However elucidation of exact mechanism of action and beneficial effects of these formulations needs further investigation. More randomized controlled trials in large patient populations have to be carried out before determining the status of these drugs in the therapy of anxiety and depression.

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A CLINICAL CASE REPORT ON THERAPY RELATED GULLIAN BARRE SYNDROME-STAGE-IV

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ABSTRACT

Gullian barre syndrome is an immune-mediated disorder, which is triggered by infectious and non-infectious matter. The annual incidence rate of gullian barre syndrome is about 1-2 in 1,00,000 population. Guillain-Barré syndrome is rare, affecting 1 in every 100,000 Americans. We present a case of 66 years male patient admitted with lower and upper limb weakness with other complaints of fever and breathlessness. The major diagnostic test that supports gullian barre syndrome is a nerve conduction study report. patient is treated with IV Ig -10mg/kg, and other symptomatic treatments.

KEYWORDS: *Gullian barre syndrome, Neurological Disorder, stroke, immune-mediated disorder, infectious and non-infectious matter*

INTRODUCTION

Gullianbarre syndrome is an immune-mediated disorder of PNS which is triggered by either infectious or non infectious matter ⁽¹⁾. It is manifested with progressive proportionate weakness as well as a loss of sensation. The annual incidence rate of GBS according to the national institute of neurological disorder is about 1-2 in 1,00,000 population⁽⁴⁾. Aetiology is not clearly understood. Pathophysiology includes demyelination of nerve cells or axonal damage. There are different types of GBS like acute inflammatory demyelinating polyradiculo neuropathy, miller-Fischer syndrome, acute motor axonal neuropathy, acute motor-sensory axonal neuropathy⁽³⁻⁵⁾. signs, and symptoms include difficulty in walking, chewing, speaking, and inability to walk difficulty in eye movements, unpleasant sensation of fingers, toes, weakness in legs that spend to upper body⁽⁴⁾. Diagnosis includes neurological manifestations, nerve conduction test, electromyography, CSF examination, spinal tap test ⁽³⁾ treatment includes plasma exchange, IV immuno globulins to block antibodies, pain-relieving medication, steroids can also be used.

CASE STUDY

BACKGROUND

Chief complaints: Acute onset progressive ascending type of weakness in all four limbs, breathlessness and fever days back.

History of present illness

A 66yrs old male patient admitted in hospital history of fall from stairs 25 days back. He had a history of diabetes and hypertension on regular medication. As symptoms are progressing admitted in the local hospital and was diagnosed to have ALDP variant of Gullian Barre syndrome and was referred to the higher center due to no improvement in course. During his stay in a local hospital he was treated with IV IG for 3 days, the patient develops respiratory distress along with bifacial weakness hence, incubated and ventilated followed by tracheostomy done because of quadriplegia and respiratory muscle weakness. On day 4 patients also developed dysautonomia. In local hospital complete blood count was done in which Neutrophils (71.7%), lymphocytes (19.5%), monocytes (8.4%), eosinophils(0.2%), Hb(11.1g/dl), HCT(36%), MCH(26.7pg), MCHC(30.7g/dl) after admission in to hospital, initially treated with IV Ig10gm-3vials each vial for four hrs infusion. The parameters are shown in Table 1,2,3 and 4.

Table 1. PHYSICAL EXAMINATION VALUES DAY WISE REPORT

PARAMETER	D1	D2	D3	D4	D5	D6	D7	D8
Respiratory rate	23	21	21	141	22	25	31	41
Blood pressure	130/90	130/70	130/70	130/80	120/70	140/90	150/90	210/110
Heart rate	115	91	112	87	72	94	117	100
Blood glucose (mg/dl)	341	354	309	300	245	283	260	292

System examination

CNS-E4V5M1 (initial assessment).

Laboratory investigations and tests :

SGOT-41IU/L, ALP-149IU/L, CRP-49.23mg/dl, total proteins -6.2IU/L, sr.albumin - 1.8g/dl , Sr. Globulin - 4.4g/dl, ALB/ GLOB ratio-0.41g/dl.

Social history

Non-smoker and non-alcoholic.

Table 2.Culture test

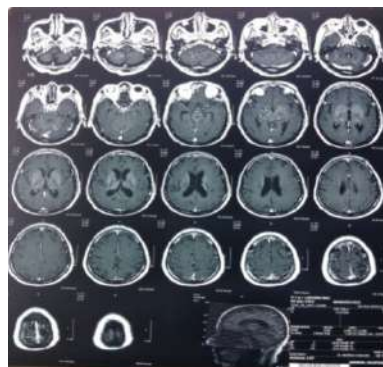
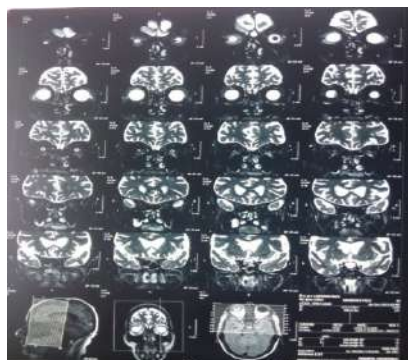
CULTURE SAMPLE	ISOLATED ORGANISM
Endo tracheal secretions	Klebsiella species
Blood culture	Enterococcus
Urine sample	Pseudomonas aeruginosa
Eye swab	Salmonella species

Urine analysis

Color yellow, specific gravity - 1.020, ph-6.5, protein/ albumin ratio - present, glucose - present 0.25gm

Table 3. BLOOD PROFILE DAY WISE REPORT

Parameter	D1	D2	D3	D4	D5	D6	D7	D8
Hemoglobin	9.3	9.9	9.6	9.0	8.2	8.0	9.6	9.6
WBC	10,200	11,500	10,900	12,700	10,700	9,500	8,300	7,900
Sr.creatinine	0.2	0.2	0.2	0.2	0.1	0.1	0.3	0.3
Sr.potassium	4.4	4.5	4.3	4.1	4.1	4.0	4.0	4.2

MRI SCAN REPORTS**Fig:1 shows MRI scan –Eye orbits Fig:2 shows MRI scan -Brain****IMPRESSION**

Increase flair signals in the vitreous chamber of the right globe with vitreous detachment.

Thickening and enhancement of sclera are seen predominantly in the anterior half of the globe. The defect in the cornea is seen and pansoptical rupture. Preseptal inflammation on left side, bilateral frontal sinusitis

Table 4. NERVE CONDUCTION STUDY:

PARAMETRES	NERVE	REPORT 1(Day-1)			REPORT 2(Day-3)		
		NCV	F-Min	CB	NCV	F-min	CB
MNCS	RT.MEDIAN	43.6	33.25	-ve	27.0	39.25	-ve
	RT.ULNAR	41.6	30.88	-ve	26.3	39.50	-ve
	RT.CPN	35.0	35.62	-ve	--	--	--
	RT.PTN	29.5	42.88	-ve	--	--	--
	LT.MEDIAN	40.8	31.00	-ve	24.3	40.88	-ve
	LT.ULNAR	44.4	31.00	-ve	28.6	41.12	-ve
	LT.CPN	33.9	36.88	-ve	--	--	--
	LT.PTN	35.2	42.12	-ve	--	--	--
		CV			CV		
SCNS	Rt.MEDIAN	44.83					
	Rt.ULNAR	45.28			37.5		
	Rt.SUCRAL						
	Lt.MEDIAN	42.27					
	Lt.ULNAR	48.98			34.78		
	Lt.SUCRAL						

IMPRESSION: S/O Severe demyelination type of sensory with neuropathy involving both upper and lower limbs. When compared Day1 report to Day3 report there is a drastic change in NCV and F-min values and CV values in the SCNS report.

DISCUSSION

Many of the patients have neuromuscular skeletal problems^[3]. Especially some patients present with complaints of weakness in both lower and upper extremities. The neuropathy is mainly seen. Alcohol, diabetes and poor diet also cause neuropathy. In extremities, numbness and sclerosis occur. GBS is a heterogeneous autoimmune disease. Auto antibodies are present against various antigens in peripheral nerves. It starts from the fingers or fingertips of the foot. After sometimes it becomes worsen. Usually, patients with Guillain-Barré Syndrome (GBS) of subtype acute ting poly radiculo neuropathy (AIDP) presents 2-4 weeks following a respiratory or gastrointestinal illness with complaints of altered sensation in the distal part of extremities and proximal muscle weakness. The weakness of ascending and symmetrical affecting lower limb preferentially may progress over hours to days to involve the arms, truncal muscles, cranial nerves, and muscles of respiration^[7,9]. The most common and symptoms are the weakness of the limbs is areflexia and paralysis. The diagnosis of these diseases based on clinical examination and history. The electrodiagnostic testing and cerebrospinal fluid analysis provide supportive diagnosis^[10]. In Our case patient is known to the case of diabetes and hypertension. History of weakness in both upper and lower extremities and developed Respiratory Distress (RD) along with bifacial weakness. Due to Respiratory distress patient was electrically incubated and ventilated followed by tracheostomy. On Day 4 patients have Dysautonomia and Glasgow coma scale score is 10. During treatment, he also developed eye keratitis. In general, GBS is diagnosed with cerebrospinal fluid and Nerve conduction studies. In our patient, the main diagnostic criteria are nerve conduction studies. Demyelination involves slowing of nerve conduction, extension of the distal latencies, Extension of the F-waves and blockage of conduction. Even a normal CSF protein level does not rule out GBS especially in the early part of the disease. During stay in the Hospitals, patient developed with Gluteal pressure sore (Bedsore) and also corneal ulcer. Eye Evisceration is also done to the patient for complete loss of vision. Standard treatment for GBS is Intravenous immuno globulins & plasma exchanges are two options available to clear out immuno globulins & immune complexes present in the blood. In our case, the patient was given IV immunoglobulins 1gm/kg. The choice between them is often hospital dependent. Intravenous immunoglobulin causes the risk of transmitting infections. In such a case, antibiotics are prescribed which may worsen the situation by inhibiting auto-antibodies. IV immunoglobulin

also blocks the binding of Fc gamma receptors, preventing phagocyte damage from macrophage and the patient is treated With Fortified Ceftadizime One Drop, One Hourly Intraocular, Fortified Colistin One Drop, One Hourly, Fortified Oflox Intraocular One Hourly, Moxifloxacin Ointment.

CONCLUSION

Guillain-Barré syndrome is a neurological disorder comprising of primarily muscle paralysis. It is important to recognize the severity of the neurological symptoms associated with Guillain-Barré syndrome, Patient should have follow-up within 2 weeks after the acute syndrome to evaluate for relapse, followed by repeat intravenous immunoglobulin. Subsequently, follow up is necessary for every 4 to 6 weeks continued for 6 months, subsequently 6 months for 1 year and then yearly. The patient is advised to continue physiotherapy and occupational therapy. Patients should contact their physician with any debilitating symptoms of weakness, numbness, paraesthesia, facial weakness and difficulty with swallowing or breathing, or bladder function.

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TISSUE ENGINEERING- A NOVEL TECHNOLOGY IN STEM CELL THERAPY

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ABSTRACT

Tissue regeneration is a process of restoring the tissue by transferring or delivering specific type of cells or cell products to injured tissue or organs. Stem cell therapy has drawn significant attention since transplantation of stem cells overcomes the limitations of autologous transplantation of patient's tissue. However it is not much useful for treating the diseases to overcome the problems associated with stem cells tissue engineering techniques have been developed. Tissue engineering substitutes are produced by developing tissue stem therapy with tissue engineering. These combinations intensify cell viability, differentiation and therapeutic efficacy of transplanted stem cells or organ functions. The stem cells that are used for tissue regeneration are mesenchymal stem cells, embryonic stem cells and induced pluripotent stem cells. Injured tissue exhibits low therapeutic efficacy and less regenerative activity is observed when injured tissue is transplanted with stem cells alone. In this review the therapeutic efficacy of stem cells is increased by using scaffolds biomaterials and techniques of tissue engineering.

KEYWORDS: Tissue engineering, Stem cell therapy, Transplantation, Biomaterials.

1.INTRODUCTION

The growing tendency of increased life expectancy as well as increased incidence of age-related degenerative diseases and tissue damage requires the use of allogeneic or autologous grafts for tissue repair. Although transplantation of tissues or cells is innovative and has been applied to a lot of treatments, its application in clinical settings is still limited¹. The assembled evidence suggests that stem cells by using various mechanisms. It has reported that variety of stem cells such as mesenchymal stem cells, embryonic stem cells and induced pluripotent stem cells can promote the regeneration of damaged tissue although the stem cells promotes tissue regeneration. The limited clinical applications are observed due to poor life span and potential differentiation of stem cells. To overcome these limitations, tissue engineering technology has been used to improve the viability and proliferative capacity of stem cells. Tissue engineering is the use of a combination of cells, biomaterials, biochemical and physicochemical factors, and engineering technologies to improve or replace biological tissues². In this paper we will review what types of stem cells are present, what is their role in various tissues and tissue regeneration using stem cells engineering. Other kind of stem cells are also available for tissue engineering however in this review we focus on above mentioned stem cells for tissue regeneration.

2. TYPES OF STEM CELLS FOR TISSUE REGENERATION

Adipose tissue, tonsil, and bone marrow comprises of mesenchymal stem cells (MSCs) however they can be isolated from various tissues. Under normal culture they exhibit plastic adherent properties and they have fibroblast like morphology. They express specific cell surface markers including CD73, CD90, and CD105. MSCs have the potential for self-renewal and differentiation potential into mesodermal lineages, including adipocytes, muscles, chondrocytes, and osteoblasts³. The increased evidence suggested that mesenchymal stem cells have immunomodulatory functions and proangiogenic activity which are very useful for tissue regeneration. MSCs interfere with dendritic cell and T-cell function and generate a local immunosuppressive environment by secreting various immune-modulatory cytokines⁴. By permitting angiogenesis by secreting pro-angiogenic factors for various diseases such as cardiovascular, bone and cartilage, neuronal and inflammatory diseases several MSC based clinical trials are conducted world widely. Several therapeutic is commercially available but their therapeutic efficacy is still in research. Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass of blastocysts, and they can differentiate to specific cell types by controlling culture conditions⁵. Recently clinical trials are initiated in several diseases such as

spinal cord injury, muscular regeneration, diabetes and heart diseases to test the safety and efficacy of human embryonic stem cells. In 2010, Geron Corporation transplanted hESC-derived oligodendrocyte precursors, GRNOPC1, into five patients with spinal cord injury, and the clinical trial data suggest long-term safety of the therapy as well as reduced spinal cord cavitations in four of the five patients⁶. In addition, Advanced Cell Technology (MA, USA) tested human ESC-derived retinal pigment epithelium for age-related macular degeneration and Stargardt disease, a juvenile form of macular degeneration, and the clinical trial data have shown positive safety data with no tumorigenicity and improved clinical data in some patients⁷. Several hidden obstacles are there in clinical applications of embryonic stem cell therapeutics. Because as they are isolated from embryo. They are allogenic to the patients and therefore may lead to immune rejections. Secondly, it is difficult to induce differentiation into a desired cell type with 100% efficiency, thus a small fraction of undifferentiated cells might remain and form teratomas⁸. As they are derived from embryo there are some ethical issues with these embryonic stem cells. These ESC-associated issues were alleviated by the work of Yamanaka and colleagues on somatic cell reprogramming⁹. They stated that by introducing four pluripotency induced transcription factors the somatic cells could be reprogrammed to primordial cells. However the ipcs can be obtained from the somatic cells as they are free from ethical issues. Although iPSCs do not negate the risk of generating tumors, transplantation of autologous iPSC-derived cell therapeutics could help solve the immunological problem associated with transplantation of ESC-derived cells¹⁰. The world's first induced pluripotent stem cell ipsc -derived therapy was transplanted into age -related macular degeneration patients by Japanese Institute successfully. However, there is a risk of neoplastic development from cells differentiated from IPSCs, because reprogramming factors are associated with the development of tumors¹¹.

3.DEVELOPMENT OF STEM CELLS BY USING GROWTH FACTORS AND PEPTIDES

Stem cells can differentiate into different kinds of cell types in response to specific ligands or growth factors¹¹. In animal models the direct transplantation of stem cells is found to be effective .however there is a possibility of local ischemia or thrombosis are observed as it is stated before stem cell based cell therapy has various limitations due to the poor life span of transplanted stem cells In vivo. In particular, BMPs have been shown to exert novel effects on cartilage and bone regeneration in several animal experiments. It has been reported that bone morphogenetic proteins (BMPs) and bone-forming peptide-3 stimulated differentiation of MSCs to osteoblasts¹²⁻¹³. There are various types of BMP'S are available among those BMP'S, both betaMP2 and betaMP7 have been shown to play an important role in bone and cartilage regeneration. It is observed that the regeneration potentials of stem cells can also be promoted by using extracellular matrix proteins. Periostin, an extracellular matrix protein that is expressed in the periosteal and periodontal ligaments, has been identified as a secreted protein of MSCs. Recombinant periosteal protein stimulates proliferation, adhesion, and survival of MSCs in vitro, and co-implantation of MSCs and recombinant periosteal n protein significantly accelerates bone regeneration by increasing angiogenesis in a caldaria defect animal model¹⁴. The angiogenesis and chemotaxis of endothelial forming cells is stimulated by periostin by involving the beta3 and beta 5 integrins. Periostin stimulates angiogenesis and chemotaxis of endothelial colony forming cells through a mechanism involving $\beta 3$ and $\beta 5$ integrins. Recently, a short peptide sequence (amino acids 142–151), which is responsible for periosteal-mediated angiogenesis, has been identified by serial deletion mapping of the first fasciclin I domain¹⁵. The above results stated that periostin can be used in all cell therapy by invigorating the pro-angiogenic and tissue regenerative potentials of msc's. In addition, it has been reported that co-transplantation of N-acetylated proline-glycine-proline, a peptide produced by the degradation of collagen, accelerates repair of dermal wounds by stimulating migration and engraftment of transplanted endothelial colony forming cells¹⁶.

4.STEM CELLS ENGINEERED USING NANO MATERIAL

The biological functions of stem cells can be affected by growth factors and cytokines from outside. However there can be several ways to manipulate by using gene therapy viral expression systems. However, viral expression systems have been reported to induce immune and inflammatory reactions in host tissues, and genetic mutations in host DNA can occur¹⁷. Therefore it is important for stem cells research to develop highly efficient nonviral expression system. For instance, reprogramming or direct conversion of somatic cells by using non-viral gene expression system have great potential for clinical application of the reprogramming cells¹⁸. The tumor formation can be reduced by replacing virus with alternative extra cellular chemicals nonviral methods encompass electroporation of cell membrane or delivering of genes in a form complexed with liposomes or cationic polymers for nonviral delivery of reprogramming factors into cells.

Several types of nano particles are developed. Several types of nano particles have been developed for non-viral delivery of reprogramming factors into cells. These nano particles are composed of mesoporous silica, calcium phosphate, chitosan, cationic polymers, and magnetic nano particles¹⁹. Recently, graphene oxide-polyethylenamine complexes have been reported to be an efficient and safe system for mRNA delivery for direct reprogramming of somatic cells to induced neurons²⁰. Thus remodeling of somatic cells will be achieved by improving gene delivery efficiency using nano particles.

5. BIOMATERIALS IMPROVING THERAPEUTIC EFFICACY OF STEM CELLS

Tissues are mainly composed of two components they are cells and their surrounding extra cellular matrix (ECM), which mediates cell proliferation and cell differentiation. The ECM mainly promotes cell growth and supplements of essential components of the cell. ECM has been reported to create a framework for cell growth and to efficiently provide the nutrients or growth factors needed for cells²¹. The cells and the ECM are lost if we use them alone for repairing the large size damaged tissues. Thus to promote tissue regeneration the artificial ECM environment should be created for transplanted cells, the useful substitute for ECM are biomaterials. These biomaterials are also useful in cell therapy. For this infiltration of cells and supply of oxygen and nutrients to cells scaffold biomaterial what we use should be porous in nature. In addition, the scaffold should be biodegradable for proper replacement of damaged tissues with the transplanted cells²². As synthetic and natural biomaterials are available. The stem cells in combination with scaffold biomaterials are used for skin wound healing. In terms of biomaterials, a variety of synthetic and natural materials have been developed. In particular, biodegradable polymers, such as collagen, gelatin, fibrin, hyaluronic acid, and poly (lactic-co-glycolic acid) are highly useful for tissue engineering²³. In addition to this by using 3D alginate hydrogels the enhancement of chondrogenic differentiation of msc's was experimentally proved. Neural stem cells have been used for treatment of neurodegenerative disease or stroke in pre-clinical and clinical studies; however, differentiation of neural stem cells to functional neurons, reconnection with host neural cells, and correct transmission of nerve signals are still obstacles to overcome²⁴. Therefore, to enhance the survival and differentiation potentials of transplanted stem cells, it is necessary to combine biomaterials with growth factors, cytokines, and cell adhesive substances²⁵.

6. 3D BIOPRINTING FOR TISSUE ENGINEERING

Biomaterial scaffolds are often used as structural elements for various components of tissues, like blood vessels, skin, and tissue layer tissues²⁶⁻²⁷. The regenerative activity of stem cells for broken bone and gristle are often improved by culturing stem cells within the presences of 3Dscaffolds. Most of the tissues for the development of engineering tissues victimization stem cells requires multi layered 3Dscaffolds. Currently, 3D bio-printing has drawn attention within the field of biotechnology for manufacturing multilayered structure²⁸. Victimization 3D bioprinting, numerous cell sorts are often positioned in specific locations in multi-layered structures for constructing completely different tissues or organs²⁹. In bio-printing technology we have a tendency to use inkjets and optical device depolarization. Several problems arise while using inkjet printers because the cells are printed in the same way as common printer. For example, in order to print stem cells through an inkjet printer, the material that is added to the cells must be in a liquid form and, subsequently, have a 3D structure after injection³⁰. Impaired cellular viability can be observed by using cross linking agents to form 3D structures. However in order to rectify these draw backs the laser printer have came to existence. The laser printers for 3D bio-printing are developed to maintain liquid from these laser printers do not require any additives. The viability of cells is higher than 95% after being printed, and apoptosis and cell proliferation are not affected³¹ for the printing of stem cells into 3D structures bio-inks are required. The commonly used bio-inks are hydrogels. Each bio-ink has its own characteristics and is used for specific purpose³². Natural bio-inks; alginate, gelatin, collagen, fibrin synthetic bio-inks comprises of b polyethylene glycol and pluronic gels. In order to mimic the ECM in vivo, de-cellularized extracellular matrix (dECM) scaffold has been developed. Thus for stem cells the dECM is widely used in maintaining the viability of tissues not only the regeneration of blood vessels play a important role. While maintaining the viability, the regeneration of tissue as well as blood vessel is important factor in maintaining blood vessel. The blood vessels fabrication is controlled by the sufficient resolution of printer and there should be no deformation of bio inks during the printing conditions. In another study, enhanced angiogenesis and bone marrow formation was observed on treatment with angiogenic. Thus there is possibility of adding pro-angiogenic factors during 3D bio printing to make the blood vessel formation in 3Dprinted tissues.

7. CONCLUSION

Most of the therapies or treatments eventually try to enhance tissue regeneration, Current status of technologies of stem cells, biomedical engineering and nano technology for tissue; however the biomaterials will produce toxicity when applied to human body. However the biocompatibility of biomaterials can be enhanced by using several methods. The various tissues can be developed by using tissue engineering. New structures, new methods, novel surface modifications should be known and developed to improve stem cell technology or engineering. We should have deep understanding on the concept of cell and relationship between cell and biomaterials.

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ASSESSMENT OF ANTIHELMINTIC ACTIVITY OF ETHANOLIC EXTRACTION OF *GOMPHRENA serrata* L

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ABSTRACT

Helminths are parasitic worms. Helminthic infections occur worldwide, especially in developing countries. About one-quarter of the world's population, i.e.; 1.5 billion are infected with helminthiasis which is chronic and debilitating in nature. The parasitic gastroenteritis is caused by mixed infection with several species of helminths like ascarids, whipworms, hookworms and schistosomes etc; .the interaction between helminths and the host's immune system provokes particular immunomodulatory and immunoregulatory mechanisms that ensure their survival in the host for years, which results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. The aim of the present study was to evaluate the anthelmintic activity of ethanolic and aqueous extract of leaves of *Gomphrena serrata* using *Pheretima posthuma* as test worms. The time of paralysis and time of death were studied and the activity was compared with albendazole as a reference standard. The alcohol and aqueous extract of leaves of *Gomphrena serrata* exhibited significant anthelmintic activity as evidenced by decreased paralyzing time and death time. The results thus support the use of *Gomphrena serrata* as an anthelmintic agent.

KEYWORDS: Anthelmintic activity, *Pheretima posthuma*, albendazole, *gomphrena serrata*.

1. INTRODUCTION

According to the world health organization (WHO) infectious diseases are the key cause of death. These infections are usually transmitted through contaminated water/food, unwashed hands, feces or contact with contaminated objects. Helminths infections are among the most widespread infections in humans especially in Poverty, stricken and developing countries with warm moist environments and poor sanitary conditions¹. The Majority of infections due to helminths are generally restricted to tropical regions and lead to the prevalence of under nourishment, anemia, eosinophilia and pneumonial². Worldwide prevalence annually lies between 500 million to 1.5 billion approximately³. Traditional medicinal plants are linked to traditional culture with traditional healers and herbalists acting as important stake holders³. It is estimated that two thirds of the world population still rely on traditional medical remedies, mainly plants due to limited availability and lack of pharmaceutical medicines⁴. More than 70% of the people in Africa refer to traditional healers concerning health issues⁴. The use of natural plant extracts as de-wormers for humans and livestock has long been practiced; however scientific validation of these practices and identification of active compounds has been lacking⁵. Moreover helminthes are increasingly showing resistance to classical drugs⁶ which creates a great need and demand for new antihelmintic agent, preferably with novel mode of action⁷. The present study was designed to investigate the anthelmintic activity of ethanolic and aqueous extract of leaves of *Gomphrena serrata* using *Pheretima posthuma* as test worms. The time taken for paralysis and death was noted and comparison is done with albendazole as standard drug.

2. PLANT PROFILE

Binomial name: *Gomphrena serrate*

Synonyms: English: Globe amaranth
Malayalam: Wadapu
Tamil: Vatamalli
Hindi: Makhmali.



2.1. VERNACULAR CLASSIFICATION

Kingdom : Plantae
Class : Angiosperm
Order : Caryophyllus
Family : Amaranthaceae
Genus : Gomphrena
Species : serrata

2.2. DESCRIPTION

Gomphrena serrata is a topical annual plant with weak erected stems grows 2 feet tall. The round-shaped flower inflorescences are a visually dominant feature and cultivars have been propagated to exhibit shades of magenta, purple, red, orange, white, pink, and lilac. Within the flower heads, the true flowers are small and inconspicuous⁹. *G. serrata* is native to Central America including regions of Brazil, Panama, and Guatemala, but is now grown globally¹⁰. As a tropical annual plant, *G. serrata* blooms continuously throughout summer and early fall. It is very heat tolerant and fairly drought resistant, but grows best in full sun and regular moisture¹¹. The plant fixes carbon through the C₄ pathway¹². At maturity, the flower heads are approximately 4 cm long and the plant grows up to 24 inches in height⁹. *G. serrata* is an out crossing species that is pollinated by butterflies, bees, and other insects. Floral volatiles likely play a significant role in the reproductive success of the plant by promoting the attraction of pollinators⁹.

3. MATERIALS AND METHODS

3.1. Collection Of Plant Material

Leaves of the plant *Gomphrena serrata* were collected from garden soil of Nirmala College of Pharmacy, Atmakuru; shade dried and blended into fine powder. The plant was authenticated by Dr.P.Satyanarayana Raju, Taxonomist, Department of Botany and Microbiology, A.N.U., Guntur, INDIA with authentication number ANU/SV/019.

3.2. Preparation Of Extract

Powdered plant material was defatted with petroleum ether, followed by successive solvent extraction with ethanol and water in continuous hot percolator for 48 hours. The solvent was filtered and evaporated to make solvent free results in marc (extracts). The extracts were stored in air tight containers until use.

3.3. Phytochemical Screening:

The extracts are subjected to preliminary phytochemical screening to identify the nature of components present in them namely alkaloids, glycosides, carbohydrates, phenolic compounds, proteins, steroids, saponins, tannins, terpenoids and flavonoids.

3.4. Experimental Animals

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity in vitro. Earthworms were collected from moist soil and washed with normal saline to remove all soil particulates used for the anthelmintic study. The earthworms of 3-6 cm in length and 0.1-0.3 cm in width were selected for experimental protocol.

3.5. Anti-Helminthic Activity

The leaf extracts of *Gomphrena serrata* has been evaluated for Anthelmintic activity by using earthworms. The activity has been evaluated on adult Indian earthworm. The earthworms are collected from soil and cleaned with saline water to make free from soil particles. Healthy earthworms of length 3-6 cm and width 0.1-0.3cm are selected and placed in petridish with 10ml of saline water. Distilled water was also taken in a separate petridish. Aqueous and ethanolic extracts were taken at 100, 200 and 400mg/ml in saline and placed in petri dish. Albendazole is taken as standard drug. All the earth worms are placed in petridish and time is noted. The time taken to paralyze the worms was observed. The living or viable worms were kept under close observation and the time taken for complete death was recorded. The death of worms was ascertained by transferring the motionless worms to warm water at 40°C. The warm environment stimulates and induces movement in the worms, if alive¹³.

Grouping of animals is as follows:

Group 1- control - petridish containing saline water

Group 2- standard- petridish containing albendazole

Group 3- test- petridish containing Ethanolic Extraction of *Gomphrena serrata* Leaves (EEGGL)

4. RESULTS

Table 1. Result showing Preliminary Phytochemical Screening for extract

S.No	Nature of Phyto Constituents	Ethanolic Extract	Aqueous Extract
1	Alkaloids	++	+
2	Glycosides	+	++
3	Carbohydrates	+	+
4	Phenolic Compounds	++	+
5	Amino acids	+	++
6	Proteins	++	+
7	Steroids	++	+
8	Saponins	+++	+
9	Tannins	+++	+
10	Terpenoids	-	-
11	Flavonoids	+	++

+=present and - = absence of chemical constituent

Table 2. Anthelmintic Activity

Groups	Time taken in minutes		
	Concentration	Paralysis	Death
Control	-	-	-
Standard (Albendazole)	100mg/ml	28.32 ± 0.04**	50.44 ± 0.03**
EEGGL	100mg/ml	35.36 ± 0.03**	65.36 ± 0.05***
EEGGL	200mg/ml	20.16 ± 0.02*	40.24 ± 0.03*
AGGGL	100mg/ml	75.36 ± 0.03**	95.36 ± 0.05***
AEGGL	200mg/ml	50.16 ± 0.02*	71.24 ± 0.03*

P: values, *p : <0.001 - Highly significant, **P: < 0.01 – Significant p value > 0.05 – Non significant**

5. DISCUSSIONS

DISCUSSION ON PRELIMINARY PHYTOCHEMICAL TESTS

The preliminary phytochemical tests as mentioned in table no:1 showed the presence of alkaloids, steroids, tannins, phenolic compounds and flavonoids in *Gomphrena serrata* leaves.

DISCUSSION ON ANTIHELMINTHIC ACTIVITY

Gomphrena serrata is a topical annual plant with weak erected stems grows feet tall. Literature survey supported the use of leaves for anti-helminthic activity. The leaves are collected and extracted using methanol and aqueous solvents. The extract was screened for preliminary phytochemical constituents as mentioned in table no:1 which resulted in presence of alkaloids, glycosides, carbohydrates, phenolic compounds, amino acids, proteins, steroids, saponins, tannins and flavonoids with absence of terpenoids in extracts. Screening for anthelmintic activity using earthworms for both aqueous and ethanolic extract resulted ethanolic extract showing better results compared with aqueous extract as mentioned in table no: 2. Ethanolic extract at dose of 200mg/ml showed better activity with paralysis time $20.16 \pm 0.02^*$ in respect to albendazole with paralysis time $28.32 \pm 0.04^{**}$. The time taken for death of earthworms was also closely with standard drug which clearly gives idea that ethanolic extract at dose 200mg/ml shows good antihelmintic activity. Tannins and saponins were shown to possess anthelmintic activity. Tannins bind with free proteins in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of the parasite and cause death of worms. The presence of more amount of tannins and saponins may justify screening result showing good activity. The result from the present investigation demonstrated the anthelmintic activity of ethanol extract of *Gomphrena serrata* leaves justifies the use of this plant in traditional Indian medicine system for helminthic infections.

6. CONCLUSION

Ethanolic extract of *Gomphrena serrata* leaves proved significant anti-helminthic effects in vitro at the dose investigated. The results support the traditional claim for the use of this plant in the treatment of several alignments in humans. More detailed Phyto chemical studies are necessary to identify the active principles and exact mechanism of action. Further comprehensive pharmacological and phytochemical investigations are needed to elucidate the specific chemical compounds responsible for anti-helminthic activity and their mode of actions. The long term toxic effect and its protective effect should elucidate.

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REVIEW ON DIABETIC WOUND HEALING THERAPY

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ABSTRACT

One of the major complications in diabetes is impaired wound healing. Diabetes causes impairment in the production of body or insulin sensitivity. Factors like nrf2 are responsible for wound formation in diabetics, which regulates the expression of phase2 detoxifying enzyme and antioxidant genes. Levels of cytokines are regulated by glucose levels in humans. It has been postulated that type 2 diabetes mellitus represent a disease of innate immune system responsible for ongoing cytokine mediated acute phase response. Diabetes is associated with poor outcomes following acute vascular occlusive events (delayed re-epithelialization). Role of oxidative stress in diabetic bone marrow derived angiogenic cell (BMAC) dysfunction and reactive oxygen species determined by flow cytometry. Anti-inflammatory and antioxidant activity shown by HO-1. The protecting effects of HO-1 related to granulation tissue maturation in the wounds of diabetic patients. TSP-2, a multicellular protein with a demonstrated role in response to injury was associated with delayed wound healing in diabetes. Many species of plants and herbs with wound healing activities have been identified in Africa and other developing countries.

KEY WORDS: Diabetes, wound healing, phytopharmaceuticals-2, NRF-2, TSP-2, HO-1.

INTRODUCTION

Heterogeneous group of metabolic disorders that share phenotype of hyperglycemia. One of the major complication in diabetes is impaired wound healing. Development of foot ulcers observed in ~4% to 10% of the patients with diabetes mellitus. Because of these ulcers infections occur frequently along with these infections great morbidity, lower extremity amputation and heavy financial burden and lower extremity amputation¹. The prevalence rate of diabetes in world is increasing day by day. It is common in male and females, and it is age related disease. Age less than 20 years the prevalence rate is 0.19 percent. Age greater than 20 years the prevalence rate is 8.6 percent. Age greater than 65 years the prevalence rate is 62.5 percent. Wound healing is long lasting in diabetes patients. It causes impairment in the production of body or insulin sensitivity, due to this hormone these cells take and use glucose from the bloodstream for energy. It is more difficult for the body to manage blood glucose levels due to this insulin disruption. When glucose levels in blood remains too high, it damages the white blood cells functioning. White blood cells play major role in the immune system. When a white blood cell doesn't work properly, the body is less able to fight against bacteria and it wills effects on wound healing properties. Poor circulation observed in patients with uncontrolled diabetes. Due to poor circulation, blood flows slowly, which makes it more difficult for the body to provide nutrients to wounds. This result, then slow healing observed in injuries, or may not heal at all. Neuropathy (nerve damage) also caused by diabetes, this effects wound healing. Nerves are damaged due to uncontrolled glucose levels in blood, numbing sensations in the area. During trauma conditions people with diabetes unable to notice the foot injury. Other ways diabetes might affect wound healing include less production of growth, healing hormones, new blood vessels repairing, weakened skin barrier, collagen production will be reduced.² In Type 1 Diabetes mellitus: Beta-cell production, usually leads to absolute insulin deficiency. Idio-pathic Type-1 refer to rare form the disease with no known cause. Immune mediated diabetes-an autoimmune disorder in which the body immunes system destroys or attempts to destroy the cells in the pancreas that produce insulin. In Type 2 diabetes mellitus: predominantly insulin resistance with relative insulin deficiency or predominantly an insulin secretory defect with or without insulin resistance.³

Pathophysiology of wound healing in diabetic patients

Non-healing wounds are open wounds that fail to close in a reasonable amount of time and usually ulcers that fail to heal after a 12-week period are characterized as chronic diabetes.

Chronic diabetic wounds begin as acute wounds, but the healing process is interrupted and stalled in different phases. Thus, normal acute ulcer repair fails to occur. Several growth factors, cytokines, proteases, as well as cellular and extracellular components play important roles during the wound healing process. This wound healing is impacted by hyperglycemia, chronic inflammation, micro and macro-circulatory dysfunction, hypoxia, autonomic and sensory neuropathy and impaired neuro peptide signaling in diabetic patients⁴.

Responsible factors for wound formation in diabetes

The key transcription factor is Nuclear factor erythroid-2–related factor-2 (Nrf2) that regulates the expression of phase II detoxifying enzyme and antioxidant genes.(2) Nrf2 belongs to the CNC (cap 'n' collar) family of b-Zip transcription factors, together with p45 NF-E2, Nrf1 and Nrf3, and acts through the heterodimer formation with one of the small Mafproteins. The regulation of ARE-dependent genes was examined in-vivo by gene targeting by the contribution of the four CNC proteins p45, Nrf1, Nrf2 and Nrf3. The regulation of erythroid-specific organic phenomenon originally known p45 NF-E2 as a molecule interacting with the NF-E2 (nuclear factor erythroid-2) binding motif. After, Nrf1, Nrf2 and Nrf3 were known. All of those factors share a preserved motif placed within the N-terminal facet of the b-Zip structure. as a result of this motif is found within the pomace fly cap 'n' collar (CNC) transcription issue, the four factors p45, Nrf1, Nrf2 and Nrf3 are classified because the CNC family of transcription factors.

Among these four members, Nrf2 may be a potent transcriptional substance. Nrf2 is expressed in metabolic and detoxification organs, like the liver and excretory organ, and in organs that are ceaselessly exposed to the setting, like the skin, respiratory organ and canal. it absolutely was so assumed that Nrf2 acts as a transcriptional substance interacting with the antioxidant-responsive part (ARE) Germline cause of the mouse nrf2 factor was performed, and Nrf2 clothed to be a key regulator of the bulk of phase II detoxifying and inhibitor enzymes.⁵

Inflammatory cytokines

It has been postulated that type 2 DM might represent an illness of the innate system liable for associate degree current cytokine-mediated acute part response. A prospective study found that 2 current markers of general inflammation, serum globulin and interleukin-6 (IL-6), were determinant of risk for development of type 2 DM in apparently healthy middle aged women. Moreover, many studies have incontestable, elevated levels of IL and tumournecrosis factor- (TNF) among people each with options of the endocrine resistance syndrome and with clinically obvious type 2 DM. Because periodical hyperglycemia is also additional deadly for epithelium cells than continuous hyperglycemia, we have a tendency to conjointly assessed the cytokine behavior in response. Finally, we have a tendency to tested the impact of the inhibitor glutathione on protein responses to acute hyperglycemia; there's proof that the discharge of TNF- elicited by high glucose in vitro is also mediate by oxygen species¹³ which aerophilous stress can be concerned in promoting a inferior general inflammation in patients with sort two diabetes to consecutive glucose pulses.⁶

Delayed re-epithelialisation

Diabetes is related to poor outcomes following acute tube-shaped structure occlusive events. This ends up in half from a failure to make adequate antagonistic microvasculature in response to anemia. Since tube-shaped structure epithelium protein (VEGF) is a necessary intermediary of neovascularization, we tend to examined whether or not hypoxic up-regulation of VEGF was impaired in diabetes. Each fibroblast isolated from kind two diabetic patients, and traditional fibroblasts exposed inveterately to high glucose, were defective in their capability to up-regulate VEGF in response to drive. In-vivo, diabetic patient's incontestable associate degree impaired ability to extend VEGF production in response to soft tissue anemia. High glucose-induced decrease in transactivation by the transcription issue hypoxia-inducible factor-1 (HIF-1), that mediates hypoxia-stimulated VEGF expression. Shriveled HIF-1 practical activity was specifically caused by impaired HIF-1 binding to the coactivator p300. We tend to establish valiancy modification of p300 by the dicarboxylic substance methylglyoxal as being liable for this shriveled association. Abrogated methylglyoxal conjugation administration of deferoxamine, normalizing interaction each HIF-1/p300 and transactivation done by HIF-1, neovascularization and increased wound healing promoted by deferoxamine.⁷

Over generation of reactive oxygen species (ROS)

ROS levels increases in various tissues in diabetic patients. This elevation is possible through some of the combination mechanisms that increase ROS production and reduce antioxidant defenses. Thus, diabetic wounds are characterized and results in high levels of ROS, particularly O_2^- and H_2O_2 . Accumulation of ROS in diabetic wounds, several pathological mechanisms contribute to all of them secondary to hyperglycemia. When considered with the central role of redox signaling in wound repair, the redox imbalance in diabetic wounds described hereunder has major implications for the pathogenesis of delayed healing in diabetes. Many mechanisms involved in increasing ROS level in diabetes. This results increases in mitochondrial superoxide production, formation of advanced glycation end products, decreased expression of antioxidant enzymes and small molecules and increase the activity of ROS generating enzymes such as NADPH oxidase.⁸

Diabetic inflammation mediated by hemeoxygenase (HO-1)

In the regulation of hyperglycemia mediated inflammatory cytokines HO-1 plays major role. Hyperglycemia induced gene expressions like IL1 β , IL6 and MCP1 suppressed by Heme oxygenase-1. On the other side it will knock down the effects of hyperglycemia by increasing the gene expression of cytokine. HO-1 plays major role in both *In-vivo* and *In-vitro* cell structure. There is no evidence showing direct regulation of HO1 on the expression of those proinflammatory cytokines. On the other hand, HO1 may indirectly modulate the gene expression of those cytokines, including IL6, IL1 β , and MCP1.

Promotion of delayed wound healing in diabetic patients by HO-1

Anti-inflammatory and antioxidant activity shown by HO-1. The protecting effects of HO-1 related to granulation tissue maturation in the wounds of diabetic patients. Sulphide production also concerned with in the result of HO-1 in diabetic wound healing.⁹

Action of thrombospondin

The expression of thrombospondin a pair of (TSP2), a matricellular super molecule with an incontestable role in response to injury, was related to delay healing in diabetes. First, we have a tendency to found that TSP2 expression was elevated in diabetic mice and skin from patients with polygenic disorder. Then, to work out the contribution of TSP2 to impaired healing in diabetes, we have a tendency to develop a completely unique diabetic TSP2-deficient. Though the TSP2-deficient mice developed fat and hyperglycemia comparable diabetic management mice, they exhibited considerably improved healing, characterized by accelerated re-epithelialization and increased connective tissue formation, formative cell migration, and vessel maturation. We have a tendency to any found that hyperglycemia increased TSP2 expression in fibroblasts, the key cellular supply of TSP2 in wounds. Mechanistically, high glucose increased activation of the hexosamine pathway and nuclear factor- κ B signal to elevate TSP2 expression. Our studies demonstrate that hyperglycemia-induced TSP2 expression contributes to impaired healing in diabetes.¹⁰

Major role of medicinal plants in wound healing in diabetic patients

Plants and herbs also involved in wound healing activities. These species found in Africa and different developing countries. Using the medicinal plants in wound healing and care involves medical aid, surgical process, and also the provision of adequate setting for natural healing method. The ingredients of medicinal plants are less toxic and have less side effects when compared with orthodox therapeutic agents; thus, the increased and renewed interest within the use and application of medicinal plants in the wound healing method each in diabetic and non-diabetic conditions. Impairment within the healing of diabetic wound is seen as a significant health challenge for health professionals globally, and this is often connected to the non-specific etiology in some cases, so one among the therapeutic approaches for treatment is that the application of medicinal plants significantly in poor resource setting. Some of the medicinal plants with their activities given below.¹¹

Medicinal plants Effects/Activities¹¹

S.No	Plant name	Activity
1	Rosmarinus officinalis	Antioxidant, antibacterial, and inflammatory effects
2	Carica papaya	Proteolytic enzymatic and antimicrobial activities
3	Radix Rehmanniae	Tissue regeneration, angiogenesis, and anti-inflammatory activity

4	<i>Annona squamosa</i>	High phenolic content, collagen contraction
5	<i>Catharanthus roseus</i>	Astringent and antimicrobial activities leading to increased rate of epithelialization
6	<i>Centella asiatica</i>	Antioxidant and anti-inflammatory activities
7	<i>Acalypha langiana</i>	Increased tissue regeneration
8	<i>Punica granatum</i>	Antibacterial and antifungal effects
9	<i>Aloe vera</i>	Antidiabetic, anti-inflammatory, and antibacterial activities
10	<i>Martynia annua</i>	Antibacterial and antioxidant activities

CONCLUSION

From this review we want to conclude that diabetes is a metabolic disorder produces different pathological condition in body leads to cell lysis or necrosis. Information on inflammatory mediators will help in fast wound recovery especially in diabetic conditions. Knowledge of phyto-pharmaceuticals will help in reduction of diabetic wound formation. This review will be useful for the persons who are doing research on diabetic induced wound healing through phyto pharmaceuticals.

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DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ESTIMATION OF IMATINIB MESYLATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Imatinib mesylate is a tyrosine kinase inhibitor. It binds to ATP binding site of enzymes and inhibits the binding of the BCR –ALB protein which produces abnormal tyrosine kinase and inhibits cell signalling for stimulation of the cell division. It inhibits tumour growth of cancerous cells. It is used to treat leukemia, tumours. HPLC (High performance liquid chromatography) method is an analytic method used to separate, identify and quantify the component. HPLC method is important in the quality control of drugs and drug products. The main objective is that to develop a simple, rapid, precise and accurate and sensitive. In HPLC pressure is applied to the column by using the pumping system forcing mobile phase through at much higher rate. The pressure used normally range from 30-200nm depending on the column. This system consists of RP-C18 column and the detection was performed at 260nm. HPLC method for the analysis of imatinib mesylate in pharmaceutical dosage form by using solvent system of buffer: ACN in the ratio 78:22 and Xterra RP-8(150mm ×4.6mm), 5 μ stationary phase. The mobile phase consists of tetrabutyl amino ammonium hydroxide and 0.1 M ammonium dihydrogen orthophosphate the chromatographic condition is optimised at flow rate of 1ml/min with UV detection at 230nm. The column temperature should be maintained at 40°C ,the auto sampler temperature is 25°C ,the flow rate is 1.0Ml /minute an cd wave length is 230nm,the injector volume is 10 μ L.in this method the % of assay should be within range of 98 – 102%. The % of assay of the performed sample was found to be 100.6% and the % value is within the range only. The % RSD values for the standard, assay and peak areas of the solution stability were not be more than.

KEY WORDS: *HPLC, VALIDATION, imatinib mesylate.*

INTRODUCTION

Imatinib mesylate was synthesized from 1-(pyridine -3-yl) ethanone and 2-methyl-5-nitrobenzeneamine by condensation ,cyclization ,hydrogenation ,acetylation and salification with methane sulfonic acid. The chemical name of imatinib mesylate is 4-[(4-Methyl piperazinyl) methyl] –N-[4-methyl -3[[4-(pyridinyl)-2-pyrimidinyl] amino]- phenyl]benzamide methyl sulfonate .

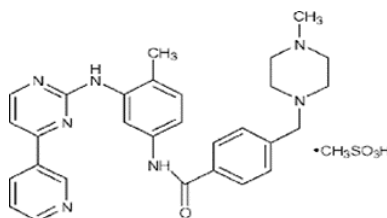


Fig.1 Imatinib mesylate

The molecular weight of imatinib mesylate is 589.7g. It is in white to white off to brownish or yellowish tinged crystalline powder .It is freely soluble in dimethyl sulfoxide, methanol and ethanol, but is insoluble in n-octanol, acetone and acetonitrile .Imatinib mesylate is a tyrosine kinase inhibitor that blocks the constitutive abnormal tyrosine kinase, Bcr-Abl tyrosine kinase that is created by the Philadelphia

chromosome abnormality found in leukemia. It works to prevent the growth inducing signals sent by enzymes found in some cancerous cells. ²In HPLC high pressure is applied to the mobile phase present in the column by using pumping system .pressure should be applied based on the type of column used. The pressure is varied to provide the optimum linear flow rate of the mobile phase⁴. It is that pressure which gives the smallest theoretical of plate height .Resolving a power of a chromatographic column increases with column length and the number of theoretical plates per length. The no of theoretical plates is related to the surface area of stationary phase. High performance liquid chromatography is a technique in analytical chemistry used to separate, identify, and qualify each component in a mixture. HPLC is also being automated which involves automated sampling, separation, detection, recording, calculation and printing of results. Due to high selectivity and sensitivity achieved by HPLC methods, it becomes the most selective method for the analysis in wide range of drugs. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages are speed(20 min or less), greater sensitivity ,improved resolution, reusable columns, ideal for the substance of low viscosity ,easy sample recovery ,handling and maintenance, instrumentation leads itself to automation and quantification, precise, integrator itself does calculations^{5,6}. HPLC is used for the detection of impurities in pharmaceutical industries, research and analysis of sample. HPLC instrument consists of solvent reservoir, pump, injector, column, detector, data acquisition and control system.³

MATERIALS AND METHOD

Chemicals

Acronitrile, ammonium di hydrogen phosphate, ortho phosphoric acid, Triethyl amine, Hydrochloric acid, water. These chemicals are purchased from MERCK system (India) Ltd.

Equipment

The instrument used for the analysis of imatinib mesylate is HPLC Waters, 295 separation module ,the software used for the chromatographic system is Empower, version 2.0 and the detectors used in the chromatographic system is UV-Visible detector.

Chromatographic conditions

The column of the chromatography is Hypersil BDS 18(250nm × 4.6mm, 5μ) or equivalent, the auto sample temperature is 25°C, the column temperature should be maintained at 35°C.The mixture of buffer: Acronitrile at a flow rate of 1ml/min with UV detection at 230nm. The injector volume should be 10μL. The run time is 30 minutes.

Selection of wave length

The wave length of the imatinib mesylate solution was ranged from the 200-400 nm by using UV detector and spectra were recorded.

Preparation of mobile phase

Prepare a mixture of pH 6.00 ammonium dihydrogen phosphate buffer and acetonitrile in the ratio of 78:22 v/v and degassed.

Preparation of standardSolution

Weigh accurately and transferred about 24mg of imatinib working standard into a 25ml clean and dry volumetric flask ,added about 10mL diluent of 0.1N hydrochloric acid ,sonicated to dissolved and diluted to volume with diluent and mixed well. Transfer 5.0ml of imatinib mesylate stock solution to 100ml clean and dry volumetric flask and diluted to volume with mobile phase and mixed well.

Preparation of sample solution

Weighed and transferred 10 imatinib tablets into 500ml clean and dry volumetric flask and added 350ml of diluents and sonicate for 30 minutes with intermediate shaking (sonicate time may be extended to ensure complete disintegration of tablets) .Made up to volume with diluent and mixed well. Filtered through 0.45μ PVDF filtered and discarded first 2-3 ml of filterate .Diluted 2-3 ml of filterate. Dilute 2-3ml of above

sample solution to 100ml clean and dry volumetric flask and diluted to volume with mobile phase and mixed well.

Validation Procedure

The validation studies are carried out by using freshly prepared solutions as per ICH requirements. The validation parameters of imatinib mesylate by HPLC is linearity, system precision, intermediate precision, method precision, accuracy, specificity, solution stability, robustness, limit of detection, and forced degradation.

System Suitability

The system suitability parameters were evaluated from standard chromatograms by calculating the %RSD from six replicate injections of imatinib mesylate retention times and peak areas.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excipients), which may be expected to be present in the sample and standard solution. To establish non-interference of the blank, sample diluent was prepared in duplicate and analysed along with standard and sample solutions. Blank interference was checked by examine the chromatographs of blank samples and sample with drug.

Accuracy

The accuracy of a measurement how close a result comes to true value. Accuracy for imatinib mesylate by taking three different concentrations of 50%, 100%, and 150% as per the test method. The API s with concentration of 20, 40, 60µg/ml were prepared and taken into each volumetric flask for each spike level and assay was performed. The average % of recovery was found to be 100. The values are in Table no-1

Precision

The precision of analytic method was studied by analysis of multiple sampling of homogenous sample. It was checked by analysing the samples at different time intervals of the same day as well as on different days. According to ICH, precision should be performed at three different levels. The proposed method having standard solution and sample solution chromatograms are shown in Fig. 2 and 3. Precision was investigated by the injection repeatability, six injections from the same standard preparations were made and the relative standard deviation for the replication injections was calculated. The % RSD of retention time and peak areas are within the limits. Precision values was placed in the table no.4

Linearity

Linearity of an analytical method is its ability to obtain test results directly proportional to the concentration of the analyte in the sample within a definite range. Six concentrations of different standard Solutions are prepared and ranging from 20% (0.05ppm) to 150% of (0.038 ppm) of the target concentration i.e 25 µg/mL. The final concentration of each solution is calculated and plotted against area response. The equation of calibration curve is $Y=57103+ 19899$, the correlation coefficient was found to be 0.999.

Forced degradation studies

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, thermal and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient. The forced degradation values are placed in the table no.2

Robustness

Robustness is the evaluation of an analytic method where the results obtained are found to be reliable even when performed in a slightly varied condition. This method was determined by analysis of aliquots from homogenous lots by differing physical parameters. In robustness method the % RSD value was found to be less than 1. The flow rate was 0.8ml/min and 1.2ml/min the effect is evaluated, variation of mobile phase is from 68:32 to 78:22 and 88:12 variation of wave length is from 248 and 238nm. The flow rate, mobile phase composition, wavelength which may differ at limits of assays. The values are placed in the table no.3

System suitability

Standard imatinib solutions was prepared and injected six times into the HPLC system and suitability parameters were evaluated from standard chromatographs by calculating %RSD by retention time and peak areas. All parameters within the limit. The USP plate count for imatinib is 4388 and tailing factor is 1.1. All parameters were found to be within the limit.

Table 1. Accuracy results imatinib mesylate

s.no	Concentration	%recovery
1	50%	20µg/ml
2	100%	40µg/ml
3	150%	60µg/ml

Mean=100.1

Table 2. Force degradation condition of imatinib mesylate

Stress condition	% of degradation
Acid	38.2
Alkali	42.4
Oxidation	0.3
Reduction	0.7
Thermal	5.8
Hydrolysis	5.8

Table 3. Roubstness results of imatinib mesylate

Name of drug	Flow plus	Flow minus	Organic plus	Organic minus	Wave length plus	Wave length minus
Imatinib mesylate %RSD	0.06	0.2	0.46	0.38	0.094	0.107

Table 4. Precision results of imatinib mesylate

Type of precision	sample	area	USP Plate count
Systemic precision	Imatinib mesylate	0.456	0.9759
Intermediate precision	Imatinib mesylate	0.385	1.79
Method precision	Imatinib mesylate	0.298	0.7996

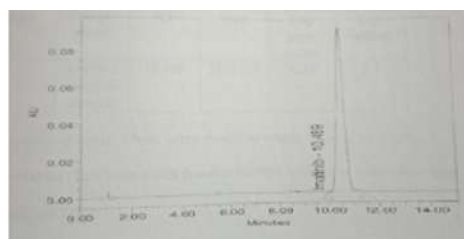
Fig 1. chromatography of standard

Fig 2. chromatography of sample

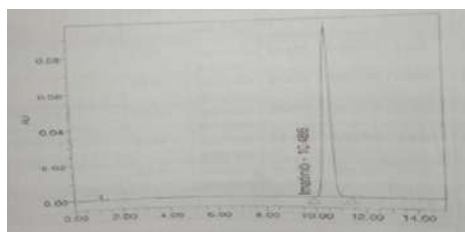
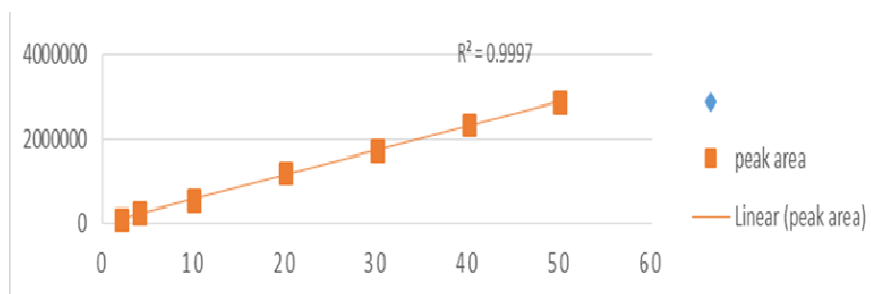


Fig no:3 Linearity graph



STATISTICAL ANALYSIS

The data obtained were analysed by Graph pad prism software version 8. The data is subjected to regression analysis to obtain the line of equation in linearity studies.

RESULTS AND DISCUSSION

The linearity range of the imatinib mesylate is 0.999, the %RSD of systemic precision, intermediate precision, method precision was 0.456, 0.385, 0.298. Accuracy 100.4%, solution stable up to 24 hrs. , and robustness of RSD% is not more than 2

CONCLUSION

In the present investigation new analytical method was developed for the quantitative estimation of potent drug imatinib mesylate. Hence the two methods such as UV and RP-HPLC can be useful to estimate imatinib mesylate in bulk and pharmaceutical dosage form.

ACKNOWLEDGEMENT

I thank Nirmala College of Pharmacy for providing all facilities

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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL RP-HPLC METHOD FOR THE ESTIMATION OF TOPIRAMATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Topiramate, "2,3:4,5 -Bis-0-(1-methylethylidene)-beta-D-fructopyranose sulfamate" is an Antiepileptic drug used for treatment of epilepsy and prevent migranes. Topiramate has also been used in the alcohol dependence. And it is a first line migraine preventive drug. It works by blocking of voltage – dependent sodium and calcium channels and also inhibits the excitatory glutamate pathway while enhancing the inhibitory effect of GABA. Moreover inhibits carbonic anhydrase. Its possible effects a mood stabiliser and inhibits maximal electrical shock and pentylenetetrazol-induced seizures. Its action on mitochondrial permeability transition pores has been proposed as a mechanism. The objective of the present work is to develop simple, accurate, economic, selective and precise High performance Liquid Chromatography (HPLC) method has been developed and validated for rapid and simultaneous quantification of Topiramate in tablet dosage form. Mobile phase contained a mixture of methanol and HPLC grade water is used in the ratio of (40:60), column was Zodiac C₈ 100×4.6mm, 5µm, flow rate 1.0ml/min, Band it shows a good linearity in the concentration range of 50-3000µg/ml and correlation coefficient was found to be within the limits i.e 0.999. The developed method has been validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD should not be more than 2. So, the planned methodology was found to be straight forward, specific, precise, accuracy, linear, and robust. Hence it can be applied for routine analysis of Topiramate in bulk drug and the pharmaceutical formulations.

KEYWORDS : P-HPLC, Rivastigmine tartrate, Validation.

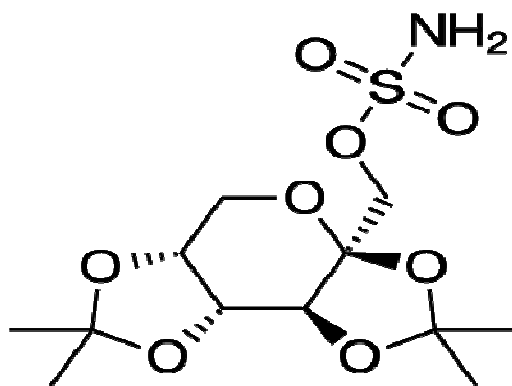


FIG-1 STRUCTURE OF TOPIRAMATE

INTRODUCTION

Topiramate drug¹ chemical formula is C₁₂H₂₁NO₈S. Its chemical name is 2,3:4,5-Bis-0-(1-methylethylidene)-beta-D-fructopyranose sulfamate. And its category is anti convulsant (anti epilepsy) & anti migraine. Topiramate drug is quickly observed after oral use. The exact mechanism is unknown, but 4 properties that may contribute to topiramate's anti epileptic and anti migraine efficacy includes blockage of voltage dependent Na channels, an augmentation of gamma-aminobutyrate acid activity at some subtypes of the GABA- A receptors, antagonism of AMPA/kinas subtype of the glutamate receptor and inhibition of

the carbonic anhydrase enzyme, particularly isoenzymes II & IV. It inhibits maximal electrical shock & pentylenetetrazol-induced seizures. Topiramate blocks action potential elicited by sustained depolarisation. Blockade of sodium channels, increases the frequency of GABA and activation of its receptors enhance induced chlorine ion influx, antagonizes kainite/AMPA subtype of glutamate receptors and inhibit carbonic anhydrase isoenzyme. bioavailability is 80% not affected by food². The pharmacokinetics of topiramate are linear over the dosage range of 200 to 800mg/day. The plasma elimination of half life is 21hrs, it weakly (13%-17%) binds to plasma proteins. It is also FDA approved for prevention of migraines. Given to Psychiatrist to treat the bipolar disorder³. This drug has been investigated for use in treating alcoholism, methamphetamine addiction, and obesity, especially to reduce binge eating. Other medical uses are to treat bulimia nervosa, smoker cessation, idiopathic intracranial hypertension, cluster headache. Adverse effects⁴ are paresthesia, diarrhea, nausea, anorexia, memory problems, fatigue, and confusion. Topiramate carries an increased risk of congenital malformations. Symptoms of overdose are agitation, depression, loss of consciousness, muscle weakness, bone pain. Topiramate impairs heart regulations⁵ especially in children. It should not be suddenly discontinued, as this may cause increased seizure activity.

MATERIALS AND METHODS

Topiramate working standard, HPLC grade water, topiramate API, topiramate tablets, and HPLC grade methanol were purchased from Merck, Germany.

EQUIPMENTS: Waters HPLC with EMPOWER2 software, refractive index detector, Zodiack C₈ were used.

CHROMATOGRAPHIC CONDITIONS: Ambient temperature was maintained, sample size is 50 µl and diluent used is methanol: water (40:60), flow rate is 1.0 ml/min, run time is 10 min, sample retention time is 7 to 8 min. It is observed that RT, peak intensity, number of theoretical plates was found to be in limits.

PREPARATION OF STANDARD SOLUTION

Weigh accurately about 200 mg of topiramate working standard taken in 100 ml volumetric flask. Add 70 ml of diluents; sonicate to dissolve to volume diluent. Shown graph in fig 2

PREPARATION OF SAMPLE SOLUTION

Weigh 10 tablets and crush the tablets weigh powder then take powder equivalent of 50 mg of topiramate into a 25 ml volumetric flask. Add 20 ml of diluent, sonicate for 20 min and dilute to volume diluent. Filter through 0.45 µm Nylon syringe filter. Shown graph in fig 3

VALIDATION

SYSTEM SUITABILITY ESTABLISHMENT

PROCEDURE: Inject 50 µL of standard preparation 5 times and sample preparation in the chromatographs. Record it and measure the peak responses for topiramate.

EVALUATION OF SYSTEM SUITABILITY & ACCEPTANCE CRITERIA: relative standard deviation (RSD) of 5 replicate injections of standard preparation for topiramate peaks and retention time should not be more than 2.0%. The tailing factor for topiramate peaks should be more than 2.0 and plate count will be not less than 2000. The %RSD of peaks of topiramate was found to be 0.650. Hence the system suitability test was passed. Shown in table no 1

LINEARITY: Linearity of detector response for topiramate was established for 9 concentrations ranging from 2.5% (50 µg/ml) to 150% (3000 µg/ml). The final concentration of each solution in µg/ml was calculated and plotted against area response. The slope, y-intercept, correlation coefficient (r) were calculated. The acceptance criteria; correlation coefficient should be not less than 0.999. Correlation coefficient was found to be within the limits i.e 0.999 shown in graph fig 4

ACCURACY: Accuracy was conducted for topiramate assay in triplicate (50%, 100%, 150%) as per test method with equivalent amount of drug containing topiramate into each volumetric flask for each spike level

to get the concentration of topiramate equivalent. The average % recovery was calculated. The acceptance criteria of % recovery of the topiramate each level should not be less than 98% and not more than 102%. This method has an acceptable of accuracy shown in table no 2.

PRECISION: The preciseness of the analytical technique was studied by analysis of multiple sampling. It is expressed as standard deviation for the replicate injections was calculated

SYSTEM PRECISION: For injection repeatability, 6 injections from same standard preparations were made, and the relative standard deviations for the replicate injections were calculated. The relative standard deviations of R_t and peak areas should not be more than 2.0%. The observation is that all parameters are within in limits. Shown in table 3

METHOD PRECISION: 6 individual preparations of topiramate drug substance was prepared with a target concentration of about 2000ppm. All parameters are within the limits. Shown in table 4

INTERMEDIATE PRECISION: 6 Sample solutions are prepared and injected on the next day (2000ppm) into the HPLC system as per test procedure. The observations of intermediate precision were given in the table below. All parameters are within the limits. Shown in table 5

LIMIT OF DETECTION

Calibration curve was repeated for 3 times and the standard deviation of the intercepts is calculated.

LOD= 3.3 σ / S

LIMIT OF QUANTIFICATION

Calibration curve was repeated for 3times and the standard deviation of the intercepts is calculated

LOD = 10 σ /S

ROBUSTNESS

It was determined by the analysis of aliquots from homogeneous lots of differing physical parameters like to flow rate, mobile phase composition, temperature which may differ but the responses are still within the specified limits.

EFFECT OF VARIATION FLOW RATE:

A standard solution (2000ppm) was prepared and injected into the HPLC system by keeping flow rates 0.8ml/min and 1.2ml/min the effect is evaluated. And all the parameters are within limits i.e should not be more than 2.0% shown in table 6

FORCED DEGRADATION

The specificity of the tatic was incontestable through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, thermal and photolytic degradation. The sample was exposed to these conditions and the main peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredients. Regulatory steerage in ICH Q2A, Q2B, Q3B, and FDA 21 CFR section 211 needs the event and validation of stability indicating effeciency assays. Weigh accurately concernig 200mg of topiramate operating standard taken into 100ml volumetric flask and add 70ml of diluent,sonicate to dissolve. From this working standard 5 ml was taken into 50 ml volumetric flask, 1ml of 5 N HCL was added and mixed, kept for 1hr and followed by neutralization with 1 ml of 5 N NaOH and made up to volume with mobile phase.same is done with alkali degradation studies.oxidation is done by adding 1ml of 5% H_2O_2 . And the solution was heated at 75^0C and cooled at room temperature.reduction is done by adding 1ml of 55 sodium bisulphate. At last photostability is done by 80 mg of topiramate was taken into a petridish and kept in photostability $200Wh/m^2$ in uv light and 1.2 million lxh in visibl light for 36hrs. Purity angle is found to be less than threshold ange in all forced degradation studies without having signs of purity flags.

RESULTS AND DISCUSSION

There are many analytical methods appeared in the literature of determination of TOPIRAMATE which includes spectrophotometry and HPLC methods. The object of the proposed work to develop an simple accurate, precise, rapid HPLC method for topiramate in bulk and pharmaceutical dosage form by using the solvent system methanol: water (40:60) zodiac C₁₈100×4.6mm, 5µm as stationary phase with flow rate 1ml/min (selection was based on peak parameters height, tailing, theoretical plates, capacity or symmetry factor, run time, resolution) with refractive detector (µ). The HPLC method for the determination of topiramate, and to validate the methods according to USP and ICH guidelines and applying the same for its estimation in marketed formulations. The refractive index detector is used for better results because this drug has low uv absorbance. The retention time for risperidone was found to be 7-8 min. According to USP XXIV (621), system suitability tests are in integral part of chromatographic method. The calibration was linear in concentration range of 50-3000µg/ml with regression 0.999 respectively. The low values of %RSD indicate the method is precise and accurate. Mean recoveries were found in the range of 98-102%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slit in different laboratories. %RSD reported was found to be less than 2% and proposed method was validated in accordance with ICH parameters.

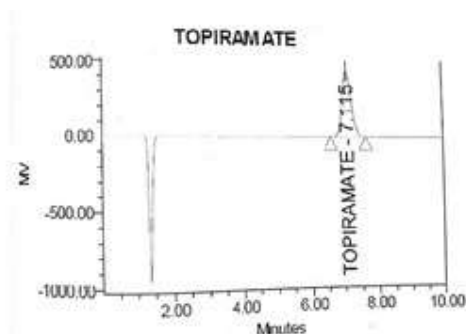


FIG:2 A representative chromatogram of standard

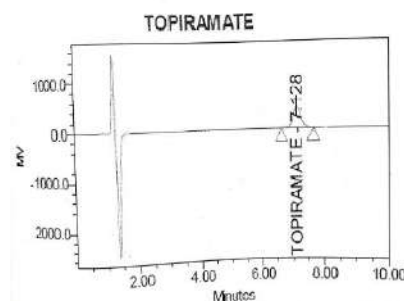


FIG:3 A representative chromatogram of sample

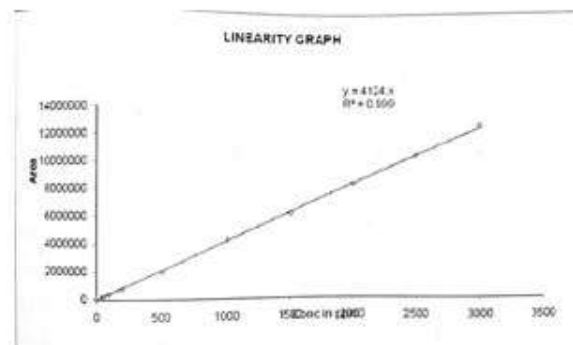


FIG:4 Linearity graph of topiramate

TABLE:1 Results of system suitability

S.NO	Sample	Peak area	USP tailing	USP plate count
1	Standard 1	8122718	1.211	3746
2	Standard 2	8198955	1.213	3696
3	Standard 3	8219155	1.212	3714
4	Standard 4	8247280	1.216	3699
5	Standard 5	8266425	1.216	3699
6	Standard 6	8258453	1.207	3678
Mean		8218831		
%RSD		0.650S		

TABLE:2 Accuracy results of topiramate by HPLC Mean=99.7; SD=0.231; %RSD=0.230

S.No	Accuracy	Conc (ppm)	Area	Amount recovered	%Recovery	Results
1	50%	25.50	4206550	25.62	100.5	Mean=99.6
2	50%	25.90	4210116	25.64	99.0	SD=0.78
3	50%	26.10	4256138	25.62	99.3	%RSD=0.780
1	100%	44.10	7233473	44.05	99.9	Mean=99.6
2	100%	47.10	7718264	47.01	99.8	SD=0.470
3	100%	51.20	8326281	50.71	99.0	%RSD=0.470
1	150%	65.20	10644851	64.83	89.4	Mean=100
2	150%	68.10	11204052	68.23	100.2	SD=0.47
3	150	71.20	11725129	71.41	100.3	%RSD=0.470

TABLE:3 Systemic precision values of Topiramate

S.No	RT	Area	USP count	Plate	USP Tailing
1	7.323	7795925	3912		1.163
2	7.410	7837532	3907		1.142
3	7.373	7875232	3907		1.160
4	7.281	7767493	3888		1.153
5	7.287	7859603	3855		1.154
6	7.296	7897845	3898		1.154
	Mean=7.289 %RSD=0.298873	Mean=7838938 %RSD=0.629	Mean=3901 %RSD=0.179		Mean=1.152 %RSD=0.6541

TABLE:4 Method Precision value of Topiramate

S.No	RT	AREA	USP plate count	Usp tailing
1	7.209	8122718	3476	1.211
2	7.254	8198955	3969	1.213
3	7.261	8219155	3714	1.212
4	7.294	8247280	3699	1.216
5	7.306	8266425	3661	1.203
6	7.292	8258453	3678	1.207
	Mean = 8218831 %RSD= 0.650	Mean= 8218831 %RSD= 0.650	Mean= 3686 %RSD= 0.9759	Mean=1.210 %RSD=0.170 6

TABLE:5 Intermediate precision of Topiramate

S.No	RT	Area	USP Plate count	USP tailing
1	7.108	7759563	3693	1.169
2	7.112	7924545	3715	1.176
3	7.112	7916915	3735	1.169
4	7.101	7939289	3728	1.180
5	7.099	7966115	3722	1.179
6	7.101	7960133	3676	1.185
	Mean=7.100 %RSD=0.126	Mean=7911093 %RSD=0.969	Mean=3726 %RSD=0.7996	Mean=1.173 %RSD=0.229

TABLE:6 Robustness data of Topiramate

S.n o	Flow rate ml/mi n	RT	Area	Plate count	USP Tailing	%Label claim
1	1.2	6.196	6730221	3458	1.102	100.70
2	1.2	6.211	6274812	3485	1.107	100.2
3	1.2	6.223	6997380	3478	1.107	100.8
		%RSD=0.5037	%RSD=0.1483	%RSD=0.1422	%RSD=0.4553	%RSD=0.321
1	0.8	8.898	10107593	4011	1.176	99.2
2	0.8	8.889	10967795	3975	1.181	99.8
3	0.8	8.860	10494301	3987	1.178	100.5
		%RSD=0.00858	%RSD=0.4094	%RSD=0.352665	%RSD=0.065521	%RSD=0.650

TABLE:7 Results of forced degradation

S. NO	Stress conditions	RT	Peak area	USP TAIL ING	PLAT E COUN T	%De grade d	Purity angle	Purity threshol d
1	Standard drug	7.316	8106527	1.177	2794	0	0.502	1.044
2	Acid	7.389	5284482	1.111	4188	19.4	0.712	1.043
3	Base	7.378	5182493	1.112	4306	19.2	0.216	1.075
4	Peroxide	7.488	5659247	1.114	4157	18.6	0.281	1.048
5	Reduction	7.403	5162824	1.111	4249	18.9	0.198	1.060
6	Thermal	7.546	5277636	1.113	4316	19.5	0.162	1.049
7	Photo	7.517	5198501	1.114	4327	19.6	0.159	1.050
8	Hydrolysis	7.545	4720418	1.097	4380	19.9	0.100	1.050
9	Humidity	7.485	5098228	1.100	4242	18.6	0.612	1.041

TABLE NO 8: The acceptance criteria for HPLC method

S.No	PARAMETERS	ACCEPTANCE CRITERIA	RESULTS OBTAINED	INFERENCE
1	Suitability	%RSD-2	0.650	complies
2	Linearity	Correlation coefficient NLT-0.999	0.999	complies
3	Precision	%RSD NMT 2	system	complies

			method	Complies	
			intermediate	Complies	
4	Accuracy	Recovery of the spiked drug(98-102%)	50%	complies	
			100%	complies	
			150%	complies	
5	Specificity	1)No interference from blank, placebo and other degradation products with the main peak		No interference	complies
		2)purity angle < Threshold angle		Peak pure	complies
6	Robustness	RSD NMT 2% in modified conditions Flow minus(0.8) Flow plus(1.2) Organic minus(75:5:20) Organic plus(55:25:20) Temperature(+5) Temperature(-5)		0.650 0.321 0.4 0.64 0.35 0.98	complies

CONCLUSION

In the present investigation a simple sensitive, precise and accurate, linear & robust RP-HPLC method was developed for the quantitative estimation of Topiramate in bulk and pharmaceutical dosage forms. The results expressed in tables. These were validated according to the ICH guidelines. The results obtained with proposed methods confirm the suitability of these methods for pharmaceutical dosage form. By these methods topiramate can be used in the both bulk drug and pharmaceutical formulations.

ACKNOWLEDGMENTS

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STABILITY INDICATING RP- HPLC METHOD DEVELOPMENT AND VALIDATION OF PARACETAMOL, CHLORPHENIRAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE AND CAFFEINE SIMULTANEOUSLY IN BULK AND COMBINED DOSAGE FORM

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ABSTRACT

A simple, Precise, Accurate method was developed for the estimation of Paracetamol, Phenyl ephrine hydrochloride, Chlorpheniramine Maleate and Caffeine by RP-HPLC technique. Chromatographic conditions are stationary phase-Inertsil C8 250 mm x 4.6 mm, 5 μ , mobile phase- water: acetonitrile in the ratio of 60:40v/v and flow rate at 1ml/min, detection wave length was 233nm, and diluent was mobile phase. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 5% to 100 % levels, R² value was found to be as 0.9996, 0.9994, 0.9991 and 0.9994 for Paracetamol, Chlorpheniramine Maleate, Phenyl ephrine hydrochloride and Caffeine respectively. LOD values are 2.425, 2.504, 2.763, 2.253 respectively and the LOQ values were found to be 5.412, 10.811, 10.553 and 10.125 respectively. By using above method assay of marketed formulation was carried out and 100.01% was present. Degradation studies of Paracetamol, Phenyl ephrine hydrochloride, Chlorpheniramine Maleate and Caffeine were done, in all condition purity threshold was more than purity angle and within the acceptable range.

KEYWORDS: HPLC, Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine Maleate and Caffeine. Method validation, ICH Guidelines.

INTRODUCTION

A combination of Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine maleate and Caffeine is widely used for analgesic, antipyretic, antihistamine and antitussive activity. Chlorpheniramine maleate (CPM) is used as an antihistaminic agent in allergic reactions, which prevents muscular response of histamine and thereby reducing cough and common cold ¹ is chemically 2-[p-chloro-[2- dimethyl amino) ethyl] benzyl] pyridine maleate. Various analytical methods such as Spectroscopy ²⁻⁵ have been reported for estimation of Chlorpheniramine maleate, Paracetamol, phenylephrine hydrochloride, and caffeine. Paracetamol (PARA) is a centrally and peripherally acting non-opioid analgesic and antipyretic is chemically N-(4-hydroxyphenyl) acetamide. Phenylephrine HCl (PEH) acts as nasal and sinus decongestant ¹ is chemically 3-[1-hydroxy-2-(methyl amino) ethyl] phenol hydrochloride form. Caffeine (CAF) is an addictive stimulant ¹ is chemically 1, 3, 7-trimethyl-3, 7-dihydro-1H -purine -2, 6- Dione. In humans it stimulates the central nervous system, heart rate, and respiration and has psychotropic properties and acts as mild diuretic. Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine maleate and Caffeine are commercially available in tablet dosage form. Literature survey revealed that one HPLC method is available for estimation of these four actives in combination, but it needs improvement. So efforts were taken to make available simultaneously evaluating, optimized, simple and cost effective HPLC method for estimation of these four actives in pure and in tablet dosage form as per International Conference on Harmonisation (ICH) guidelines. In the present study the combination of Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine maleate and Caffeine were used under the brand name of SUDACEF. The objective of the present study is to develop and validate a RP-HPLC method for the simultaneous estimation of Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine Maleate, Caffeine in combined dosage form.

MATERIALS AND EQUIPMENT

MATERIALS

Instruments used- EutechPH meter, Denver weighing balance, UCA 701 unichrome Ultrasonicator, Empower software HPLC LC Waters 2695. Flow rate 1mL/min. Isocratic model Pump

Chemicals And Reagents: HPLC grade water, HPLC grade acetonitrile.

Drugs: Paracetamol, Chlorpheniramine Maleate, Phenylephrine hydrochloride and caffeine

METHODOLOGY

METHOD DEVELOPMENT

The developed RP-HPLC method for the simultaneous estimation of Paracetamol, Chlorpheniramine Maleate, Phenylephrine hydrochloride and caffeine was carried out on with Inertsil C8 (250mm x 4.6mm, 5µm) column. Water and acetonitrile in the ratio (60:40 v/v) used as mobile phase and flow rate of 1.0 ml/min. The detection was carried out at 233nm and ambient column temperature was maintained.

Chromatographic condition

Column: Inertsil C8,

Injection volume: 20µL

Column Temperature: Ambient

Flow rate: 1.0 mL/min

Mobile phase: Buffer and Acetonitrile in the ratio of 60:40v/v

Preparation of Diluent: Mobile phase was used as diluents.

Retention time of Phenylephrine HCl, Paracetamol, CPM and Caffeine is about 3.0 min, 4.0 min, 5.6 min, 7.3 min respectively.

Preparation of standard solution

(a) Phenylephrine Hydrochloride: 10 mg of Phenylephrine HCl was weighed accurately into a 200 mL volumetric flask. 70 mL of diluent was added, and sonicated to dissolve the powder and made up to the volume. This gives solution A

(b) Paracetamol: 500 mg of Paracetamol was weighed accurately into a 100 mL volumetric flask 70 mL of diluent was added and sonicated to dissolve the powder, made up to the volume. This gives solution B.

(c) CPM: 20 mg of CPM was weighed accurately into a 100 mL volumetric flask. 70mL of diluent was added and sonicated to dissolve the powder, made up to the volume. Further dilute 5 mL to 50 mL with the diluent. This gives solution C.

(d) Caffeine: 30 mg of Caffeine was weighed accurately into a 100 mL volumetric flask. 70 mL of diluents was added and sonicated to dissolve the powder, made up to the volume. This gives Solution D. Then further dilute each 5mL Solution-A, Solution-B, Solution- C and Solution- D to 50 ml with the diluent.

Preparation of Sample solution: 10 tablets were weighed and powdered, then take 5 tablets equivalent powder into a 250 mL volumetric flask. 100 mL of diluents was added and sonicated to dissolve the powder, made up to the volume by using diluent. Then further dilute 5 mL to 50 mL with the diluent. Filter through 0.45µ Nylon syringe filter.

ASSAY OF FORMULATION

%Assay was carried out in tablet formulation

The results were calculated by using the formula given below and reported.

$$\% \text{ Assay} = \frac{\text{Test area} \times \text{STD weight} \times \text{test dilution} \times \text{average weight} \times \text{potency} \times 100}{\text{STD area} \times \text{test weight} \times \text{STD dilution} \times \text{label claimed} \times 100}$$

METHOD VALIDATION

A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely. There are several parameters that are considered in the method validation process as per International Conference of Harmonization (ICH) guidelines and the values for these parameters are as follows.

Figure-01: A Representative chromatograph of Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine maleate and Caffeine.

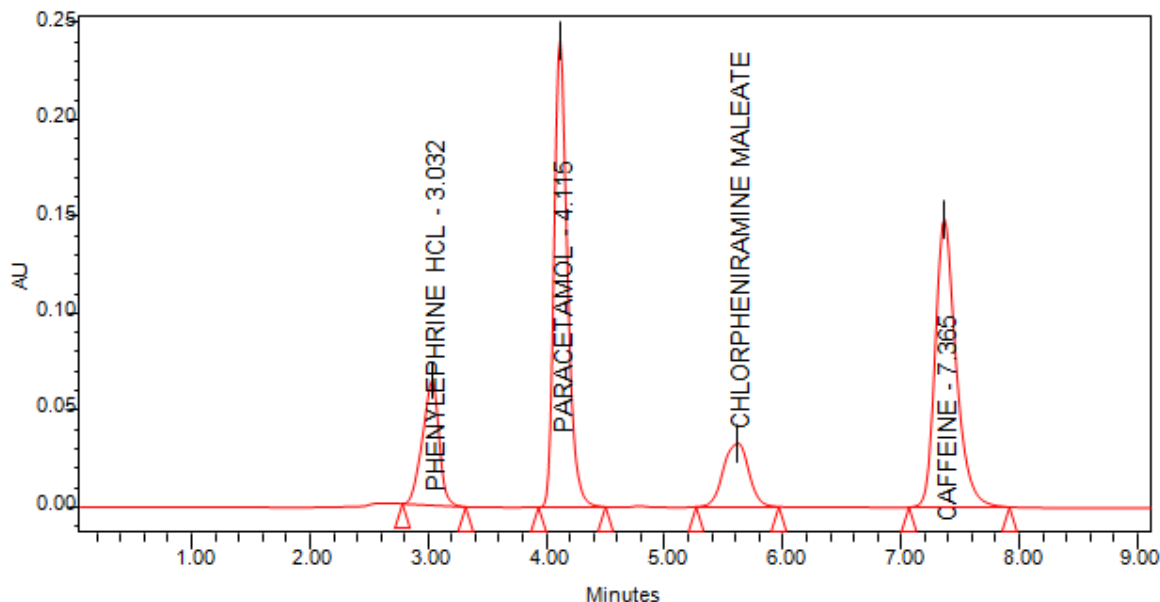


Table: 1 Assay Calculations

DRUG	LABEL CLAIMED FOR SAMPLE TAKEN (mg)	CALCULATED VALUE (mg+SD)	%OF ASSAY
Phenyl ephrine hydrochloride	5	5.15	100.2
Paracetamol	500	501.23	100.4
Chlorpheniramine Maleate	2	2.05	99.9
Caffeine	30	30.12	100.1

Table: 02 Validation parameters for Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine maleate and Caffeine.

PARAMETERS	PARA	CPM	PHE	CAFF
LINEARITY (UG/ML)	60-750	0.25-4.50	0.5-7.5	3-45
CORRELATION COEFFICIENT	0.9996	0.9994	0.9991	0.9994
PRECISION				
(I)METHOD PRECISION	0.128	1.052	0.846	1.012
(II) INTERMEDIATE PRECISION	0.154	1.11	0.182	0.855
(III)SYSTEM PRECISION	0.338	1.436	0.480	0.994
LOD	2.425	2.504	2.763	2.253
LOQ	5.412	10.811	10.553	10.125

ACCURACY (% OF RECOVERY)	50%	0.897	0.546	0.251	0.146
	100%	0.757	0.457	0.357	0.257
	150%	0.646	0.345	0.285	0.145
ASSAY (%)		100.4	99.9	100.2	100.1
SOLUTION STABILITY		100.4	100.1	100.2	100.3
ROBUSTNESS RSD NMT 2% IN MODIFIED CONDITION		Complies	Complies	Complies	Complies
FLOW PLUS		0.141	1.137	0.712	0.220
FLOW MINUS		0.336	0.712	0.134	0.256
ORG PLUS		0.956	1.137	0.739	0.615
ORG MINUS		0.299	0.671	0.188	0.088
WAVE LENGTH PLUS		0.108	0.300	0.023	0.137
WAVE LENGTH MINUS		0.299	0.610	0.428	0.616

STABILITY STUDIES

Table: 03 Results of forced degradation for Paracetamol

		PARACETAMOL				PEAK PURITY		
	SAMPLE WEIGHT IN mg	AREA COUNTS	MEAN AREA COUNT	% LABEL CLAIM	% DEGRAD ATION	PURITY ANGLE	PURIT Y THRES HOLD	PASS /FAI L
		INJECTIONS						
CONTROL	600.8	1893440	1893440	100.4	0.00	5.316	20.357	PASS
ACID	600.4	1364982	1364982	79.5	20.5	7.053	28.549	PASS
ALKALI	600.7	1204922	1204922	78.8	21.2	4.919	21.126	PASS
PEROXIDE	601.2	1215947	1215947	77.3	22.7	5.105	21.235	PASS
REDUCTION	602.1	1211758	1211758	72.5	27.5	5.099	20.121	PASS
THERMAL	600.9	1281799	1281799	71.9	28.1	5.228	20.163	PASS
PHOTO	601.1	1281799	1281799	72.5	27.4	5.228	20.163	PASS
HYDROLYSIS	602.1	1222629	1222629	74.6	25.4	5.022	20.566	PASS
HUMIDITY	605.5	1211758	1211758	73	27	5.099	20.121	PASS

Table: 04 Results of forced degradation for Chlorpheniramine maleate.

		CPM				PEAK PURITY		
	SAMPLE WEIGHT IN mg	AREA COUNTS	MEAN AREA COUNT	% LABEL CLAIM	% DEGRA DATION	PURITY ANGLE	PURITY THRES HOLD	PASS FAIL
		INJECTIONS						
CONTROL	600.8	487932	487932	100.2	0.00	16.869	90.00	PASS
ACID	600.4	347194	347194	78.8	21.2	19.689	90.00	PASS
ALKALI	600.7	305580	305580	77.3	22.7	18.238	90.00	PASS
PEROXIDE	601.2	310948	310948	75.5	24.5	18.209	90.00	PASS
REDUCTION	602.1	304286	304286	72.9	27.1	17.750	90.00	PASS
THERMAL	600.9	323287	323287	73.5	26.5	17.688	90.00	PASS
PHOTO	601.1	323287	323287	75.6	24.4	17.688	90.00	PASS
HYDROLYSIS	602.1	308467	308467	78.2	22.8	17.851	90.00	PASS
HUMIDITY	605.5	304286	304286	77.3	22.7	17.750	90.00	PASS

Table: 05 Results of forced degradation for Phenylephrine HCL.

		PHENYLEPHERINE HCL				PEAK PURITY		
	SAMPL E WEIGH T IN mg	AREA COUNTS	MEAN AREA COUN T	% LABE L CLAI M	% DEGR A DATIO N	PURIT Y ANGL E	PURITY THRESHOL D	PAS S/ FAIL
		INJECTIO NS						
CONTROL	600.8	627255	627255	100.1	0.00	20.145	48.493	PASS
ACID	600.4	497261	497261	78.8	21.2	12.946	62.666	PASS
ALKALI	600.7	412641	412641	77.7	22.3	9.380	41.979	PASS
PEROXIDE	601.2	441811	441811	75.5	24.5	11.534	52.361	PASS
REDUCTIO N	602.1	438268	438268	76.5	23.5	11.451	50.827	PASS
THERMAL	600.9	461396	461396	74.8	25.2	10.900	51.159	PASS
PHOTO	601.1	461396	461396	76.8	23.2	10.900	51.159	PASS
HYDROLYS IS	602.1	450422	450422	79.1	22.9	11.808	51.066	PASS
HUMIDITY	605.5	438268	438268	77.5	22.5	11.451	50.827	PASS

Table: 06 Results of forced degradation for Caffeine.

		CAFFEINE				PEAK PURITY		
	SAMP LE WEIG HT IN mg	AREA COUNT S	MEAN AREA COUNT	% LABEL CLAIM	% DEGRA DATIO N	PURIT Y ANGLE	PURIT Y THRES HOLD	PASS/ FAIL
		INJECT IONS						
CONTROL	600.8	1875341	1875341	100.8	0.00	3.373	16.128	PASS
ACID	600.4	1327782	1327782	78.8	21.2	3.481	15.253	PASS
ALKALI	600.7	1159035	1159035	75.5	24.5	3.400	16.158	PASS
PEROXIDE	601.2	1166879	1166879	78.2	21.8	3.428	15.631	PASS
REDUCTION	602.1	1149133	1149133	77.7	22.3	3.414	15.352	PASS
THERMAL	600.9	1230334	1230334	76.4	23.6	3.395	15.681	PASS
PHOTO	601.1	1230334	1230334	75.5	24.5	3.395	15.681	PASS
HYDROLYSIS	602.1	1167315	1167315	77.8	27.2	3.678	15.786	PASS
HUMIDITY	605.5	1114913 3	11149133	79.3	22.7	3.414	15.352	PASS

CONCLUSION

The proposed RP-HPLC method is suitable for the determination of Paracetamol, Phenyl ephrine hydrochloride, Chlorpheniramine Maleate and Caffeine indosage forms. All the parameters of developed methods met the criteria of ICH guidelines for method validation. Hence, the developed chromatographic method for Paracetamol, Phenyl ephrinehydrochloride, Chlorpheniramine Maleate and Caffeine is said to be rapid, simple, precise, accurate, specific and cost effective that can be effectively applied for the routine analysis.

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BIOSYNTHESIS OF SILVER NANOPARTICLES BY AZADIRACHTA INDICA AND MENTHA ARVENSIS LEAF EXTRACT IS CHARACTERIZED AND APPLICATION FOR ANTIMICROBIAL AND ANTIBACTERIAL FINISH ON FABRIC

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ABSTRACT

Silver nanoparticles (AgNPs) are used in various medical and commercial products because of their experimental and microbial and anti-odor properties. Studies are needed to assess their impact on the environment as well as on human body. Several reports shown that chemically synthesised AgNPs could cause some serious neurotoxic effects. So, green nanoparticles are usually considered safer than their chemically synthesized silver nanoparticles. According to this research work, we have assessed the effect of AgNPs synthesized from aqueous-leaf-extract of *azadirachta indica* and *menthe arvensis*. Products made from neem trees have been used in india for over two millennia for their medicinal properties. Neem products are believed by Siddha and Ayurvedic practitioners to be anthelmintic, antifungal, ant diabetic, antibacterial, antiviral, contraceptive, and sedative. Neem oil is also used for healthy hair, to improve liver function, detoxify the blood, and balance blood sugar levels. Neem leaves have also been used to treat skin diseases like eczema, psoriasis, etc. In ayurveda, Pudina is considered as appetizer and useful in gastric troubles, and it was traditionally used to treat flatulence, digestive problems, gall bladder problems and coughs. Nowadays it is used in many countries for various ailments. Mint extracts and menthol-related chemicals are used in food, drinks, cough medicines, creams and cigarettes.

KEYWORDS: *Azadirachta indica and menthe arvensis, Escherichia coli and staphylococcus aureus, Uv-visible spectrophotometry, nanoparticles.*

INTRODUCTION

SILVER NANOPARTICLES

Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into product that range from photovoltaics¹ to biological and chemical sensors. Based on antimicrobial activity and availability neem (*Azadirachta indica*) was selected for present research work. In these work investigations emphasizes on screening of herbs with potent antimicrobial activity, treatment of woven and nonwoven fabrics with herbal leaf extracts, assessment of their antimicrobial efficacy². The pomegranate, neem and turmeric extracts coated on fabrics exhibit more antimicrobial efficiency on gram negative bacteria than gram positive bacteria³. It is important to take into account the impact of stress strain, thermal and mechanical effects on the finished substrates. The following requirements need to be satisfied to obtain maximum benefits out of the finish⁴. The study centered on the development of biofunctional polyester/cotton blend cloth using a natural product. An antimicrobial agent extracted from the seeds of Neem tree (*Azadirachta indica*) was used for imparting antibacterial property to the blend fabric⁵. The use of natural antimicrobial agents for bioactive textiles is a rising area of research. Neem (*Azadirachta indica*), an evergreen tree of india, is one of the promising sources of biologically active compounds with chemicals known as limonoids which are bitter in taste⁶. To determine the antimicrobial effect of Mint stem. To optimise and evaluate the parameters for antimicrobial finishing. To treat the cotton fabric with selected source and evaluation⁷, the antimicrobial effect of the synthesized nanoparticles. *Azadirachta indica*, which is a common plant known as Neem is found abundantly in India and in nearby Indian subcontinents. It belongs to Meliaceae family and is known for its various applications especially its medicinal property.

Azadirachta indica leaf extract is employed in the synthesis of various nanoparticles like gold, zinc oxide, silver etc. The phytochemicals present in neem are namely terpenoids and flavanones, which act as reducing as well as capping agent and helping in stabilizing the nanoparticles. When silver salt is treated with neem leaf extract, the silver salt is reduced to AgNPs. The synthesized nanoparticles, which are capped with neem extract also, exhibit enhanced antibacterial activity⁸. The field of nanotechnology has proved to be one of the most active areas of research⁹. Synthesis of nanoparticles is increasing exponentially because of its wide range of applications in the field of optoelectronics, biosensors, bio-nanotechnology, and biomedicine etc¹⁰. All the above methods are reported on Neem combined with different plants on cotton fabric no suggested methods are reported for the neem and pudina leaf extracts for the comparison of antifungal, antimicrobial activity on both cotton and silk fabrics. So the present research work is performed for comparison of neem and pudina leaf extracts for antifungal and antimicrobial activity.

MATERIALS AND METHODS

PREPARATION OF BIO EXTRACT

EXTRACTION BY INFUSION

Twenty grams fresh leaves of neem and pudina were washed with tap water and then washed with distilled water, air dried and then they were finely cut and soaked in 100 ml boiling distilled water for 5–10 min and filtered through Whatman filter paper no. 42. This extract was used for generating silver nanoparticles.

PREPARATION OF SILVER NANOPARTICLES (SNP)

5ml of leaves extract was added into 45 ml 0.002 M AgNO₃ solution in 100 ml conical flasks at room temperature in dark. After 1 h, formation of silver particles started to appear in the flask.

CHARACTERISATION BY FTIR SPECTRA

IDENTIFICATION OF FUNCTIONAL GROUPS WITH IR SPECTROSCOPY

FT-IR spectral analysis was carried out to categorize the probable biomolecules involved in the reduction of the Ag⁺ ions and the capping of the AgNPs synthesized by pudina, silver nitrate, neem extract. The chemical composition of silver nanoparticles was analyzed using FTIR spectrometer after bio reduction with silver nitrate. 2 ml of bio synthesized liquid silver nanoparticles were scanned using infrared in the range of 400-4000 cm⁻¹ using 'nujol' method for sample introduction.

PREPARATION OF POTATO DEXTROSE AGAR PLATES

PDA was accurately weighed and they were dissolved in distilled water, and transferred into a conical flask. Before going to sterilization conical flask was plugged with the cotton and then placed it in the autoclave for sterilization. After sterilization, 20 ml Agar was transferred in each sterilized Petri plates and then allows the plates to cool, then the agar gets solidified and then inoculation was done.

PREPARATION OF SILVER NANOPARTICLES BY DYEING OF COTTON AND SILK

Pre-washed cotton and silk fabrics dyed with the neem and mentha leaf extracts are used as control fabrics. whereas silver nanoparticles-treated cotton and silk pieces were used as sample fabrics to assess durable textile finishing by subsequent washing method and further for the antibacterial activity. The control samples are prepared by dipping the fabrics in 20% aqueous extract of neem and mentha arvenses leaves at 60-70°C for 2 h keeping the material in liquor ratio 1:25. Dyed fibres are dried in shade without squeezing. similarly pre-washed cotton and silk fabrics are dipped in silver nanosolution generated by neem and mentha leaves for 4 hours and then they were taken out and dried in the shade. The cotton treated fabric is in grayish brown and silk treated fabric is in greenish brown in colour.

DURABLE TEXTILE FINISH TEST

The coated silver nanoparticle pieces of cotton and silk dyed fabrics with the dimensions of 4x 3cm are used for wash continuously to estimate the results of durable textile finish. 5 continuous washings are carried out thorough wetting of treated fabrics by distilled water and the samples were left for 4 h at room temperature. After drying, changes in sample color and bleeding to white fabric were determined. These samples were further used for estimation of antibacterial and anti microbial activity

DETERMINATION OF ANTIMICROBIAL, ANTIBACTERIAL ACTIVITY OF TEXTILE MATERIAL

PARALLEL STREAK METHOD

Plant extracts of *Azadirachta indica* and *Mentha arvensis* were checked against two different bacteria, namely *Staphylococcus aureus*, *Escherichia coli*. Four different concentrations of plant extract were tested for anti-bacterial and anti-microbial activity using potato dextrose agar medium by streak plate method test organisms are inoculated, after 18-24 hours lower colony areas are counted along with the sides of fabrics indicating antibacterial, antimicrobial activity.

QUANTIFICATION OF ANTIBACTERIAL ACTIVITY

The micro-organisms were inoculated in 100ml flask containing potato dextrose agar broth. These flasks were incubated at 37 for 24hrs. Media was prepared using N-agar, test microorganisms were then spread over the solidified plates and A Bacterial positive control and antibiotic control were kept for comparative study. According to optimum temperature required for bacterial species anti-bacterial activity was obtained by determining the zone of inhibition around the cloth.

Zone of inhibition was measured in cm.

RESULTS

Characterization of silver nanoparticles by uv-visible spectroscopy

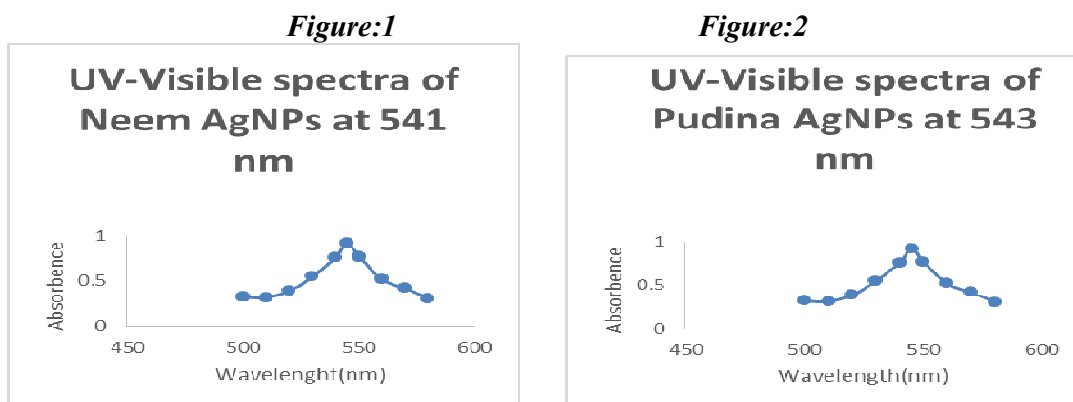


Table: 1 *Uv-visible spectra of agnp's with azadirachta indica and mentha arvensis*

s.no	absorbance	azadirachta indica	mentha arvensis
		wavelength(nm)	wavelength(nm)
1	500	0.325	0.325
2	510	0.32	0.32
3	520	0.39	0.39
4	530	0.55	0.55
5	540	0.765	0.765
6	545	0.92	0.890

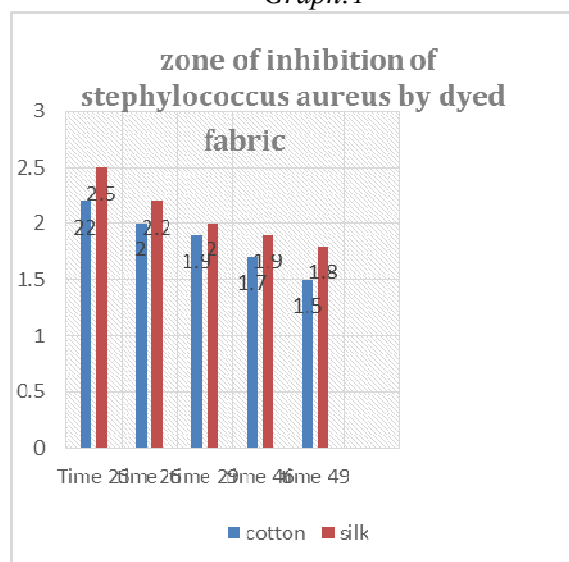
Table: 2 *antibacterial, antimicrobial activity of azadirachta indica plant extract mentha arvensis dyed cotton and silk fabrics*

S.No	Time (hrs)	Zone of inhibition for plant extract(neem) dyed silk fabric(cms)	Zone of inhibition for plant extract (neem)dyed cotton fabric(cms)	Zone of inhibition for plant extract(mint) dyed silk fabric(cms)	Zone of inhibition for plant extract(mint) dyed cotton fabric(cms)
1	23	2.5	2.2	2.3	2.5
2	26	2.2	2.0	2.1	2.3

3	29	2.0	1.9	2.0	2.2
4	46	1.9	1.7	1.9	2
5	49	1.8	1.5	1.7	1.8

Zone of inhibition of stephylococcus aureus,e.coli on dyed fabric.

Graph:1



Graph :2

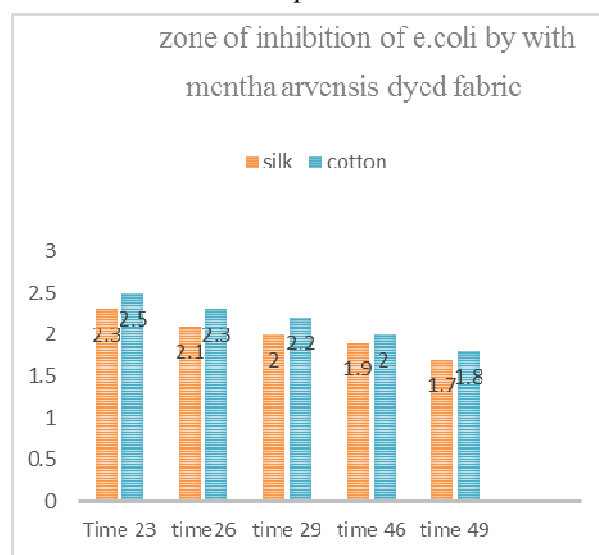
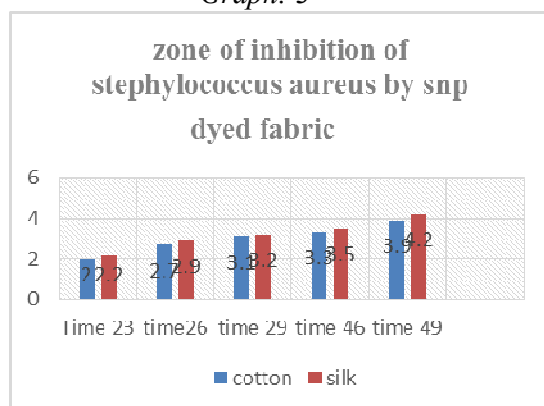


Table:3 Antibacterial,antimicrobial activity of snp of neem and menthe on cotton and silk fabrics

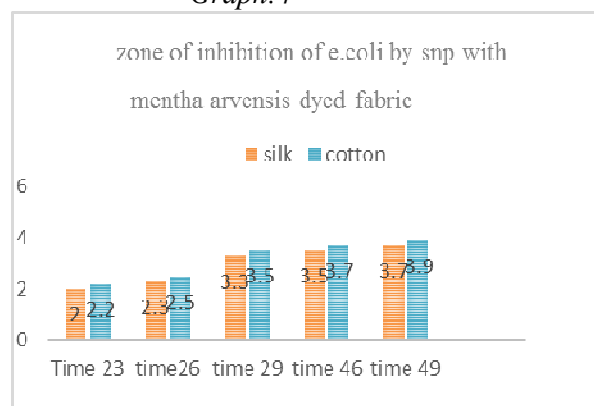
S.No	Time (hrs)	Zone of inhibition for plant extract (neem) snp dyed silk fabric(cms)	Zone of inhibition for plant extract (neem) snp dyed cotton fabric(cms)	Zone of inhibition for plant extract (mint) snp dyed silk fabric(cms)	Zone of inhibition for plant extract (mint) snp dyed cotton fabric(cms)
1	23	2.2	2.0	2.0	2.2
2	26	2.9	2.7	2.3	2.5
3	29	3.2	3.1	3.3	3.5
4	46	3.5	3.3	3.5	3.7
5	49	4.2	3.7	3.7	3.9

zone of inhibition of micro organisms by snp azadirachta indica dyed fabric

Graph: 3



Graph:4



CONCLUSION

Biosynthesis of silver nanoparticles by *azadirachta indica* and *mentha arvensis* leaf extract is characterized and application for antimicrobial and antibacterial finish on fabric. Silver nanoparticles synthesized by the green chemistry approach reported in this study using *Azadirachta indica* and *Mentha arvensis* leaves extract shows potent applications it has been demonstrated that use of a natural, renewable and low-cost biological reducing agent, such as *Azadirachta indica* and *Mentha arvensis* leaves can produce metal nanostructures in aqueous solution at ambient temperature, avoiding the presence of hazardous and toxic solvents. The antifungal, antimicrobial activity of SNP derived from *Azadirachta indica* shows enhanced activity when compared to *Mentha arvensis*. By incorporating nano scale silver into textiles the manufactures can make materials that use a small amount of silver to kill the microbes present on the surface of the clothing material it helps to prevent spoilage from the microbial growth.

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BIOANALYTICALTECHNIQUE FOR ESTIMATION OF FENOFIBRIC ACID BY LC-MS/MS: A REVIEW

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ABSTRACT

A simple, selective and rugged quantitative method for the determination of Fenofibric acid the active and metabolite of fenofibrate in human plasma (Na₂EDTA) using liquid chromatography–tandem mass spectrometric (LC-MS/MS) method has been developed and validated with 200µL human plasma. Carbamazepine was used as an internal standard. Analyte and the internal standards were extracted from human plasma by liquid-liquid extraction using ethyl acetate as extraction solvent and HPLC grade Acetonitrile: 0.1% Formic acid (80:20, V/V) as extraction buffer. The reconstituted samples were chromatographed on a C18 column Hypersil (BDS-4.6×100mm, 5µm) by using isocratic mobile phase. The method was validated over the concentration range of 100.0ng/mL to 20004.2 ng/mL, the API 3000 MDS SCIEX mass spectrometer was operated under the multiple reaction –monitoring mode (MRM) using the electro spray ionization technique for quantification of ion transition at m/z – 319.20 (parent) and 233.10 (product) and m/z -237.20 (parent) and 194.10(product) for the drug and the internal standard respectively. The method was validated for precision, accuracy, stability, matrix effect, dilution integrity, ruggedness, selectivity and extraction efficiency and method developed has been proved to be simple, sensitive, selective, rugged and reproducible. A run time of 2.00 min for each sample made it possible to analyze more than 400 plasma samples per day. The proposed method can be applied for the estimation of the Fenofibric acid in real time plasma samples for pharmacokinetic, drug-drug interaction and toxicological studies.

KEY WORDS: LC-MS/MS, Fenofibric acid, Carbamazepine, Ethyl acetate, Acetonitrile, Multiple Reaction Monitoring mode (MRM), Electro spray ionization technique.

INTRODUCTION

Fenofibric acid is a metabolite of drug Fenofibrate¹. It has role as marine Xenobiotic metabolite and a drug metabolite. Fenofibric acid is a lipid regulating agent used to treat high cholesterol and triglycerides levels². Fenofibric acid belongs to the class Fenofibrates³. Fibric acids are amphipathic carboxylic acids characterized by the presence of carboxyl group at the terminal end. It is a PPAR stimulator and can also influence the other transcriptional factors such as liver X receptors and ANGPTL which also plays a key role in lipid biology. It has a LXR agonist activity to up regulate ABCA1. The Fenofibric acid is virtually insoluble in aqueous media suitable for injection. The lipid modifying effects of Fenofibric acid in clinical practice have been explained in vitro in human hepatocyte cultures by the activation of PPAP. It increases lipolysis and eliminates triglyceride rich particles by stimulating LPL and decreases apoprotein C-III production. The decreased triglycerides levels causes alteration of LDL from small dense particles to large buoyant particles, which are having greater affinity for cholesterol receptors and are metabolized rapidly. It is mainly uses in severe hypertriglyceridemia and primary hypercholesterolemia [4-6]. This article gives information of analysis by LC-MS/MS of Fenofibric acid where as HPLC methods also done in previous days.

MATERIALS AND METHODS

CHEMICALS: (HPLC grade), Acetonitrile HPLC (grade), milli-Q-water, ammonium acetate (GR grade), Fenofibric acid, Carbamazepine, formic acid (GR grade).

EQUIPMENT : Analytical balance, micro balance, API 3000 LC-MS/MS system 1.4.2 version, HPLC of Shimadzu DGU 20A3, Pump A of LC- 20 AD, Auto sampler of SIL-HTC ,oven of CTSI-10 ASvp, Receptor shaker, turbo evaporator, and centrifuge.

CHROMATOGRAPHIC CONDITIONS: HPLC Column: Hypersil (BDS – $4.6 \times 10 \times 1000$ 5 m), Mobile Phase: HPLC grade Acetonitrile- 0.1%, formic acid (80:20,v/v), Rinsing Solution: Acetonitrile-milli Q or HPLC grade, Flow rate: 1.000mL/min, Split ratio: 50 ratio, Sample cooler temperature: 10°C, Injection volume: 10L, Needle rinsing solution: 500l, Column oven temperature: 40°C, Rinsing mode: Before and after aspiration, Retention time: Fenofibric acid 0.88+/- 0.3 min, Run time: 2.00 min.

METHOD DEVELOPMENT: The method for analyzing the drugs by HPLC and LC-MS/MS can be developed about the nature of sample, its molecular weight, polarity, ionic character, pka value and stability⁷.

MS/MS PARAMETERS OPTIMIZATION: Selection of the ion source and ion mode will be based on chemical properties of the compound. Suitable stock solutions are prepared and injected for optimizing the MRM Conditions. Sample should be injected in Q1 ion scan mode, then parent ion intensity should be increased by altering the decluttering potential (DP), Focusing Potential (FP) and Ion spray voltage (IS) parameters in Q1 multiple ion scan mode. Then intense stable product can be attained. Then by altering the collision energy, collision cell exit potential and collision activated dissociation has in MRM mode the stable product ion can be attained. Then the source parameters will be optimized by injecting the sample through HPLC using suitable mobile phase⁸⁻⁹.

CHROMATOGRAPHIC PARAMETERS OPTIMIZATION: Mobile phase selection and column selection are the two steps in optimization of chromatographic parameters. By optimizing stable and intense peaks are attained.¹⁶

FENOFIBRIC ACID STOCK SOLUTION: About 5mg of Fenofibric acid working standard is accurately weighed and transferred to 5mL volumetric flask, dissolved in HPLC grade methanol and make up to 1mg/mL solution. The stock solutions were diluted to suitable concentrations for spiking in to plasma to obtain the calibration curve standards, quality control, sample and DIQC samples.⁹⁻¹⁰

CARBAMAZEPINE STOCK SOLUTION (INTERNAL STANDARD): About 10mg of Carbamazepine reference standard is weighed accurately and transferred to a 10mL volumetric flask dissolved in HPLC grade methanol and made the volume to produce 1mg/mL of Carbamazepine¹¹. The stock solution was diluted to suitable concentration for internal standard dilution and mobile phase for system suitability dilution.

SAMPLE PREPARATION

A 200µL of plasma samples was pipetted into a 15ml polypropylene centrifuge tubes. 20µL of 15080.0 ng/mL. Carbamazepine dilution was added to it vortexed, except in blank sample where 20 µL diluent was added and vortexed. Then add 100µL of 20% formic acid solutions prepared in HPLC grade or milli Q water and vortexed¹². The samples were placed on a reciprocating shaker at 200 rpm for 20 minutes and centrifuges at 4000rpm at 4°C for 10 minutes. Supernatant or organic layer was transferred to pre-labelled RIA vials and evaporated to dryness in turbo evaporator under nitrogen at 45°C. The residue was reconstituted in 1000µL of mobile phase & injected.¹⁴⁻¹⁵

RESULTS AND DISCUSSION

The selectivity, sensitivity, matrix effect, linearity, stability, accuracy and precision are validated of Fenofibric acid.

Analyte selectivity:It was established by checking the interference at analyte retention time caused due to IS and by injecting six replicates of matrix blank with IS. No significant interference was observed.

Sensitivity: The lowest limit of quantification for fenofibric acid was set at the concentration of LLOQ, 100.1 ng/mL. The precision and accuracy for fenofibric acid at this concentration was found to be 58.4% and 88.86% respectively.

Linearity: A regression equation with a weighting factor of $1 / (\text{concentration})^2$ of drug to IS concentration was judged to produce the concentration- detector response relationship for fenofibric acid in human plasma

Precision: The precision of the assay was measured by the percent coefficient of variation over conc. Of LLOQ, QC, LQC, MQC1, MQC2 & HCQ samples.

Accuracy: The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the LLOQ, low, middle, high-quality control samples.

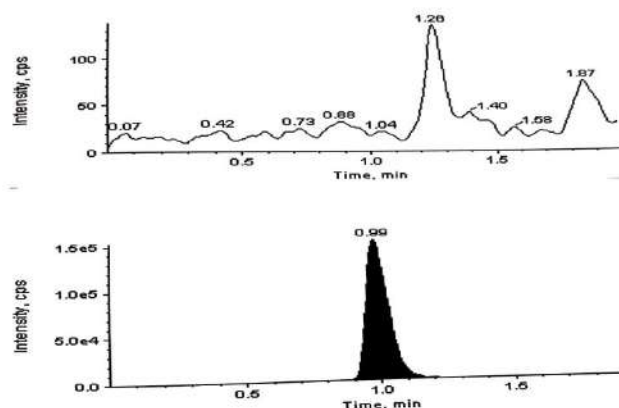


Fig.1 Blank sample with internal standard

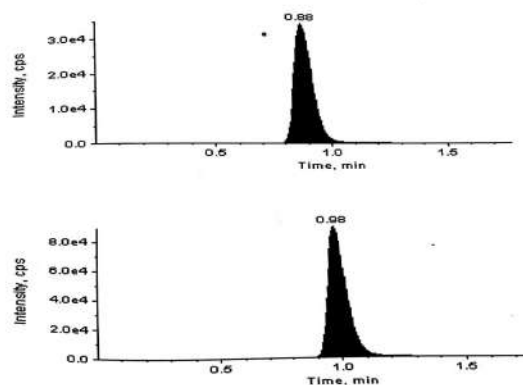


Fig.2 Standard solution with internal standard

Stability: Room temperature stock solution stability was carried out at 0 and 6 hours for fenofibric acid by injecting 6 replicates of prepared stock dilutions of fenofibric acid and equivalent middle concentration.

CONCLUSION

The result of selectivity, carry over test, matrix effect, sensitivity, linearity, precision, accuracy and stability presented in this report are within the acceptance range for Bioanalytical batch acceptance criteria given in the 'Bioanalytical Method Validation' and USFDA acceptance range as per 'Guidance for Industry' given by CDER. Method was validated over a concentration range of 100ng/mL to 20004.2ng/mL for fenofibric acid. The analytical method described above is valid for the estimation of fenofibric acid, in human plasma over a range of 100.ng/mL -20004.2ng/mL with detection of Fenofibric acid m/z -319.20 and 233.10 and internal standard Carbamazepine m/a- 237.20 (Parent) and 194.10(Product).

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CARDIOVASCULAR RISK ASSESSMENT IN TYPE2 DIABETES PATIENT AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Diabetes is a complex heterogeneous group of metabolic diseases that are characterized by Hyperglycemia that occurs when no longer able to produce insulin production or when unable to use insulin from the pancreas. To prevent cardiovascular disease, risk prediction models are important in clinical practice; a risk prediction model can identify people who are at high risk individually. To provide practical safe goals for reduction of cardiovascular complications and prevent cardiovascular morbidity and mortality, probably used prediction model in South Asia is WHO/ISH based on the prediction charts risk are categorized into <10%, 10-<20%, 20-<30%, 30-<40%, $\geq$ 40%. A prospective observational study, carried out in the Multispeciality Hospital for a period of 3months. Both males(15%) and females(15%) are at high risk, maximum number of people are at low risk are 188 females are slightly low when compared to males. Maximum People who are having Hypertension at high risk, obese people(25%) are more at low risk. Alcoholics(32%) are at high risk than smokers(19%). physical activity people are more at low risk. Study among 418 populations, based on the WHO risk prediction charts, the Conclusion of this study indicates on average 57 years are at risk of cardiovascular complications. Absolutely ten years of Cardiovascular risk people who are having Hypertension, alcohol, and comorbidities are at high of cardiovascular disease.

KEYWORDS: *WHO/ISH risk prediction charts, cardiovascular risk factors, Diabetes Mellitus.*

1.INTRODUCTION

Diabetes is a complex heterogeneous group of metabolic diseases that are characterized by Hyperglycemia that occurs when no longer able to produce insulin production or when unable to use insulin from the pancreas¹ and diabetes is also called Iceberg disease². India has the highest population living with diabetes and had become "the Diabetes Capital of the World."¹ Diabetic patients have a pre-existing cardiovascular disease at the time of diagnosis and over half die from macrovascular and microvascular complications³. Cardiovascular disease is growing burden in India. The number of people with Diabetes, increasing day by day due to urbanization physical inactivity, lifestyle changes, increase in prevalence of obesity.⁴ Persons with Diabetes are two to four times at high risk than the general population⁵. Non-diabetic patients have low ejection factor than diabetic patients⁶. People with Diabetes Cardiovascular disease is leading cause of mortality and morbidity⁷. Complications of Diabetes mellitus are divided into Microvascular and Macrovascular complications. Microvascular complication consists of Diabetic Retinopathy, Neuropathy, Nephropathy and Diabetic food disorders. Macrovascular complications consist of Cardiovascular disease and Stroke¹. If HbA1c increases it is strongly linked to microvascular complications. Coronary artery disease, Stroke, Obesity are of non communicable disease these are increasing in number than the communicable disease, if 1% rises in HbA1c it is associated with 18% increases the risk of Cardiovascular disease and 12 to 14% increases in the risk of mortality⁸. In 2040 it was estimated that 640million adult people are with diabetes⁹. Based on multicentric survey done by Indian Council of Medical Research (ICMR) thirty years ago in urban Diabetes prevalence is around 2% and in Rural 1%⁹. To prevent Cardiovascular disease, risk prediction models are important in clinical practice, risk prediction model can identify people who are at high risk individually¹⁰. Cardiovascular risk prevention is an appropriate and economic burden, so cardiovascular risk approach is recommended The National Cholesterol Education

Program Adult Treatment Panel.III (NCEP-ATP III)listed diabetes as Coronary heart disease equivalent which needs stratification of risk it indicated clearly not all diabetic patients have the same Cardiovascular risk estimation of cardiovascular risk approach is needed. Furthermore,high prevalence of cardiovascular diseases⁷. Risk prediction models of Cardiovascular disease are WHO, Pooled cohort equations, SCORE, The INTERHEART,QRISK2,ASSIGN,QRISK lifetime,are used¹¹.A risk assessment tool should be validated and evaluates relevant modifiable and non-modifiable risk factors¹¹. To provide practical safe goals for reduction of cardiovascular complications and prevent cardiovascular morbidity and mortality³, Probably used prediction model in South Asia is WHO/ISH based on the prediction charts risk are categorized into <10%, 10-<20%, 20-<30%, 30-<40%, ≥40%. Among these countries the age of onset of cardiovascular disease is on an average 10 years earlier than other countries⁵. South Asian Indians have low HDL and high Triglycerides. LDL particles are smaller and denser. Lipoprotein, C-reactive protein, homocysteine, plasminogen activator inhibitor -1 level tends to be higher in South Asian Indians¹². Early detection of cardiovascular events is early intervention to reduce clinical cardiovascular disease onset¹³. Risk factor primarily derived from age, risk modification takes place by changing their lifestyle because individuals who are at high risk leads to rapidly rising absolute Cardiovascular risk¹⁴. Persons who are having many risk factors in young age has same level of Cardiovascular risk to older people with no risk factors.¹⁴Hypertension is extremely Comorbidity condition in Diabetes, affecting 20-60% of patients with diabetes. Lack of control in Blood glucose level related to progression of many factors like loss of beta-cell function, Comorbidities, lifestyle factors, and finally glucotoxicity¹⁵. Hypertension substantially increases microvascular and macrovascular complications¹⁶. Patients who are obese or overweight recommended weight loss based on short term studies showing numerous benefits of weight loss which improves quality of life, glycemic control¹⁷. Persons who are Hypertensive are more at risk of cardiovascular disease. Persons who are taking alcoholic are at high risk than persons having habit of smoking of cardiovascular disease. Persons who are having 1 or 2 comorbidities are at high cardiovascular complications.

2.METHODOLOGY

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Naresuan University Institute Review Board (IRB Ref. No: PD011/IRB/NRML/19-20)

2.1 Study design

This study is a hospital-based Prospective cross-sectional descriptive study.

2.2 Study area

This study was carried out in the inpatient department at a tertiary care hospital in Vijayawada, Andhra Pradesh.

2.3 Study population

All patients attending the diabetic outpatient department

2.4 Study period

The study was carried out for 6 months from June to November 2019

2.5 Inclusion criteria

The inclusion criteria for including participants in the study were known patients of type 2 diabetes mellitus who attended the diabetic out-patient and who consented to participate in the study.

2.6Exclusion criteria

The exclusion criteria for not including the individuals in the study were those patients who were not willing to give consent to participate in the study and patients with a known case of cardiovascular disease

2.7 Data collection

Data was collected using a pre-tested structured questionnaire consisting of socio-demographic profile and WHO-ISH cardiovascular risk scoring indicators which includes risk factors such as tobacco use, family

history of diabetes, family history of premature myocardial infarction, systolic blood pressure. All the blood parameters were recorded from recently taken investigation reports available with the study participants

3. RESULT

Updated risk prediction charts assessment of Cardiovascular diseases for 10 years⁽¹⁸⁾. Based on the WHO/ISH risk prediction charts patients who are known to be diabetic were categorized as 10%, 10-20%, 20-30%, 30-40%, $\geq 40\%$

3.1 Severity of Cardiovascular risk index in Diabetes Mellitus patients

Among the 418 study population, the main findings in this population are according to WHO/ISH⁽¹⁹⁾ mean average age of males and females 57yr. Table 1 shows males are 219 & females are 199. Gender difference were found in participants who are at Cardiovascular risk both males and females are at high risk ($\geq 40\%$) are 15% (Males = 32, Females = 32), who are at low risk ($< 10\%$) slightly females (22.7%) are more than males (22%).⁽²⁰⁾

Table 1 indicates the severity of the cardiovascular risk index in Diabetes mellitus patients.

Cardiovascular risk Index (%)	Male N=219	Female N=199	Total % N=418
<10	93(22.2%)	95(22.7%)	188(44.9%)
10-<20	52(12.4%)	21(5%)	73(17.4%)
20-<30	27(6.4%)	45(10.7%)	72(17.1%)
30-<40	15(3.5%)	6(1.4%)	21(5%)
≥ 40	32(7.6%)	32(7.6%)	64(15.2%)

3.2 Clinical parameters along with cardiovascular risk index

Summarize the category of risk factors in Table 2 shows based on risk factors social activity like Smoking, Alcohol, when compared to smokers, alcohols are at high-risk. High Body mass index is risk prediction for Cardiovascular disease, Overweight, Obese with diabetes can cause Cardiovascular disease that can lose weight (as participants who are obese are at low risk are 104 (45%), and at high-risk are 18 (7.8%).⁽²³⁾ Risk in overweight was maximum which includes 20 (23%) are in 20-30% and the minimum was found in 11 (12.9%) are in $\geq 40\%$. Based on physical activity the maximum cases were 54 (12.9%) are at low risk (10%), minimum was found to be 16 (3.8%) are in $\geq 40\%$. Participants who are having 1 or 2 Co-morbidities are 188 (44%) are at low risk (10%) and 64 (15%) are in high risk ($\geq 40\%$). More than 3 Co-morbidities are 22 (5.2%) in ($\geq 40\%$) who are at low risk (10%) is 60 (14%).⁽²⁴⁾

Table 2 Reveals Clinical parameters along with risk index.

Clinical parameters	<10%	10-<20%	20-<30%	30-<40%	>40%
Age					
30-60	101(50%)	29(14%)	27(13%)	13(6%)	29(14%)
60-80	87(40%)	44(20%)	43(20%)	7(3.2%)	32(15%)
>80	0	0	0	1(25%)	3(75%)
(n=200)					
Gender					
Male(n=219)	93(22%)	52(12%)	27(6.4%)	6(1.4%)	32(7.6)
Female(n=199)	95(22.7%)	21(5%)	45(10.7%)	15(3.5%)	32(7.6%)
Risk factors					
Smoking(n=47)	15(31%)	12(25%)	4(8%)	7(14%)	9(19%)
Alcohol(n=43)	15(31%)	9(20%)	3(9%)	2(4%)	14(32%)

BMI mg/kg					
18-22(105)	30(28%)	23(21%)	17(16%)	15(14)	9(11%)
23- 25(85)	31(36%)	15(17%)	20(23%)	8(9%)	20(23%)
>25(288)	104(45%)	36(15%)	35(15%)	35(15%)	18(7.8%)
No. of co-morbidities					
1(418)	188(44%)	73(17%)	72(17%)	21(5%)	64(15%)
2(418)	188(44)	73(17%)	72(17%)	21(5%)	64(15%)
>2(208)	60(28%)	22(10%)	23(11%)	81(38.9%)	22(10%)
No Physicalactivity (n=105)	54(51%)	24(22.8%)	9(8%)	2(1%)	16(15%)

Hypertension is one of the modifiable risk factors for cardiovascular complications ⁽²¹⁾. all the participants the number of cases having a high risk ($\geq 40\%$) include Hypertensive patients 68(13.3%) and the least in patients having BMI (23-25) is 11(2.6%). The highest number of cases having low risk (10%) were found in Hypertension and patients having more than 2 Comorbidities 188(44%) and the least cases were seen in patients having BMI (18-22) are 33(7%). ⁽²⁴⁾

4. CONCLUSION

A study among 418 population based on the WHO/ISH risk prediction charts age between 30-60 people is more at low risk of Cardiovascular disease in diabetes patients like Atherosclerosis, Angina Pectoris, Myocardial Infraction, Stroke. Males are slightly more at low risk compared to the females, because males are smokers, alcoholics. People who are Hypertensive are more at high risk of cardiovascular disease. Maximum cases of low risk in BMI are in obese patients. As a clinical pharmacist should provide Patient counseling based on the cardiovascular risk.

6. Acknowledgment

We are sincerely Thanks to Dr. G. Murali Krishna, Endocrinologist, Manipal hospital, Vijayawada for their continued support.

6. Conflict interest: Authors do not have any conflict of interest

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NOVEL METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC OF TEZACAFTOR, IVACAFTOR IN HUMAN PLASMA

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ABSTRACT

A simple, precised, accurate method was developed for the estimation of Tezacافتor, Ivacaftorin human plasma using the Stavudine as internal standard by RP-HPLC (Reverse phase-High performance Liquid Chromatographic) technique. Chromatographic conditions used are stationary phase Kromasil c18 (250 x 4.6 mm x 5µm), Mobile phase 0.01N Di Sodium hydrogen phosphate (pH: 3.0): Acetonitrile in the ratio of 65:35(v/v) and flow rate was maintained at 1.0ml/min, detection wave length was 255nm, column temperature was set to 30°C and Diluent used is water: Acetonitrile (50:50). Conditions were finalized as optimized Conditions. Retention time of Ivacaftor and Tezacافتor were found to be 4.83min and 7.88min. %CV of the Tezacافتor and Ivacaftor were found to be 0.19% and 1.14%. % Recovery was obtained as 99.19% and 96.91%. The linearity concentration is in the range of 0.3-12µg/mL of Tezacافتor, 0.06-2.4µg/mL of Ivacaftor. The lower limits of quantification were 0.3µg/mL of Tezacافتor and 0.06µg/mL of Ivacaftor which reach the level of both drugs possibly found in human plasma.

KEYWORDS: *Stavudine, Tezacافتor, Ivacaftor, RP-HPLC, Method development, ICH Guidelines, validation.*

INTRODUCTION

Tezacافتor is chemically known as 1-(2,2-difluoro-2h-1,3-benzodioxol-5-yl)-n-{1-[(2r)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1h-indol-5-yl} cyclopropane-1-carboxamide¹; Tezacافتor is a drug of the cystic fibrosis transmembrane conductance regulator (cftr) potentiator class²; Tezacافتor is metabolized extensively in humans by the action of cyp3a4 and cyp3a5. There are three main circulating metabolites; m1, m2, and m5. The m1 is an active metabolite with similar activity to the parent drug (tezacافتor). The m2 metabolite is significantly less active and m5 is considered an inactive metabolite³; Ivacaftor is chemically known as n-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3carboxamide⁴; Ivacaftor (Trade name kalydeco) is a drug used to treat cystic fibrosis in people with certain mutations in the cystic fibrosis transmembrane conductance regulator (cftr) gene (primarily the g551d mutation), who account for 4–5% cases of cystic fibrosis⁵; Development of HPLC and LC-MS/MS methods for the analysis of Ivacaftor, its major metabolites and Lumacaftor in plasma and sputum of cystic fibrosis patients treated with orkambi or kalydeco in which it deals with the method development with HPLC and LC-MS/MS⁶; Stability indicating RP-HPLC method for simultaneous estimation of Lumacaftor and Ivacaftor in bulk and pharmaceutical dosage form⁷; A new validated RP-HPLC method for simultaneous estimation of Lumacaftor and Ivacaftor in pharmaceutical dosage form⁸; are some of the studies conducted other than RP-HPLC method.

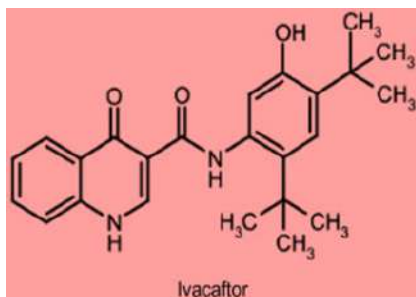


Figure-1 Ivacaftor

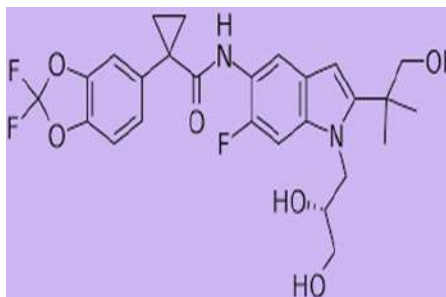


Figure-2 Tezacافتor

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

Pure drug of Tezacaftor and Ivacaftor were purchased from Selleckchem LLC supplied by pro labs. HPLC grade Acetonitrile and Methanol were taken from Merck chemical division. Throughout the study HPLC grade water was used and is purchased from Milli-Q purification system.

Instrumentation

In Instrumentation Chromatography Technique was performed. It was performed with 2695 HPLC water with a quaternary pump, column oven, high speed autosampler, degasser and PDA detector to provide a compact and Empower-2 Software was used.

Chromatographic conditions

The separation was performed by using Kromasil C18 (250×4.6mm×5µm) column with a Mobile phase consisting of 0.01N Di Sodium hydrogen phosphate (pH: 3.0): Acetonitrile in the ratio of 65:35(v/v) and Flow rate was maintained at 1.0ml/min, Detection wave length was at 255nm. The sample dilution was carried out by using water and acetonitrile (50:50) ratio as diluents.

METHOD DEVELOPMENT

Preparation of internal standard

Internal standard was prepared by transferring 50mg of Stavudine into 100ml of volumetric flask and makeup the volume with diluents. From the prepared stock solution, 10µg/ml solution was prepared for further dilutions.

Preparation of calibration and quality control solutions

The stock solutions of Tezacaftor and Ivacaftor were prepared individually by dissolving 60 mg&12mg of the drug in 100 ml of diluent to obtain 600 µg/ml concentration each. The prepared stock solution is again diluted with diluents for getting the spiking solution to obtain the calibration curve standards. The working concentration of Analytes were Tezacaftor (0.3 to 12µg/ml), Ivacaftor (0.06 µg/ml to 2.4 µg/ml). Calibration curve and quality control samples are obtained by 10µl spiking above prepared solution of each analyte to 750µl of plasma and 500µl of internal standard and 250µl of Tezacaftor.

Sample preparation and extraction

The prepared spiking solution of analytes each 750µl of plasma +500µl of internal standard, 250µl of Tezacaftor. To the spiked plasma, 1 ml of acetonitrile was added and vortexed for 2min. The resulting solutions were centrifuged at 3200 RPM for 5 min. The resultant organic layers were used for analysis.

RESULTS AND DISCUSSION

System suitability

System suitability was performed by using the MQC level sample as six homogenous Injections. The % coefficient of variation for retention time and response was calculated. Obtained results shows, less than 2% of suitability for the analysis of combination by using human plasma.

Specificity

Specificity of drug was performed by doing batches. It was performed by testing six batches of K₂EDTA blank plasma solution. The mass detection of extracted blank plasma has given good selectivity for drug and internal standard. The chromatogram results are given in Figure 3.

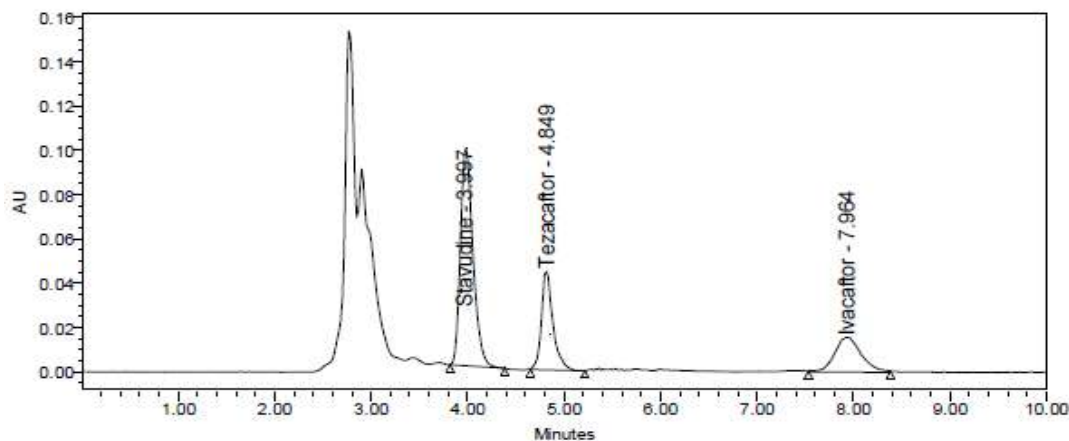


Figure 3-Chromatogram of drugs and internal standard

Precision and Accuracy

Accuracy and precision was evaluated by performing the six replicates of analytes of Tezacaftor and Ivacaftor for 4 concentrations level each, i.e., 0.3 µg/ml (LLOQ), 0.9 µg/ml (LQC), 6 µg/ml (MQC) and 9.6 µg/ml (HQC), Ivacaftor at 0.06 µg/ml (LLOQ), 0.18 µg/ml (LQC), 1.2 µg/ml (MQC) and 1.92 µg/ml (HQC). % accuracy was obtained with range varied from 98.86%-100.44%, and 98.63%-100.37% for intraday and 100.01%-101.06 and 98.85%-102.04 for inter day respectively.

Recovery

Recovery was obtained by calculating the peak areas obtained by the prepared plasma samples and the extracted blank spiked plasma with the standards in same area with the known amount of Ivacaftor and Tezacaftor. The recovery results were given in the Table-1.

Table 1- Recovery of Tezacaftor, Ivacaftor

Acquisition Batch ID						
Replicate No	HQC		MQC1		LQC	
	Extracted response (Tezacaftor)	Extracted Response (Ivacaftor)	extracted Response (Tezacaftor)	Extracted Response (Ivacaftor)	extracted Response Tezacaftor)	Extracted Response (Ivacaftor)
1	1021337	50695	524601	25132	79137	3874
2	1013338	50249	525523	24445	79320	3946
3	1010606	51824	522251	25125	79124	3827
4	1036547	50788	523577	24967	79273	3959
5	1044999	51404	521882	25644	78804	3871
6	1038754	51613	522359	25752	79209	3979
N	6	6	6	6	6	6
Mean	1027597	51096	523366	25178	79145	3909
SD	14412.57	610.77	1461.64	476.10	183.24	60.27
% CV	1.40	1.20	0.28	1.89	0.23	1.54
% Mean Recovery	98.19		97.44		95.12	
Overall % Mean Recovery	99.193(Tezacaftor)		96.915 (Ivacaftor)			

Sensitivity

Sensitivity was performed by using LLOQ level samples with six numbers of replicates to obtain the lowest limit for the detection, % coefficient of variation, % mean accuracy.

Linearity

Linearity indicates the excellent correlation between the peak areas of each concentration for analytes. A representative calibration curves is shown in Figure 4, 5.

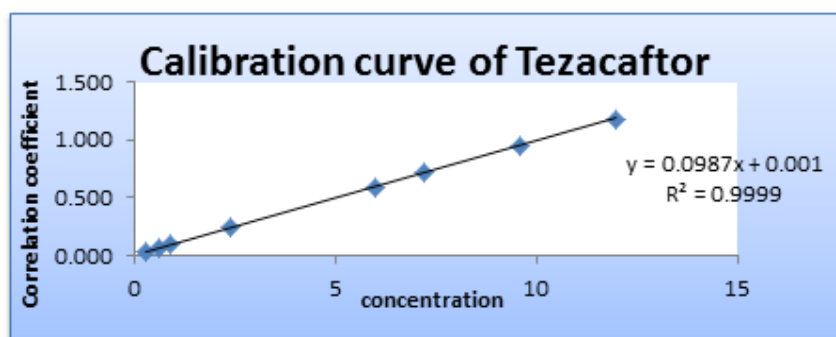


Figure 4 - calibration curve of Tezacaftor

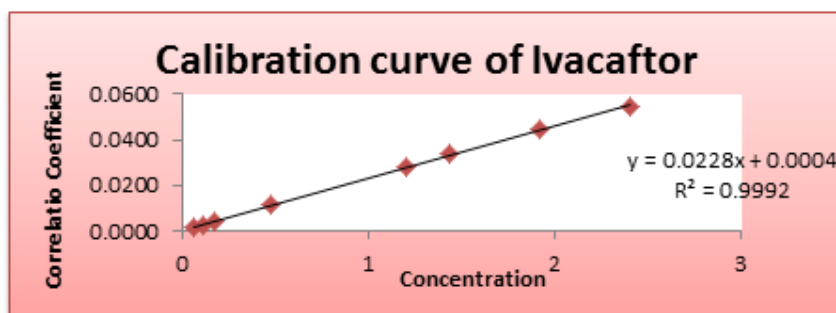


Figure 5 - calibration curve of Ivacaftor

Stability

Matrix samples stability at -28 ± 5 °C for 37 days & -80 ± 5 °C was determined for tezacaftor and Ivacaftor. At a concentration of LQC-HQC level The % mean stability of the Tezacaftor was found to be 98.87%, 99.33% at 28 ± 5 °C and 100.68%, 102.29% at 80 ± 5 °C and 100.01%, 96.31 at 80 ± 5 °C respectively.

CONCLUSION

A simple, accurate, precise method was developed for the estimation of the Tezacaftor and Ivacaftor in human plasma using the Stavudine as internal standard. Retention time of Tezacaftor and Ivacaftor were found to be 4.83min and 7.88min. %CV of the Tezacaftor and Ivacaftor were found to be 0.19% and 1.14%. %Recovery was obtained as 99.19% and 96.91%. The linearity concentration is in the range of 0.3-12µg/mL of Tezacaftor, 0.06-2.4µg/mL of Ivacaftor. The lower limits of quantification were 0.3µg/mL of Tezacaftor and 0.06µg/mL of Ivacaftor, which reach the level of both drugs possibly found in human plasma. As per the ICH guidelines the results obtained are within the accepted limit range.

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The authors are thankful to Nirmalacollege of pharmacy for providing the facilities.

CONFLICT OF INTEREST

There is no conflict of interest.

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NOVEL RP- HPLC METHOD DEVELOPMENT AND VALIDATION OF DARUNAVIR AND RITONAVIRIN BULK AND PHARMACEUTICAL FORMULATION USING HUMAN PLASMA

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ABSTRACT

A simple, precised, accurate method was developed for the estimation of Ritonavir and Darunavir and in human plasma using the Lamivudine as internal standard by RP-HPLC (Reverse phase-High performance Liquid Chromatographic) technique. Chromatographic conditions used are stationary phase Symmetry c18 (250 x 4.6 mm, 5 μ), Mobile phase 0.01N potassium di hydrogen phosphate (pH: 3.0): Acetonitrile in the ratio of 55:45(v/v) and flow rate was maintained at 1.0ml/min, detection wave length was 278nm, column temperature was set to 30°C and diluent was mobile phase Conditions were finalized as optimized method. Retention time of Ritonavir and Darunavir, were found to be 3.256min and 4.240min. % CV of the Ritonavir and Darunavir were found to be 0.22% and 0.6%. % Recovery was obtained as 98.28% and 98.11%. The linearity concentration is in the range of 0.0375 to 1.5 μ g /m of Ritonavir, 0.25 to 10 μ g /ml of Darunavir. The lower limits of quantification were 0.0375 μ g/mL of Ritonavir and 0.25 μ g/mL of Darunavir which reach the level of both drugs possibly found in human plasma.

KEYWORDS: Ritonavir, Darunavir, Lamivudine, RP-HPLC, Method development, ICH Guidelines, validation.

INTRODUCTION

Darunavir is an antiviral drug used to treat HIV. Darunavir (DRV) is a Protease inhibitor that blocks the activity of the protease enzyme and it is necessary for the assembly of HIV particles. Ritonavir is an antiretroviral protease inhibitor which is widely used in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). It is available in both liquid formulation and capsules. It is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B virus. Bioanalytical techniques, employed for the quantitative determination of drugs and their metabolites in biological fluids and creates a specific procedure to enable a coalesce of interest to be identified and at the same time to be quantified in a matrix. A coalesce is measured by several procedures¹. The choice of analytical procedures involves many considerations, such as: concentration levels, chemical properties of the analyte, specimen matrix, cost of the analysis, experimental speed, quantitative or qualitative measurement, required precision and necessary equipment². Bioanalytical method validation comprises all criteria determining data quality, such as selectivity, accuracy, precision, recovery, sensitivity, and stability³. Liquid liquid extration process is based on the theory of differential solubility and partitioning equilibrium of sub atom between aqueous (the original specimen) and the organic part⁴. Liquid-liquid extraction process generally involves the extraction of a particular substance from one liquid part to another liquid part⁵. They act as viral replication by interfering with the HIV protease enzyme⁶. The novel reversed-phase high-performance liquid chromatographic (HPLC) method is for the quantitative determination of darunavir ethanolate⁷. A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of Darunavir in pure and tablet dosage forms⁸. Darunavir, an anti-HIV drug was subjected to forced degradation under acid, base, thermal and neutral hydrolysis, oxidation and photolysis as prescribed by ICH guidelines⁹. A sensitive ultra high performance liquid chromatography with tandem mass spectrometry method was developed for the simultaneous determination of darunavir, ritonavir and tenofovir in human plasma¹⁰.

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

Ritonavir and Darunavir API sample was obtained from Spectrum pharma research solutions. Distilled water HPLC water, Acetonitrile, Phosphate buffer, Methanol, Sodium dihydrogen phosphate, Ortho-phosphoric acid obtained from Rankem, Avantor performance material India limited.

INSTRUMENTATION

Chromatographic techniques was performed by using HPLC waters, quaternary pump, high-speed autosampler, column oven, degasser and PDA detector and by using Empower-2 software.

CHROMATOGRAPHIC CONDITIONS

The separation was achieved by using Kromasil C18 (250×4.6μ×5mm) column with a mobile phase consisting of 0.01N potassium di hydrogen phosphate (pH: 3.0): Acetonitrile in the ratio of 55:45(v/v) and flow rate was maintained at 1.0ml/min, detection wave length was at 278nm. The sample dilution was carried by using water: acetonitrile (50:50) ratio as diluents.

METHOD DEVELOPMENT

Preparation of Ritonavir Stock solution (75μg/ml):

Take 7.5 mg of Ritonavir in 100 ml volumetric flask and make the volume with diluent to produce 75μg/ml.

Preparation of Ritonavir Spiking Solutions

From the above Ritonavir stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.375μg/ml, 0.75 μg/ml, 1.125 μg/ml, 3 μg/ml, 7.5 μg/ml, 9 μg/ml, 12 μg/ml and 15μg/ml. Calibration standards and quality control (QC) samples were prepared by using spiking blank plasma with working stock solution of analyte to produce 0.0375 μg/ml, 0.075 μg/ml, 0.1125 μg/ml, 0.3 μg/ml, 0.75 μg/ml, 0.9 μg/ml, 1.2 μg/ml and 1.5 μg/ml.

Preparation of Darunavir Stock solution (500 μg/ml)

Take 50 mg of Darunavir in 100 ml volumetric flask and make the volume with diluent to produce 500 μg/ml.

Preparation of Darunavir Spiking Solutions

From the above Darunavir stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.0025 μg/ml, 0.005μg/ml, 0.0075μg/ml, 0.02 μg/ml, 0.05 μg/ml, 0.06 μg/ml, 0.08 μg/ml and 1.0 μg/ml. Calibration standards and quality control (QC) samples were prepared by using spiking blank plasma with working stock solution of analyte to produce 0.025 μg/ml, 0.05 μg/ml, 0.075 μg/ml, 0.2 μg/ml, 0.5 μg/ml, 0.6μg/ml, 0.8μg/ml and 1.0 μg/ml.

Preparation of internal standard Solution (Lamivudine)

Stock-1: Take 50 mg of Lamivudine in 100 ml volumetric flask and dilute to produce 500μg/ml solution.

Stock-2: From the above solution, take 1ml of solution into 10 ml volumetric flask and again dilute to produce 50μg/ml solution.

Final concentration: From the above solution, take 0.5ml of solution and spiking blank plasma with working stock solution and dilute the analyte to produce 10μg/ml ISD concentration.

Extraction procedure:

750μl of plasma +500μl of internal standard +250μl of Ritonavir+250μl of Darunavir taken in a beaker and mix well and add 1ml of acetonitrile and vortex for 2min and centrifuge for 5min at 3200rpm and collection of supernatant samples and filter the sample and inject 50μL into HPLC

RESULTS AND DISCUSSION

METHOD VALIDATION

Selectivity/Specificity

Specificity is the ability to measure the desired Analyte in the presences of components such as excipients and impurities which are expected to be present. Specificity should be conducted during the identification test, determination of impurities and also in assay. This result was shown in figure number 3.

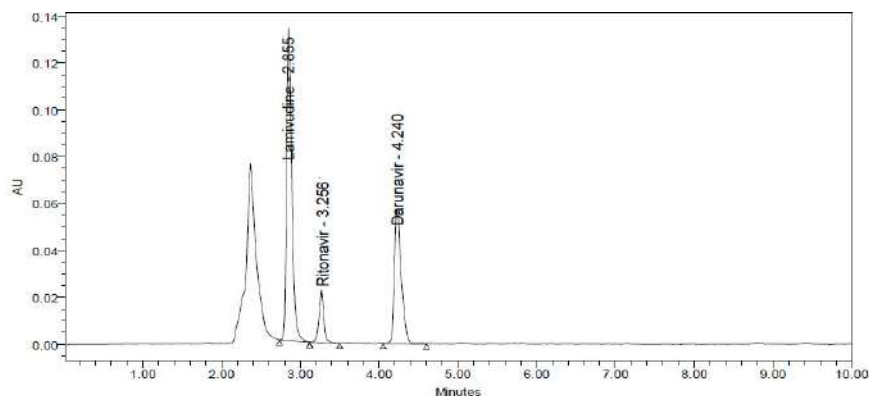


Figure-3 Chromatogram of darunavir and ritonavir

Linearity

Calibration was found to be linear over the concentration range of 0.0375 to 1.5 $\mu\text{g/ml}$ for Ritonavir, 0.25 to 10 $\mu\text{g/ml}$ Darunavir. The coefficient correlation (r^2) value was found consistently greater than 0.999 in all the cases. This indicating linearity of results and an excellent correlation between peak area ratios for each concentration of analytes. This result was shown in figure number 4, 5.

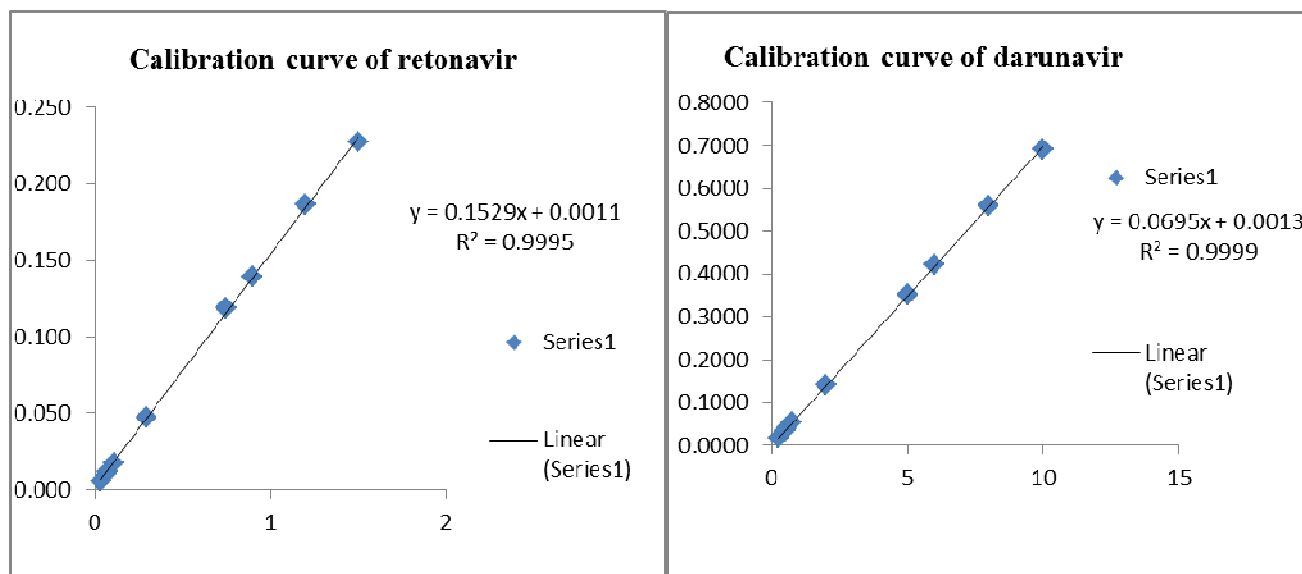


Figure-4 Calibration curve of ritonavir Figure-5 Calibration curve of darunavir

Precision and Accuracy

The intraday and inter day accuracy and precision was assessed by analysing six replicates at five different QC levels like LLOQ, LQC, MQC and HQC. Accuracy and precision method performed by using different analyzing quality control levels of ritonavir are 0.0375 $\mu\text{g/ml}$ (LLOQ), 0.125 $\mu\text{g/ml}$ (LQC), 0.75 $\mu\text{g/ml}$ (MQC) and 1.2 $\mu\text{g/ml}$ (HQC), Darunavir at 0.25 $\mu\text{g/ml}$ (LLOQ), 0.75 $\mu\text{g/ml}$ (LQC), 5.0 $\mu\text{g/ml}$ (MQC) and 8.0 $\mu\text{g/ml}$ (HQC). The intra-day and inter day accuracy of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 99.77-101.33%, and 100.22%-101.29% for intraday and 99.11%-100.53 and 98.73%-100.56 for inter day respectively. The %CV of analytes and plasma samples of intraday precision was found to be 0.63-10.01% and 0.90%-3.28% and interday precision was found to be 1.33%-8.23% and 0.72 %-2.01%.

Recovery

Recovery was obtained by calculating the peak areas obtained by the prepared plasma samples and the extracted blank spiked plasma with the standards in the same area with the known amount of ritonavir and darunavir. The overall % mean recovery for Ritonavir and Darunavir was found to be 98.28% and 98.11%. This results was shown in table number 1.

Table 1: Recovery of ritonavir and darunavir

Acquisition batch ID						
Replicate No	HQC		MQCI		LQC	
	Extracted response (ritonavir)	Extracted response (Darunavir)	Extracted response (ritonavir)	Extracted response (Darunavir)	Extracted response (ritonavir)	Extracted response (Darunavir)
1	1021337	54832	524601	34273	79137	5209
2	1013338	54453	525523	34366	79320	5211
3	1010606	55036	522251	34123	79124	5203
4	1036547	53821	523577	34247	79273	5210
5	1044999	54117	521882	34911	78804	5209
6	1038754	54353	522359	34688	79209	5215
N	6	6	6	6	6	6
Mean	1027597	54435	523366	34435	79145	5210
SD	14412.57	448.10	1461.64	301.42	183.24	3.89
%CV	1.40	0.82	0.28	0.88	0.23	0.07
Mean recovery	99.19	97.54	98.09	98.28	100.30	98.52
Overall % Mean Recovery	98.0					

Stability

Long term stock solution stability for the Ritonavir was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C & -80°C in refrigerator. The % mean stability of the Ritonavir was found to be 99.85%, 99.78% at $28 \pm 5^\circ\text{C}$ and 99.76%, 100.17% at $80 \pm 5^\circ\text{C}$ respectively. Long term stock solution stability for the Darunavir was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C & -80°C in refrigerator. The % mean stability of the Darunavir was found to be 96.22, 99.99 at $28 \pm 5^\circ\text{C}$ and 98.07%, 100.17 at $80 \pm 5^\circ\text{C}$ respectively.

CONCLUSION

A simple, accurate, precise method was developed for the estimation of the Ritonavir and Darunavir in human plasma using the Lamivudine as internal standard. Retention time of Ritonavir and Darunavir were found to be 3.256min and 4.240min. %CV of the Ritonavir and Darunavir were found to be 0.22% and 0.6%. %Recovery was obtained as 98.28% and 98.11%. The linearity concentration is in the range of 0.0375 to 1.5 $\mu\text{g}/\text{mL}$ of Ritonavir, 0.25 to 10 $\mu\text{g}/\text{mL}$ of Darunavir. The lower limits of quantification were 0.0375 $\mu\text{g}/\text{mL}$ of Ritonavir and 0.25 $\mu\text{g}/\text{mL}$ of Darunavir, which reach the level of both drugs possibly found in human plasma. As per ICH guidelines the results are within the acceptable range.

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The authors are thankful to Nirmala College of pharmacy for providing the facilities.

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AN OVERVIEW OF METHEMOGLOBINEMIA: AN UNCOMMON BLOOD DISORDER

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ABSTRACT

Methemoglobin is a bluish chocolate brown coloured metalloprotein in which Iron in the heme is oxidized to ferric state and leads to the decreased O₂ carrying capacity of the blood to the body parts and its normal level in the blood ranges from 0.14-0.175 g/dl. When the range exceeds the normal range, the condition is called as Methemoglobinemia which is a serious, uncommon blood disorder. Due to decreased O₂ saturation in the blood, skin turns blue and thus this condition can also be referred as BLOOD BLUES. It is mainly of two types: Hereditary which is caused due to NADH Cytochrome b₅ reductase enzyme deficiency and acquired condition, major etiological factors include exposure of skin/mucous membrane to the oxidizing agents like Acetanilide, Arsine, Benzene, Benzocaine, Chlorates, Chloroquines, Dapsone etc. Till date there is no exact treatment for hereditary methemoglobinemia, more research is yet to be carried out. In case of acquired methemoglobinemia, Methylene blue therapy is used as first line where ascorbic acid follows. Physicians should be aware while prescribing drugs that induce methemoglobinemia in normal/ high doses. Proper monitoring should be done in case of presence of any signs & symptoms as earlier diagnosis provides better relief to the patients.

KEY WORDS: *Methemoglobin, Methemoglobinemia, Blood blues, Cyanosis*

INTRODUCTION

Methemoglobin is a bluish chocolate brown coloured metalloprotein in which Iron in the heme is oxidized to ferric state and leads to the decreased binding of O₂ or decreased O₂ carrying capacity of the blood to the body parts^{1,2}.

Methemoglobin normal level ranges from 0.14-0.175 g/dl^{1,3}. General physiologic level of MetHb ranges from 0-2% and when the range exceeds the normal level, the condition is called as Methemoglobinemia^{4,5}. Some patients can even tolerate up to 10-20% of MetHb levels but levels above 70% are highly fatal⁵.

General skin colour alters from pale to pinkish colour based upon the levels of Hb and O₂ present in it. Cyanosis is bluish/purplish discoloration of skin which is main symptom in this Methemoglobinemia condition. Due to decreased O₂ saturation in the blood, skin turns blue and thus this condition can also be referred as BLOOD BLUES³.

EPIDEMIOLOGY

In case of Congenital methemoglobinemia which is a rare condition, exact incidence is not known but increased frequencies are found in Yakuts, Athabaskans, and Eskimol & Novajo⁶. Whereas in case of acquired methemoglobinemia, it is mainly due to the excessive exposure to chemicals either topically/ local anaesthetics. Most of these cases are due to unintentional exposure to Benzocaine compounds⁶. In around 30,000 transoesophageal echocardiograms, almost 0.067% of methemoglobinemia conditions were observed. Almost 2/3rd of these cases are mainly due to Benzocaine exposure^{6,7}.

ETIOLOGY

1. Hereditary methemoglobinemia: It is rare and caused mainly due to the deficiency of NADH cytochrome b₅ reductase enzyme⁵. Gene that regulates this enzyme synthesis is 22q13qter. Due to number of mutations in this gene, methemoglobinemia condition is observed⁵. This can be classified into 2 types. In type1, this enzyme is absent only in erythrocytes and leads to cyanosis. Whereas in case of type 2,

membrane bound form of enzyme is absent which leads to the deficiency of enzyme in almost all the tissues & enzymes leading to premature deaths and developmental abnormalities^{3,5,6}.

2. Acquired methemoglobinemia: This can be treated with pharmacological agents like Methylene blue, Ascorbic acid etc^{3,5,6}. In acquired condition, major etiological factors include exposure of skin/mucous membrane to the oxidizing agents like Acetanilide, Aniline dyes, Arsine, Benzene, Benzocaine, Chlorates, Chloroquines, Dapsone, Nitroglycerins, Sodium nitroprusside, Phenacetin, Phenol, Phenytoin, Rifampin, Silver nitrate, Sulfonamides⁵. Some of these agents oxidize directly to form Methemoglobin complex while some indirectly reduces free O₂ to oxygen radicals which directly oxidize Hb to MetHb⁵.

Methemoglobinemia is also seen in younger infants who are presented with symptoms of acidosis. Intestinal pH in infants is high which promotes growth of gram negative bacteria which is responsible for the conversion of dietary nitrates to nitrites. Nitrate containing foods such as beets, spinach, carrots may also leads to methemoglobinemia³.

INTERESTING FACTS

PARACETAMOL can be presented as a rare cause of Methemoglobinemia. Based on some case studies, we have come through a 60 year old woman who has a history of HTN, asthma & CHF. For pain relief effect, she is using acetaminophen 14gm/day (over dosage). On admission patient was positive for influenza & so liver cannot be transplanted. Her SPO₂ levels ranged in mid 80's and her lab reports proved her hypoxic. On further investigations, her Methemoglobin levels were found to be 7.8% and on day-2, it led to 14.8%. Then methemoglobin was confirmed due to the excessive usage of PCM and G6PD deficiency. Then she was transferred to emergency care and methylene blue treatment was started. On day 4 her methemoglobin fell to 7% again and then she expired. Later her lab reports confirmed that G6PD deficiency as a primary cause for methemoglobinemia. This case majorly illustrates oxidative stress from acetaminophen led to methemoglobinemia^{8,9}.

BENZOCAINE also plays a major role in development of methemoglobinemia. Local anaesthetics stood first in the etiology of methemoglobinemia. Based on some case studies, patient did not develop any cyanosis/ ant other methemoglobinemia symptoms but with lidocaine usage alone. It was after lidocaine along with benzocaine. Benzocaine led to methemoglobinemia in many infants. Other local anaesthetics also stood as major etiological factors. Cyanosis develops after 500mg administration of drug (for nerve block)¹⁰⁻¹⁵.

PHENAZOPYRIDINE rarely causes methemoglobinemia which is best OTC drug for UTIs¹¹. It was termed as its incidence is 10 cases from past 35 years. A patient was presented with symptoms like lethargy, diarrhea, lightheadedness and she was using Phenazopyridine for Dysuria. Later after the usage of this, she developed symptoms of hemolytic anemia and on further investigations it was diagnosed as methemoglobinemia. Later on investigations, phenazopyridine stood the main reason for development of methemoglobinemia¹¹. One of the major metabolites of phenazopyridine is aniline, which is a well known cause of methemoglobinemia. Aniline induced methemoglobinemia cannot be treated with methylene blue so alternative therapy like ascorbic acid can be used. Based on some etiological factors, phenazopyridine has also proved to cause Sulfhemoglobinemia^{8,11}.

DAPSONE which is a potent antibiotic and anti inflammatory agent also leads to methemoglobinemia. Based on some case studies, Dapsone when administered in standard doses like 100mg/day may lead to methemoglobinemia or it would even accelerate the pre-existing hematologic conditions. Thromboxane & prostaglandin synthesis in response to infection puts pressure on erythrocytes in the form of oxidative stress & thereby leads to methemoglobinemia^{8,11,12}.

ANTI MALARIAL AGENTS which are potent i.e., Chloroquine also rarely leads to methemoglobinemia. Some investigations revealed that these drugs decreases the concentration of NADH MetHb reductase in RBC & inhibits erythrocytes to convert MetHb to Hb. Thereby produces MetHb and which led to evacuation of 6 soldiers in Vietnam^{8,13}.

PATHOPHYSIOLOGY

Due to deoxygenation of hemoglobin or due to Reactive Oxygen Species (ROS) like superoxide or peroxide, the ferrous ion in the heme group gets oxidized into ferric state³. Then oxidized heme group would be unable to bind with sufficient O₂, thereby the RBC cannot carry sufficient amount of O₂ to the body organs^{3,16}. According to normal physiology, Iron oxidizes to ferric state during normal physiologic functions. By the action of Cytochrome B5 reductases, its levels are maintained below 1%^{1,14}. Thus cytochrome b5 reductases utilizes NADH which is formed during glycolysis and reduces Methemoglobinemia to hemoglobin^{1,14}.

NADPH-MetHb reductase also contributes for the reduction of MetHb. It utilizes NADPH that is formed by G6PD in the HMP shunt. Under oxidative stress & some electrons, the function of this reductase can be enhanced.

CLINICAL MANIFESTATIONS

Symptoms mainly depend upon the rapidity of the formation of MetHb content in the blood. Levels up to 10-20% are tolerated.

Percentage of MetHb	Symptoms
15-20%	Cyanosis, chocolate brown blood
20-50%	Dysopnea, Nausea, Tachycardia, weakness, dizziness
>70%	Prefound hypoxia, death

Drug induced – Hemolytic anemia⁵

DIAGNOSIS

1. MetHb (Male) <0.14-0.175 g/dl
2. MetHb (Female) 0.123-0.153 g/dl

KCN test: To differentiate between MetHb and SulfHb. In case of acquired methemoglobinemia, increased levels of methemo globinemia is found but with normal enzymatic activity. But in congenital/ hereditary condition, decreased enzymatic activity is seen. ABG and co-oximetry to detect hypoxemia, saturation gap^{5,19}.

TREATMENT

First line therapy is administration of methylene blue as it produces a great relief from Methemoglobinemia as it is an oxidant drug⁵. Methylene blue produces its effects even orally as parenterals doses are not widely available⁵. The action of methylene blue depends upon the levels of NADPH in the RBCs⁵.

Treatment should be started in the condition where patient is asymptomatic with methemoglobin levels at 30% / in patients with symptoms and levels of 20%⁵. In case of patients with already any chronic ailments like cardiovascular disorders, management for MetHb should be carried out even at some lower levels (<20%)⁵.

After entry into the body, methylene blue gets converted into leukomethylene blue which gets excreted through urine and appears in green to blue colour and when excreted through feces, blue colored stools are observed. So the patient should be counseled before the administration of this therapy²⁰. Some of the majorly presenting side effects are green/blue discoloration of urine, Nausea, Vomiting, Diarrhea, abdominal pain, bluish saliva, headache, dysopnea^{3,20}. Patient may also develop rashes, burning pain at the site of injection in vase of IV administration^{3,20}. Increased doses of methylene blue may also leads to bluish discoloration of skin which may confuse physicians that it may be cyanosis¹. Both in cases of inherited and acquired methemoglobinemia, management should be carried out at the level not exceeding 20-30%^{1,20,21}. Normal/ standard dosage of methylene blue is 1-2 mg/Kg/dose or 0.1-0.2ml/Kg of 1% solution. Maximum dose is 7mg/kg for 24 hours. Dosage pattern can be repeated in every 30 minutes interval, if the symptoms exceed the treatment threshold^{1,20,21}. G6PD deficient patients and asymptomatic patients can be mainly

treated with O₂ supplementation. Some of the other alternative drug therapies include- Ascorbic acid - 200 to 500 mg/day ORAL³. Major side effects include increased urinary excretion of oxalate, renal failure due to hyperoxalatemia¹. Dextrose can be given as a major source as it is the major supplement of NADH in RBC's⁵. Blood transfusions are also helpful in patients who cannot take medications. Fully packed RBCs are transfused to the patient, but the underlying physiological defect or the pathological condition cannot be treated with this transfusions. Hyperbaric supplemental O₂ also produces some better effects in case of patients who are not responding to methylene blue and ascorbic acid²¹.

CONCLUSION

Drugs that induce methemoglobinemia should be carefully used. Any particular signs and symptoms should be monitored with attention and further research is to be carried out in order to decrease the mortality rates. In most of the cases, we may suspect patient with acquired metHb instead have congenital methemoglobinemia. So careful monitoring should be done. In case of congenital, levels raise after administration of methylene blue. In such conditions, Ascorbic acid/riboflavin can be given.

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ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICE OF THE PHARMACY STUDENTS TOWARDS PHARMACOVIGILANCE

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ABSTRACT

Pharmacovigilance is the evaluation of the benefit-risk profile of the drug for better efficacy and safety for use in patients. Pharmacovigilance has been known to play a key role in the rational use of drugs by providing information about the adverse drug reactions occurring in patients. A cross-sectional KAP based questionnaire study was carried out in 225 students of a Pharmacy Institute, Guntur. The response of KAP questionnaires were analyzed in percentage and tabular form. Nearly 79.5% of participants heard about Pharmacovigilance, 97.7% know how to identify rare ADRs in clinical trials. 97% people feel that reporting an ADR is a professional obligation, but only 80% came across ADRs. Less than 63% of students know the classification of ADRs.

KEYWORDS: *Adverse Drug Reaction, Pharmacovigilance, Adverse Drug Monitoring Center, Spontaneous Reporting, Knowledge Attitude Practice.*

1. INTRODUCTION

Pharmacovigilance is defined by WHO as "the science and activities relating to the detection, understanding and prevention of adverse effects or any other drug related problems"¹ The major aim of Pharmacovigilance is the evaluation of the benefit-risk profile of the drug for better efficacy and safety for use in patients.³ Pharmacovigilance (PV) was officially introduced in December 1961 in Lancet by W.McBride¹. In 1968, the World Health Organization (WHO) promoted the "Programme for International Drug Monitoring", a pilot project aimed to centralize world data on adverse drug reactions (ADRs)³ Healthcare professionals play a crucial role in the Pharmacovigilance system. Spontaneous adverse reaction reporting is the main backbone of Pharmacovigilance and is required to create hypotheses about potential harms of medicines that need further evaluation.⁴ Lack of awareness about Pharmacovigilance is one of the most important causes of under-reporting. Spontaneous reporting system is considered the main mechanism of Pharmacovigilance study for gathering information about ADRs. Hence our study was undertaken to assess the knowledge, attitude and practice regarding Pharmacovigilance among Pharmacy students in a tertiary care hospital.⁵ To promote drug safety WHO started International Drug Monitoring in 1961 and subsequent to that it promoted Pharmacovigilance program at country level in collaboration with Center for International Drug Monitoring, Uppsala. To identification and reporting of ADRs to ensure the patient safety and outcomes, National Pharmacovigilance Program was initiated in India in the year 2004. It is now renamed as Pharmacovigilance Program of India (PVPI) and operational since July 2010 under the aegis of Central Drug Standard Control Organization. The Uppsala Monitoring Centre (UMC), Sweden maintains the international database of ADR report received from different countries.²

2. METHODOLOGY

2.1 Study Design - It is a prospective cross-sectional Questionnaire based study. There are 32 questions in all (13 questions related to knowledge, 9 questions related to attitude, and eight related to practice). These questions were designed based on earlier studies for assessing KAP of ADR reporting.⁶

2.2 Study Site -A total of 225 Professional students from a Pharmacy College, Guntur.

2.3 Inclusion Criteria – 1) Professional Students 2) Both the gender.

2.4 Exclusion Criteria –1) Those who are not willing to participate in our study.

2.5 Study Process –An Awareness program was conducted with the support of Adverse drug reaction monitoring centre (AMC), Guntur under the supervision of Dr.ZahedaBano, M.D., Professor and HOD of Pharmacology, Guntur Medical College,(AMC Member) Guntur. Pre and Post examination was conducted to assess the knowledge, Attitude and Practice of Pharmacy students towards Pharmacovigilance and ADR reporting.

2.6 Data collection - 32 pretested questionnaires were distributed among pharmacy students. A time of 1 day was given for collection of the filled forms.

3. RESULTS & DISCUSSION

3.1. Demographic Details

S.No	Demographic Factors	Categories	Total	%
1	Age	<20	32	14.2
		20-25	171	76
		26-30	22	9.7
2	Gender	Male	71	31.5
		Female	154	68.4
3	Qualification	Undergraduate	30	13.3
		Graduate	110	48.8
		Masters	85	37.7

Table – 3.1: Demographic Details of Study Sample

Table 3.1 indicates the demographic details of the study sample. Our study subjects were divided into 3 groups based on the Age criteria, in which major participants were between 20-25 Age groups. In gender-wise distribution Females contributed to 154 among 225 participants. Based on Educational Qualification the participants are sub divided into 3 groups in which 30 Undergraduates which includes Diploma in Pharmacy students, 110 Graduates which includes B.Pharmacy students and 85 Maters which includes M.Pharmacy and Pharm.D students have participated in our study ⁷

3.2:Responses of Students to Knowledge based questions

S.No	Description of the Question	Pre				Post			
		Positive	%	Negative	%	Positive	%	Negative	%
1	Define Pharmacovigilance.	179	79.5	46	20.4	207	92	18	8
2	The most important purpose of Pharmacovigilance is	164	72.8	61	27.1	198	88	27	12
3	The most common method employed by the pharmaceutical companies to monitor the ADRs of new drugs once they are launched in the market.	153	68	72	32	213	94.6	12	5.3
4	A serious ADE in India should be reported to the regulatory body within	104	46.2	121	53.7	175	77.7	50	22.2
5	The International center for ADR monitoring is located in	98	43.5	127	56.4	178	79.1	47	20.8
6	Which agency in USA is involved in drug safety issues	190	84.4	35	15.5	217	96.4	8	3.5
7	Which of the following is the	100	44.4	125	55.5	150	66.6	75	33.3

	major risk factor for the occurrence of maximum ADRs?								
8	In India which regulatory body is responsible for monitoring of ADR's	184	81.7	41	18.2	220	97.7	5	2.2
9	Which of the following scales is most commonly used to establish the causality of an ADR	190	84.7	35	15.5	222	98.6	3	1.3
10	Match the ADR reporting systems to respective countries	86	38.2	139	61.7	186	82.6	39	17.3
11	Which among these is a regional Pharmacovigilance center	111	49.3	114	50.6	198	88	27	12
12	Which one of the following is the WHO online database for reporting ADR's	116	51.5	109	48.4	144	50.6	18	8
13	Rare ADR's can be identified in the following phase of a clinical trial	200	97.7	25	11.1	220	97.7	5	2.2

Table – 3.2: Responses of Students to Knowledge based questions

Table-3.2 explains the Pre and Post responses of students regarding Knowledge. In Pre-trial the highest number of positive responses was given to Identification of ADRs in the stages of Clinical Trials, the highest number of negative response was given to ADR reporting systems in different countries. In Post responses the highest number of positive responses was given to commonly used causality scales for establishing ADRs and highest negative response was given to major risk factor for the occurrence of maximum ADRs. It indicates that low knowledge of students on Pharmacovigilance⁸

Table - 3.3: Responses of Students to Attitude based questions

S.No	Description of the Question	Pre				Post			
		Positive	%	Negative	%	Positive	%	Negative	%
1	The health care professional responsible for reporting ADR in a hospital is	218	96.8	7	3.11	221	98.2	4	1.7
2	Which among the following factor discourage you from reporting an ADR	210	93.3	15	6.6	212	94.2	13	5.7
3	Do you think ADR reporting is a professional obligation for you	219	97.3	6	2.6	223	99.1	2	0.8
4	What is your opinion about establishing ADR monitoring center in every hospital	220	97.7	5	2.2	220	97.7	5	2.2
5	Do you think reporting an ADR is necessary	222	98.6	3	1.3	223	99.1	2	0.8
6	Do you think Pharmacovigilance should be taught in detail to health care professionals	216	96	9	0.4	220	97.7	5	2.2
7	Have you any time read any article on prevention of ADR	165	73.3	60	26.6	165	73.3	60	26.6

8	Have you come across an ADR	193	80.17	32	14.2	193	85.7	32	14.2
9	Have you ever been trained on how to report an ADR	60	26.6	165	73.3	225	0	0	0

Table - 3.3: Responses of Students to Attitude based questions

Table-3.3 explains the Pre and Post responses of students regarding Attitude. The maximum number of students agrees that reporting an ADR is necessary to all the health care professionals but most of the students have not ever been trained on how to report an ADR. Many of the students have not reads any article regarding the reporting of ADR. All the students have positive attitude towards reporting of ADRs⁹

Table – 3.4: Responses of Students to Practice based questions

S.No	Description of the Question	Pre				Post			
		Positive	%	Negative	%	Positive	%	Negative	%
1	National Pharmacovigilance Program (NPP) of India was officially inaugurated in the year	169	75.11	56	24.8	212	94.2	13	5.7
2	Pharmacovigilance program in India comprises of how many members	196	86.5	29	12.8	212	94.2	13	5.7
3	AIIMS New Delhi is a	98	43.55	127	56.4	129	57.3	96	87.1
4	Hierarchy of Pharmacovigilance centers in India comprises of the following	110	48.8	115	51.1	163	72.4	62	27.5
5	Peripheral centers in National Pharmacovigilance India	139	61.7	86	38.2	193	85.7	32	14.2
6	The chairman of Pharmacovigilance program in India	176	78.2	49	21.7	218	96.8	07	3.1
7	ADR reporting forms are periodically reviewed by	154	68.4	71	31.5	215	95.5	10	4.4
8	According to Wills and Brown, ADR's are classified into how many classes	140	62.2	85	37.7	210	93.3	15	6.6
9	ADR's which are independent can be treated by which of the following	89	39.5	136	60.4	173	76.8	52	23.1
10	Which among the following is a Augmented drug reaction	109	48.4	116	51.5	207	92	18	8

Table – 3.4: Responses of Students to Practice based questions

Table-3.4 explains the Pre and Post responses of students regarding Practice. In Pre-trial the highest number of positive responses was given to the composition of PVPI, the highest number of negative response was given to treatment of independent ADRs. In Post responses the highest number of positive responses was given to the Chairman of PVPI and highest negative response was given to details of AIIMS New Delhi¹⁰

Table - 3.5: Mean scores of Respondents- Pre KAP Evaluation

Variable/Status of Outcomes	Mean $\pm$ Standard Deviation	Confidence Interval	T-Value	P-Value
Positive	155.56 $\pm$ 48.09	51.44 – 120.80	5.06	< 0.0001**
Negative	69.43 $\pm$ 48.09			

Table - 3.6: Mean scores of Respondents-Post KAP Evaluation

Variable/Status of Outcomes	Mean $\pm$ Standard Deviation	Confidence Interval	T-Value	P-Value
Positive	198.81 $\pm$ 26.15	516.83 – 192.22	20.11	< 0.001*
Negative	24.28 $\pm$ 24.13			

Table 3.5 explains the mean scores of respondents. Our study was applied to Statistics; it reveals pre and post evaluation. The outcomes are shown in the form of Mean and Standard Deviation in pre-trial in both positive and negative responses 155.56 $\pm$ 48.09, 69.43 $\pm$ 48.09 respectively. Similarly the post-trial results of both positive and negative responses are 198.81 $\pm$ 26.15, 24.28 $\pm$ 24.13 respectively. The significant value of Pre and Post is highly significant, Pre (< 0.0001) and Post (< 0.001).

4. CONCLUSION

Our study concludes that the knowledge and awareness of professional students towards ADR reporting is good. Even though majority of the students have positive attitude towards reporting of ADRs, identifying and reporting among them is low, this is mainly due to lack of awareness regarding ADRs. The Practice towards ADR reporting showed a positive trend towards improving ADR reporting and safeguarding the safety of the patients. To improve the knowledge, attitude and practice of ADR reporting in India, widen the teaching programs during undergraduate period itself, simplify the reporting process, and make ADR reporting a mandatory work for health care professionals, which may have a favourable influence on PV in India.

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6. CONFLICT OF INTEREST- Authors of our study are not havingany Conflict of Interest.

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A PROSPECTIVE OBSERVATIONAL AND COMPARATIVE STUDY ON EFFECTIVENESS OF DRUGS USED IN TYPE II DIABETES MELLITUS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Diabetes is a globally predominant disease characterized by hyperglycemia, abnormal lipid and protein metabolism. It mainly occurs when the cells in the body are unable to intake and utilize the glucose due to the insufficiency in the insulin production by the pancreas. The treatment options for type 2 diabetes include, ORAL HYPOGLYCEMIC AGENTS (OHA's) and INSULIN. Insulin is added to the therapy when the glucose levels are alarming and the OHA's alone cannot do the job. Generally, the physicians prescribe different classes of drugs for the treatment of Diabetes Mellitus. Each drug, of different classes, has varied levels of glucose reducing capability. In our study, with a sample size of 60, we have collected data from the patient case files in the Inpatient department of a tertiary care hospital. We calculated the percentage reduction in the glucose levels for different classes of drugs prescribed to patients with comorbidities. The Objective of this study is to assess the most effective Oral Hypoglycemic agents prescribed to patients of both the genders (male and female) with different comorbidities, in the form of Monotherapy, Dithierapy, Trithierapy and a combination with Insulin for the treatment of Type 2 Diabetes. This study finds that, Metformin as monotherapy, combination of Sulphonylureas and Biguanide as Dithierapy, combination of Sulphonylureas, Biguanids and α -glucosidase inhibitors as Trithierapy and Human Actrapid Insulin with different classes of OHA's showed the best reduction in plasma blood glucose levels.

KEYWORDS: *Diabetes, Insulin, Oral Hypoglycemic Agents, Monotherapy, Dithierapy, Trithierapy*

1. INTRODUCTION

Diabetes is a globally predominant disease, characterized by the high blood glucose levels (HYPERGLYCEMIA) or when the cells in the body are unable to intake and utilize the glucose due to the insufficiency in the insulin production by the pancreas.¹ Diabetes is of two types, Type1 (Insulin dependent) and Type 2 (Non-Insulin dependent).² Millions of people around the world live with diabetes. In the past 3 decades the prevalence of diabetes has raised substantially in countries at all income levels, mirroring the global increase in the number of people who are overweight or obese. The global prevalence of diabetes has grown from 4.7% in 1980 to 8.5% in 2014.³ Coming to the Indian scenario; it is the major hub for the global diabetic epidemic with the second highest diabetes population in the world (~69 million as of 2015). With this trend, India would be home to 123.5 million people with diabetes by 2040.⁴ The treatment options for type 2 diabetes include, ORAL HYPOGLYCEMIC AGENTS (OHA's) and INSULIN. Insulin is added to the therapy when the glucose levels are alarming and the OHA's alone cannot do the job.⁵ Generally, the physicians prescribe different classes of drugs for the treatment of Diabetes Mellitus. Each drug, of different classes, has varied levels of glucose reducing capability. But, the prescriber may not have an idea on the percentage of blood glucose levels that a specific drug can reduce. In most of the instances, the prescribed drugs may not fulfill the intentions of the prescriber and the needs of the patient. Because, each drug has different doses, mechanisms of action, potency and variability in its action depending on the existing comorbid conditions in the patient and other drugs that are already being used to treat the comorbidities. This aspect has laid the path to conduct this Prospective, Comparative and observational study, where we have calculated the percentage glucose reducing capability of different classes of drugs, which helps in

optimizing the appropriate and efficient therapy for Type 2 diabetes patients. It may help the physician to choose between these drugs, for the therapy. The Objective of this study is to assess the most effective Oral Hypoglycemic agents prescribed to patients of both the genders (male and female) with different comorbidities, in the form of Monotherapy, Ditherapy, Tritherapy and a combination with Insulin for the treatment of Type 2 Diabetes.

2. METHODOLOGY

This Prospective Observational and Comparative study was conducted in the Manipal Super Specialty Hospital, Vijayawada for duration of 6 months. The data obtained was from the case sheets of the patients with Type 2 diabetes in the In Patient (IP) department of different wards in the hospital.

Inclusion criteria : Patients of the Inpatient department who were above 18 years of age and of both the genders (Male and Female), diagnosed with Type 2 Diabetes irrespective of their co morbidities, admitted in the department during the study period.

Exclusion criteria: Patients of the Outpatient (OP) department were excluded.

Study design: Type 2 diabetes mellitus cases were collected from different wards of the IP department. The collected data were filled in the pre designed diabetic profile forms. The form includes data like the demographic details, past and present medical conditions and co-morbidities, medication history, duration of disease, category of drugs prescribed to treat the co-morbid condition and hyperglycemia. It also includes GRBS and FBS values which were collected from the diabetic chart in the patient case sheets from the hospital.

2.1. Data Analysis

The obtained data was thoroughly analyzed and it was divided into age and gender wise distribution of diabetic cases, co-morbid condition wise distribution, type of therapy provided, type of therapy received in patients who were already on oral hypoglycemic therapy, prescription frequency of oral hypoglycemic agents, effect of insulin + oral hypoglycemic agents on glycemic control in patients, patients who had achieved glycemic control by oral therapy, patients achieved optimal glycemic control by oral hypoglycemic agents+Insulin, patients had not achieved optimal glycemic control, optimal glycemic control in patients based on their co-morbid conditions to compare the effectiveness of oral anti-diabetic medications used in the treatment of TYPE 2 diabetes mellitus.

2.2. Study Material

All the necessary and relevant data were collected during ward rounds from case sheets and by interviewing the patients.

<i>S.no</i>	<i>Source</i>	<i>Data collected</i>
1	Patients history record	Relevant history with diagnosis, laboratory report of investigations/ disease duration, ordered and past history regarding anti-diabetic drugs are collected
2	Treatment chart	List of all the medications prescribed, along with dose, frequency, route of administration
3	In patient follow up records	Disease course, current treatment, clinical laboratory reports fasting blood glucose levels and random blood glucose levels.

3. RESULTS

Figure.1 Age and gender wise distribution of diabetes

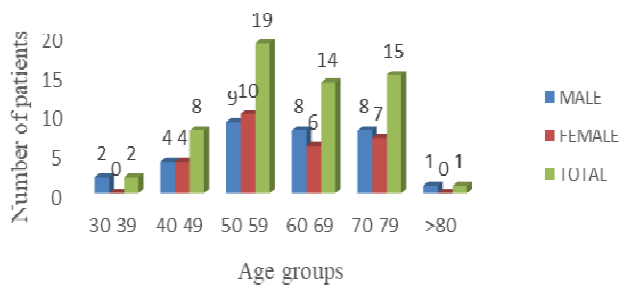


Figure 2: Co-morbid condition wise distribution of diabetic cases(n=60)

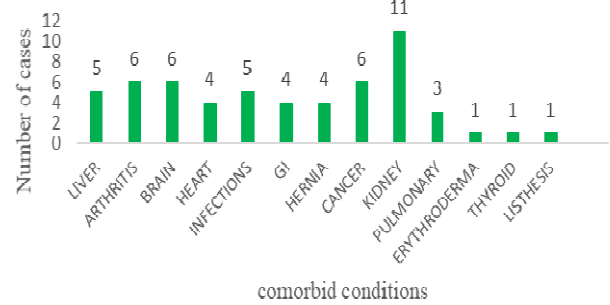


Figure.3 Type of therapy provided (n=60)

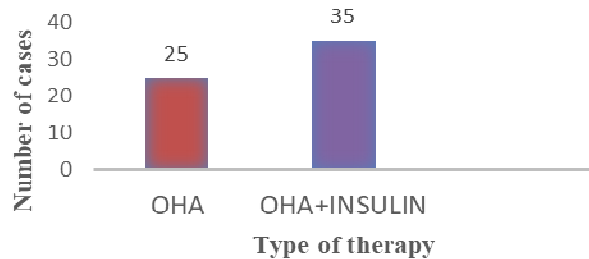


Figure.4 Type of therapy received in patients among who were only on OHAs

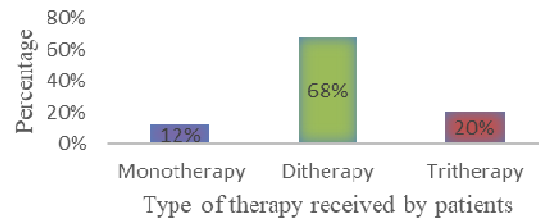


Table 1: Prescription frequency of oral hypoglycemic agents

S no.	Prescription frequency of oral hypoglycemic agents	Number (out of 60 cases)	Percentage
1	Metformin	8	13.3 %
2	Glimepiride	5	8.3 %
3	Gliclazide	5	8.3 %
4	Linagliptin	3	5 %
5	Teneligliptin	3	5 %
6	Glipizide	2	3.3 %
7	Pioglitazone	2	3.3 %
8	Dapagliflozin	1	1.66 %
9	Glibenclamide	1	1.66 %
10	Vildagliptin	2	3.3 %
11	Metformin + Voglibose	1	1.66 %
12	Metformin + Sitagliptin	8	13.3 %
13	Metformin + Glicazide	1	1.66 %
14	Metformin + Glibenclamide	1	1.6 %
15	Metformin + Linagliptin	1	1.6 %
16	Metformin + Teneligliptin	1	1.6 %
17	Metformin + Glipizide	2	3.3 %
18	Metformin + Glimepiride	23	38.33 %
19	Glimepiride + Metformin + Voglibose	6	10 %
20	Glimepiride + Metformin + Pioglitazone	3	5 %

Table 2: Prescription frequency of Insulins with OHA

<i>Types of Insulins prescribed</i>	<i>Percentage of insulins prescribed</i>	<i>Prescribed class of OHA's along with insulin</i>
Long acting(20-24hrs) Inj.Lantus	5.71%	DPP-4 inhibitors
Short acting (2-3hrs) Human insulin (HAI , Insugen R)	68.57%	DPP-4 inhibitors, Biguanides, sulfonylureas, Thiazolidinediones, α -glucosidase inhibitors.
Fast + long acting • Human mixtard	17.14%	DPP-4 inhibitors, Biguanides, sulfonylureas
• Novomix	14.28%	DPP-4 inhibitors, Biguanides

4. DISCUSSION

60 patients were selected according to inclusion and exclusion criteria. Among 60 patients 33 were males and 27 were females. Age group of 50 to 60 are most vulnerable for type 2 DM as shown in Figure.1.⁶ Total number of cases collected were 60 from tertiary care hospital. Among those, 31 cases achieved optimal glycemic control. From 31 cases, 13 cases were treated only with oral hypoglycemic agents (OHA) in which 61.5% of males and 38.46% of females achieved optimal glycemic control. Here the greater percentage of class of drugs prescribed as mono therapy are Sulphonylureas and Biguanides (33.3%), as ditherapy the combination of Biguanides and Sulphonylureas (71.4%), as tri therapy the combination of Biguanide's, Sulphonylureas and α -glucosidase inhibitors (66.6%) and 18 cases were treated with both oral hypoglycemic agents (OHA) and Insulin in which 50% of males and 50% of females achieved optimal glycemic control. Here the greater percentage of class of drugs prescribed as mono therapy are dipeptidylpeptidase-4 inhibitors(50%), as ditherapy the combination of Biguanide's and sulphonylureas (66.6%), as tri therapy the combination of Biguanide's, sulphonylureas and thiazolidinedione(66.6%). 95 percent of diabetic patients were observed with comorbid condition. In co-morbid wise distribution of diabetic cases, kidney problems (11 cases) were observed the most as shown in Figure.2. Most of the adult diabetic patients will develop atleast one comorbid condition. Up to 75 percent of patients with diabetes additionally have hypertension. Other basic comorbidities of diabetes are hyper lipidaemia, cardiovascular illness, kidney problems, and obesity and liver problems. The hazard factors for diabetes may also cause specific sorts of malignant growth.⁷ The oral hypoglycaemics alone and the combination of OHAs plus insulin were used in the management of type II DM. Out of 60 patients, 25 patients received only OHAs and remaining 35 patients received combination of OHAs and Insulin as shown in Figure.3. Efficacy of both types of therapies was assessed. Treatment of type II diabetes mellitus only with oral hypoglycaemic agents are categorised into monotherapy, ditherapy, tritherapy. The highest percentage occupied by ditherapy such as 68percent as shown in Figure.4. Insulin resistance is the leading factor for type II diabetes. Insulin resistance is observed in most of the overweight patients. Body gets energy to perform daily activities, when insulin allows body cells to take glucose. Due to some factors like overweight, the insulin fails to take glucose into the body cells known as insulin resistance. In that event blood glucose levels will raise and gets accumulated in circulatory system and cause hyperglycaemic condition.⁸ As monotherapy metformin has showed good reduction of blood glucose levels. Other studies also showed that metformin has gotten one of the most generally utilized medications in the treatment of type 2 diabetes mellitus since its endorsement in the UK. It is the main line treatment for patient with T2DM as indicated by the ADA. Metformin works by diminishing intestinal glucose retention, improving glucose take-up, bringing down fasting plasma insulin levels and expanding insulin affectability, which bring about a decrease of blood glucose focuses without causing clear hypoglycaemia.^{9,10} As ditherapy the combination of sulfonylureas and biguanides showed good reduction of blood glucose levels. The sulfonylureas are considered as second line drug of choice to treat typeII diabetes mellitus. Combination of sulfonylureas and biguanides shows good reduction for the patients who are having baseline glycated haemoglobin or if the blood sugar levels are not getting to normal by using metformin alone and these class of drugs as combination may reduce over production of glucose from hepatic cells by decreasing hepatic gluconeogenesis.¹¹ As tritherapy the combination of sulfonylureas,

biguanides and alpha-glucosidase inhibitor showed good reduction of blood glucose levels. Alpha-glucosidase inhibitors show significant reduction in post prandial hyperglycaemic condition. The post prandial hyperglycaemic condition leads to CVD risk. In this condition there is a need of add on therapy using voglibose in ditherapy that includes sulfonylureas and biguanides. Alpha-glucosidase inhibitors will reduce total cholesterol (TC), triglyceride (TG) and low density lipoproteins (LDL) levels and increase HDL levels.¹² Human actrapid insulin (HAI) in combination with different classes of OHAs showed the best reduction of plasma blood glucose levels. The combination therapy of insulins along with OHA's are preferred when the diabetic patients does not respond to OHA's or if the patients are having HbA1c levels are greater than 9%.¹³ Combination of Insulins and OHAs therapy is most commonly used. This showed effectiveness in improving glycemic control. Addition of Insulin therapy reduces the risk of microvascular complications in type 2 DM. Insulin therapies, in initial stage of type 2 DM may minimise the risk of macrovascular complications.^{14, 15} The combination therapy of Insulins and OHAs will maintain the HbA1c levels at less than 7%.¹⁶

5. CONCLUSION

This study found that, the Metformin as monotherapy; Sulfonylurea's and Biguanide's as di therapy; Sulfonylurea's, Biguanide's, Alpha-glucosidase inhibitors as tri therapy and Human actrapid insulin (HAI) with different classes of OHA's showed the best reduction of plasma blood glucose levels.

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A PROSPECTIVE STUDY ON THE HEALING OUTCOMES OF DIABETIC FOOT ULCER AFTER THE IMPLEMENTATION OF CLINICAL PHARMACIST INTERVENTION GUIDELINE

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ABSTRACT

Diabetes and its complications are rapidly becoming the world's most significant cause of morbidity and mortality. Diabetic Foot Ulcer (DFU) causes major health issues in diabetes patients. 15% to 25% of diabetic patients get affected by DFU in their life time. Diabetic foot ulcers are the cause for physical disability and poor quality of life among diabetic patients which leads to the loss of limb, major health as well economic sufferings and even death. Clinical pharmacy is a health care discipline which focuses on clinical pharmacist's involved patient care programmes to provide rationality in drugs use, to improve health and prevention and management of diseases. The aim of the study was to identify the essential domain in the management of DFU in comparison with the international standards and to implement guidelines for the effective management of Diabetic foot ulcer. The prospective study was conducted on determination of wound ulcer outcome among 50 DFU patients admitted in the surgery wards of tertiary care hospital by wound classification, switching over of antibiotics, culture tests, hospital stay, and recurrence of DFU and wound healing rate after the implementation of clinical pharmacist intervention guideline. The healing of ulcers improved outcome percentage was found to 16% in the control group and 32% in the interventional group. The ulcer healing impression was found to be healed in (4 vs. 8 patients), partially healed (10 vs. 11 patients), requiring surgical intervention (7 vs. 5 patients) and unhealed (4 vs. 1 patient) in the control vs. Intervention group, respectively. There was improved healing impression in the control vs Intervention group. Implementation of clinical pharmacist's intervention guideline and antibiotic policy brought a significant improvement in terms of reduction in the number of ulcers, size of ulcers, ulcer grade and improved ulcer healing duration.

KEYWORDS: *Diabetic foot ulcer, Clinical pharmacy services, patient education, wound care.*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder caused by acquired and additionally procured insufficiency in the insulin secretion by the pancreas, or by the incapability of the insulin produced¹. It leads to expanded convergences of blood glucose, which can harm many body structures and organs like the veins and nerves. 15% to 25% of diabetic patients get influenced in their lifetime²⁻⁴. Diabetes and its complications are rapidly becoming the world's most significant cause of morbidity and mortality. It is predicted that by 2040 there will be over 642 million people with diabetes in the world^{5,6}. Foot ulcer prognoses to physical disability and poor quality of life⁷⁻¹¹, leading to loss of limb, major sufferings and cost¹²⁻¹⁵ and even death.¹⁸⁻²³ Patients with DM are prone to multiple complications such as diabetic foot ulcers (DFU). DFU is a common complication of DM that has shown an increasing trend over previous decades²⁵⁻³². In total, it is estimated that 15% of patients with diabetes will suffer from DFU during their lifetime³¹. Although accurate figures are difficult to obtain for the prevalence of DFU, the prevalence of this complication ranges from 4%-27%³²⁻³⁸. Clinical pharmacy is a health care discipline that focuses on clinical pharmacists involved patient care programmes to provide rationality in drug use, to improve health and prevention and management of diseases^{39,40,41}. The aim of the study was to identify the essential domain in the management of DFU in comparison with the international standards and to implement guidelines for the effective management of Diabetic foot ulcers.

MATERIAL AND METHODS

The prospective study was performed to evaluate the improvement in healing outcomes of diabetic foot ulcer viz., reduction in the number and size of ulcers, healing duration, status at the time of discharge, change of antibiotics and reduction in wound grade in the control and clinical pharmacist intervened group. The study was conducted among the DFU patients admitted in the departments of surgery of a tertiary care hospital. The study protocols were approved by the Institutional Ethical Committee and every patient's consent was obtained.

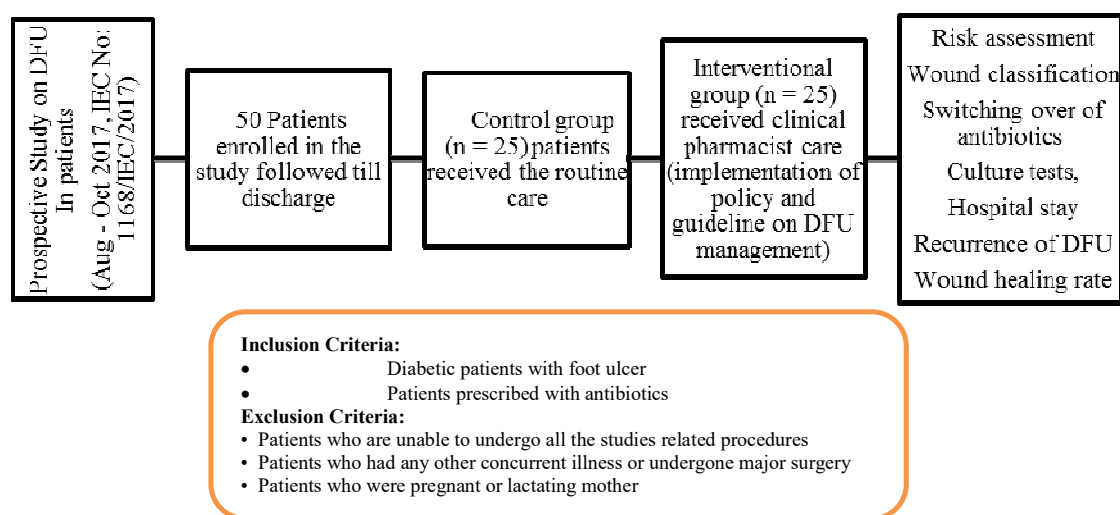


Figure 1 Prospective study design on wound ulcer outcome in DFU patients

Infectious Diseases Society of America (IDSA) and IWGDF Diabetic Foot Infection classifications were adopted in the clinical pharmacist guideline to characterize the injury and recommend anti-infection agents⁴²⁻⁴⁴. The University of Texas grouping is considered as a thorough scale and incorporates hazard stratification and communicates tissue breakdown, contamination and gangrene independently⁴⁵⁻⁴⁷. In spite of the fact that Texas arrangement depicts the review of the injury in more subtleties, it does exclude proportions of neuropathy or ulcer zone.

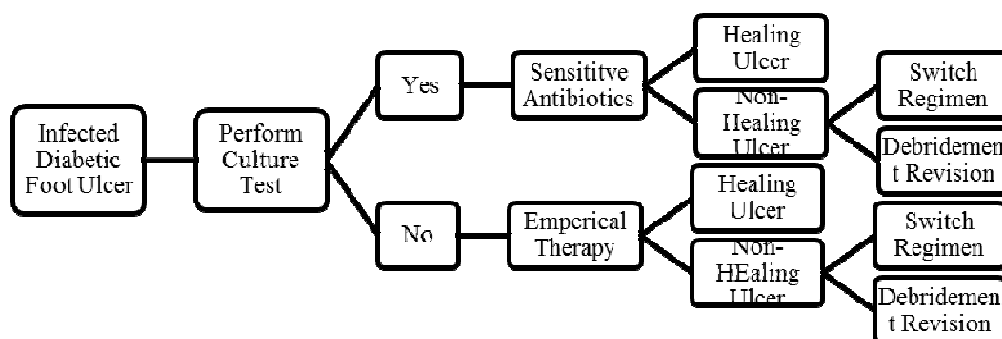


Figure 2 Guideline on Treatment Algorithm for Treating Diabetic Foot Ulcer

Table 1 The University of Texas Wound Classification System

Stage	Grade			
	0	I	II	III
A	Pre or post ulcerative completely epithelized lesion	Superficial wound	Wound penetration up to tendon or capsule	Wound penetration up to bone or joint
B	Infection	Infection	Infection	Infection
C	Ischemia	Ischemia	Ischemia	Ischemia

D	Infection and Ischemia	Infection and Ischemia	Infection and Ischemia	Infection and Ischemia

The IDSA rule demands profound tissue acquired as the sample for performing culture test to get exact outcomes than the swab samples from insufficiently debrided wounds^{48,49}. Type1 and 2 diabetic patient's foot care by National Institute of Clinical Excellence (NICE) rule demand understanding focused consideration as indicated by per quiet individual needs⁵⁰⁻⁵⁵. Data collected for the study was tabulated and the result analysis was done using GraphPad Prism 7.0. Descriptive statistics includes the calculation of mean, standard deviation and percentage. Paired T test and ANOVA was used to compare the significance between the baselines and follow up within the control as well as in interventional group. Unpaired T test was performed to compare the significance between the control and interventional group. The effects were found to be significant to the least significant difference at 5% level of significance.

RESULTS

The ulcer size was found to be ≤ 1 cm in 7 and 6 patients, ≤ 3 cm in 13 and 11 patients and > 3 cm in 5 and 8 patients, in the control and interventional group, respectively during the admission and the ulcer size was found to ≤ 1 cm in 5 and 9 patients, ≤ 3 cm in 7 and 2 patients and > 3 cm in 3 and 1 patient, in the control vs. interventional group, respectively during the discharge. There is notable wound size reduction in the interventional group from > 3 cm to ≤ 3 cm or ≤ 1 cm in the interventional group compared to control group (Fig 3).

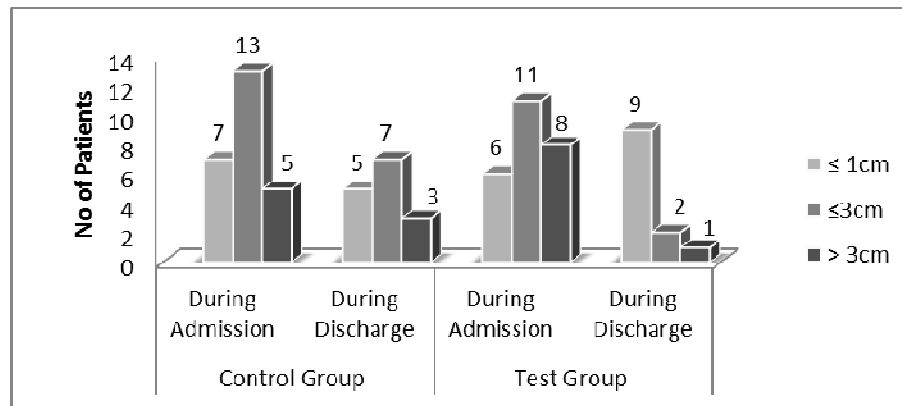


Figure 3 Distribution of ulcer size in the study population

The healing of ulcers improved outcome percentage was found to 16% in the control group and 32% in the interventional group. The ulcer healing impression was found to be healed in (4 vs. 8 patients), partially healed (10 vs. 11 patients), requiring surgical intervention (7 vs. 5 patients) and unhealed (4 vs. 1 patient) in the control vs. intervention group, respectively. There was improved healing impression in the control vs. intervention group (Table 2).

Impression	Control group (n= 25)	Test Group (n= 25)	Intervention VS Control Improved Outcome %
Healed	4	8	16 ↑
Partially Healed	10	11	4 ↑
Surgical Intervention	7	5	8 ↓
Unhealed	4	1	12 ↓

Table 2 Distribution of ulcer healing impression in the study population

The DFU Healing Duration was 1 week for 6 and 9 patients, 2 weeks for 6 and 7 patients, 3 weeks for 6 and 3 patients, 4 weeks for 7 and 4 patients and 5 weeks for 4 and 2 patients in the control vs. intervention group respectively. There was improvement in the healing duration in the intervention group compared to control group (Fig 4).

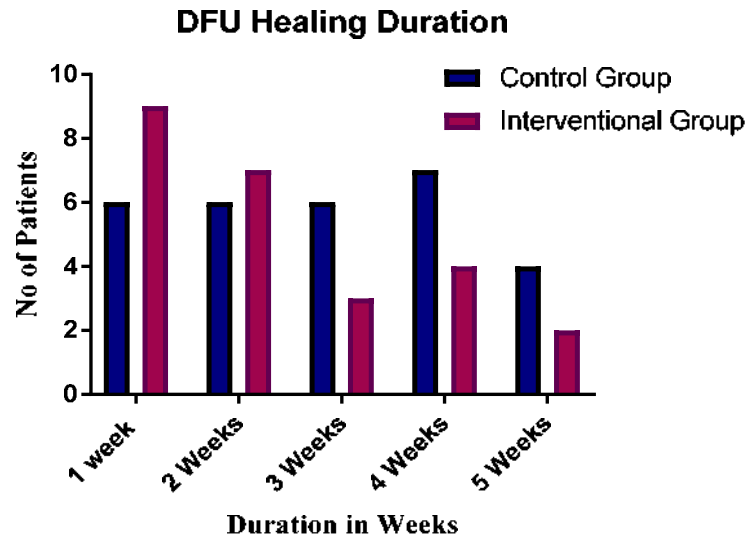


Figure 4 Comparison of DFU Healing Duration

The number of switching over of antibiotics was found to be once in 7 vs. 3 patients and 18 vs. 1 patient in the test group compared to interventional group. There was improved outcome percentage in the intervention compared to control group (**Fig 5**).

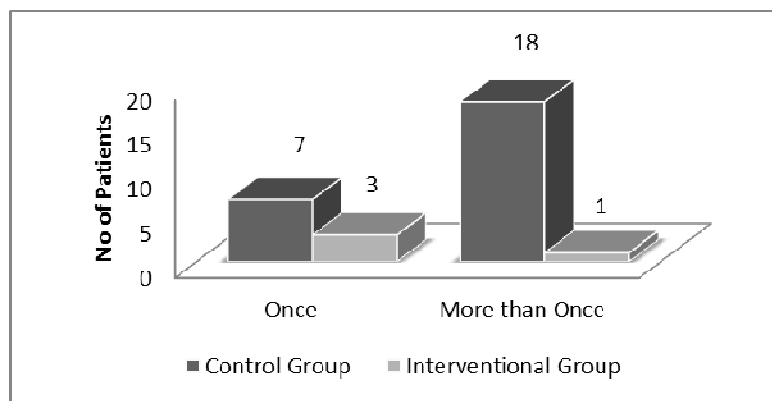


Figure 5 Comparisons on Number of Antibiotics Switching Over

DISCUSSION

Development of Guideline and its Outcome

Clinical practice is continuously evolving because of significant changes in the incidence; increasing consequent clinical evidences on new and existing treatments especially in non-communicable diseases will influence the disease occurrence clinically. Belatti, D.A.³⁰, Markowitz, J.S.⁵⁶ researches identifies the highly effective treatment and the ineffective treatment influences on basis of guidelines adopted. This is one of the pioneer studies which explains on the effective guideline that focuses on the management and prevention of diabetic foot ulcer. Importantly this is the first if its kind for the clinical, hospital and community pharmacist prepared by the clinical pharmacist. This guideline is prepared on the base of existing NICE guideline on DFU and the present scenario revealed by our retrospective study. Understanding the implementation of the evidence obtained through the studies by Aydin, K.⁵⁷, and Lepäntalo, M.⁵⁸, and clinical pharmacists practice skill the practical guideline was framed from the perspectives, 1) improving patient health safety outcomes, 2) designing the policies to encourage use of appropriate antibiotics, culture sampling techniques and effective management, 3) advising community and clinical pharmacist on evidence based patient education and counseling on DFU prevention and management. The guideline implementation was enormously welcomed by the community and clinical pharmacists and was evaluated by the clinical pharmacist experts for the quality and feasibility. This guideline highlighted points over the wound classification, first line and

second line antibiotics choice and patient education were well suited for the current scenario. This will help in minimizing the switching over of antibiotics, effective treatment outcome, reduction of stay in hospital and thereby reducing the considerable direct and indirect expenses. The prospective study followed the guideline policies and proved the best results.

Wound outcome

As per the literature reviews and our previous studies, it was found that the guideline implementation on antibiotics use and treatment is very important. IDSA and NICE guidelines were found to be the best guidelines as they were developed with the outputs from the various study reports. A complete guideline on antibiotics use and clinical pharmacist role was prepared carefully under the supervision of a Diabetologist and it was revised by a Clinical Pharmacologist. The guideline was adopted while treating the patients and the outcomes were recorded until the patients were discharged. The ulcer size was found to be ≤ 1 cm in 7 and 6 patients, ≤ 3 cm in 13 and 11 patients and > 3 cm in 5 and 8 patients, in the control and interventional group, respectively during the admission and the ulcer size was found to be ≤ 1 cm in 5 and 9 patients, ≤ 3 cm in 7 and 2 patients and > 3 cm in 3 and 1 patient, in the control vs. interventional group, respectively during the discharge. There is notable wound size reduction in the interventional group from > 3 cm to ≤ 3 cm or ≤ 1 cm in the interventional group compared to the control group. The healing of ulcers improved outcome percentage was found to 16% in the control group and 32% in the interventional group. The ulcer healing impression was found to be healed in (4 vs. 8 patients), partially healed (10 vs. 11 patients), requiring surgical intervention (7 vs. 5 patients) and unhealed (4 vs. 1 patient) in the control vs. intervention group, respectively. There was an improved healing impression in the control vs. intervention group. The DFU Healing Duration was 1 week for 6 and 9 patients, 2 weeks for 6 and 7 patients, 3 weeks for 6 and 3 patients, 4 weeks for 7 and 4 patients and 5 weeks for 4 and 2 patients in the control vs. intervention group respectively. There was an improvement in the healing duration in the intervention group compared to the control group. The study by Warriner R.A⁵⁹, in 2012, worked on the study on foot ulcer outcomes when the patients were monitored closely through the follow-ups. They found that 63.8% of wounds healed in patients who visited hospital weekly for one month were compared with 2.0% of patients who visited every other week, patients who had vascular lower ulcers 78 of 105 wounds (52%) closed in week group compared with 0% in the every-other-week group ($P = 2.40 \times 10$). The number of switching over of antibiotics was found to be once in 7 vs. 3 patients and 18 vs. 1 patient in the test group compared to interventional group. There was improved outcome percentage in the intervention compared to control group.

CONCLUSION

Implementation of clinical pharmacist prepared guideline and antibiotic policy brought a significant improvement in terms of reduction in the number of ulcers, size of ulcers, ulcer grade and improved ulcer healing duration. Effective implementation of antibiotic policy in empirical and therapeutic use among DFU patients reduces the switching over of antibiotics which in turn reduce the resistance to antibiotics. Teamwork of uniting various departments comprising physician, surgeon, nurse, pathologists, and clinical pharmacologists along with clinical pharmacist enhances the management of DFU management.

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GENETICAL DISORDER PROGERIA

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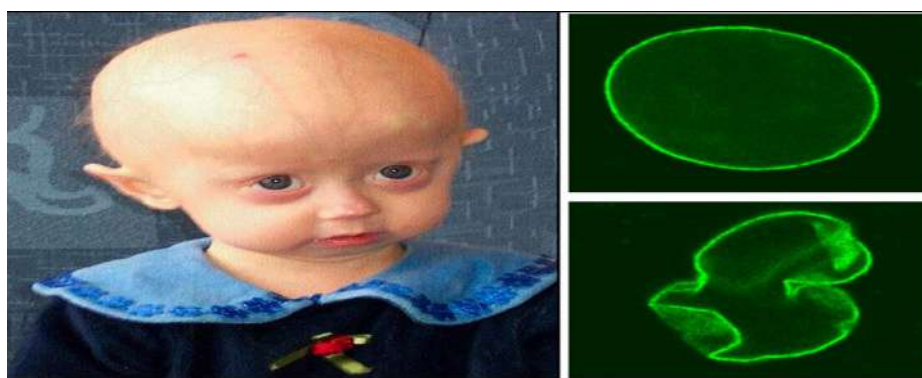
ABSTRACT

During recent years, our understanding of pathogenesis of inherited microcytic anemias has gained from the identification of several genes and proteins involved in systematic and cellular iron metabolism and heme syntheses. Numerous case reports illustrate that the implementation of these novel molecular discoveries in clinical practice has increased our understanding of the presentation, diagnosis, and management of these diseases. Integration of these insights into daily clinical practice will reduce delays in establishing a proper diagnosis, invasive and/or costly diagnostic tests, and unnecessary or even detrimental treatments. To assist the clinician, we developed evidence-based multidisciplinary guidelines on the management of rare microcytic anemias due to genetic disorders of iron metabolism and heme synthesis. These genetic disorders may present at all ages, and therefore these guidelines are relevant for pediatricians as well as clinicians who treat adults. This article summarizes these clinical practice guidelines and includes background on pathogenesis, conclusions, and recommendations and a diagnostic flowchart to facilitate using these guidelines in the clinical setting.

KEYWORDS: Hutchinson-Gilford syndrome, premature aging, laminA gene mutation

INTRODUCTION

Progeria is an extremely rare autosomal dominant genetic disorder in which symptoms progeroid syndromes. Those born with progeria typically live to their midteens to early twenties. It is a genetic condition that occurs as a new mutation, and is rarely inherited, as carriers usually do not live to reproduce children¹. Although the term progeria applies strictly speaking to all diseases characterized by premature aging symptoms, and is often used as such, it is often applied specially in reference to HGPS



Disease name and synonyms

Progeria

Hutchinson-Gilford progeria syndrome (HGPS) Progeria is a term recognized by many physicians as applying to individuals who appear prematurely aged. Misdiagnosis of HGPS is frequently made in patients presenting with some of the features of the syndrome, *i.e.* alopecia and skin with an aged appearance².

STEPS IN NORMAL CELL	STEPS IN PROGERIA CELL
The gene LMNA encodes a protein called prelamin A	The gene LMNA encodes a protein called prelamin A
Prelamin A has a farnesyl group attached to its	Prelamin A has a farnesyl group attached to its

end	end
Farnesyl group is removed from prelamin A	Farnesyl group remains attached to prelamin A
Normal form called PRELAMIN A	Abnormal form of prelamin A called PROGERIA
Prelamin A is not anchored to the nuclear rim	Progeria is anchored to the nuclear rim
Normal state of the nucleus	Abnormally shaped nucleus

Diagnosis criteria / Definition

Association of:

- Prematurely aged phenotype of postnatal onset
- Rapidly progressive growth failure
- Characteristic facies, alopecia, loss of subcutaneous fat, hypotrichosis, thin, wrinkled skin with prominent superficial veins
- Stiffness of joints, osteolysis
- Early death by atherosclerosis or cerebrovascular disease

Prevalence

The estimated birth prevalence is one in four million births.

Causes

Progeria is caused by mutations that weaken the structure of the cell nucleus, making normal cell division difficult. In normal conditions, the LMNA gene codes for a structural protein called prelamin A, which undergoes a series of processing steps before attaining its final form, called lamin A. In one of these steps, after prelamin A is made in the cytoplasm, an enzyme called farnesyltransferase attaches a farnesyl functional group to the protein's carboxyl-terminus³. The farnesylated prelamin A is then transported through nuclear pore to the interior of the nucleus. The farnesyl group allows prelamin A to attach temporarily to the nuclear rim. Once the protein is attached, it is cleaved by protease, which removes the farnesyl group along with a few adjacent amino acids. Failure to remove this farnesyl group permanently attaches the protein to the nuclear rim⁴. After cleavage by the protease, prelamin A is referred to as lamin A. Lamin A, along with lamin B and lamin C, makes up the nuclear lamina, which provides structural support to the nucleus

Before

Clinical description

Clinical manifestations are evident in the first or second year of life. The failure to grow and to gain weight at a normal rate usually appears abruptly during the first year of life and can recur later at times of illness. Linear growth tends to be about half the normal rate and shows no rapid increase at prepubertal or pubertal ages. The weight deficit is greater than the height deficit. By the time growth failure can be noticed, the patients usually have a cranium which appears to be large in comparison with the face and body. Intelligence and brain development is not impaired. The mean age of death is 14 years. This death is often secondary to coronary artery disease.⁵ Consistent clinical features include lower weight and less subcutaneous fat, craniofacial disproportion, micrognathia, prominent scalp veins, alopecia, "plucked-bird" appearance, abnormal teeth, pyriform thorax, short clavicles, "horse-riding" stance, wide-based gait, coxavalga, thin limbs, prominent and stiff joints, and distal osteolysis. Other anomalies frequently present in Hutchinson-Gilford progeria are thin, wrinkled, sclerodermatous and brown-spotted skin, hypotrichosis, absence of eyebrows and eyelashes, patent fontanels and sutures, beaked nose, circumoral cyanosis, thin lips, protruding ears with absent lobes, and dystrophic nails with short terminal phalanges. Autopsy reports have described varying degrees of generalized atherosclerosis, mainly involving the larger arteries. Coronary occlusions with myocardial infarctions were found more frequently than cerebral vascular lesions. Nephrosclerosis was sometimes described.⁶

Differential diagnosis

Many other premature aging syndromes, which are called progeroid syndromes and which also mimic senescence, need to be distinguished from progeria. Neonatal progeroid syndromes are evident at birth and

include Wiedemann-Rautenstrauch syndrome, Hallerman-Streiff syndrome and De Barsy syndrome. Others, including Mandibuloacral dysplasia or Cockayne syndrome are diagnosed later in life, although they may have a neonatal onset. Werner syndrome and acrogeria can be also mistaken with HGPS.

Diagnostic methods

Diagnosis currently depends upon the recognition of clinical and radiographic findings. The finding of the common *LMNA* truncating mutation could also be helpful in the diagnosis. The characteristic radiological abnormalities are to be found in the skull, thoracic cage, long bones and phalanges. The cranial bones tend to be hypoplastic and the fontanelles and sutures remain open longer than expected. Wormian bones are common. Thinning and resorption of the distal clavicles is the most consistent abnormality to be found in the thorax. Narrowing of the posterior ribs is frequent. The long bones are slender with thin cortices. Severe cox vara is a consistent finding, and moderate genu valgum is frequently present. The progressive bone loss from the distal phalanges of the fingers and/or toes is one of the hallmarks of the disease.

Etiology

The etiopathogenesis of progeria has been slow to emerge. Extensive metabolic investigations have failed to detect the defect in HGPS. Growth hormone (GH) responses are normal, elevated GH level is frequent, with an elevated metabolic rate. No abnormality of the thyroid, parathyroid, pituitary or adrenal gland function has been reported. There is a degree of insulin resistance. Abnormalities of skin collagen and elastin have been described, with markedly elevated elastin and collagen type IV production. Skin cultures are sometimes difficult because of early cell death. Hyperlipidemia has never been very high or marked. Some authors suggested defective vitamin E metabolism, others demonstrated an increased fraction of heat-labile enzymes and other altered proteins. One patient had an inverted insertion in the long arm of chromosome 1 in 70% of cells, which suggested that a gene for progeria may be located on chromosome 1. Studies of aneuploidy in skin fibroblasts of patients with HGPS showed that the rate was significantly higher than in controls, but without a significant pattern of chromosome-specific aneuploidy. Studies of the DNA maintenance indicate faithful transcription in HGPS. Another study showed reduced DNA repair in progeria cells. In 2003, mutations in *LMNA*, the gene encoding Lamin A (1q21.2), have been evidenced. It is to date the only gene associated with HGPS, and point mutations are found in 90% of individuals. A recurrent *de novo* point mutation within exon G608G is found in most of the individuals, leading to abnormal splicing truncated protein. Individuals with the common mutation G608G present a remarkably similar phenotype, whereas patients with other point mutations have unusual phenotypes. Other individuals with HGPS have been found to have uniparental isodisomy of chromosome 1 and a deletion of the *LMNA* gene. The protein product of this gene coats and organizes the interior surface of the nuclear envelope. Immunofluorescence of HGPS fibroblasts with antibodies directed against lamin A reveals that 40-50% of the cells show an irregular shape of the nuclear envelope. The common mutation appears to act as a dominant negative mutation affecting nuclear morphology. Interestingly, mutations in *LMNA* have also been reported in other conditions, named laminopathies, including autosomal dominant and recessive Emery-Dreifuss muscular dystrophy, autosomal recessive restrictive dermopathy, autosomal dominant dilated cardiomyopathy with conduction system defects, autosomal dominant Dunnington-type partial lipodystrophy, autosomal dominant limb-girdle muscular dystrophy 1B, autosomal recessive Charcot-Marie-Tooth type 2B1, autosomal recessive mandibuloacral dysplasia and atypical Werner syndrome.

Genetic counseling

De novo dominant mutations have been evidenced in HGPS. Most cases are sporadic and mean paternal age is increased. The risk for the sibs of a proband is small, limited to the possibility of germinal mosaicism. Nevertheless, genetic counseling requires clinical expertise in order to rule out other differential diagnoses and therefore different modes of inheritance. Molecular analysis of the *LMNA* gene could be helpful in some cases. Parent to child transmission has never been observed since patients with HGPS do not reproduce.

Antenatal diagnosis

Antenatal early molecular diagnosis is available if the mutation has been identified in the proband, although the risk of recurrence is small, limited to the unlikely possibility of germline mosaicism in one of the parents. Careful ultrasound monitoring searching for intrauterine growth retardation could also be proposed,

but normal growth cannot rule out the diagnosis with certainty since birth weight varies from 1 to 3.8 kg.

Management including treatment

No effective therapy is currently available to cure the disease. Nevertheless, symptomatic treatment should be proposed for all its complications, including orthopedic complications. The main complication of progeria is coronary artery disease, and monitoring for cardiovascular disease should be obtained at least annually. The possibility of coronary artery bypass surgery or percutaneous transluminal angioplasty has been reported. Low doses of aspirin could be discussed in the prevention of heart attacks or strokes. Anesthetics should be used with particular caution. Non aggressive nutritional therapy was proposed in a study and slightly improved weight gain and growth. Combined nutritional therapy and GH treatment improved growth velocity, levels of growth factors and lowered the basal metabolism rate. Since delayed loss of primary teeth is common, extractions may be required to avoid crowding or development of two rows of teeth.

Unresolved questions

A unique consanguineous family with autosomal recessive HGPS and homozygous mutation within the LaminA gene has been reported, with overlapping features with mandibuloacral dysplasia. Further reports should confirm the existence of an autosomal recessive entity in HGPS. A patient was reported to have a neonatal form of HGPS, but this diagnosis is discussed since the postnatal character of the premature aging is part of the definition of HGPS. Although patients with HGPS have an aged appearance, they usually do not demonstrate senile cataracts, senile presbycusis, presbyopia, arcussenilis, osteoarthritis, or senile personalities. This syndrome may only mimic certain aspects of the aging process.

CONCLUSION

It is concluded that to assist the clinician, we developed evidence-based multidisciplinary guidelines on the management of rare microcytic anemia's due to genetic disorders of iron metabolism and heme synthesis. These genetic disorders may present at all ages, and therefore these guidelines are relevant for pediatricians as well as clinicians who treat adults.

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VITAMIN 'D' DEFICIENCY LEADS TO DIABETES

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ABSTRACT

Vitamin D is a fat soluble which is essential for the several reasons, including maintaining healthy bones teeth. It regulates insulin level and supporting diabetes management. Vitamins are nutrients that the body cannot create and so a person must consume them in diet .vitamin D is not a vitamin but a prohormone, or precursor of a hormone. Vitamin D is believed to help improves body sensitivity to insulin – the hormone responsible for regulating blood sugar levels – thus reduce the risk of insulin resistance, which is a precursor to type 2 diabetes.

KEYWORDS: Vitamin D, diabetes, inflammation, hyperglycemia, insulin

INTRODUCTION

Vitamin D plays a pivotal role in calcium metabolism, and vitamin D deficiency may be associated with a range of serious diseases, including cancer, cardiovascular disease, and type 2 diabetes . The underlying biological mechanisms are poorly understood, the association of low serum 25-hydroxyvitamin D3 [25(OH)D3] concentrations with type 2 diabetes may be mediated through effects on glucose homeostasis and, in particular, a direct effect of vitamin D on the b-cell function, and thus insulin secretion¹ . Several studies have suggested that low vitamin D status also contributes to insulin resistance². Low vitamin D status is associated with markers of impaired glucose metabolism, such as glycosylated¹ hemoglobin (HbA1c)

VITAMIN D EFFECT ON TYPE 1 AND 2 DIABETES

In Type 1 diabetes mellitus, pancreatic islet beta cells are destroyed as a result of autoimmune processes over weeks to months, causing severe insulin deficiency. Blood glucose concentrations rise, without insulin to cause glucose uptake into muscle [to provide energy] and the liver [for storage as Glycogen]. Hepatic gluconeogenesis also continues unabated without insulin to suppress The blood glucose increases rapidly leading to marked symptoms and requiring insulin replacement treatment from an early stage. Ketone bodies and keto-acids accumulate leading to acute metabolic crises if insulin provision is inadequate. Type 2 diabetes mellitus increases in insulin resistance, for whatever reason, lead to increased demands for insulin production. Beta cells hypertrophy, beta cell damage and fibrosis follow, with reductions in insulin secretion. Mechanisms causing islet cell damage include increased rennin angiotensin system [RAS] activity and excessive free radical production. Continuing islet damage explains why insulin treatment is so often needed in T2DM and why acute upsets in diabetic control in T2DM usually require insulin treatment³. Diabetes is common and presents enormous self-care problems to all the people have sedentary lifestyle.

Vitamin D and diabetes mechanisms

The higher levels of vitamin D might lower the risk of type 2 diabetes. One study, done in mice, revealed that lack of the vitamin D receptor was tied to higher rates of diabetes. A receptor is a cell protein that receives specific chemical signals in the cell's environment ⁴ . Other studies have found that insulin-producing cells in the pancreas have vitamin D receptors and that by products of vitamin D in the body "stimulate the pancreas to produce insulin." They also cite other animal studies that have demonstrated that "active metabolites of vitamin D" can protect insulin-producing cells in the pancreas from receiving certain signals that trigger inflammation and cell death⁷. Vitamin D levels should ideally be between 20-56 ng/ml (50-140 ng/mol)*, with anything below 20 mg/ml considered deficient. It is known that

raising the amount of vitamin D in your body to around 60-80 ng/ml can help keep blood glucose levels under control, which is vital for people with diabetes⁸.

VITAMIN D DEFICIENCY

- Osteoporosis
- Increased risk of death from cardiovascular
- Cognitive impairment
- Severe asthma in children
- Cancer
- Type 1 and 2 diabetes
- Multiple sclerosis'

MECHANISMS OF ACTION

The vitamin D deficiency could be contributing factor in the development of both type 1 and 2 diabetes. The beta cell in the pancreas that secretes insulin as shown to contain VDRs as well as the alpha hydroxylase enzyme⁵. Vitamin D deficiency leads to reduce insulin secretion vitamin D treatment improves glucose tolerance and insulin resistance. It contributes to normalization of extra cellular calcium ensuring normal calcium flux through cell membranes. Vitamin D and diabetes include improving insulin action by stimulating expression of the insulin receptor enhancing insulin responsiveness for glucose transport, having an indirect effect on insulin action potentially via a calcium effect on insulin secretion and improving systemic inflammation by a effect on cytokines.

TYPES OF VITAMIN D

There are two main forms of vitamin D

- Vitamin D2 [Ergocalciferol] which is synthesized by plants and is not produced by the human body
- Vitamin D3 [Cholecalciferol] which is synthesized in skin when sunlight strikes bare skin it can also ingested from animal source.

FOODS THAT PROVIDE VITAMIN D INCLUDE

- Fatty fish, like tuna, mackerel, and salmon
- Foods fortified with vitamin d, some dairy product orange juice, soymilk, and cereals
- Beef liver
- Cheese, egg yolk

To get vitamin D from food, fish is a good option 3 ounces of cooked salmon has more than 450IU

VITAMIN D LEVELS

1. Optimal – 40-59ng/ml
2. Adequate – 30-39ng/ml
3. Therapeutic – 60- 100ng/ml
4. Excess - >100ng/ml
5. Deficient - < 30ng/ml

TREATMENT OF VITAMIN D DEFICIENCY

The person with vitamin D deficiency treatment may include oral ergocalciferol [vitamin D2] at 50,000IU once for eight weeks. After vitamin D is equivalent, 6000IU of [vitamin D3] daily to achieve a blood level of 25[OH]D above 30ng/ml followed by maintenance therapy of 1,500-2,000IU/day

The dose 600 IU/day for pregnant and lactating women.

CONCLUSION

Vitamin D status appears to play a role in the development and treatment of diabetes. It is possible that optimal levels of serum vitamin D may be different for people at risk for developing diabetes those with diabetes, and without diabetes. Both animal and human study support notion that adequate vitamin D supplementation may decrease the incidence of type 1 and possibly also of type 2 diabetes mellitus and may improve the metabolic control in the diabetes state.

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HERBAL PRODUCTS USED TO TREAT PSORIASIS WHICH IS THE MOST COMMON DISEASE IN THE WORLD SKIN DISEASE

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ABSTRACT

In-day-to-day life, because the pollution will increase there are a unit possibilities of increase of skin diseases. There are units many sorts of skin diseases like skin condition, eczema, herpes simplex, lupus, acne, and acne rosacea and carcinoma. Among all the illness disease of the skin Psoriasis is that the most typical skin disease in the world. By using steroidal drug min Psoriasis treatment there are many side effects like nasopharyngitis, upper respiratory tract infection, headache, fatigue, back pain, dizziness, pharyngolaryngeal pain, pruritus, skin rashes etc.

KEYWORDS: *skin condition, skin sensation illness, and sorts of skin eruptions.*

INTRODUCTION

PSORIASIS DEFINATION

Psoriasis could be a common skin condition that changes the life cycle of skin cells. This cause cells to make up chop-chop on the surface of the skin. The additional skin cells type thick, silvery scales and fidgety, dry, red Patches that area unit generally painful.

1. PLAQUE PSORIASIS

80%-90% of Patients area unit affected by Plaque skin condition⁴ in Plaque skin condition fast cell growth creates silvery scale, on the highest of the irregular formed spots of red. These silvery scales area unit found on scalp, knees, elbows and lower back that will crack and bleed⁴⁻⁵



TREATMENT

COUNTER TREATMENT

1. Hydroxyl acid
2. Pitch soaps
3. Moisturizers

2. INVERSE PSORIASIS

This type seems on creases and skin folds on the body, there's very little to no scales and is sleek, however still inflamed Patches of skin and red⁶⁻⁷ Heat, trauma, and infection area unit thought to play a job within the development of this atypical variety of skin condition⁸



TREATMENT:

Phototherapy

3. PUSTULAR PSORIASIS: A non-contagious, non-infection form of Pustules with inflammation. It will cover giant sections of the body or smaller regions.

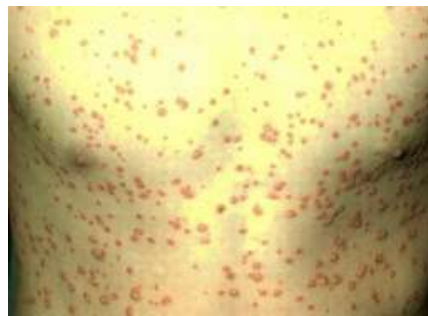
TREATMENT:

Topical treatment

Photo medical aid ⁸

4. GUTTATE PSORIASIS:

Guttate skin condition seems on the trunk and limbs of individuals below 30 ⁴ and looks like chicken Pox. These lesions often appear on the scalp or face or ears. ¹⁰⁻¹¹



TREATMENT:

Topical creams

Systemic medication treatment

5. ERYTHRODERMIC PSORIASIS

Erythrodermic skin condition is seen within the Patient WHO area unit suffering with chronic Plaque skin condition. ¹¹⁻¹² It is affecting most of the expanse of the body; wide unfold of redness on larger areas of the body with Pustules and with intense skin sensation and Pain. In line with the national skin condition foundation says "if someone is suffering with erythrodermic skin condition ought to consult doctor as a result of this type are often life threatening. ¹³⁻¹⁴ This illness might cause swelling, hot and cold flashes, symptom coronary failure.



TREATMENT

Topical steroid creams

Phototherapy

6. PSORIATIC ONCHODYSTROPHY :(NAIL DISEASE)

In this condition five hundredth skin condition cases finger nails area unit concerned and thirty fifth in toenails.¹⁸



This type of skin condition is sequencetic¹⁹ though it's not familiar that gene is concerned.

CAUSES OF PSORIASIS

The cause skin problem isn't totally understood, however variety of things are concerned that cause's skin problem.

1. Genetics
2. Lifestyle
3. HIV
4. Microbes
5. Diet

HERBAL PRODUCTS USED TO TREAT PSORIASIS

There may medicinal used to cure Psoriasis in Ayurveda system siddha system, etc. The medicinal Plants used to treat Psoriasis are anti-inflammatory agents, moisturising agents, immunosuppressant's etc. The medicinal agents used to treat Psoriasis are as a follow:

1. Flaxseed Oil

Flaxseed and flaxseed oil are rich source of essential fatty acid alpha-linolenic acid (ALA). In body ALA is converted into omega – 3 fatty acids²⁰⁻²¹ High amount of omega -3 fatty acids are Present in flaxseeds. Proximately 11.5 ml of omega -3 fatty acids are Present in the flaxseeds. Omega -3 fatty acids are polysaturated fats which reduce the alleviated inflammation from arthritis. As it is having anti-inflammatory property it will be helpful to cure Psoriasis²²

2. Aloe Vera gel

Aloe Vera gel is to keep the sin moisturizer and Pain reliever. As the dryness of skin is one the reason for the cause of Psoriasis alovera gel is used to keep the skin moisture and Prevent the skin dryness²³⁻²⁴ And scalps Present on skin is sometimes Painful it is used to reduce Pain. Alovera gel also makes the skin soothing and cooling²⁵⁻²⁶

3. Tea tree oil

Tea tree oil is used to treat many skin diseases. It is used to fight against acne, improve dry scalp, smooth skin irritation, and to treat Psoriasis caused by microorganisms. It is also having anti-inflammatory property. It Prevent the skin from dryness²⁷⁻²⁸

4. Banana Peel Powder

Banana Peel Powder is used as moisturizes skin naturally.²⁹⁻³⁰ As the Psoriasis Patient is having scalps on body it is used to improve collagen and elastin levels in the skin which causes skin whitening and Prevent spots Permanently³¹⁻³²

5. Turmeric Powder

Turmeric Powder is used to cure wounds. The antioxidants and anti-inflammatory qualities may help to treat Psoriasis by controlling flares and symptoms³³

6. Wrightiatinctoria oil

Shwetakutaja or WrightiaTinctoria when applied externally helps to heal stubborn wounds.³⁴ It stimulates the growth of granulation tissue and hastens the healing Process. Decoction and oil of this herb is useful in healing Psoriasis³⁴

7. Olemcocosnucifera:

It is used in many traditional medicines. It is used to treat many skin diseases. It act as moisturising agent for the skin. It is used to Prevent the skin from dryness³⁵

8. HydnocarPuswightiana

This oil is thought to Possess antibacterial properties and has been used for ages for treating various health conditions, including eczema, skin inflammations, sprains, arthritis and bruises³⁶

9. Gokshuradiguggulu

Gokshura is very popular in Ayurveda medicine to cleanse the skin from within. Young teenagers or even adults suffering from acne and breakouts can use Gokshura to remove it and Prevent it from occurring in the future³⁷ It also treats wounds, hives, itchiness, and skin inflammation and skin eruptions³⁷

10. Coal tar

Coal tar topical (for the skin) is used to treat the skin symptoms of Psoriasis, including dryness, redness, flaking, scaling, and itching. Coal tar is not a cure for Psoriasis, and it will Provide only temporary relief of skin symptoms³⁸

11. Black pepper

Pepper helps cure Vitiligo, a skin disease that causes certain areas of skin to lose Pigmentation and turn white³⁹ The piperine content of pepper can stimulate the skin to Produce Pigment, and topical treatment of piperine combined with ultraviolet light therapy is much better than harsher, more chemically-based treatments. It also reduces the chances of skin cancer due to excessive ultraviolet radiation.⁴⁰

12. Neem oil

There's nothing more annoying than a dry, itchy scalp. Combining neam oil with Powerful essential oils such as eucalyptus oil (an excellent anti-inflammatory), and Peppermint oil (to Provide soothing cooling relief) is a great way to nourish a dry scalp, curb itching and make your hair smell great⁴¹ Both eczema and Psoriasis are skin conditions that cannot be cured but only controlled. Being rich in Vitamin E, neem oil creates a thin layer to protect the skin and reduce moisture loss and being a natural Pain killer (analgesic), neem oil can Provide immediate relieve from discomforting skin irritation. This can reduce itching and scratching and actually Prevent further complications⁴²

13. Narayan oil

It is used to relive Pain from Psoriasis wounds.
It is mostly used to treat arthritic Psoriasis⁴²

14. Bavchi oil

Psoralea Oil (Bavchi Oil in India) is Processed from its seeds by steam distillation method⁴³ It is used to treat many Skin Diseases like Skin Rashes, Leucoderma, Psoriasis, Eczema, and much more. It Promotes Pigmentation and stimulates Melanin in the skin. It enhances the appearance of Skin colour, hair and nails by Promoting Blood Circulation⁴³

15. Vitamin D

It helps in regulating the absorption of calcium and Phosphorus, and facilitating normal immune system

function⁴⁴ Vitamin D is sometimes called the "sunshine vitamin" because it's Produced in your skin in response to sunlight.⁴⁵

16. Vitamin E

Vitamin e can aid in the treatment of various skin diseases or skin conditions. Topical applications such as those used to treat Psoriasis is one of the benefits of vitamin e^[45] orally ingested vitamin e can help treat erythema (a skin inflammation that results in reddish, Painful, and tender lumps)^[45]

CONCLUSION

From the higher than data I conclude there are a unit several plants area unit to treat skin problem and that we are a unit reaching to kindulate the dose form by victimisation all natural products without any side effects.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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IMPACT OF NANOPARTICLES ON PLANTS

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ABSTRACT

An increasing would like of applied science in varied industries might cause an enormous atmosphere dispersion of nanoparticles in returning years. A concern regarding nanoparticles interaction with flora and fauna is raised thanks to a growing load of it impact of nanoparticle on plant in relevance its size, concentration, and exposure methodology. This review, focuses on the role of nanoparticles in plant growth and development and also an plant mechanism. Hence, it is necessary to minimize nutrient losses in fertilization, and to increase the crop yield through the exploitation of new applications with the help of nanotechnology and nanomaterials. This review summarizes this understanding and also the future prospects of plant nanoparticle analysis.

KEYWORDS: *nanoparticles, plant growth and development, photosynthesis.*

Abbreviations: ISO- International standards of organisation, SiO₂- Silicon dioxide nanoparticles, AgNp – Silver nanoparticles, AuNp- Gold nanoparticles,

INTRODUCTION

According to ISO Technical Specification 80004, a nanoparticle is outlined as a nano-object with all 3 external dimensions within the nanoscale, whose longest and shortest axes don't disagree considerably, with a significant difference typically being factor of at least three.¹ Nanoparticles are classified as a material in which at least one dimension is less than 100nm in diameter.⁵ They are also classified based on morphology, physical and chemical properties. Some of them are silicon dioxide nanoparticle, metal nanoparticles, ceramic nanoparticles etc., Nanoparticles are not new to the environment and occur naturally in the form of minerals, clays, and products of bacteria. It has been used since ancient times as a colorant for metals but the systematic design and engineering of nanoparticles for various uses has started only in the last few decades that is now a days, pottery from the middle ages and Renaissance often retains a distinct gold-or copper-colored metallic glitter. This luster is caused by a metallic film that was applied to the transplanted surface of a glazing. The luster will still be visible if the film has resisted region oxidation and different weathering.²⁻³ Nanoparticles will exhibit size-related properties considerably completely different from those of either fine particles or bulk materials.⁴⁻⁶ The nanoparticles square measure created by the artisans by adding copper and silver salts and oxides in conjunction with vinegar, ochre, and clay on the surface of previously -glazed pottery. The object is then placed into a kiln and heated to about 6000C in a reducing atmosphere.⁶ Nanoparticles often possess unexpected physical and optical properties, as they are small enough to confine their electrons and manufacture quantum effects.⁷ Nanoparticles have unique physiochemical properties and the potential to boost the plant metabolism. According to Galbraith and Toreny et al engineered nanoparticles are able to enter into plant cells and leaves, and also can transport DNA and chemicals into plant cells. Engineered nanoparticles are designed to have the properties which are not present in bulk samples of the same materials.⁵ Engineered nanoparticles are composed of a variety of materials and occur in different sizes and shapes with a suite of synthetic surface molecules, which makes them distinct from naturally occurring materials.⁸ There are different nanoparticles, metal and metal oxide nanoparticles are manufactured by addition of reducing or oxidizing / precipitating agents during their synthesis, respectively.⁹ Several factors are responsible for nanoparticles reactivity with biomolecules which includes nanoparticles size, core composition, shape, surface properties, purity, stability, and method of manufacturing.¹⁰⁻¹¹

SYNTHESIS OF NANOPARTICLES

There are several methods for creating nanoparticles, They are;

- including gas condensation,
- attrition,
- chemical precipitation,
- ion implantation,
- pyrolysis and
- hydrothermal synthesis.
- Inert gas condensation is frequently used to produce metallic nanoparticles.

Silicon dioxide Nanoparticles

Silicon dioxide nanoparticles, additionally referred to as silicon oxide nanoparticles or nano-silica, are used in a wide range of medical specialty analysis thanks to their stability, low toxicity and ability to be functionalized with a range of molecules and polymers. Plant growth and development starts from the germination of seeds followed by root elongation and shoot emergence as the earliest signs of growth and development. Nano-silica particles are divided into P-type and S-type; the latter also has a comparatively smaller surface area. The lower concentrations of nano SiO₂ improved seed germination of tomato. According to Suriya _ Prabha et al. Nano SiO₂ hyperbolic seed germination by providing higher nutrients availability to maize seeds, and pH scale and conduction to the growing medium. Nano SiO₂ improved seedling growth and quality, including mean height, root collar diameter, main root length, and the number of lateral roots of seedlings and also induced the synthesis of chlorophyll. Under salinity stress, nano- SiO₂ improves leaf fresh and dry weight, chlorophyll content and proline accumulation. An increase within the accumulation of amino alkanolic acid, free amino acids, content of nutrients, antioxidant enzymes activity due to the nano SiO₂ thereby improving the tolerance of plants to abiotic stress. Inductively coupled plasma mass spectroscopy is one of the most reliable techniques for the detection of nanoparticles.

Impact of silicon dioxide nanoparticles

Various effects of silicon dioxide have been observed in plant yield, and biotic and abiotic resistance.²¹⁻²⁵ It acts as a physical mechanical barrier, and is placed on the walls of epidermis and vascular tissues of the stem, leaf sheath of most plants especially monocots.²¹ It also controls the physiological activities in plants. Nano-SiO₂ improved seedling growth and quality, including mean height, root collar diameter, main root length, and the number of lateral roots of seedlings and also induced the synthesis of chlorophyll. The lower concentration of nano-SiO₂ enhances seed germination of tomato (*Lycopersicum esculentum*). Mixture of nanoscale SiO₂ (nano-SiO₂) and TiO₂ (nano-TiO₂) could increase nitrate reductase in soyabean (*Glycine max*), enhance its abilities of absorbing and utilizing water and fertilizer, stimulate its antioxidant system, and apparently hasten its germination and growth.

Gold Nanoparticles

Gold may be a soft, malleable, transition metal with the electronic configuration painted by (Xe) and is one in all the smallest amount with chemicals active parts.¹⁹ whereas usually used as a valuable for jewellery, colloidal gold, unlike bulk gold, is considered to be highly reactive. Gold nanoparticles melt at much lower temperature (3000C for 2.5 nm size) than bulk gold (10640C)²⁰ Synthesis of gold nanoparticles has investigated utilizing Fusarium,¹⁸ Integrated gold nanoparticles with plant biological systems offer, and provide profit inspiration models and bio-assembled parts to be studied and applied. Genotoxicity and plant safety concerns can be improved with gold nanoparticles.⁸ Growth of leguminous plant seedlings in chloroaurate solution resulted within the accumulation of gold with the formation of stable gold nanoparticles in plant tissues. Gold nanoparticles ranged from 0.5 to 100nm in diameter have been reported inside the many plant tissues due to metal nanoparticles exposure. Few studies have been done on the interaction of gold nanoparticles with plants. Some researchers found AuNps induce toxicity in plants by inhibiting aquaporin function, a group of proteins that help in the transportation of wide range of molecules including water. AuNps improve the quantity of leaves, leaf area, plant height, pigment content, and sugar content that result in the higher crop yield. AuNps have a significant role on seed germination and

antioxidant system in *Arabidopsis thaliana* and altered levels of micro RNAs expression that regulates various morphological, physiological, and metabolic processes in plants.¹²⁻¹⁴

Silicon Nanoparticles

Mesoporous silicon nanoparticles have also been used to deliver DNA, proteins, and other chemicals in plants.¹⁵ Use in agriculture includes nanoparticles, such as, nanozeolites as well as the hydrogels which helps in the improvement of soil quality, and Nano sensors (for monitoring plant and soil health). Silica nanoparticles were observed to be non toxic to plant, but some authors observed the toxic effect due to decrease in PH of the media after addition of nanoparticles.

Silver Nanoparticles

A soft, white, lustrous transition metal, it exhibits the best electrical physical phenomenon, thermal physical phenomenon, and reflectivity of any metal. Synthesis of silver nanoparticles has been investigated utilizing many ubiquitous fungal species including *Trichoderma* which is a genus of fungi family Hypocreaceae, that is present in all soils.¹⁶⁻¹⁷ Among different nanoparticles, AgNps are fetching more attention because of their intensive uses in various products, which includes their uses as antimicrobial agents, shampoo, soap, toothpaste, fabrics, room sprays, waste water treatment, food packing materials, etc.,

Impact of silver nanoparticles

Based on the analysis of AgNPs accumulation in plant tissues of rice seed (*Oryza sativa*), it implied that the higher uptake was found when the seeds were treated with smaller AgNPs, 20nm diameter AgNPs, however it absolutely was cornered within the roots instead of transported to the leaves. These resulted within the less negative effects on phanerogam growth, when compared to the seed soaking with the larger AgNPs with 150nm diameter. The AgNps of 25 nm at high concentration was observed to break the cell wall and damage the vacuoles of root cells of *Oryza Sativa*, thus causing a toxic the root cells of *oryza sativa* when present in low concentration (up to 30 microgram / ml), AgNps increased plants growth profile (shoot and root length, leaf area) and biochemical attributes (chlorophyll, carbohydrate and protein contents, antioxidants enzymes) of *Brassica juncea*, common bean and corn. By this we can say, that the impact of AgNps on morphology and physiology of plants depends on the size and shape of nanoparticles. Syu et al (2014) studied the result of 3 completely different morphologies of AgNps on physiological and molecular response of *Arabidopsis* and instructed that decahedral AgNp's showed the very best degree of root growth promotion, however the spherical AgNps had no effect on RGP and triggered the highest levels of anthocyanin accumulation in *Arabidopsis* seedlings.

Role of nanoparticles in photosynthesis

We know that photosynthesis is a key process for plants on earth that changes light energy to chemical energy. Based on the scientific works performed, it is evident that most of the nanoparticles are toxic to the plants in high concentration. It is hypothesized that, for exhibiting the toxic effect the uptake of nanoparticles by plant and their translocation into different tissues is needed. Further, based on their transportation, properties, and reactivity, the nanoparticles may interfere with different metabolic activity to produce an impact on plants. Nanoparticles either boost up the photosynthesis process by improving LHC (Light Harvesting Complex) in plants or hinder their pathways by blocking ETC (Electron Transport Chain) and they affect photosynthetic rate by change in several genes and enzymes like Carbonic anhydrase, RUBISCO and PEP carboxylase. The entered nanoparticles can interfere with electron transport chain of mitochondria and chloroplast, which may result into the oxidative burst. A very high concentration of nanoparticles may affect the photosynthesis which may result in plant growth suppression or plant death.

CONCLUSION

In modern age, nanoparticles are used intensively and becoming a part of the human life. But due to the need of present and modern life, environment cannot be neglected. It is evident from the studies that metal and metal oxides nanoparticles in excess are harmful to plants, whereas present in traces it can be beneficial for plants. Silver nanoparticles interaction is very complex and by optimizing the silver nanoparticles concentration, plant growth promotion can be achieved without causing harm to the environment. The use of such nanomaterial's is more acceptable since they are safe for plants and cause less environmental pollution when used in trace amounts in comparison to conventional chemical pesticides. This review took out clear

information from known literature to show an influence of metal and metal oxide nanoparticle on the plant, but there are needs of research to understand the molecular mechanism of plant nanoparticle interaction. Nanoscience is attracting lot of research funding, some of which need to be diverted for the awareness of the people about the proper disposal of nanoparticle products. The research is also needed to be performed in the area.

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NEPHROPROTECTOR ACTIVITY OF ETHANOLIC EXTRACT OF PODS OFCANAVALIAGLADIATA

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ABSTRACT

Nephrotoxicity is a major limiting factor in cisplatin treatment. In the present study, hydro-ethanolic pod extract of *canavliagladiata* was investigated for its protective role in cisplatin induced nephrotoxicity. The experiment was designed for 15 days and healthy adult albino rats were divided into 5 groups. Group 1 received 1% carboxy methyl cellulose(CMC), in distilled water for 15 days, group 2 received cisplatin (6mg/kg b.w) on 5th day, group 3 received the low dose of *C.gladiata* (200mg/kg b. w) suspended in the vehicle for 15 days, group 4 received the high dose of *C.gladiata* (400mg/kg b. w) suspended in the vehicle for 15 days, group 5 received only *C.gladiata* suspended in the vehicle for 15 days, and animals belongs to group 3 and 4 were received cisplatin (6mg/kg b. w) on the day 5. At the end of the experiment urine samples and blood samples were collected from all the groups and were sacrificed to study renal functional parameters. Treatment with the *C.gladiata* pod extract significantly (0.05) attenuates renal damage by decreasing serum creatinine and blood urea nitrogen(BUN), enhanced the activities of catalase,GSH, LPO,UTP, CLcr, levels compared with cisplatin treatment group. Our results suggest that, pod extract of *C.gladiata* may ameliorate renal damage caused by cisplatin.

KEYWORDS: *Nephrotoxicity, cisplatin implication, Canavaliagladiata, anti-oxidant parameters.*

Abbreviations: CAT-catalase, CLcr-creatinine clearance, GSH- Glomerular filtration rate, LPO-lipid peroxidation, UTP- urinary total protein.

INTRODUCTION

NEPHROTOXICITY

Nephrotoxicity is the toxicity in the kidneys. It is a poisonous effect of some substance, both toxic chemicals and medications, on renal function. Nephrotoxicity is an intrinsic adverse effect of certain anti cancer drugs. Anticancer drugs have therapeutic effect but it may also produce nephrotoxicity. ^{1,2}Multiple risk factors exist in majority of patients with acute renal failure. glomerular filtration rate declined beyond the age of 50, may be because of vascular changes. ^{3,4}

CISPLATIN

Cisplatin is used in the treatment of several solid tumours unfortunately it is also one of the most toxic anti-cancer drug its dose limiting toxicity being nephrotoxicity.⁶ Cisplatin produces an acute, mainly proximal tubular functional impairment within hours administration.^{7,8}

CISPLATIN NEPHROTOXICITY

The most serious and usually dose limiting and usually dose limiting toxicity of cisplatin renal experimental cisplatin induced nephrotoxicity was first reported in 1971. Nephrotoxicity of cisplatin is associated with its metabolism^{9,10} Nephrotoxic effects of cisplatin has been well documented in all species studied including mice, rats,dogs,and humans. Acute tubular necrosis is a prominent feature of cisplatin nephrotoxicity and is clinically manifested by elevation of blood urea nitrogen (BUN) and serum creatinine (SC), proteinuria and hyperuricemia.¹¹

CANAVLIA GLADIATA

Canavalia gladiata is a vigorous, deep-rooted, and annual to perennial climbing plant. The stems, which are often slightly woody, can grow up to 10 meters long, scrambling over the ground or twining into other plants for support^{12,13,14}

ACTIVITIES OF CANAVLIA GLADIATA:

- ANTI MICROBIAL ACTIVITY
- HEPATO PROTECTOR ACTIVITY
- ANTI HELMENTIC ACTIVITY
- ANTI BACTERIAL ACTIVITY

MATERIALS AND METHODS

Preparation of extract

The hanging aerial pods were washed thoroughly with distilled water and shade-dried. Ethanolic extract of the dried roots of *canavalia gladiata* was prepared by soxhelt method using^{15,16,17}. The ethanolic extract thus obtained was dried under reduced pressure at room temperature not exceeding 40°C to get a yield of 90% from the crude extract.^(18,19)

CHEMICALS

All the chemicals used were of analytical grade, from SD, merk [INDIA] chemicals^{20,21}

ANIMALS

Healthy adult male albino rats [100-150mgbw] of wistar strain aged 60-90 days were used for the study. The rats were housed in polypropylene cages maintained at standard conditions. The animals had free access to standard lab chow and tap water²²

TREATMENT PROTOCOL

Animals were divided into 5 groups each group consisted of six rats [n=6]

- The animals of group-1 received 1% carboxy methyl cellulose [CMC] in distilled water for 10 days.
- the animals of group-2 received cisplatin [6mg/kg b.w.] on 5th day
- The animals of group-3 received the low dose of *canavliagladiata* [200mg/kg b.w.] suspended in the vehicle for 10 days.
- The animal of group-5 received only *canavliagladiata* suspended in the vehicle for 10 days.
- Animals which belong to group-3 and 4 were received cisplatin [6mg/kg b.w, i.p] on day 5. Group -1 received normal saline instead of cisplatin^{23,24}

BIO CHEMICAL DETERMINATION

On the day 9, urine was collected with the help of metabolic cages and the urine samples were subjected for estimation of urinary functional parameters. The animals were sacrificed by cervical decapitation and blood samples were collected by cardiac puncture and were used for estimation.^{25,26}

RESULTS

Table - 1
ESTIMATION OF SERUM PARAMETERS

S.NO	GROUP	BUN	SC
1.	Control	22.08±0.80	0.6±0.05
2.	Ccl4	42.05±1.10	2.25±0.20
3.	200mg of extract	27.01±0.10	1.90±0.10
4.	400mg of extract	29.05±0.05	1.14±0.05

Table - 2
ESTIMATION OF URINARY PARAMETERS:

S.NO	GROUP	UTP	CLcr
1	Control	07.6±0.15	18.80±1.6
2.	Ccl4	20.1±0.12	0.510±1.5
3.	200mg of extract	08.4±0.01	13.05±1.2
4.	400mg of extract	0.64±0.15	11.00±0.2

Table-3
ESTIMATION OF TISSUE HOMOGENATION PARAMETERS:

S.NO	GROUP	GSH	LOP	CAT
1.	Control	145.12±0.1	11.12±0.1	01.25±0.12
2.	Ccl4	72.15±2.3	15.12±0.1	08.10±0.57
3.	200mg of extract	85.42±2.7	12.12±0.5	12.05±1.3
4.	400mg of extract	90.10±1.90	12.0±0.0	14.0±1.00

*Each value represents mean±SEM from six animals in each group.
P<0.05 is significant when compared with normal group.*

DISCUSSION

Cisplatin is one of the most important anti-tumour agents and is highly effective against a diverse spectrum of malignancies. However, the use of this agent in combating cancer is limited by the development of nephrotoxicity along with various untoward side effects including nausea, vomiting, diarrhea and myelosuppression.^{33,34} The present study was focused on the effects of ethanolic extracts of pods of *Canavalia gladiata* on the renal damage induced by cisplatin. The precise mechanism of cisplatin induced nephrotoxicity has not been elucidated. The basic effect of cisplatin induced toxicity on the cellular membrane is believed to be peroxidation of membrane lipids. Cisplatin caused renal failure characterised by increase of serum marker levels, decline in the renal functional parameters indicated by increased the urinary protein excretion and decreased the CLcr. The nephroprotector activity in rats was further more concluded by viewing the results of the following parameters.

1. SERUM MARKERS

BUN: In the cisplatin induced rats the BUN levels are vigorously increased and when the extracts of *C. gladiata* were administered the level are lowered and has attained the normal level in case of (400 mg/kg) dose.

SC: In the cisplatin induced rats the SC levels are vigorously increased and when the extracts of *C.gladiata* was administered the levels are lowered significantly.

2.URINARY FUNCTIONAL PARAMETERS

UTP: In the cisplatin induced rats the UTP levels are vigorously increased and when the extracts of *C.gladiata* were administered the levels are significantly lowered.

CLcr: In the cisplatin induced rats are the CLcr levels are lowered down and when the extracts of *C.gladiata* were administered the levels are significantly increased.

3. TISSUE HOMOGENATE PARAMETERS/ESTIMATION OF ANTIOXIDANT ENZYME ACTIVITY

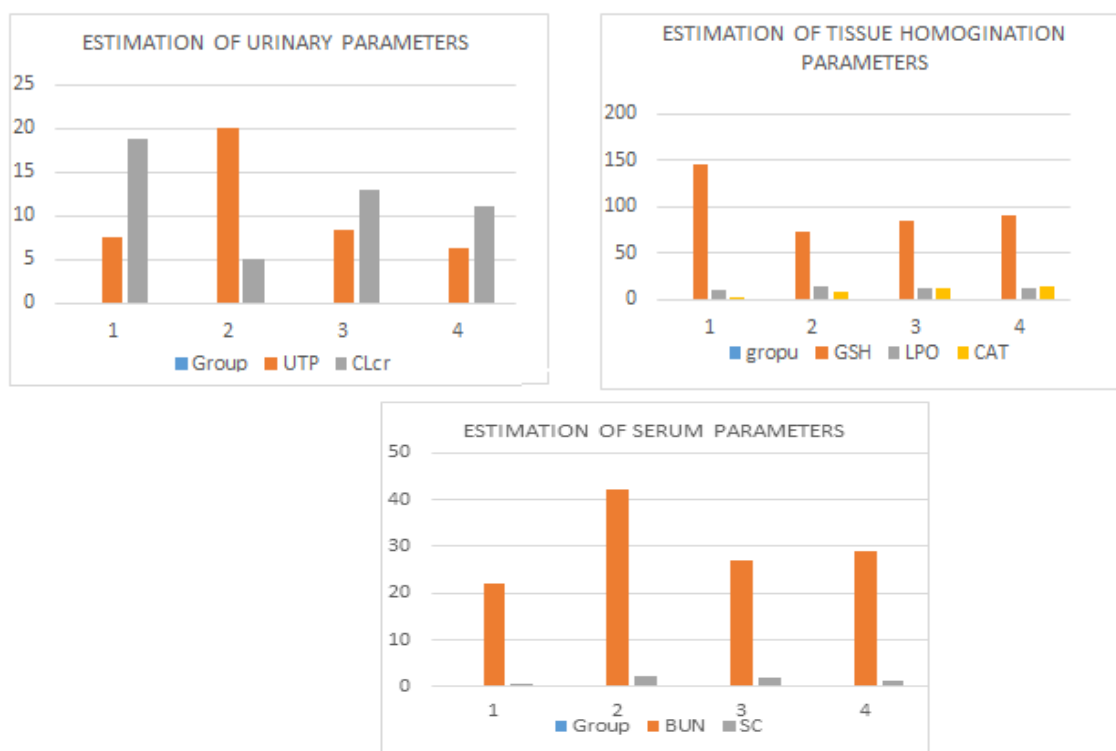
LPO: In the Cisplatin induced rats the LPO levels are increased than the normal level and when the extracts of *C.gladiata* were administered the levels are significantly decreased.

GSH: In cisplatin induced rats the GSH levels are decreased than the normal level and when the extracts of *C.gladiata* were administered the levels are significantly increased.

CAT: In the cisplatin induced rats the CAT levels are decreased than the normal level and when the extracts of *C.gladiata* were administered the levels are significantly increased.

Hence by viewing the above results we can conclude that, *C.gladiata* attenuates the nephrotoxicity of cisplatin in rats and confirm the antioxidant potential of *canavaliagladiata*.

Graphical Representation



CONCLUSION

When the high doses (400mg/kg) were administered to the rats, no toxicity was observed. So the ethanolic extract of pods of *C. gladiata* have no side effects and also it has the protective effect against cisplatin induced nephrotoxicity.

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ANTIBACTERIAL ACTIVITY OF SYZYGIUM CUMINI IN HERBAL TOOTHPASTE

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ABSTRACT

The objective of the present research work is to study the antibacterial activity of *Syzygium cumini* in herbal toothpaste. The herbal toothpaste is formulated by using natural ingredients and it is free from artificial ingredients like parabens, fluoride source and chlorine source. Formulations containing natural ingredients are more acceptable in belief that they are safer than synthetic drugs. The herbal toothpaste can satisfy all required conditions to keep the mouth fresh and prevent tooth decay by bacteria. In the present work *Syzygium cumini* powder is the active pharmaceutical ingredient. The antibacterial activity of *Syzygium cumini* is evaluated by performing the micro biological assay. The microbiological assay against *staphylococcus aureus* reveals that formulated herbal tooth paste exhibited notable antimicrobial activity. *Syzygium cumini* has antimicrobial property and also used as dental analgesic. Other ingredients used are clove it is a dental analgesic, soap nut powder acts as foaming detergent, stevia leaves are used as sweeteners, eucalyptus oil acts as flavouring agent and also acts as dental antimicrobial agent. Sodium benzoate is used as preservative. Calcium carbonate and chalk are used as abrasives and are responsible for whitening of teeth; microcrystalline cellulose is used as polymer. Titanium dioxide is used as opacifier. The results of the evaluating parameters show better results as compared to marketed formulations. Hence the lab made herbal toothpastes was found to be of good quality.

KEYWORDS: *Antibacterial, opacifier, Abrasive, Dental analgesic, Microbiological assay.*

INTRODUCTION

HISTORY OF TOOTHPASTE

In ¹ early 17th & 18th centuries, people made the tooth paste for their convenience to use. The first tooth powder was formulated by Chinese and Romans, mainly their formulation contained charcoal, ginseng, mint and salt as the common ingredients. However, they believed that toothpastes are useful to fix the loose teeth, strengthening the gums and whitening teeth as well as provide relief from toothache. A dentist named Dr. Washington Wentworth Sheffield probably invented first toothpaste around 1850.¹

DEFINITION

Tooth paste² is a paste or gel dentifrice, a semisolid dosage form used with toothbrush as an accessory to clean and maintain the aesthetics and health of teeth. Toothpaste is used to promote oral hygiene. It serves as an abrasive that aids in removing the dental plaque and food particles from the teeth, assists in suppressing halitosis, toothache and gum diseases. Most of the cleaning achieved by mechanical action of the toothbrush, and not by the toothpaste².

TYPES OF TOOTHPASTES

Toothpastes are mainly categorized by 2 types

1. Organic toothpaste
2. Herbal toothpaste

1. Organic Toothpaste

Organic toothpastes are the oral hygienic products containing organic ingredients which are meant for the removal of food materials, strengthening of the teeth as well as protection from gems.

APPLICATIONS

- Organic toothpastes are containing fluorine source that is useful in strengthening of teeth.
- Organic toothpastes are having good gums killing activity.
- Calcium source present in organic toothpaste gives marked good mouth freshening.

DISADVANTAGES

- Sodium lauryl sulfate present in organic toothpaste causes the drying of tissue present in mouth and ultimately responsible for mouth cancer.
- Fluoride source present in organic toothpaste is responsible for fluorosis and overdose leads to cancer, renal failure.

2. HERBAL TOOTHPASTE

Herbal ⁽³⁾ toothpastes are toothpastes containing mostly plant products for protecting the gums and strengthening the teeth without causing any irritate and harmful effects. Moreover herbal toothpastes are free from parabenes fluoride source, chloride source and sodium saccharin.

APPLICATIONS

- Herbal toothpastes are nontoxic and non irritant in nature.
- Herbal toothpastes reserve the perfect gem killing and dental analgesic properties.
- Moreover the herbal product donnot cause any sort of dangerous consequences ³.

METHODS FOR FORMULATION OF HERBAL TOOTHPASTE

While ⁴ formulating herbal toothpaste the following methods are recommended.

Method 1:-Mix humectants and polymer (MCC) for 1 minute in a mortar and pestle then add surfactant or detergent to the above mixture present in a mortar and mix the contents evenly. Add vehicle and preservative to the mixture and stir well. To this solution API (active pharmaceutical ingredient) are added and mixed uniformly. After 15 minutes trituration add sweetener and mixed well. Now abrasives are added to the above mixture during which the consistency of paste was first time observed. To the above mixture add flavouring agent and opacifier and mix well.

Method2:-Dissolve the preservatives in water at 72 °C in a beaker. Mix humectants and the polymer for 1 minute in a stainless steel bowl. Add solution S, mix for 2 minutes. Homogenize for 3 minutes. Transfer into GUEDE homogenizer at 20° C. Add sweeteners, API and the flavour, mix for 90s under vacuum. Add the surfactant or detergent, mix for 90s under vacuum.

Method3:-Mix binder or polymer, humectants and vehicle in a mortar and pestle. Here a liquid dispersion is formed where the binder is in slightly swollen state. To this mixture APIs are added and mixed for 2 minutes. Then abrasive and surfactant are added, uniform trituration was facilitated. Now remaining quantity of humectant are added followed by preservative. To this mixture sweetener was added and mixed well. Then flavouring agents, opacifier and remaining portion of vehicle were added and triturated uniformly ⁴. Of all these methods method 3 is best suitable for formulation of herbal toothpaste.

FORMULATION OF HERBAL TOOTHPASTE

Sl no	Ingredients	Category	M1	M2	M3
1	Syzygium cumini	API, Abrasive	5 ml	5 ml	5 ml
2	Clove oil	Dental analgesic	-	-	2 ml
3	Propylene glycol	Binder	3 ml	3 ml	3 ml
4	Calcium carbonate	Abrasive	2 ml	2 ml	2 ml
5	Chalk	Abrasive	5gm	5 gm	5 gm
6	Micro crystalline cellulose	Polymer	4 gm	-	-
7	Carboxy methyl cellulose	Polymer	-	4 gm	-

8	Methyl cellulose	Polymer	-	-	4 gm
9	Soap nut powder	Foaming detergent	3 gm	3 gm	3 gm
10	Stevia leaves	Sweetening agent	3 gm	3 gm	3 gm
11	Sodium benzoate	Preservative	0.1 gm	0.1 gm	0.1 gm
12	Titanium dioxide	Opacifier	0.6 gm	0.6 gm	0.6 gm
13	Glycerine	Moisturiser	4 gm	4 gm	4 gm
14	Eucalyptus oil	Flavor	3 drops	3 drops	3 drops
15	Purified water	Vehicle	q.s	q.s	q.s

MICROBIOLOGICAL ASSAY

Take ⁵ the petridish containing medium and 3 wells were made using the sterile metallic template, with rubber teat in each plate. The inoculums were prepared and adjusted to 0.5 McFarland turbidity standards guidelines. The agar plates were streaked with respective stock culture micro organisms. Using the sterile inoculating needle, the toothpastes were dispersed into wells. At the point, the plates were incubated at 37⁰ C for 48 hours in respective environments. That is, E.coli and staphylococcus aureus are inoculated in the medium. After incubation, zones of inhibition were examined around the wells that contain the dentifrice. These appeared as a clear, circular hallow surrounding the wells. Diameters of the zones were measured with the scale. The mean diameter of the well measurements represented the inhibition value of tested product. The zone of inhibition of toothpaste is found to be 6 mm in diameter⁵.

EVALUATION OF HERBAL TOOTHPASTE

Evaluation ⁶ of toothpaste was done according to "Bureau of Indian standards". These tests include

DETERMINATION OF HARD AND SHARP EDGED ABRASIVE PARTICLES

The paste was extruded about 15-20 cm length from collapsible tube of each sample on a butter paper. Then all the samples were tested by pressing it entire length by finger for the presence of hard and sharp edged abrasive particles for all samples.

DETERMINATION OF SPREADABILITY

About 1 gm of each sample was weighed and placed at the center of glass plate and another glass plate 2 kg weight was placed at the center of the plate avoid sliding of the plate. The diameter of paste in cm was measured, after 30 minutes, for all samples. The experiment was repeated 3 times and the averages were repeated for all samples.

DETERMINATION OF FINENESS

10 gm of each sample was accurately weighed and placed in a 100 ml beaker. To this 50 ml water is added and allowed to stand for 30 minutes with occasional stirring. This solution was passed through 150 micron Indian standard sieve. Then the sieve was washed with running tap water. After washing the residue remains on sieve were collected and dried in an oven at 105⁰ C.

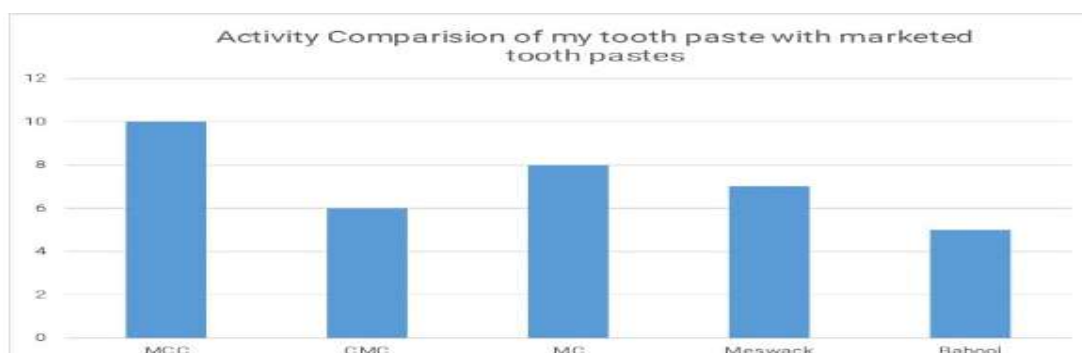
DETERMINATION OF FOAMING POWER

About 5 gm of each sample was weighed in a 100ml glass beaker. To this 10 ml water was added and allows standing for 30 minutes. The contents of beaker were stirred and transferred into 250 ml graduated measuring cylinder. The content of the cylinder was adjusted to 250ml by adding sufficient water & maintained at 30⁰C. Stir the contents of cylinder with glass rod to ensure uniform suspension. As soon as temperature of content reached 30⁰C the cylinder was stoppered and 12 complete shakes were given to it. The cylinder was noted for all samples were allowed to stand for 5 minutes and volume of foam with water⁶

RESULTS AND DISCUSSION

Sl no	Evaluation	F1	F2	F3	Meswac	Babool	Standard values
1	Hard and sharp edged abrasive particles	Absent	Absent	Absent	Absent	Absent	Absent
2	Spreadability(cm)	5.4	5.7	6.2	5.9	5.7	Max 8.5

3	Fineness (%)	0.42	0.38	0.39	0.40	0.42	Max 0.5
4	Foam formation(ml)	54	52	57	54	55	55min 50 ml



CONCLUSION

Herbal products are having great eminence and elegance fortifying in our daily healthy life. Because of their enormous engage of herbal products in our daily life, the current trend espouses towards herbal products. From the micro biological essay it is seen that the toothpaste can easily inhibit the growth of bacteria. Hence it shows good anti bacterial activity when compared to marketed preparations. PH test shows that our product is within neutral range. The herbal toothpaste is successfully whitens the teeth, kill gems, imparts freshness feel inside the mouth.

ACKNOWLEDGEMENT

I have prepared this presentation with the help of many articles and websites. I pay my sincere gratitude to principal sir and my guide of Nirmala College of pharmacy for giving me this opportunity.

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FORMULATION AND FINDING THE PIROXICAM MICROEMULSION BY TERNARY PHASE DIAGRAM

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ABSTRACT

The current study deals with the construction of ternary phase diagram by using ProSim software. The ultimate aim is to find the suitable region of microemulsion by considering the mixture of surfactant, co-surfactant and oil phase. Depending on the highest solubility the surfactant (SLS), co-surfactant (PG 5%) were selected and different ratios of surfactant and co-surfactant mixtures were prepared. The different ratios like (S: CS) 1:0, 1:1, 1:2, 1:3, 2:1, 3:1 are prepared. Pseudo ternary phase diagrams were constructed using the software ProSim ternary diagram. The amount of S, CS mixture, oil and weight of water added were entered into the software ProSim ternary diagram. As the range lies between 0-1 the data entered must be normalized. Directly from the software we can convert the values to normalized values. After that from the normalized values of S, CS mixture, oil, water within the range 0-1 total six pseudo ternary phase diagrams were constructed which contains the 3 phases as S, CS mixture, oil, and water. The region was found and a trial batch of 100 ml was prepared and stability study was conducted.

KEYWORDS: Ternary, microemulsion, surfactant, oil phase, Pseudo ternary.

INTRODUCTION

In pharmaceutical world, there has been a huge demand on microemulsion, improves the bioavailability, enhancement of permeability. Microemulsions are mixtures of oil, surfactant and water that form spontaneously on bringing the three components together¹. Microemulsions are thermodynamically stable, and their formation is facilitated by the ultralow interfacial tension of these systems, which leads to the formation of extremely small droplets of the dispersed phase. These microemulsions can take different forms which include, among others, oil, water, and oil/water bicontinuous microemulsions^{2, 3}. There are three kinds of microemulsions: oil dispersed in water (o/w), water dispersed in oil (w/o), and bicontinuous. The presence of o/w droplets is likely to be a characteristic of microemulsions where the amount of oil is low. In contrast, the existence of w/o droplets is a characteristic of microemulsions where the water fraction is low. Droplet diameter varies in the range of 10–140 nm⁴. Ternary phase diagram is an optimization technique⁵, which led to find the possible region of microemulsion without being carried out a large number of experiments.

MATERIAL AND METHODS

The pure drug (piroxicam) was received as gift sample. Sodium lauryl sulphate (SLS), Tween 80, Span 60 and co-surfactants like Polyethylene glycol, Propylene glycol and oils like coconut oil, oleic acid purchased from loba chemical, Chennai. All the chemicals are of high analytical grade.

Calibration curve

Initially the drug (10mg) was taken and dissolved in the particular solvent (10ml) and shaken well which gives 1000µg/ml (stock solution). From 1000µg/ml solution 1ml was taken and volume was made up to 10ml with the particular solvent which gives 100µg/ml solution (working standard solution). And the 100µg/ml solution was scanned in UV visible spectrophotometer and the $\lambda_{\max}$ was observed for the drug in particular solvent. Then the serial dilutions were done and checked for the absorbance at the particular $\lambda_{\max}$ in UV visible spectrophotometer. Like this the calibration curve were done for all the solvents {water, SLS,

Tween80 (10%, 5%), Span 60 (0.25%, 0.5%), Polyethylene glycol (PEG5%, 10%), Propylene glycol (PG 5%, 10%), coconut oil, oleic acid}

Solubility determination

The drug (Piroxicam) solubility was checked in different solvents such as surfactants like Sodium lauryl sulphate (SLS), Tween 80(5%, 10%), Span 60 (0.25%, 0.5%) and co-surfactants like Polyethylene glycol (PEG5%, 10%), Propylene glycol (PG 5%, 10%) and oils like coconut oil, oleic acid. The drug solubility was also checked in distilled water. The drug was added to the solvent in excess amounts with continuous shaking in order to get solubilised or dissolved in the particular solvent. The drug was added in excess amounts until the super saturation was attained. And the drug which is dissolved in the solvent was shaken in an orbital shaker for about 24 hours. And after that the solution was kept aside for some time and the supernatant liquid was collected and checked for the absorbance in UV Visible Spectrophotometer at the particular wavelength depending upon the drug in particular solvent. When absorbance was not present within the range then dilutions were done and then absorbance was checked. And from the observed absorbance value and standard absorbance and standard concentration from the calibration curve the solubility was calculated for the drug in particular solvent. The drug solubility was checked in all the solvents i.e., in SLS, Tween 80, Span60, PEG, PG, Coconut oil, Oleic acid ^{6, 7}. Depending upon the highest solubility the Surfactant (SLS), Co- surfactant (PG5%), Oil (Coconut oil) phases were selected for the preparation of emulsions.

RESULTS AND DISCUSSION

Depending on the highest solubility the surfactant (SLS), co-surfactant (PG5 %) were selected and different ratios of surfactant and co-surfactant mixtures were prepared 8. The different ratios like (S: CS) 1:0, 1:1, 1:2, 1:3, 2:1, 3:1 are prepared. For 1:0 one part of surfactant and zero part of co-surfactant were taken in a beaker and mixed well by placing them on magnetic stirrer at low speed without foam formation. Like this all 6 different S, CS mixtures were prepared.

Table 1
Preparation of surfactant and co-surfactant mixture (S, CS)

SURFACTANT: CO-SURFACTANT	
1:0	
1:1	
1:2	
1:3	
2:1	
3:1	
SURFACTANT	CO-SURFACTANT
1	0
1	1
1	2
1	3
2	1
3	1

Mixing of S, CS mixture with oil phase (S, CS: oil):

Each S, CS mixture were taken and 15 different ratios (S, CS: Oil) were prepared. 1:0 S, CS mixture was taken and 15 different ratios(S, CS: Oil) like 15:0 (15 parts S, CS mixture and zero part of oil), 14:1 (14 parts S, CS mixture and 1 part oil), 13:2 (13 parts S, CS mixture and 2 parts oil), 12:3 (12 parts S, CS mixture and 3 parts oil), 11:4(11 parts S, CS mixture and 4 parts oil), 10:5 (10 parts S, CS mixture and 5 parts oil), 9:6 (9 parts S, CS mixture and 6 parts oil), 8:7 (8 parts S, CS mixture and 7 parts oil), 7:8 (7 parts S, CS mixture and 8 parts oil), 6:9 (6 parts S, CS mixture and 9 parts oil), 5:10 (5 parts S, CS mixture and 10 parts oil), 4:11 (4 parts S, CS mixture and 11 parts oil), 3:12 (3 parts S, CS mixture and 12 parts oil), 2:13 (2 parts S, CS mixture and 13 parts oil), 1:14(1 part S, CS mixture and 14 parts oil) were mixed. Like this

remaining S, CS mixtures were taken and for each mixture 15 different ratios were prepared. A total of 90 different ratios were prepared.

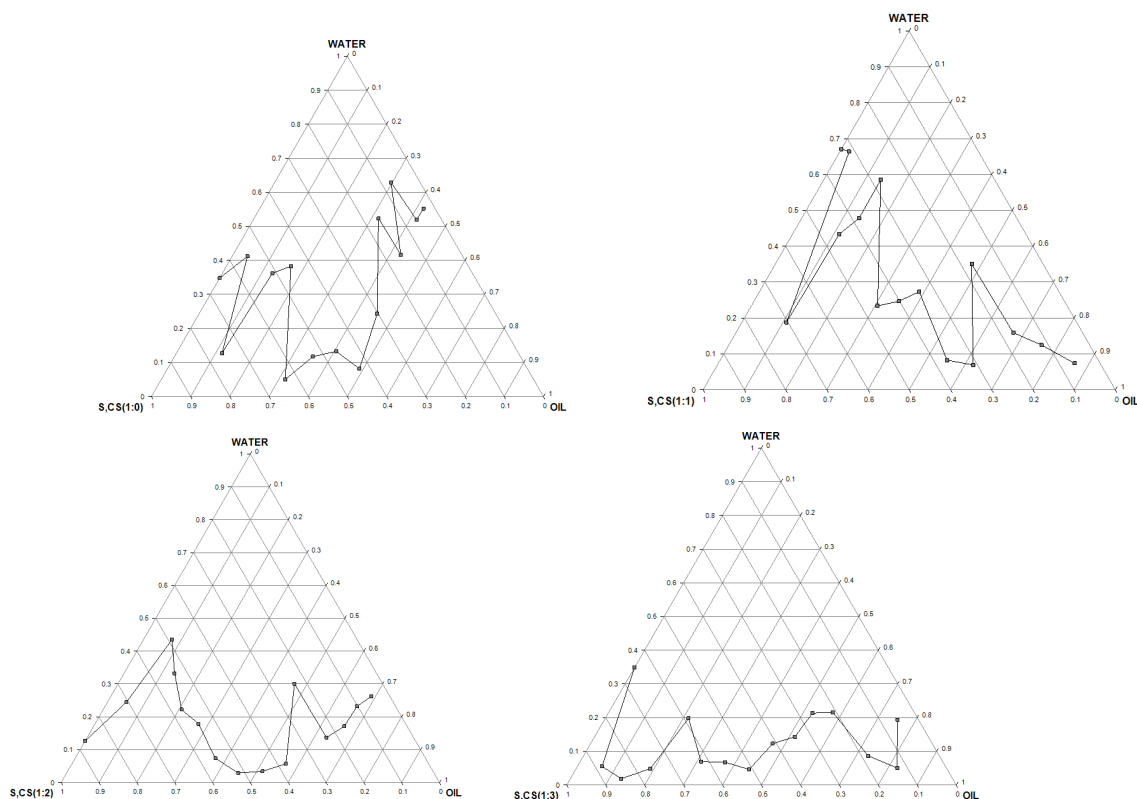
Preparation of emulsion by water titration method

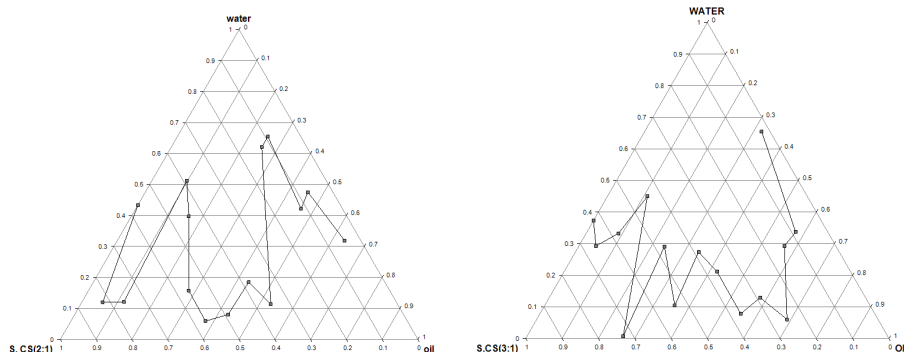
After mixing the S, CS mixture with the oil phase each ratio was taken and 10mg of drug was added. Initially the beaker containing S, CS mixture, oil, drug was weighed on a weighing balance. The initial weight was noted. Then that beaker containing 3 phases along with drug was kept on a magnetic stirrer at high speed and water was added drop by drop to that mixture. Water was added until the mixture becomes translucent that is the point where emulsion forms and the mixture becomes milky white. The final weight of that beaker was noted and time taken for the formation of emulsion was noted. The difference between the final weight and initial weight gives the weight of water added to form the emulsion⁸. Like this all different ratios of emulsions were prepared by water titration method. A total of 90 emulsions were prepared. All the data like different ratios of S, CS mixture, oil and weight of water added was tabulated.

Construction of pseudo ternary phase diagrams

Pseudo ternary phase diagrams were constructed using the software ProSim ternary diagram. The amount of S, CS mixture, oil and weight of water added were entered into the software ProSim ternary diagram. As the range lies between 0-1 the data entered must be normalized^{9, 10}. Directly from the software we can convert the values to normalized values. After that from the normalized values of S, CS mixture, oil, water within the range 0-1 the pseudo ternary phase diagrams were constructed which contains the 3 phases as S, CS mixture, oil, and water. 6 different pseudo ternary phase diagrams were constructed¹¹.

Figure 1
Ternary phase diagram by considering suitable concentration of ingredients





On successful finding the region the microemulsion of piroxicam for 100 ml was prepared and tested for stability study.

CONCLUSION

The study concluded that, with effective implementation of ternary phase diagram by ProSim software the microemulsion region plotted. Also the percentage of S, CS, S-CS mixture and oil effectively defined the region also studied. The study also claims with a limited number of experiments, the ideal percentage of above mentioned can be found. Moreover, a trial batch was also prepared and passed the stability study.

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ASSESSMENT OF PRESCRIPTION PATTERN AND MEDICATION ADHERENCE IN GERIATRIC PATIENTS AT A TERTIARY CARE HOSPITAL: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

In the present days, India has the elderly population which accounts for 7.4% of total population; it is projected to rise to 12.4% of the population by the year 2026. India occupies the third place after the China and USA in terms of elderly population. To identify the potentially inappropriate medications (PIMs) using Beers criteria 2019 & to assess the medication adherence in geriatrics and to categorize according to their adherence. The current study is a prospective observational study in which a total number of 150 geriatrics who were aged 55 years and above were included in the study. Data was collected and recorded in data collection forms after obtaining the consent from the patient. Among the 150 patients males were 89 (59.33%) and females were 61(40.66). A total number of 22 inappropriate medications were found fewer than 5 categories of Beers criteria 2019. Majority of the patients showed lower adherence (36%) in which male patients showed predominance in low adherence. The present study showed the prevalence of disease pattern, medication use, polypharmacy, potentially inappropriate medications and medication adherence. The report of the study showed increased use of potential medications and low adherence was seen in about 54 in which males are predominant.

KEY WORDS: *Medication adherence, PIMs, polypharmacy, AGS Beers criteria, MMAS-8 item scale.*

1. INTRODUCTION

In the present days, India has the elderly population which accounts for 7.4% of total population; it is projected to rise to 12.4% of the population by the year 2026. India occupies the third place after the China and USA in terms of elderly population. We commonly observe the geriatrics suffering with a number of co-morbidities and they are also hospitalized for several times.¹ This population also shows physiological changes ascribed to aging and this may influence on the pharmacokinetics and pharmacodynamics of drugs increasing the risk for the drug therapy.⁴ With aging, there are changes in absorption, metabolism, distribution and excretion, some changes are clinically more relevant.¹⁴ By considering all the above factors there is an increased chance for the occurrence of polypharmacy and drug-related issues, which needs to be addressed.¹ The American Geriatric society (AGS) Beers criteria for potentially inappropriate medication (PIM) is intended for the use in the older adults in all ambulatory, acute and institutionalized settings of care except for the hospice and palliative care settings. It is released in 1997 and updated in 2002, 2012, 2015 and 2019. According to BEERS criteria, medications which are prescribed inappropriately are classified into the following categories.¹⁴

- Category - 1: Medications which are considered to be potentially inappropriate in older adults
- Category - 2: Medications which may exacerbate existing disease or syndrome
- Category – 3: Medications to be used with caution in older adults.
- Category - 4: Medications combination that may lead to harmful interactions.
- Category - 5: Medications that should be avoided or have their dosage adjusted for those with Decreased kidney function.

Medication adherence usually refers to whether patients taking their medications as prescribed by the physician, as well as whether they continue to take the prescribed medication. Medication non adherence is a growing concern to clinicians and health care systems.¹ Poor compliance among older persons is a public health concern, as it accounts for adverse outcomes, medication wastage with increased cost of healthcare, and substantial worsening of the disease with increased disability or death.² Because of the mounting evidence that it is prevalent and associated with adverse outcomes and higher costs of care.¹ Hence our present study was undertaken to assess the prescription pattern and medication adherence in geriatrics.

2. METHODOLOGY

2.1. STUDY DESIGN

A prospective observational study, involving the human participants according to the standards of the Nirmala college Institutional review board (PDD12/IRB/NRML/19-20) who are aged 55 and above 55 years over a period of 6 months was conducted from June 2019 to November 2019 in Manipal hospital, Tadeballi. All the studies were done according to the ICMR guidelines.

2.1.1. Sample size & sample selection

150 patients have been enrolled in the study during the study period. Patients of either gender aged above 55 years and who appear for the consultation or admission in the department of Cardiology, Neurology, Nephrology, Orthopedics and General medicine were included in the study.

2.2. STUDY POPULATION

2.2.1. Inclusion criteria:

- Patients who are aged 55 years and above 55 years.
- Patients of either gender admitted to the respective study site during the study duration.
- Patients having co morbid conditions and previous medication history.
- Patients who are willing to participate in the study.

2.2.2. Exclusion Criteria

- Patients who are not willing to participate.
- Terminally ill patients requiring ICU admission.
- Mentally unstable patients.

2.3. STUDY PLAN

During the study period, 150 geriatric patients were identified based upon the inclusion and exclusion criteria. Informed consent was obtained from the patients who are willing to participate in the study. They were assessed for medication adherence using Morisky 8 item medication adherence questionnaire (MMAS-8) and PIM's were identified using American Geriatric Society updated Beers Criteria 2019.

2.4. STATISTICAL ANALYSIS

Based on the patient demographic details, major diagnosis observed in the study population, no. of medications per prescription, category of drugs prescribed, patients with co morbid conditions and previous medication history were statistically analyzed and evaluated by using Beers criteria 2019, errors identified in prescriptions, categorization of medications according to Beers criteria, common categories of PIMs in geriatrics, safer alternatives in geriatrics. Evaluation of medication adherence was done by using MMAS-8 questionnaire and sex distribution in low medication adherence was done.

3. RESULTS AND DISCUSSION

Our study was carried out at Manipal hospitals for about 6 months period from June 2019 to November 2019. Within the study duration 150 patients were included in our study and they were evaluated and analyzed for

the identification of potentially inappropriate medications by using Beers criteria 2019 and medication adherence among those patients was analyzed by using MMAS-8 questionnaire and scoring was given accordingly. Among the 150 patients males were 89(59.33%) and females were 61(40.66) and majority of the patients were under the age group of 65-70 years (33.33%), followed by 55-60 years and 60-65 years (21.33%), then 70-75 years (14.66%) and the least number of people were found in ≥ 75 yrs (9.33%). According to category of disease diagnosed the majority diagnosis are under category of Hematological diseases (28%) followed by miscellaneous diseases (17.33%), C.V.S and infectious diseases (16%), Musculoskeletal and G.I diseases (12.66%) and least diagnosis of Renal diseases (7.33%). Based on the duration of stay in hospital majority patients stayed up to 0-5 days (72%), followed by 5-10 days (27.33%), 10-15 days (0.66%) and no patient stayed ≥ 15 days, as shown in table-3.1. A total number of 215 medications were prescribed under different categories. Out of these the commonly prescribed medications were anti infective medications (22.7%), Cardiovascular and C.N.S medications (11.62%), analgesics and anti inflammatory medications (9.3%), Anti diabetic medications (6.04%), G.I medications (6.04%), Renal medications (6.04%), respiratory medications (5.11%), medications acting on hematological system (4.65%), anti epileptic medications (3.25%), as shown in Figure-3.1. According to Beers criteria 2019 we have found about 12 PIMs under the category-1, 3 inappropriate medications under the category-2, 5 medications under category-3, 2 potential drug interactions under category-4 and no medications were found in category-5, as shown in Figure-3.3. We have provided the alternate medications for the inappropriate drugs in geriatrics identified in our study, as shown in Table-3.4. Among 150 patients high medication adherence was found in 51(34%) persons, medium adherence in 45(30%) persons and low adherence was found in 54(36%) in which 32(59.25%) were males and 22(40.74%) were females, as shown in Table-3.5.

3.1. Demographic characteristics of enrolled patients

Table-3.1: Demographic characteristics of enrolled patient

Characteristics		Data
1. No. of patients	Male	89 (59.33%)
	Female	61 (40.66 %)
2. Age in years	55 – 60 yrs	32 (21.33%)
	60 –65 yrs	32 (21.33%)
	65 – 70 yrs	50 (33.33%)
	70 – 75 yrs	22 (14.66%)
	≥ 75 yrs	14 (9.33%)
3. Category of disease Diagnosed	Respiratory system diseases	11(7.33%)
	C.V.S diseases	24 (16%)
	Musculoskeletal diseases	19 (12.66%)
	Gastro intestinal disease	19 (12.66%)
	Infectious diseases	24 (16%)
	C.N.S diseases	12(8%)
	Blood diseases	4 2 (28%)
	Renal diseases	11 (7.33%)
	Miscellaneous diseases	26 (17.33%)
4. Duration of stay	0 – 5days	108(72%)
	5 – 10 days	41 (27.33%)
	10 – 15 days	1 (0.66%)
	≥ 15 days	-

3.1. Category of drugs prescribed in each category

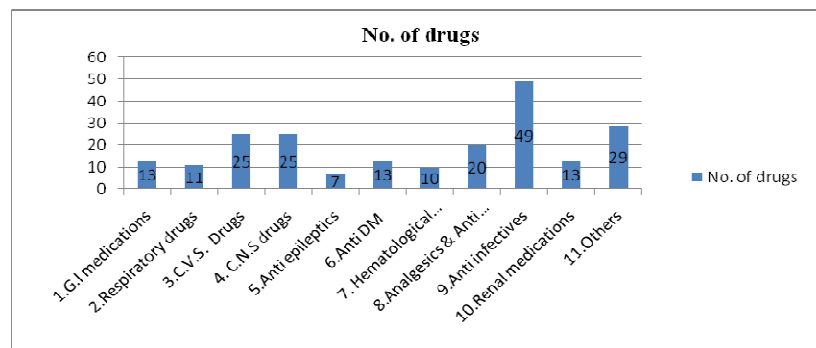


Figure-3.1: No. of drugs in each Category

3.3. Categorization of PIMs according to Beers criteria

Category - 1	Category - 2	Category - 3	Category - 4	Category - 5
1. Alprazolam 2. Zolpidem 3. Nefedipine 4. Prazocin 5. Dimenhydrinate 6. Digoxin 7. Amiodarone 8. Amitriptylline 9. Diazepam 10. Diclofenac 11. Ibuprofen 12. Keterolac	1. Alprazolam 2. Pioglitazone 3. Ranitidine	1. Dabigatran 2. Aspirin 3. Torsemide 4. Furosemide 5. Oxcarbazepine	1. Theophylline + Ciprofloxacin 2. Morphine + Keterolac	Nil

Tabel-3.3: Categorization of PIMs according to Beers criteria

3.4: Alternatives for PIMs in geriatrics

Medications	Indication	Rationale	Alternatives
1. Alprazolam, Zolpidem	Insomnia	Increased sensitivity to Benzodiazepines, increased risk of cognitive impairment, delirium, accidents and fractures.	Tab. Ramelteon – 8mg can be taken 1hr. before going to bed
2. Diclofenc, Ibuprofen, Keterolac	To treat moderate to severe pain.	Increases the risk of G.I bleeding and peptic ulcer disease.	Avoid chronic use. They can be used along with gastro protectants like proton pump inhibitors.
3. Amiodarone	Atrial fibrillation and to maintain sinus rhythm.	It has greater toxicities compared to other anti arrhythmics.	Tab. Metoprolol-25mg-O.D can be given.
4. Digoxin	Heart failure and Atrial fibrillation	Higher doses may cause toxicity.	Dose should not exceed >0.125mg/day
5. Amitriptylline	Pain	Causes hypotension and highly anticholinergic effects(sedation)	Nortriptylline – 30mg to 50mg per day in divided dose.

6.Prazocin	Hypertension	High risk of orthostatic hypertension	Calcium channel blockers(Amlodipine),ARBs(Telmisartan) can be used
7. Dimenhydrinate	To treat nausea & vomiting.	Causes high anticholinergic effects like sedation, confusion, dizziness e.t.c.	Tab.Ondansetron- 4mg-TID can be used.
8. Nefidipine	Hypertension	Risk of precipitating myocardial ischemia.	Calcium channel blockers or ARBs can be used instead.
9.Diazepam	To treat cardiac muscle spasms.	Higher anticholinergic effects and increased sensitivity to benzodiazepines.	Tab.Clonidine – 0.1 to 0.2mg/day Can be used.
10.Theophylline+Ciprofloxacin	To treat insomnia and treat bacterial infections	Increase risk of Theophylline toxicity.	Albuterol along with other beta-2 agonist can be used instead of Theophylline.
11.Morphine + Keterolac	To treat moderate to severe pain.	Increased risk of G.I bleeding and peptic ulcer disease.	Avoid the combination or use them along with G.I protection.
12. Torsemide, Furosemide and Oxcarbamazepine	Edema and seizures.	May exacerbate or cause SIADH or hyponatremia.	Monitor the sodium levels closely when starting or changing the doses.
13. Aspirin, Dabigatran	Prevention for cardiac diseases.	Risk of major bleeding increases	Use with caution in patients with age >70yrs.

Table-3.4: Alternative medications for PIMs in geriatrics**3.5. Categories of Medication adherence based on MMAS-8 questionnaire & scores:**

S. no.	Categories of Adherence	No. of patients	Gender distribution	
			Male	Female
1.	High adherence	51(34%)	29 (64.4%)	22(43.13%)
2.	Medium adherence	45 (30%)	29 (64.4%)	16 (35.5%)
3.	Low adherence	54 (36%)	32 (59.25%)	22 (40.74%)

Table-3.5: Categorization of medication adherence based on MMAS-8 questionnaire & scores**4. CONCLUSION**

The current study report shows that the prevalence of potentially inappropriate medications is steadily increasing based on Beers criteria 2019. Before dispensing the medicine to geriatrics, the pharmacist should assess the appropriateness of prescription so that the quality and efficacy of the medical care given to geriatrics can increase. As number of drugs to take increases, patients may be overwhelmed and psychologically burdened. Hence, as a professional, educate patients about ways to recognize and cope with them, enabling their confidence. This study has shown about 54 patients having low adherence among 150 geriatrics by calculating the score for MMAS-8 questionnaire.

5 ACKNOWLEDGEMENT

The authors are extremely obliged to the co-operation of superintendent and other staff of Manipal hospitals for granting permission to carry out this project at their hospital. We also acknowledge our gratitude to the guide and our institution Nirmala College of pharmacy for providing support and guidance to carry out our work.

6. CONFLICT OF INTEREST

Our work has been carried out in a peaceful manner without any problems. The authors do not have any conflict of interest.

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ASSESSMENT OF RATIONAL USAGE OF PROTON PUMP INHIBITORS IN A TERTIARY CARE REFERRAL HOSPITAL: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Proton pump inhibitors are among the most widely recognized classes of drugs prescribed. Proton pump inhibitors signify a progressive advancement in gastroenterology. These medicines irreversibly inhibit the gastric H⁺/k⁺ ATPase pump and reduction both basal and stimulated gastric acid creation. The study aimed to assess the rational usage of proton pump inhibitors & to evaluate the rationality of PPI use. The study was a prospective observational study which aims to assess the rational usage of PPI in Gastroenterology department. The study was conducted over a period of 6 months. The sample size is 204 patients. Most of the subjects belong to the age group more than 60 years (38.24%) followed by 20-30 (22.55%) 31-40, 51-60 (11.76) and 41 – 50 (10.78%). Age group above 60 years was predominant due to increased number of medications in geriatrics due to multiple disorders. The current study suggests that the risk of PPI may extend beyond previously studied high risk individuals. Although the proton pump inhibitors are very safe and effective drugs to reduce the acid secretion but they should use with a documented evidence of GI disorders that fails to respond for H₂ receptor antagonists. The study finally concludes that there should be the combined efforts of pharmacist and the medical profession to work together for rational use of PPI through interventions or institutional guidelines for use may be developed and implemented to help decrease the use of PPIs in the inpatient population.

KEY WORDS: Proton pumps inhibitors, gastro intestinal, rational usage.

1. INTRODUCTION

Rational use of drugs has been defined by the World Health Organization WHO in 1985 as: "Rational use of medicine requires that patients get medications appropriate to their clinical needs, in doses that meet their own individual requirements for an adequate period of time, and at the lowest cost to them and their community."^{1,2} This implies that, irrational use of drugs includes all of the greater part of the practices that make the specified procedures of appropriate medication prescribing not satisfied.^{3,4} Using many drugs associatively is known as polypharmacy, which is characterized as using 2-5 medicines all the while. However, patients with co-morbidities have a convincing need to use numerous drugs to treat their multiple diseases. There are many reasons which may lead to irrational usage of drugs in developing country. They are: Lack of data, Faulty and education of medical graduates and inadequate training, Poor communication between medical practitioners & patient, Lack of diagnostic facilities and Uncertainty of diagnosis, Patient request, Defective medication supply system & inadequate drug regulation, Promotional practices of pharmaceutical industries. Medical practitioners routinely prescribe drugs. They are relied upon experience to apply their knowledge of therapeutics to choose proper medications for their patients' condition and then recommend these in right dosages and for the correct duration so as to optimize the benefit to the patient. This is the manner by which it should occur in a perfect world, with ideal doctors who have got ideal training. The opening statements by the WHO on its 'Rational Medicine Use' website page underscore the current circumstance: "The irrational use of drugs is a noteworthy issue around the world. WHO evaluates that the 50% of all drugs are prescribed, dispensed or sold improperly, while 50% of patients fail to take

them effectively. Moreover, around 33% of the total world's population needs access to essential drugs, especially in the developing countries.^{5,6} The abuse, misuse or underuse of drugs results in wastage of rare resources and widespread health hazards.⁷ Proton pump inhibitors (PPIs) are among the most widely recognized classes of drugs prescribed. Proton pump inhibitors (PPIs) signify a progressive advancement in gastroenterology.^{8,9,10} These medicines irreversibly inhibit the gastric H⁺, K⁺ ATPase pump and reduction both basal and stimulated gastric acid creation. PPIs are useful and cost-effective for the treatment of extreme gastro esophageal reflux disorder (GERD) and other acid-related diseases.^{11,12} Treatment using PPIs have expanded extremely over the most recent decade. Proton pump inhibitors (PPI) have emerged as the main medication for GERD and peptic ulcer disease due to their highly effectiveness and less toxicity in treating these condition¹³. PPIs are first decision for the treatment of GERD and peptic ulcers for a period of 28-42 days. In association with antibiotics like Clarithromycin and amoxicillin, they are used for abolition of *Helicobacter pylori* treatment. The important factor for a broad and rare use of PPI seems to be the launch of a PPI therapy during a patient's hospitalization and its ratification, for continuation in essential care without appearing reason.¹⁴ Proton pump inhibitors (PPIs) represent a chief financial burden for the medical sector in some countries. Worries have been raised over the expanding costs related with dispensing of these medicines as they are frequently prescribed for irrelevant symptoms and denied of clear indications¹⁵. Researches from the Australia, Europe and USA have affirmed abuse of proton pump inhibitors (PPIs) in primary care and hospitalized patients. Proton pump inhibitors have some of side effects and couple of important drug interactions, and for the most part measured safe for long-term therapy.

2. METHODOLOGY: (IRB Ref .No: PD012 /IRB /NRML/ 19 – 20)

2.1 Study Site:

The study was carried out in inpatient department of Gastroenterology, Manipal hospital, Vijayawada, Andhra Pradesh. All the studies were performed according to the ICMR GUIDELINES.

2.2 Study Duration:

The study was conducted over a period of six months from July 2019 to December 2019.

2.3 Study Design:

This is a Prospective observational study which aims to assess the rational usage of PPI in Gastroenterology Department.

2.4 Study Criteria:

2.4.1. Inclusion criteria:

- All Patients irrespective of gender who are prescribed PPI
- Patient should have age of more than 18 years
- Patients who are willing to participate

2.4.2. Exclusion Criteria:

- Patients who are treating on OP basis
- Pediatric patients and Pregnant women are excluded from this study
- Patients who are not willing to participate

2.5 Sample Size:

Sample size: 204 Patients

2.6 Data resources

- ✓ Patient case sheets
- ✓ Medication charts
- ✓ Treatment reviews
- ✓ Laboratory data
- ✓ Patient interview

2.7 Statistical Analysis:

Descriptive statistics like mean, frequency, percentage were majorly used in representation of data

3.RESULTS

3.1: Age Distribution of study population (n=204)

Age	Frequency	Percentage
20-30	46	22.55%
31-40	34	16.67%
41-50	22	10.78%
51-60	24	11.76%
>60	78	38.24%
Total	204	100%

Table 3.1. Age Distribution of study population (n=204)

Table 3.1 shows age distribution of study population, a total of 204 subjects are included in this study, in this most of the subjects (38.24%) are belongs to age group more than 60 years followed by, 20-30 (22.55%), 31-40 (16.67%), 51-60 (11.76%) and 41-50 (10.78%)

3.2: Gender distribution of study population

Gender	Frequency	Percentage
Male	134	65.68%
Female	70	34.32%
Total	100	100%

Table 3.2 Gender distribution of study population

Table 3.2 shows gender distribution of study population, most of the subjects are males (65.68%) and females are (34.32%).

3.3: Educational status of Study Population

Educational status	Frequency	Percentage
Illiterate	143	70.10%
Higher secondary school	35	17.15%
Graduation	26	12.75%
More than Graduation	0	0%
Total	204	100%

Table 3.3 Educational status of Study Population

Table 3.3 shows distribution of education level among study participants. Most of the subjects are Illiterate (70.10%), followed by secondary level of education (17.15%) and graduation (12.75%).

3.4 Clinical diagnosis distribution of study population

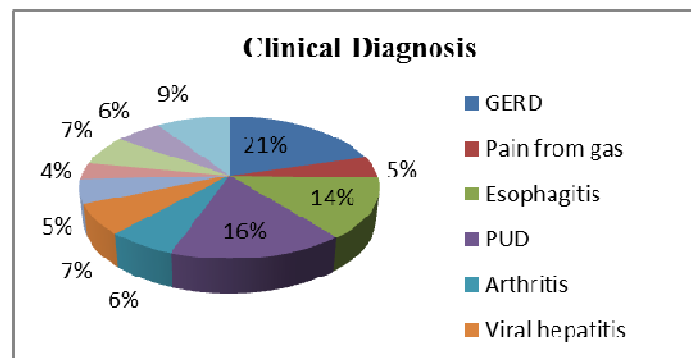


Figure 1 clinical diagnosis distribution of study population

Figure 1 shows clinical diagnosis distribution of study population, most of the patients are belongs to GERD (20.59%), PUD (16.17%), Esophagitis (13.73%), DM/HTN (9.32%), Viral Hepatitis (6.86%), LRTI (6.86%), and Pain from gas (4.90%)

3.5. Number of medicines per prescription

No. of medicines per prescription	Frequency	Percentage
0-3	68	33.33%
4-5	117	57.36%
>5	19	9.31%
Total	204	100%

Table 3. 5 Number of medicines per prescription

Table 3.5 shows distribution of subjects based on number of medicines per prescription. Out of 204 subjects, most of the patients (57.36%) prescribed 4-5 medications, followed by three or less than three (33.33%), and more than five medications (9.31%)

3.6: Prescribing Pattern of Proton Pump Inhibitors

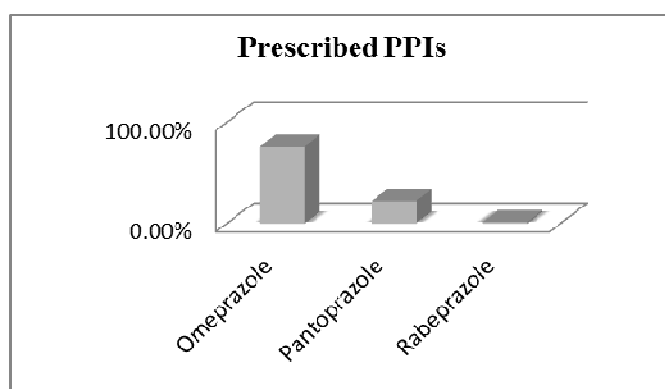


Table-3.1: Demographic characteristics of enrolled patient

Figure 2 shows prescribing pattern of Proton Pump Inhibitors in study population. Most of the common prescribing PPIs are Omeprazole (76.47%), Pantoprazole (22.06%) and Rabeprazole (1.47%)

T 3.7: Appropriateness of PPIs

Appropriateness of PPIs	Frequency	Percentage
Most Appropriate	104	50.98%
Appropriate	92	45.09%
Inappropriate	8	3.93%
Total	204	100%

Table 3.7: Appropriateness of PPIs

Table 3.7 shows appropriateness of PPIs prescribed in population. Most of the PPIs are prescribed in most appropriate manner (50.98%), Appropriate and rarely inappropriate (45.09%).

4. DISCUSSION

Most of the subjects are belongs to age group of more than 60 years (38.24%) followed by, 20-30 (22.55%), 31-40, 51-60 (11.76%) and 41-50 (10.78%). Age group above 60 years was predominant due to increased number of medications in geriatrics due to multiple disorders. PPIs use will be more in prescription containing maximum number of drugs. . Most of the study subjects were males (65.68%) followed by females (34.32%). Maximum number of study subjects are illiterates (70.10%), followed by secondary school education (17.15%) and graduation (12.75%), this is majorly due to study was conducted in rural hospital settings where most of the people are less access to formal education. Similar results were found in the study conducted by Nasrin Shahsavani et al. Most of the patients are belongs to GERD (20.59%), PUD

(16.17%), Esophagitis (13.73%), DM/HTN (9.32%), Viral Hepatitis (6.86%), LRTI (6.86%), and Pain from gas (4.90%). Most commonly used route was IV (62.74%) followed by Oral (37.26%). The current study suggests that the risk of PPIs may extend beyond previously studied high risk individuals. These findings confirm and extend the findings of Shih and colleagues, writing and implementing guidelines for the uses of ASDs, mainly PPIs, by pharmacists can be another strategy to reduce misuse. Such a practice guideline can be in the form of a verbal, written or electronic communication. Our study is subject to several limitations. Most importantly, these observational data may be subject to confounding in multiple ways, and it is possible that PPI usage is merely a marker of a sicker patient population. For example, we were unable to control for factors such as obesity and insulin resistance, and it may be that in some individuals PPIs were prescribed for angina that was misidentified as acid reflux. The study of prescribing patterns of proton pump inhibitors is a component of medical audit, which seeks monitoring, evaluation and necessary modifications in the prescribing practices of prescriber to achieve the rational medical care. It is necessary to define prescribing pattern and to identify irrational prescribing habits to drive a remedial message to the prescriber

5. CONCLUSION

Although Proton Pump Inhibitors are very safe and effective drugs to reduce acid secretion but they should use with a documented evidence of GI Disorders that fails to respond for H₂ Receptor antagonists. Inadequate recommendations for PPIs in discharge letters are frequent. This may lead to a continuation of this therapy in primary care, thereby unnecessarily increasing polypharmacy and the risk of adverse events as well as burdening the public health budget. Hospitals should therefore critically review recommendations for PPI medication and the dosage in their discharge letters as well as on inpatients among medicines unit and clearly document the reason for PPI use and the need for continuous prescription in primary care. This study lastly concludes that there should be combined efforts of pharmacists, and the medical profession to work together for rational use of PPI through intervention such as educational programs or institutional guidelines for use may be developed and implemented to help decrease the use of PPIs in the inpatient population.

6. ACKNOWLEDGEMENTS

I would like to express our special thanks of gratitude to my guide (DR . Y Malyadri .) as well as our principal sir (Sk. Abdul Rahaman) who gave me the opportunity to do this project on the topic (Assessment of rational usage of proton pump inhibitors in a tertiary care referral hospital). The authors are extremely obliged to the cooperation of superintendent and other staff of Manipal hospitals for granting permission to carry out this project at their hospital. We also acknowledge our gratitude to our institution Nirmala College of pharmacy for providing support and guidance to carry out our work.

7. CONFLICT OF INTEREST

Our work has been carried out in a peaceful manner without any problems. The authors do not have any conflict of interest.

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A NEW STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF VALSARTAN AND AMLODIPINE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS.

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ABSTRACT

This work is aimed to develop and validate a specific, accurate, precise, and simple protocol for the analysis of Amlodipine and Valsartan in bulk and its pharmaceutical dosage forms using RP-HPLC approach. As beginning, Phosphate buffer (pH 3): Acetonitrile: Water [45: 35:20,v/v/v] mobile phase was prepared, the mode was set as isocratic and 150 mm x 4.6 mm, 5 μ m symmetry C18 column was used for the separation. Flow rate fixed at 0.8 ml / min and effluents were tracked at 248 nm. After the injection of Amlodipine and Valsartan, chromatograms was developed a peak at 2.780 min and 3.945 min of retention time respectively. Amlodipine and Valsartan show the purity percentage values of 100.02 % w/v and 99.84 % w/v respectively. The method was validated for system suitability, linearity, precision, accuracy specificity, ruggedness, robustness, LOD and LOQ. Mean recovery of Amlodipine and Valsartan was found to be in the range of 100.25% and 101.26% respectively and showed linearity in the range of 1.0 -3.0 μ g / ml for Amlodipine and 32.0 – 96.0 μ g/ml for Valsartan. The LOD and LOQ for estimation of Amlodipine and Valsartan were found to be 0.048 μ g / ml, 0.149 μ g / ml, and 0.040 μ g / ml, 0.128 μ g / ml respectively. The method represents a fast-analytical procedure and stability indicating analytical method for the simultaneous estimation of Amlodipine and Valsartan in bulk and its dosage forms. The result analysis concluded that, there is no intervention observed due to the other excipients of Amlodipine pharmaceutical dosage. Also, this protocol validation exposed the higher reproducibility, specificity, reliable and rapid method. The method is amenable for the routine analysis of large numbers of samples with good precision and accuracy.

KEY WORDS: *Amlodipine besylate, Valsartan, RP-HPLC, Stability indicating method.*

INTRODUCTION

Amlodipine besylate (AMLB) is, 2-[(2-amino ethoxy)-methyl]-4-(2-cholophenyl)-1, 4-dihydro-6-methyl-3, 5- pyridine dicarboxylic acid 3-ethyl-5-methyl ester, benzosulfonate, is a potent dihydro calcium channel blocker (**Fig.1**)¹⁻⁷. Valsartan (VAT) is, N-(1-Oxopentyl)-N-[[2'-(1H-tetrazol- 5-yl) [1, 1'-biphenyl]-4-yl] methyl]-L-valine (**Fig.2**), is a potent angiotensin receptor blocker⁸⁻⁹.

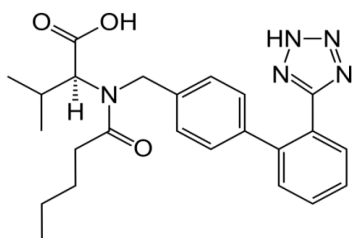


Fig. 1 Chemical structure of Amlodipine

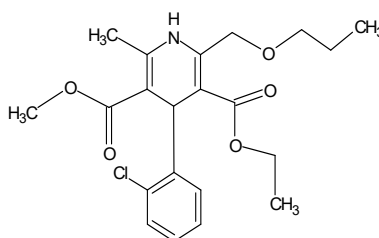


Fig. 2 Chemical structure of Valsartan

It is imperative to have safety and efficacy in drug therapy, pharmacists must take into consideration, the stability of drugs and its therapeutic values. The stability of the drug formulations can be assessed by using

stability indicating methods. A well-designed stress study is important to help develop and demonstrate the specificity of stability indicating methods. They are also useful for checking rapid and accurate drug quality during stability testing. Lack of in-depth study¹⁰⁻¹⁸ prompted us to work on the stability indicating method for simultaneous determination of Amlodipine and Valsartan in bulk and its dosage forms by RP-HPLC. The aim of the present work is to develop validated stability indicating analytical methods for the simultaneous estimation of Amlodipine and Valsartan in bulk and its dosage forms by RP-HPLC.

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents

Amlodipine and Valsartan were obtained as gift samples from Pharma Train labs, Hyderabad. Acetonitrile (HPLC grade), potassium phosphate and orthophosphoric acid were of reagent grade. Double distilled water was used in preparation of mobile phase. The commercial formulation of Amlodipine and Valsartan is available in ratio of 1:32 {Valzaarsm (2.5/80 mg)} as capsules.

2.2. Chromatographic Condition:

Waters e2695 Separation Module HPLC system and a UV-visible detector were used for Chromatographic separation. The chromatographic column utilized in the study was Symmetry C18 column having 150 mm x 4.6 mm, 5 µm in isocratic mode. Three miscible solvents phosphate Buffer (pH-.3): Acetonitrile: Water is used as a mobile phase with the ratio of 45:35:20. Orthophosphoric acid was used to maintain the buffer pH without change. Prepared mobile phase was filtered by a 0.45 micron membrane filter and sonicated for 30 min to remove generated CO₂. The isocratic flow of 0.8 ml/min was set and mobile phase was used as a diluent for test sample. The overlay spectrum of Amlodipine and Valsartan was obtained and the isosbestic point of Amlodipine and Valsartan showed absorbance maxima at 248 nm. All determinations were performed at constant column temperature 30⁰c.

2.3. Preparation of Stock Solutions of Amlodipine and Valasartan

Accurately weigh and transfer 80mg of Valsartan & Amlodipine 2.5 mg working standard into a 50 ml volumetric flask add about required diluent to 50ml and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter to give the concentration of 2.0 µg/ml of amlodipine and 64 µg/ml of valsartan respectively.

2.4. Preparation of Sample Solution Amlodipine and Valsartan

The sample powder equivalent to 80mg of Valsartan & Amlodipine 2.5 mg was placed into a 50 ml volumetric flask add about required diluent to 50ml and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter to give the concentration of 2.0 µg/ml of amlodipine and 64.0 µg/ml of valsartan respectively.

2.5. Assay

The 10 µL of standard and sample solutions were injected into the injector of HPLC and the peak areas of the drugs in standard and sample were compared, and the assay was performed. Amlodipine and Valasartan show the purity percentage values of 100.02 % w/v and 99.84 % w/v respectively.

2.6. Validation of the method

Validation of the developed protocol was carried out by the measurement of nine parameters such as limit of detection, limit of quantification, system precision, method precision, specificity, robustness, ruggedness, accuracy and linearity.

2.7 Degradation Studies

Stress testing of the drug substance can help identify the likely degradation products, the stability and specificity of the analytical procedure.

2.8 Degradation studies

2.7.1. Acidic Degradation

The sample solution was prepared with 10 ml of 0.01 M Hydrochloric acid. Reflux under heat at 60°C for one hour. The sample solution was neutralised using 0.01 M Sodium hydroxide and diluted with diluent to make the final concentration as per test method.

2.7.2. Basic Degradation

The sample solution for basic degradation studies was prepared with 10 ml of 0.1 M Sodium hydroxide. Reflux under heat at 60°C for one hour. The sample solution was neutralised using 0.1 M Hydrochloric acid and diluted with diluent to make the final concentration as per test method.

2.7.3. Oxidative Degradation

The sample solution for oxidative degradation studies was prepared with 10 ml of 3 % Hydrogen peroxide. Reflux under heat at 60°C for one hour. It was further diluted with diluent to make the final concentration as per test method.

2.7.4. Photolytic Degradation

The sample solution for photolytic stability studies was prepared with diluent as per test method. The resultant solution was exposed to natural sunlight during the daytime for 8 hours. 10 µl of the acidic, basic, neutral, oxidative and photo stability solutions were injected. The peak area for the Amlodipine and Valsartan were measured and % degradation calculated from % Assay.

3. RESULT AND DISCUSSION

In this study, attempts were made to develop a simple, accurate, rapid, and precise and stability indicating method and validate for determination of Amlodipine and Valsartan in bulk and its combined pharmaceutical formulations by HPLC. When compared to other reported methods, the flow rate in this developed method was 0.8 ml/min. So, the developed method benefits from the low volume of mobile phase and short analytical run time. Hence the developed method is economical and cost-effective. The retention times of Amlodipine and Valsartan were found to be around 2.780 min and 3.945 min respectively. Amlodipine and Valsartan show the purity percentage values of 100.02 % w/v and 99.84 % w/v respectively. All parameters of this proposed method were validated.

3.1. Method Validation

The proposed HPLC method was validated as per ICH guidelines.

3.1.1 Specificity

The peak purity of Amlodipine and Valsartan were assessed by comparing the retention time of standard Amlodipine and Valsartan. Good correlation was obtained between the retention time of standard and sample of Amlodipine and Valsartan (Fig.3)

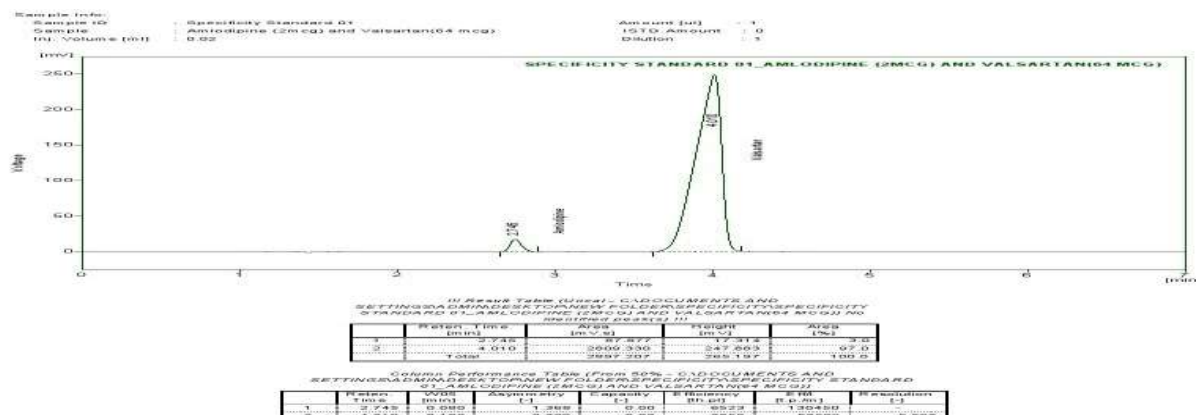


Figure 3 Chromatogram showing specificity of Amlodipine and Valsartan

3.1.2 Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Amlodipine and Valsartan were found to be 1.0 – 3.0 µg/ml and 32 – 96 µg/ml. respectively. The regression equation for AMLB and VAT were found to be $y = 47.489x - 11.303$ and $y = 45.783x - 52.482$ with coefficient of correlation (r) 0.9918 and 0.9905 respectively. The results are reported in Table-1. The calibration curves are shown in (Fig.4&5).

Table 1 Linearity results for Amlodipine and Valsartan

Parameters	Amlodipine	Valsartan
Linear Dynamic Range	1.0 – 3.0 µg/ml	32 – 96 µg/ml
Slope (m)	129.231	4265.53
Intercept	47.489	52.482
Correlation Coefficient	0.9918	0.9905

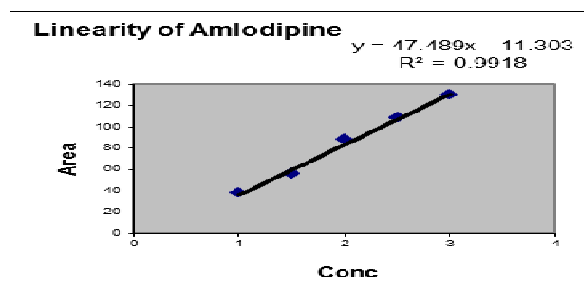


Fig. 4 Linearity Curve for Amlodipine

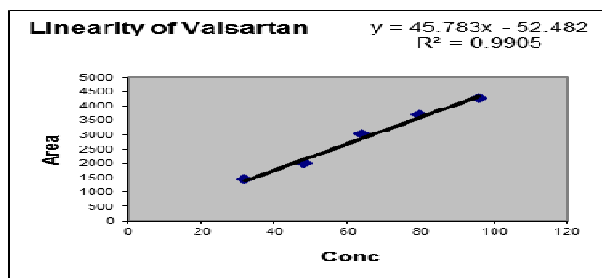


Fig. 5 Linearity Curve for Valsartan

3.1.3 System Precision

The % R.S.D of peak area was 1.96 and retention time was 0.13 for Amlodipine. The % R.S.D of peak area was 0.43 and retention time was 0.11 for Valsartan. It showed that the drug was having good precision and the % R.S.D of peak area and retention time were present within the Acceptance criteria of R.S.D < 2 %. The results are reported in Table-2.

3.1.4 Method Precision

The % R.S.D of peak area was 0.78 and retention time was 0.44 for Amlodipine. The % R.S.D of peak area was 0.34 and retention time was 0.18 for Valsartan. It showed that the drug was having good precision and the % R.S.D of peak area and retention time were present within the Acceptance criteria of R.S.D < 2 %. Thus, the proposed method was found to have a high degree of precision and reproducibility.

3.1.5 System suitability

The parameters including resolution, tailing factor, no. of theoretical plates mainly used to assess system suitability. The Tailing factor for Amlodipine and Valsartan was found to be 1.351 and 0.379 respectively. The Theoretical plates per unit for Amlodipine and Valsartan was found to be 7232 and 2427 respectively. The resolution value of Amlodipine and Valsartan was found to be 5.203. The resolution value of more than 2 indicates satisfactory results in quantitative work and the higher resolution value obtained indicates the complete separation of the drugs. The tailing factor values for Amlodipine and Valsartan indicated the

symmetrical nature of the peak. The no. of theoretical plates was high indicating the efficient performance of the column. System suitability results are presented in Table- 3. The chromatograms are shown in (Fig-6).

Table 2. System precision data for Amlodipine

AMLODIPINE			VALSARTAN	
S.NO	Rt	PEAK AREA	Rt	PEAK AREA
1	2.763	91.648	3.953	2931.615
2	2.770	94.471	3.942	2919.601
3	2.770	94.961	3.942	2932.761
4	2.770	92.82	3.942	2907.62
5	2.763	90.367	3.953	2939.538
6	2.770	91.411	3.942	2939.642
AVG	2.767	92.61	3.946	2928.463
S.D	0.0036	1.813	0.006	12.562
%RSD	0.13	1.96	0.14	0.43

Table 3. System suitability parameters of Amlodipine and Valsartan

Parameters	Amlodipine	Valsartan
Tailing factor	1.351	0.379
Resolution	5.203	
Retention time	2.770	3.948
Theoretical plates per unit	7232	2427

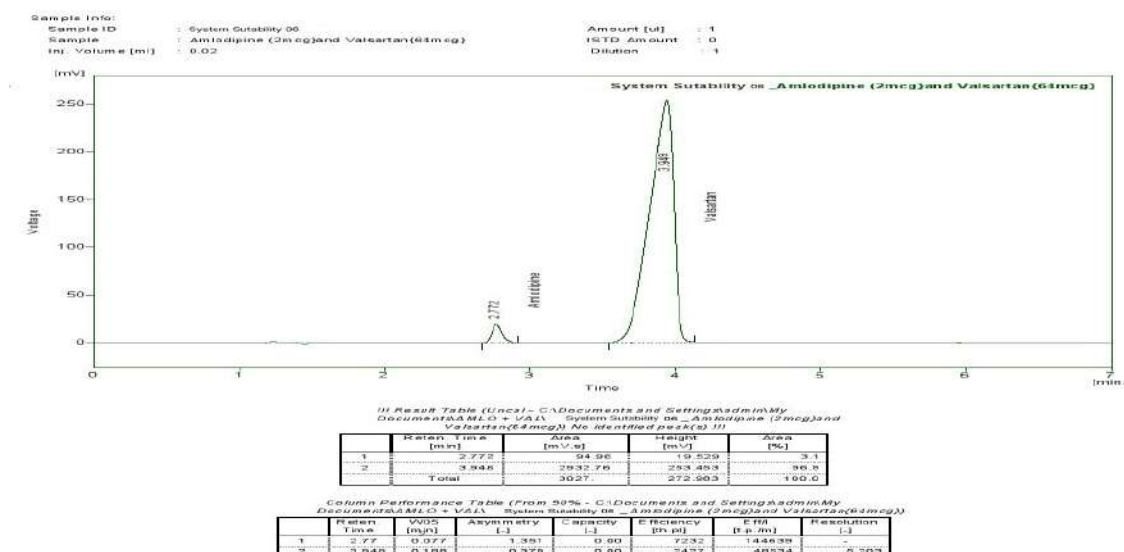


Fig. 6 Chromatogram showing system suitability of Amlodipine and Valsartan

3.1.6 Accuracy (Recovery studies)

Studies were conducted in triplicates at 50 percent, 100 percent and 150 percent by standard additional process. Registered concentrations of regular Amlodipine and Valsartan have been applied to pre-analyzed samples and subjected to proposed HPLC method. Mean recovery of Amlodipine and Valsartan was found to be in the range of 100.25% and 101.26% respectively. The obtained mean recovery values were within the acceptance criteria of 98 - 102 %. This serves as a good index of the accuracy and reproducibility of the proposed method. This shows that the method was accurate.

3.1.7 Robustness of method

Small deliberate variations in optimized method parameters were done to assess the robustness of the developed RP-HPLC method. The effect of flux rate changes, wavelength on retention time and tailing factor was studied. The method was found to be unaffected by small changes ± 0.2 ml flow rate.

3.1.8 Ruggedness of method

Ruggedness of the proposed RP-HPLC method was evaluated by comparing the results obtained by two different analysts. The % RSD for Amlodipine and Valsartan for analyst I was 0.45 and 0.32 and for analyst II was 0.42 and 0.19 respectively.

3.1.9 Limit of Detection

The LOD of Dolutegravir and Rilpivirine were 0.048 µg/ml and 0.040 µg/ml. The obtained LOD values were very less when compared to the reported method. As per the analytical point of view, the method could detect very least quantity. So, when compared to the reported methods [35-36], the developed method is most suitable to detect less quantity.

3.1.10 Limit of Quantification

The LOQ of Dolutegravir and Rilpivirine were 0.149 µg/ml and 0.128 µg/ml. The obtained LOQ values were very less when compared to reported methods.

3.2 Degradation studies

Degradation studies were carried out as per ICH guidelines. Amlodipine was not stable in acidic and oxidative conditions. Valsartan was not stable in basic and oxidative conditions. The proposed method was helpful in the separation of the two compounds without the interference of degradants, estimate the active contents. Hence the developed method can be used for quality control analysis and accelerated stability studies. The obtained data are reported in **Table - 5**.

Table 5. Results of degradation studies for dolutegravir and rilpivirine.

S. No.	Name	Sample weight	% Assay- Amlodipine	% Assay- Valsartan	% Degradation- Amlodipine	% Degradation- Valsartan
1	Acid	242	88	96	- 12	-3
2	Base	242	95	86	-5	-13
3	Peroxide	242	84	85	- 16	-14
4	Thermal	242	96	97	- 4	-2

4.CONCLUSION

A new stability indicating analytical method was developed and validated by RP-HPLC technique. The sample preparation is simple, consumes less amount of mobile phase and the required time for analysis is very short, the information given in the study will be very useful for the quality monitoring of Amlodipine and Valsartan in bulk and its dosage forms. The proposed method can be efficiently applied for the separation of the drugs from its excipients and its degradation components in the pharmaceutical formulation.

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PHARMACOECONOMICS IN INDIA: A FUTURE OUTLOOK

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ABSTRACT

The unaffordability of the health care and delivery system is one among the major concerns of all patients, health care professionals and the country. Evaluation of pharmacoeconomics is important to help clinicians and decision-makers to make choices about new pharmaceutical products and to help patients gain access to new medicines with low cost. The scientific rigor of this area has been rising over the last couple of years. In India, the government pays for around 20% of the medicines being used, however, India stands top in the world regarding the money spend on private medical sectors. Government should consider seriously a shift in health policy objectives towards quality and lifespan. In resource allocation, priority should be given to programs with higher benefits at the lowest cost to society. Preparing pharmacoeconomics guidelines will be an important step towards developing HealthTechnology Assessment (HTA) in India. HTA could be implemented in several Indian contexts such as the pricing of medications, the creation of guidelines for clinical practice and the prioritization of treatments of the highest value within a limited budget. Research on pharmacoeconomics should be strongly supported in a country like India where the majority of healthcare expenditure is taken from the patients. The purpose of this article is to provide an introduction to pharmacoeconomics in Indian scenario and its future outlook.

KEYWORDS: *Pharmacoeconomics, health care, delivery system, HTA, Clinical practice*

INTRODUCTION

The International Society for Pharmacoeconomics and Research on Outcomes (ISPOR) describes pharmacoeconomics as 'the field of study which analyses the actions of individuals, firms and market relating to the use of pharmaceutical products, services and programs, and often focuses on the costs (inputs) and results (outcomes) of that use. Pharmacoeconomics and outcome Research is a new branch of medical, pharmaceutical, social science research in India. Although in some other countries it has a long history, its use in the past is not so common especially in a country like India. Several organizations, including the ISPOR- India Regional Section, have been trying to advance this science branch in our country since 2006. Pharmacoeconomics studies find value in setting the price of a new drug and re-fixing the price of an existing drug, finalizing a drug form, creating data for medicines promotional materials, compliance with drug licensing requirements, including a drug in the medical/insurance reimbursement schemes, introduction of new schemes and programs in hospital pharmacy and clinical pharmacy, drug development and clinical trials. Guidelines for pharmacoeconomics (PE) can be used as a guideline for planning studies to be included in the reimbursement application, as a guide for designing and performing a study, or as a model for assessing the economic research results.

PRESENT SCENARIO IN INDIA

"Physicians prescribe, patients consume and, increasingly throughout, third-party purchasers (government insurance companies) pay the bill with money collected from increasingly reluctant active community members. Medicines produced in India have an international standard, but the cost of production is small. Indian pharmacy ranks third world wide in terms of production. Indian pharmacy ranks 14th with respect to turnover.¹ Just 1/3rd of the population can use such basic drugs. Urban areas are getting greater health services than rural areas. Many of the inpatient services are provided in the Indian health system. In order to obtain the insurance, it is appropriate to remain in the hospital for a day. Both the patients and the insurance companies will benefit from implementing the pharmacoeconomics theory of hospital management and treatment protocols.² At reduced costs, patients will receive better quality healthcare, and insurance companies will be able to provide their customers with improved coverage at

minimum cost.³ Indian pharmaceutical industry has a range of medicines as the third largest manufacturer of drugs by volume; however, brand name prescriptions are the law of the day. Formulary system is very weak. Treatment protocols exist only in theory. The resources are scarce and there are plenty of competing programs in healthcare. In the country, the concept of health insurance is still to be popularized. Because of the problems in the Indian healthcare system, there are many applications in pharmacoeconomics. Available in large quantities, Indian primary care providers are often bombarded with many new drugs of the same class, in addition to existing drugs. Introduction of new drugs will confuse doctors and administrators for the sound choice and appropriate use of medicines.⁴

Adapting pharmacoeconomics to shape efficient health care system in India

The Indian Pharmaceutical Industry (IPI) is the fourth largest in size in the world and is likely to lead India's manufacturing sector.⁵ The 1970 Indian Patent Act played a major role in the establishment of a base for India's manufacturing sector. Change in law in 2005 has created opportunities for exchanging expertise for both international firms and local Indian firms⁶. This has certainly created enormous employment opportunities, particularly in the field of clinical research, making room for research into health outcomes. In India, the government still does not use the concept of pharmacoeconomics to make reimbursement decisions. In addition, the pharmacoeconomics concept is not used in academic research, although cost-effectiveness studies have been carried out in different parts of India⁷. Pharmacoeconomics has yet to appear in India, where patients spend most of their healthcare expenditure from their own pockets, unlike medical insurance policies in most developed countries. It is necessary that pharmacoeconomic research be strongly enforced in India and carried out from the clinical trial so that the government can ensure that money is spent in the right direction and also that the financial burden on patients.⁸⁻⁹

WHY PHARMACOECONOMICS?

In industry it has been noted that pharmacoeconomics is necessary to decide among the different alternatives to research and development; in government to evaluate program benefits and prices paid; and in the private sector to determine insurance benefits was proposed that four points were reached in the study of pharmacoeconomics, including lower costs, better results; higher costs, better results; lower costs, worse results; and higher costs, worse results. Despite the smooth application of pharmacoeconomics in the pharmaceutical and other private sectors, pharmacoeconomic studies have faced various challenges, including setting guidelines or standards of practice; setting up a framework of trained pharmacoeconomic producers and consumers; providing education on the relevant characteristics of this discipline.⁹

PHARMACOECONOMICS: PROBLEMS ASSOCIATED & CHALLENGES

The main problem associated with pharmacoeconomic studies is the maintenance and creation of schemes for perfectly trained employers and consumers of pharmacoeconomic evaluations; the regulation of the proper characteristics of disciplined practices for practitioners, government officials and private field workers; and the lack of fixed resources to support applied pharmacoeconomics evaluation¹⁰. In addition, limitations in studies of pharmacoeconomics generate a lot of problems that reduce the use of health economics in therapy.¹¹ It has been noted that health economics is being misused primarily by health care payers in the advertising sector. Other issues can be attributed to the inadequate pharmacoeconomic study, which is evident from the fact that the clinical pharmacist chooses the wrong drug because of the marketed pressure¹². In fact, medicinal drugs are prescribed by the medicinal practitioner by the marketing executives and medical staff of pharmaceutical companies, who provide an incentive for the medical practitioner to prescribe their marketed generics or pseudo-generics.¹²⁻¹³

PHARMACOECONOMICS GUIDELINES: NEED OF THE HOUR!!

Although the government pays for about 20 percent of drugs used in India, India's private out-of-pocket spending on healthcare is one of the world's highest. To establish a Health Technology Assessment (HTA) in India, preparing guidelines for pharmacoeconomics will be an important step¹⁴. To establish a Health Technology Assessment (HTA) in India, the preparation of guidelines for pharmacoeconomics will be an important step. Areas in which HTA could be applied in the Indian context include drug pricing, the development of guidelines for clinical practice and the prioritization of interventions within a limited budget that represent the greatest value. NHT (new health technology) developer's plan to learn how to

cope with the changes brought about by HTA implementation by attending HTA meetings and exchanging feedback¹⁵. Niëns et al.'s study results found that high medical costs would drive large numbers of patients into poverty. Such findings call for action on the part of both governments and civil society organizations to prioritize access to essential medicines, not only to ensure access to appropriate medicines, but also as a component of poverty reduction¹⁶.

The possible lines of action include:

- Development, implementation and compliance of sound national and international price policies through national and international advisory committee creation.
- Promoting the use of high-quality, low-cost generics, e.g. by preferential registration procedures, is also an important strategy.
- Due to health shocks (and poor health due to poverty), the establishment of health insurance systems with outpatient medicine benefits seems crucial in the long term to avoid poverty.
- The cost of drugs can be reduced by reducing duties and narcotic taxes.
- HTA design in accordance with the guidelines for PE.
- Innovative methods, such as the use of private distribution systems to provide chronic disease patients with subsidized medicines.
- Life-saving medicines such as anticancer drugs, antimalarials and antiretrovirals should be promoted by pharmaceutical companies¹⁷

DEVELOPMENT OF GUIDELINES

India plans to be part of the scheme for universal health coverage by 2022. It is a major capacity building challenge for central and state governments to provide high-quality healthcare to health care seeker without financial hardship. Focusing on preventive and public health strategies aimed at reducing India's most significant health issues is important. Recent progress in high-quality primary health care by the State of Tamil Nadu, including maternal and child health services, is encouraging.¹⁸ Through involving all stakeholders, problems in the development and implementation of pharmaco-economic guidelines could be handled. Some recommendations are as follows:

- Establish advisory committees for federal and state drug regulators in pharmacoeconomics
- Using recommendations for pharmaco economics to incorporate HTA.
- Concentrate on both direct and indirect programs to reduce the burden of illnesses such as improving food, reducing poverty, building health and healthy living infrastructure and preventing disease transmission through patient treatment and community immunization.
- Improve access to life-saving drugs and provide essential medicines
- Implement public-private health insurance programs linked to the Aadhar card
- Collecting tax on health care and increasing spending on health budgets
- Creating public and professional awareness for better use of resources
- Considering health care as a basic necessity, individual right and responsibility
- Include the concepts of pharmaco economics in medicine, pharmacy, nursing, public health, and other professional education of healthcare¹⁹.

Health Technology assessment (HTA) in India: current status and future outlook

Health Technology Assessment (HTA) is 'any method of evaluating and reporting on the properties of a medical technology used in healthcare, such as security, efficacy, viability and use, quality and cost-effectiveness, as well as personal, economic and ethical implications, whether intended or not.'²⁰ It is a cause of serious concern as the health budget in India is very poor compared to the allocation of the international budget, resulting in OUT OF POCKET situation for many uninsured patients who fall below the poverty line. Creation of Health Technology Assessment can solve this problem by using direct expenditures to be shared in the healthcare budget, which can result in the full use of resources for patients. This is the time to develop an active HTA program from the Government of India, which can only overcome the disastrous OUT OF POCKET situations²¹. The value of money and cost-effectiveness can be achieved only after the effective HTA program has been introduced. Principles of pharmacoeconomics promote the development model of the HTA program. Different pharmaco economic instruments such as cost-effective measures provide the basis for the development of new evaluation methods and techniques²².

There is currently no organized national HTA system in India, although isolated attempts have been made in this direction by a few organizations. India's healthcare industry is affected by various factors, such as low health budget spending in India, reduced health insurance coverage, a large number of population lying in the low income group and highbills of medical care for long term disease²³. India's national health expenditure in terms of purchasing power parity per capita is half that of Sri Lanka and one-third that of China and Thailand. Many patients in India were forced below the poverty line due to health care expenditure nearly 40% of Indians who were hospitalized in 1995-1996 fell into debt due to hospital expenditure payments, resulting in almost a quarter falling below the poverty line. During hospitalization, the risk of falling into poverty ranged from 17% in Kerala to double that in Uttar Pradesh and Bihar. Only 3-5% of Indians are covered under some sort of health insurance against this backdrop, and premiums amount to only 0.3% of total health care expenditure²³. This can clearly be understood after reviewing it information that the HTA program will be a valuable to the development field of health care. In a review about quality of existing pharmacoeconomics studies conducted in India, Desai et al. proposed a standardized set of guidelines for these studies and enhanced pharmacoeconomics training for the development of skilled professionals capable of producing high-qualityresearch. There are many challenges in establishing effective health technology assessment in India ranging from lack of professional experts, inadequate reporting system, and low budget allocation. The assessment of medical technology in India will ensure the following goals:

- Efficiently and transparently analyze health interventions andtechnology.
- Build processes and structures to facilitate health technology implementation as well as effective commitment of healthpolicy.
- Distribute research findings and educate the public to make the best use of theresults.

The following goals for developing a sustainable HTA system were identified by Professor David Banda, a specialist in the creation of global HTA programs:-

- Awareness and commitment from government policymakers
- Capacity and willingness to commit public money toHTA
- Support from keystakeholders
- Technicalcapability
- Ability to research literature and search theWeb
- Involvement of teachers (HTA trainingprogram)
- Consideration of feasible options, such as regional agencies, networks, coordinating agencies²⁴.

Future implications in India

The model is being developed with regard to the complexities of Indian healthcare, as well as social, ethical and philosophical aspects important to local policy making. Such methodologies will form the basis for pricing, reimbursement and potential investment in Indian healthcare²⁵. Potential applications of HTA include, In India, there are several potential applications for HTA, including developing guidelines for public healthcare reimbursement, advanced novel drug pricing policy. Advising national health authorities to create guidelines for clinical trialstoensure consistency of evidence-based studies to maximize efficiency²⁶.

CONCLUSION

Government should seriously consider a change in quality and longevity in the health policy goals. In resource allocation, priority should be given to initiatives that yield higher benefits with the lowest cost to society. Under these rules, costs canbe regulated, quality improved, and access to healthcare can be improved. India needs to develop the forum for pharmacoeconomics with a validating methodology and appropriate training to allow insurance companies to provide better facilities at minimum cost. It is anticipated that the position of clinical pharmacists, will be more important than conventional pharmacists because they will be able to apply the economic principles in daily practice in community and hospitalpharmacy.

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IS STATINS FOR THE PRIMARY PREVENTION OF CARDIOVASCULAR DISEASE IS A SCAM?

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ABSTRACT

Cardiovascular disease (CVD) is a principal cause of morbidity and mortality worldwide. Statins are generally considered effective and well-tolerated medicines, which significantly reduce cardiovascular morbidity and death in primary and secondary prevention. Firstly, primary prevention evidence indicates that statins significantly reduce CVD morbidity but only mildly decrease CVD mortality. Secondly, people taking the drugs for primary prevention, particularly those at low risk, have less to gain from the use of statin, and this benefit may not outweigh the risk of side effects. Therefore, based on clinical assessment, statins are recommended for primary prevention in nearly all high-risk individuals, whereas an individualized approach is recommended in those at moderate or low risk.

KEYWORDS: *Cardiovascular disease, well-tolerated medicines, morbidity and mortality worldwide, clinical assessment, high-risk*

INTRODUCTION

Cardiovascular disease is a broad term that includes a variety of atherosclerotic disorders affecting the heart and blood vessels, including coronary heart disease as eventually characterized by myocardial infarction (MI) and stroke disease⁷. Statins are commonly used drugs for the treatment of CVD, which remarkably reduce cardiovascular morbidity and mortality in primary as well as secondary prevention. The objective of any primary prevention strategy is to prevent or delay the onset of disease, thereby improving the quality of life and increasing productivity. Regarding 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), the main prevention technique aims at minimizing cardiac morbidity [myocardial infarction (MI), unstable angina, infarction and arterial revascularization] as well as death (cardiovascular death and all-cause mortality)¹². Cardiovascular risk reduction achieved with statins depends on person's baseline risk of developing CVD based on, Age, Sex, Smoking Status, Cholesterol Levels, Blood Pressure. Most people in many countries are taking statins for primary prevention of CVD⁵. For example, a national cohort (age ≥ 50) study took place in Ireland and found that almost 2/3 of people taking statins were in favor of primary prevention, i.e., the increased proportion of women compared to men (73% of women versus 57% of men)⁶.

How do clinical guidelines help statin growth?

We examined changes in the European guidelines on the prevention of CVDs from 1987 to 2016 using data from a national cohort of older people in Ireland. The eligibility criteria have been extended over time to clinical guidelines³. In the 2016 guidelines, the proportion of sample eligible for statins increased from 8%(1987) to 61%(2016)³. The advancement of diagnostic criteria over this period resulted in people with lower risk becoming eligible for treatment, and also increased the number of people to be treated (NNT). That is, 40 people at the lowest risk compared to 400 (2016 Guidelines) (1987 Guidelines).

Evidence for statins in primary prevention

Statins effectively reduce atherogenic lipoproteins, leading to clinically significant decreases in cardiovascular morbidity and mortality². Most of the published systematic reviews identified studies involving several CVD background participants. Out of that, low risk patients taking statins for primary prevention had less benefit¹. Statin therapy is usually recommended for high-risk individuals who do not achieve a low-density lipoprotein cholesterol (LDL-C) target of less than 100 mg / dl with lifestyle changes (TLC)². An Intervention Trial Evaluating Rosuvastatin (JUPITER) recorded that for individuals with

comparatively low rates of low-density lipoprotein cholesterol (LDL-C) (< 130 mg / dL) and baseline levels of hs-CRP above 2 mg / L, statins significantly reduced all-cause mortality by 20%⁹. Overall, statins appear to be very well tolerated and effective in lowering cardiovascular risk among low risk population.

Balancing benefits and harms of statins

Statin use was not associated with serious adverse events such as cancer, high levels of liver enzymes or severe muscle-related harms. However, the risk of developing diabetes with the use of high-dose statins may be slightly increased. Myalgia is an adverse effect of statin that is widely reported⁷. The incidence of myopathy and rhabdomyolysis (excess incidence of 0.5 and 0.1 per 1000 treated over a span of 5 years) was extremely low and far outweighed by cardiovascular benefits of therapy⁵⁻¹³. The relationship between statin use and adverse cognitive effects is very limited, but there has been no clear increase in risk. ie, A systematic review of RCTs and observational studies on the impact of statin use on any cognition indication found no statistically significant differences in performance ratings on attention tests, visual perception, motor and processing speed, memory, cognitive performance, or executive function, and no effect on Alzheimer's disease or dementia incidence.⁸

The major limitations of the studies include,

- Characteristics of those participating in trials were not representative of real world patients.
- Access to full clinical trial data remains unavailable for independent analysis.
- Decision making with older patients is further complicated due to high prevalence of multimorbidity and related polypharmacy¹ ie, Because of limited evidence of the efficacy of Statin therapy, particularly in very old subjects (over 80–85 years of age), the decision whether to treat or not treat an elderly person with primary prevention statins should be based on good medical judgment and consideration of the situation of the individual subject with regard to comorbidity, polypharmacy and possible adverse effects¹¹⁻¹⁴.
- Larger trials are needed that focus on low risk populations: including people over 70 and women.

DISCUSSION

Overall significant risk reductions are seen among those taking statins in all-cause mortality, vascular deaths, major coronary events and vascular events. Even the side effects are rare increased risk of myopathy, rhabdomyolysis, diabetes, and haemorrhagic stroke are seen⁶. Cardiovascular risk reduction achieved with statins depends on,

- person's baseline risk of developing CVD
- based on, Age, Sex, Smoking Status, Cholesterol Levels, Blood Pressure¹⁵.

Systematic reviews and medical guidelines demonstrate that in patients with cardiovascular risk, the cardiovascular benefits of statins by far outweigh non-cardiovascular harms.¹⁰

CONCLUSION

To conclude, Absolute risk reductions for low risk patients are small and patients may not consider that the absolute benefits justify taking a daily medication or the risk of adverse effects. Based on evidence from two recent large-scale meta-analyses, we establish that current guidelines underutilize statin therapy in primary prevention populations with risk factors for CHD. Better data on harms and low risk populations are needed to facilitate shared decision making.

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THE HIDDEN LINKS THAT CLOG LUNGS AND AIRWAY WITH FAT

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ABSTRACT

We are already known of how fat build ups in the arteries and their role in developing heart problems, but now for the first time, new studies shows that clogging of fat could happen in the airway walls of the lungs particularly in people who are overweight or obese. The link between obesity and asthma has been already known for years, but the cause behind the link was not completely clear. Before it was thought the direct pressure on the lungs or additional inflammation due to obesity contributes to asthma. Recently it's found that fatty airways also play a part, too. Excess fat accumulation in the airway alters its normal structure and prompt inflammation in the lungs. The lungs also have a subpopulation of cells that form fat. To keep the airways properly lubricated during breathing, a fatty secretion is expelled into the inner lining by surfactant producing cells. Accumulation of fat in the lungs mimics the process that make fat to build up and damage the liver of alcoholics. In this article, I review how fatty airway is correlated with the body weight of individuals, why overweight increases the chances of developing asthma? And whether respiratory disease can be reversed by weight losstherapy?

KEYWORDS: *Fatty airway, Obese, Asthma*

INTRODUCTION

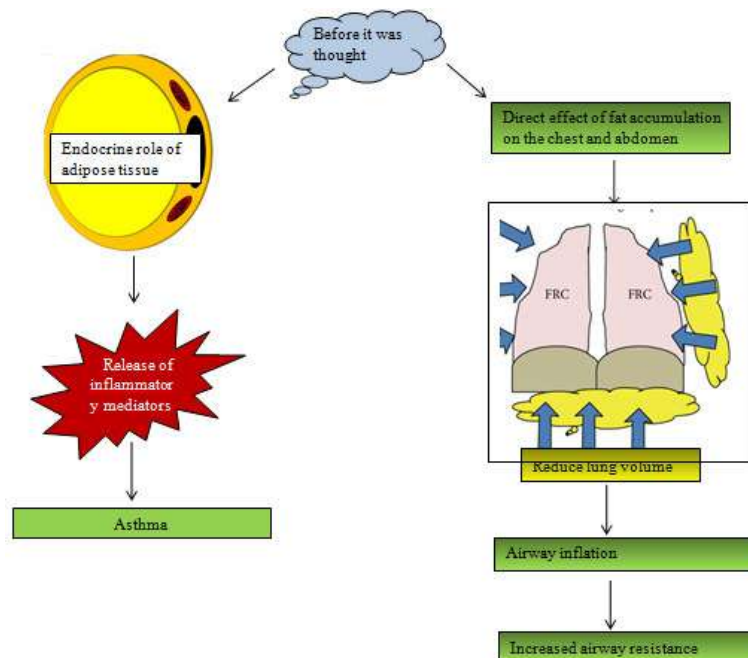
Obesity is recognized as an emerging world-wide health concern.¹ It is anticipated that by 2025 global obesity prevalence will become 18% in men and 21% in women.² The link between obesity and various diseases is well known, but for the first time, new studies shows that clogging of fat could happen in the airway walls of the lungs particularly in people who are overweight or obese.³ Epidemiological data have shown a positive association between adiposity and abnormal respiratory function, particularly asthma.⁴ In the United States the majority of patients with severe asthma are obese and they experience more symptoms compared with non- obese asthma patients.⁵ In this article, I review mysterious link between body weight and respiratorydiseases.

FATTY LUNG DISEASE?

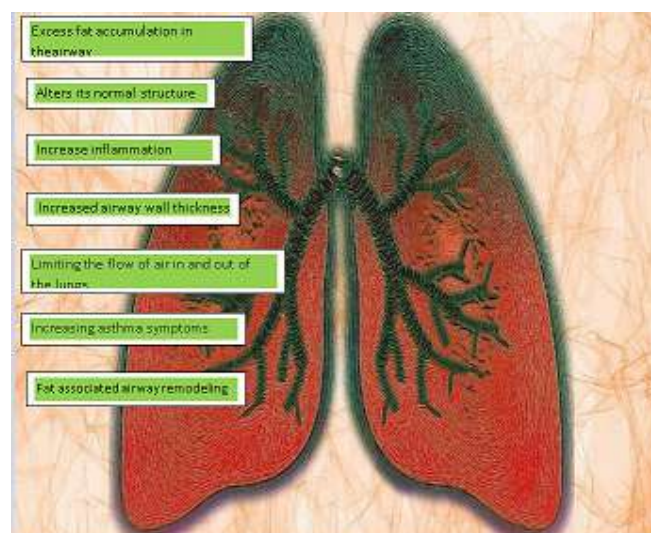
All of us are familiar with fatty liver disease, which is so common today. But fatty lung disease is a totally new topic. A study at the Thomas Jefferson University says that fat also collects in the lungs mainly in obese people. In this study, researchers examined post-mortum samples of the lungs of 52 people-15 (without asthma) and 21 (with asthma). They identified and quantified any fatty tissue present. The study concluded that fat accumulates in the walls of the airways and the amount of fat present increases in line with increasing BMI.³

CAN FAT CAUSE ASTHMA?

The link between obesity and asthma has been known for years, but the cause(s) behind the link was not completely clear.^{3, 4, 5}



Recently it's found that fatty airways also play a part, too ^{3,7}



FAT ACCUMULATION IN LUNGS IMMUNE CELLS

Accumulation of fat in the lungs mimics the process that make fat to build up and damage the liver of alcoholics. In alcoholic people, liver cells produce fat as a defense mechanism against the harmful effects of the alcohol. This fat builds up overtime and leads to fatty liver disease, which in turn because liver failure (3, 8, 9, 10). The lungs also have a sub-population of cells that form fat. To keep the airways properly lubricated during breathing, a fatty secretion is expelled into the inner lining of the lungs by surfactant producing cells (3). After being exposed to alcohol, surfactant cells seem to increase their production of triglycerides by 100 per cent and free fatty acids by 300 per cent. This fat accumulation is seen also in lung macrophages-immune cells that normally destroy the bacteria or sick cells in the lung (13). Researchers say that in order to protect the cells in the lungs, the macrophages try to engulf the excess fat. But, in the process, they become less protective against infections and disease³.

RISKS OF FAT IN THE LUNGS

Fatty airways increase the risk of several health disorders other than pneumonia and acute respiratory distress syndrome.

Fatty airways Can Cause Lipoid Pneumonia

Fatty lung disease or Fatty airways can lead to pneumonia and life-threatening acute respiratory distress syndrome in alcoholics with a weak immune system. Also inhalation through the nose or aspiration while eating can also form fat in the lungs. Here, fat particles from outside get in the body which lead to exogenous lipoid pneumonia. Also, fatty deposits in the lungs over time and causes endogenous lipoid pneumonia¹¹

Fatty airways Can Cause Fibrotic Lung Damage

Fat accumulation in the lungs over time makes it hard and form fibroids, which in turn cause pulmonary fibrosis. There is no cure for pulmonary fibrosis and management is mainly given to decrease the severity of symptoms¹²

Fatty airways can increase the risk of asthma

Obesity can cause fat accumulation in the airway altering its normal structure. This can limit airflow by blocking the airways due to increased airway thickness making it difficult for a person to breathe normally. Thus increases the risk of asthma.^{3, 5, 14, 15)}

CAN WEIGHT LOSS THERAPY REVERSE RESPIRATORY DISEASES?

Studies on the effects of weight loss in asthmatic patients are scarce. A prospective, randomized trial was conducted in obese patients with severe uncontrolled asthma, which aims to confirm the impact of weight reduction program in severe asthmatic patients associated with obesity.¹⁷ This study says that weight loss showed significant improvement in asthma control.¹⁸ The mechanism involved was not related to airway inflammation or bronchial reactivity, but by an increase in forced vital capacity. So this trial suggests that weight loss has an important role in the management of obese asthmatic patients^{19,20}

CONCLUSION

It's very urgent to understand why asthma remains a major health issue and identify new tactics to improve asthma management.

WHAT CAN WE DO?

Although some factors may be beyond our control, there are a few things that we can do to decrease the risk due to fatty lungs Drink in moderation and maintain healthy weight. Eat well-balanced meals and include a lot of colorful fruits and veggies to diet. Exercise regularly and maintain a healthy lifestyle.

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DEVELOPMENT AND VALIDATION OF A SIMPLE UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF CLOBETASOL PROPIONATE PRESENT IN PH 7.4 PHOSPHATE BUFFER

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ABSTRACT

The objective of this study was to develop and validate a novel; specific, precise and simple UV-spectrophotometric method for the estimation of clobetasol propionate present in pH 7.4 phosphate buffer. UV-spectrophotometric determination was performed with Lab India analytical 2000 U UV/Visible double beam spectrophotometer using pH 7.4 phosphate buffers. The spectrum of the standard solution was run from 200-400 nm range for the determination of absorption maximum (λ max). λ max of clobetasol propionate was found at 242 nm. The absorbance of standard solutions of 6, 9, 12, 15 and 18 μ g/ml of drug solution was measured at an absorption maximum (242 nm) against the blank. Calibration graph was plotted by taking concentration on X-axis and absorbance on Y-axis which gave a straight line. Validation parameters such as linearity and range, selectivity and specificity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness were evaluated as per ICH guide lines. Linearity for the UV-spectrophotometric method was noted over a concentration range of 6-18 μ g/ml with a correlation coefficient of 0.9995. The limit of detection (LOD) and limit of quantification (LOQ) for clobetasol propionate was found at 1.42 μ g/ml and 4.30 μ g/ml respectively. Accuracy was in between 98.89 and 99.38%. % RSD for repeatability, intraday precision and interday precision were found to be 0.192, in between 0.149 & 0.351 and in between 0.747 & 1.064 respectively. The proposed UV spectrophotometric method is found to be robust. This present work is validated according to the ICH guidelines and results & statistical parameters demonstrated that the developed method is accurate and reproducible for the determination of clobetasol propionate in pH 7.4 phosphate buffers. This developed method is useful for the determination of *in vitro* drug release from the oral controlled release dosage forms and topical preparations in pH 6.8 phosphate buffer.

KEYWORDS: UV spectroscopy, Validation, Analytical Method Development, Clobetasol propionate, pH 7.4 phosphate buffer.

INTRODUCTION

Clobetasol propionate is a derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity. Clobetasol Propionate is the propionate salt form of clobetasol, a topical synthetic corticosteroid with anti-inflammatory, anti-pruritic, and vaso-constrictive properties. Chemically, clobetasol propionate is (11 β , 16 β)-21-chloro-9-fluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-pregna-1, 4-diene-3, 20-Dione. It is white or almost white crystalline powder. It is soluble in acetone, chloroform and dioxane; sparingly soluble in methanol; insoluble in water and it has the following structural formula.¹⁻³

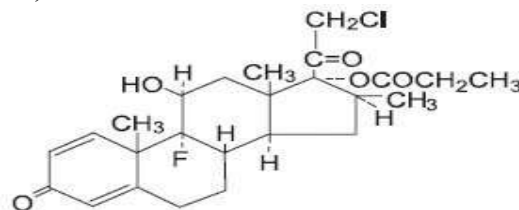


Figure 1: Chemical structure of Clobetasol propionate

Corticosteroids are thought to act by the induction of phospholipase A₂ inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of

inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid. Arachidonic acid is released from membrane phospholipids by phospholipase A₂. Clobetasol bind to the glucocorticoid receptor, these complexes, enters the cell nucleus and modifies genetic transcription (transrepression/transactivation)¹⁻³. The literature survey reveals that various analytical methods have been developed such as HPLC⁴⁻¹⁰, and UV spectroscopy methods¹¹⁻¹² for the estimation of clobetasol propionate present in bulk, single or combinational pharmaceutical formulations. Most of the reported methods are using several solvents, expensive reagents and often time-consuming. Because of simplicity of UV spectrophotometry and also precise, reliable, minimum solvent usage and requires less analysis time, it is widely used for the estimation of drug content in bulk and pharmaceutical products. A detailed review of the literature regarding the existing methods revealed that there is a need for the development of the spectrophotometric method, which is simple for the determination of *in vitro* drug release from the oral controlled release dosage forms and topical preparations in pH 7.4 phosphate buffer. An attempt was made in the present method to develop a simple, sensitive, accurate, reliable and reproducible with minimum Relative Standard Deviation (RSD) values for the determination of *in vitro* drug release from the oral controlled release dosage forms and topical preparations in pH 7.4 phosphate buffers.

MATERIAL AND METHODS

Materials

All chemicals used were of analytical reagent grade. Distilled water was used to prepare solutions wherever required. Clobetasol propionate is gift sample from Wockhardt Research Centre, Aurangabad, and India. Methanol was purchased from Lobachemie pvt ltd., Mumbai, India. Potassium dihydrogen ortho phosphate is from Lobachemie Pvt. Ltd., Mumbai, India. NaOH from Lobachemie Pvt. Ltd., Mumbai, India.

Equipment used

Spectroscopic analysis was carried out using Lab India analytical 2000 U UV/Visible double beam spectrophotometer with 10 mm path length quartz cells was used for the analytical purpose.

Preparation of stock and standard solution for calibration curve

For the method development, a stock solution of clobetasol propionate was prepared by dissolving 100 mg of the drug in 100 ml of methanol so as to obtain a final concentration of 1000 µg/ml. From the 1000 µg/ml solution, 1 ml was taken into a 10 ml volumetric flask and dissolved in methanol which gives 100 µg/ml solutions. From the above 100 µg/ml solution 0.6, 0.9, 1.2, 1.5 and 1.8 ml were pipetted out into 10 ml volumetric flask and the volume was made up to the mark with pH 7.4 phosphate buffer. These dilutions gave 6, 9, 12, 15 and 18 µg/ml concentration of clobetasol propionate. The spectrum of the standard solutions was run from 200 - 400 nm range for the determination of absorption maximum (λ_{max}). λ_{max} of clobetasol propionate was found at 242 nm using pH 7.4 phosphate buffer solution as blank solution. The absorbance of each standard solution was measured by using Lab India analytical 2000 U UV/Visible double beam spectrophotometer at wavelength 242 nm using pH 7.4 phosphate buffer solutions as blank solution. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis which gave a straight line.

Validation of developed analytical method

This developed method, for the estimation of *in vitro* drug release from the oral controlled release dosage forms and topical preparations in pH 7.4 phosphate buffer was validated as per ICH guidelines¹³.

Linearity and range

Linearity is the ability of a method to elicit test results that are directly proportional to the analyte concentration within a given range. The range is the interval between the upper and lower levels of analytes that have been demonstrated to be determined with precision, accuracy, and linearity using the method as described. 6, 9, 12, 15 and 18 µg/ml of standard solutions was selected for assessing linearity range. The calibration curve was plotted using concentration of the standard solution versus absorbance and the regression equation was calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

Detection and quantification limits

Limit of detection (LOD) represents the lowest amount of analyte in the sample which can be detected. Limit of quantification (LOQ) represents the lowest amount of analyte, which can be quantitatively determined. LOD & LOQ are calculated based on the standard deviation of the response and the slope of the calibration curve.

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

Accuracy

Accuracy of the method was evaluated by standard addition method at 3 levels. Standard quantity equivalent to 80%, 100% and 120% is to be added in sample. The result shown that best recoveries (98.89 - 99.38%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Precision

The precision of the method was performed at three different levels: repeatability, intra-day precision and inter-day precision at different concentration levels of analyte covering the concentration range. In repeatability, the absorbance of the 12 µg/ml clobetasol propionate concentration solution was measured six times and % RSD was calculated. The intra-day precision was performed by analyzing six replicate standard solutions on the same day and inter-day precision was performed by analyzing a series of standard solutions for 3 consecutive days using the proposed UV-spectrophotometric method.

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure deliberate variations in method parameters. If measurements are susceptible to variation in analytical conditions, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. In this present work absorption maxima was decreased and increased by 1 nm and the process was carried for 12 µg/ml standard solution. The % RSD was calculated.

RESULTS AND DISCUSSION

The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the estimation of clobetasol propionate present in pH 7.4 phosphate buffer solutions. The method was validated as per ICH guidelines (Q2 (R1)).

Linearity and range

A linear correlation was found between absorbance at λ_{max} and concentration of clobetasol propionate. The graph is described by the regression equation: $Y = a + bX$ (where Y=absorbance of drug solution; a =intercept; b =slope and X=concentration of drug in µg/ml). The slope (b), intercept (a) and correlation coefficient (r) were evaluated by using the method of least squares. Good linear correlations were obtained between absorbance and standard drug concentration in the selected range of 6- 18 µg/ml. Characteristic parameters such as slope is 0.0442, Y intercept is 0.0111, and correlation coefficient is 0.9995.

Table1: Calibration curve for the estimation of clobetasol propionate in pH 7.4 Phosphate buffer solution

Concentration (µg/ml)	Absorbance (Mean±SD*)(n=6)	RSD**
0	0	0
6	0.286±0.002	0.728
9	0.415±0.002	0.482
12	0.548±0.001	0.192
15	0.674±0.003	0.453
18	0.798±0.002	0.191

SD -Standard Deviation, RSD** -Relative Standard Deviation

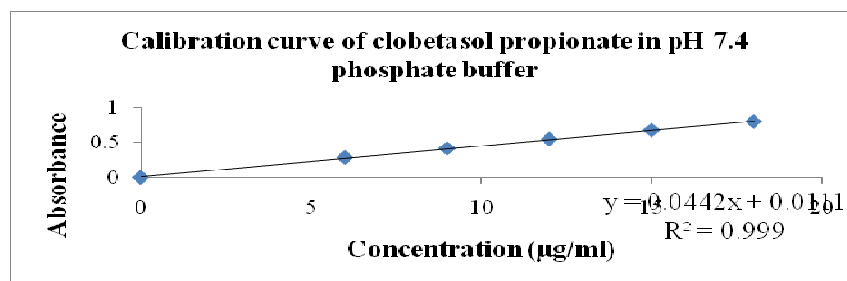


Figure 2: Calibration curve of clobetasol propionate in pH 7.4 phosphate buffer

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of clobetasol propionate were determined by using the standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The limit of detection (LOD) and limit of quantification (LOQ) for clobetasol propionate were found to be 1.42 µg/ml and 4.30 µg/ml respectively, indicating that the proposed UV method is highly sensitive. Results of regression and analytical parameters are reported in table 2.

Table 2: Regression and analytical parameters

Parameter	Value
$\lambda_{\max}$, nm	242
Beer's law limits (µg/ml)	6-18
Regression equation, Y *	$Y = 0.0442X + 0.0111$
Intercept, (a)	0.0111
Slope, (b)	0.0442
Correlation coefficient (r)	0.9995
Standard deviation of intercept	0.018917193
Limit of detection(µg/ml)	1.42
Limit of quantification (µg/ml)	4.30

* $Y=a+bX$, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in µg/ml.

Accuracy

Accuracy of an analytical method is the closeness of test results to true value. Accuracy of the method was evaluated by standard addition method at 3 levels. Standard quantity equivalent to 80%, 100% and 120% is to be added in sample. The result shown that best recoveries (98.89- 99.38%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. Results of recovery studies are reported in table 3.

Table 3: Accuracy/recovery studies for three different concentrations of clobetasol propionate by the proposed method

% Drug added	Amount of drug added to preanalysed sample (µg/ml)	Amount found (µg/ml)	% Recovery	% Average recovery(n=3), (Mean±SD*)	% RSD**
80	9.6	9.51	99.06	99.24±0.159	0.160
80	9.6	9.53	99.27		
80	9.6	9.54	99.38		
100	12	11.89	99.08	99.14±0.048	0.049
100	12	11.90	99.17		
100	12	11.90	99.17		
120	14.4	14.27	99.10	98.98±0.106	0.107
120	14.4	14.25	98.96		
120	14.4	14.24	98.89		

SD* -Standard Deviation, ** RSD-Relative Standard Deviation

Precision

% RSD for repeatability, intraday precision and interday precision were found to be 0.192, in between 0.149 & 0.351 and in between 0.747 & 1.064 respectively. The results showed an excellent repeatability, intra-

day precision and inter-day precision of the proposed method. The results obtained were presented from table 4-6.

Table 4: Repeatability studies

Concentration (µg/ml)	Absorbance	Mean ± SD* (n=6)	% RSD**
12	0.548	0.548 ± 0.001	0.192
12	0.549		
12	0.547		
12	0.547		
12	0.548		
12	0.546		

SD* -Standard Deviation, RSD -Relative Standard Deviation**

Table 5: Intra-day precision for five different concentrations of clobetasol propionate

Concentration (µg/ml)	Time			% RSD**
	10:30 a.m.	1:0 p.m.	4:30 p.m.	
6	0.286	0.285	0.284	0.351
9	0.415	0.414	0.413	0.242
12	0.548	0.547	0.546	0.183
15	0.674	0.673	0.672	0.149
18	0.798	0.796	0.794	0.251

RSD -Relative Standard Deviation**

Table 6: Inter-day precision for five different concentrations of clobetasol propionate

Concentration (µg/ml)	Day			% RSD**
	1	2	3	
6	0.285	0.282	0.279	1.064
9	0.415	0.411	0.407	0.973
12	0.548	0.543	0.538	0.921
15	0.674	0.669	0.664	0.747
18	0.798	0.792	0.786	0.758

RSD - Relative Standard Deviation**

Robustness

The robustness of this method was tested in terms of variation in wavelength change. Experimental findings proved that the change since % RSD values were found to be less than 0.4% (less than the acceptable theoretical limit of <2% RSD). The proposed UV-spectrophotometric method was found to be robust. The results obtained were presented in table 7.

Table 7: Effect of different wavelengths of light on a 12µg/ mlsolution of clobetasol propionate in pH 7.4 phosphate buffer

Wavelength (nm)	Concentration (µg/ml)	Absorbance	Mean±SD* (n=3)	%RSD**
241	12	0.544	0.544 ±0.001	0.184
		0.545		
		0.543		
242	12	0.548	0.548±0.002	0.279
		0.549		
		0.546		
243	12	0.545	0.544±0.002	0.318
		0.545		
		0.542		

SD* -Standard Deviation, RSD -Relative Standard Deviation**

CONCLUSION

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, reliable, accurate and precise. This method was validated as per ICH guidelines and can be conveniently employed for the determination of *in vitro* drug release from the oral controlled release dosage forms and topical preparations in pH 7.4 phosphate buffer. Future studies may be conducted to develop

advanced instrumental methods for the determination of clobetasol propionate present in pH 7.4 phosphate buffer which is used as a dissolution medium.

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AUTHOR CONTRIBUTIONSTATEMENT

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflict of interest.

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UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF RETINOL ACETATE PRESENT IN PH 6.8 PHOSPHATE BUFFER

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ABSTRACT

The objective of this study was to develop and validate a novel; specific, precise and simple UV-spectrophotometric method for the estimation of retinol acetate present in pH 6.8 phosphate buffer. UV-spectrophotometric determination was performed with Lab India analytical 2000 U UV/Visible double beam spectrophotometer using pH 6.8 phosphate buffers. The spectrum of the standard solution was run from 200-400 nm range for the determination of absorption maximum (λ max). λ max of retinol acetate was found at 348 nm. The absorbance of standard solutions of 6, 9, 12, 15, 18 and 21 μ g/ml of drug solution was measured at an absorption maximum (348 nm) against the blank. Calibration graph was plotted by taking concentration on X-axis and absorbance on Y-axis which gave a straight line. Validation parameters such as linearity and range, selectivity and specificity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness were evaluated as per ICH guide lines. Linearity for the UV-spectrophotometric method was noted over a concentration range of 6-21 μ g/ml with a correlation coefficient of 0.9997. The limit of detection (LOD) and limit of quantification (LOQ) for retinol acetate was found at 1.18 μ g/ml and 3.58 μ g/ml respectively. Accuracy was in between 98.68 and 99.27%. % RSD for repeatability, intraday precision and interday precision were found to be 0.319, in between 0.359 & 0.706 and in between 0.969 & 1.566 respectively. The proposed UV spectrophotometric method is found to be robust. This present work is validated according to the ICH guidelines and results & statistical parameters demonstrated that the developed method is accurate and reproducible for the determination of retinol acetate in pH 6.8 phosphate buffers. This developed method is useful for the determination of *in vitro* drug release from the oral controlled release dosage forms and topical preparations in pH 6.8 phosphate buffer.

KEYWORDS: UV spectroscopy, Validation, Analytical Method Development, Retinol acetate, pH 6.8 phosphate buffer.

INTRODUCTION

Retinol acetate is a natural form of vitamin A which is the acetate ester of retinol. The term "Vitamin A" refers to a group of substances, the retinoids, including retinol (Vitamin A1) and substances with similar structures with the biological characteristics of retinol. Retinol acetate is a pale yellow prisms or yellow supercooled melt, viscous liquid. Chemically it is all-trans-3, 7-dimethyl-9-(2, 6, 6-trimethyl-1-cyclohexen-1-yl)-2, 4, 6, 8-nonatetraene-1-yl acetate. It is soluble in most organic solvents (acetone, chloroform, ethanol, isopropanol) and in fats or oils¹.

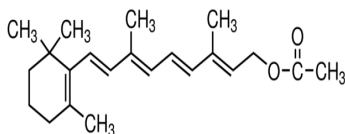


Figure 1: Chemical structure of retinol acetate

Vitamin A is used as a cosmetic ingredient at maximum use concentrations of 0.05% (retinol equivalents) in body lotions, 0.3% (retinol equivalents) in hand and faces creams. These products are usually presented as anti-wrinkle agents. Vitamin A induces biosynthesis of collagen in the skin and, at the same time, impedes the ultra violet induced synthesis of collagen-reducing enzymes. In dental products, it serves to protect the gum epithelium against marginal parodontitis². The anti-aging effect of topical retinoids is mainly linked

to the receptormediated gene activation induced by the ligand retinoic acid modulating epidermal cellproliferation and differentiation, extracellular matrix production, angiogenesis, oxidative stress and melanocyte function³⁻⁴. The literature survey reveals that various analytical methods have been developed such as HPLC⁵⁻¹¹, and UV spectroscopy methods¹²for the estimation of retinol acetate present in bulk, single or combinational pharmaceutical formulations. Most of the reported methods are using several solvents, expensive reagents and often time consuming. Because of simplicity of UV spectrophotometry and also precise, reliable, minimum solvent usage andrequires less analysis time, it is widely used for the estimation of drug content in bulk and pharmaceutical products. A detailed review of the literature regarding the existing methods revealed that there is a need for the development of the spectrophotometric method, which is simple for the estimation of retinol acetate present in pH 6.8 phosphate buffer. An attempt was made in the present method to develop asimple, sensitive, accurate, reliable and reproducible with minimum Relative Standard Deviation (RSD) values for the determination of *in vitro* drug release from oral controlled release dosage forms and topical preparationsin pH 6.8 phosphate buffers.

MATERIAL AND METHODS

Materials

All chemicals used were of analytical reagent grade. Distilled water was used to prepare solutions wherever required. Retinol acetate ispurchased fromSigma Aldrich Chemie, Steinheim, Germany.Methanol was purchased from Lobachemiepvt ltd., Mumbai, India. Potassium dihydrogenortho phosphate is from Lobachemie pvt Ltd., Mumbai, India. Na OH from Lobachemie pvt Ltd., Mumbai, India.

Equipment used

Spectroscopic analysis was carried out using Lab India analytical 2000 U UV/Visible double beam spectrophotometer with 10 mm path length quartz cells was used for the analytical purpose.

Preparation of stock and standard solution for calibration curve

For the method development, a stock solution of retinol acetate was prepared by dissolving 100 mg of the drug in 100 ml of methanol so as to obtain a final concentration of 1000 µg/ml. From the 1000 µg/ml solution, 1 ml was taken into a 10 ml volumetric flask and dissolved in pH 6.8 phosphate buffer which gives 100 µg/ml solutions. From the above 100 µg/ml solution 0.6, 0.9, 1.2, 1.5,1.8 and 2.1 ml were pipetted out into 10 ml volumetric flask and the volume was made up to the mark with pH 6.8 phosphate buffer. These dilutions gave 6, 9, 12, 15, 18 and 21 µg/ml concentration of retinol acetate. The spectrum of the standard solutions was run from 200 - 400 nm range for the determination of absorption maximum (λ max). λ max of retinol acetate was found at 348 nmusing pH 6.8 phosphate buffer solution as a blank solution. The absorbance of each standard solution was measured by using Lab India analytical 2000 U UV/Visible double beam spectrophotometer at wavelength 348 nm using pH 6.8 phosphate buffersolution as a blanksolution. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis which gave a straight line.

Validation of developed analytical method

This developed method, for the estimation of *in vitro* drug release fromthe oral controlled release dosage forms and topical preparations in pH 6.8 phosphate buffer was validated as per ICH guidelines¹³.

Linearity and range

Linearity is the ability of a method to elicit test results that are directly proportional to the analyte concentration within a given range. The range is the interval between the upper and lower levels of analytes that have been demonstrated to be determined with precision, accuracy, and linearity using the method as described. 6, 9, 12, 15 18 and 21µg/ml of standard solutions were selected for assessing linearity range. The calibration curve was plotted using concentration of the standard solution versus absorbance and the regression equation was calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

Detection and quantification limits

Limit of detection (LOD) represents the lowest amount of analyte in the sample which can be detected. Limit of quantification (LOQ) represents the lowest amount of analyte, which can be quantitatively

determined. LOD & LOQ are calculated based on the standard deviation of the response and the slope of the calibration curve.

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

Accuracy

Accuracy in which drug was evaluated by standard addition method at 3 levels. The Standard amount equivalent to 80%, 100% and 120% is to be added in sample. The obtained results shows that best recoveries (98.68 - 99.27%).

Precision

The precision of the method was performed at three different levels: repeatability, intra-day precision and inter-day precision at different concentration levels of analyte covering the concentration range. In repeatability, the absorbance of the 12 µg/ml retinol acetate concentration solution was measured six times and % RSD was calculated. Intraday precision was carried out by analysing six replicate standard solutions on same day and whereas interday precision was carried out by analysing a series of standard solutions for 3 consecutive days using the UV-spectrophotometry.

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure deliberate variations in method parameters. The estimations are susceptible to variation in analytical conditions; the analytical condition should be suitable controlled. The present work absorption maxima was decreased and increased by 1 nm and the process was carried for 12 µg/ml standard solution. The % RSD was calculated.

RESULTS AND DISCUSSION

The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the estimation of retinol acetate present in pH 6.8 phosphate buffer solutions. The method was validated as per ICH guidelines (Q2 (R1)).

Linearity and range

A linear correlation was found between absorbance at λ_{max} and concentration of retinol acetate. The graph is described by the regression equation: $Y = a + bX$ (where Y = absorbance of drug solution; a = intercept; b = slope and X = concentration of drug in µg/ml). The slope (b), intercept (a) and correlation coefficient (r) were evaluated by using the method of least squares. Good linear correlations were obtained between absorbance and standard drug concentration in the selected range of 6-21 µg/ml. Characteristic parameters such as slope is 0.0373, Y intercept is 0.0091, and correlation coefficient is 0.9997.

Table1: Calibration curve for the estimation of retinol acetate in pH 6.8 phosphate buffer solution

Concentration (µg/ml)	Absorbance (Mean±SD*)(n=6)	RSD**
0	0	0
6	0.239±0.001	0.521
9	0.350±0.002	0.472
12	0.461±0.001	0.319
15	0.567±0.002	0.300
18	0.682±0.002	0.249
21	0.786±0.001	0.159

SD* -Standard Deviation, ** RSD -Relative Standard Deviation

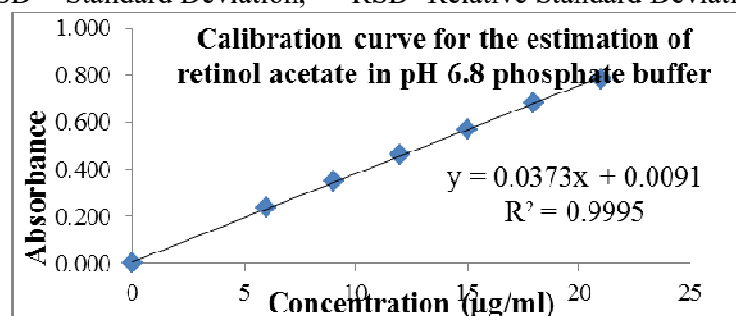


**Figure 02: Calibration curve of retinol acetate in pH 6.8 phosphate buffer
Limit of detection (LOD) and limit of quantification (LOQ)**

Limit of Detection and Limit of Quantification of Retinol acetate are determined by using the standard deviation of the response and slope as defined in International Conference on Harmonization (ICH) guidelines. The limit of detection (LOD) and limit of quantification (LOQ) for retinol acetate were found to be 1.18 µg/ml and 3.58µg/ml respectively, indicating that the proposed UV method is highly sensitive. Results of regression and analytical parameters are reported in table 2.

Table 2: Regression and analytical parameters

Parameter	Value
$\lambda_{\max}$, nm	348
Beer's law limits (µg/ml)	6-21
Regression equation, Y *	$Y = 0.0373X + 0.0091$
Intercept, (a)	0.0091
Slope, (b)	0.0373
Correlation coefficient (r)	0.9997
Standard deviation of intercept	0.013250737
Limit of detection(µg/ml)	1.18
Limit of quantification (µg/ml)	3.58

* $Y=a+bX$, where Y is the absorbance, a is the intercept,
b is the slope and X is the concentration in µg/ml.

Accuracy

Accuracy of an analytical method is the closeness of test results to true value. The accuracy of the method was evaluated by standard addition method at 3 levels. Standard quantity equivalent to 80%, 100% and 120% was added in sample. The obtained results show that recoveries (98.68 - 99.27%) of the spiked drug are obtained at each added concentration, indicating that the method was accurate. Results of recovery studies are reported in table 3.

**Table 3: Accuracy/recovery studies for three different concentrations
Of retinol acetate by the proposed method**

% Drug added	Amount of drug added to preanalysed sample (µg/ml)	Amount found (µg/ml)	% Recovery	% Average recovery (n=3), (Mean±SD*)	% RSD**
80	9.6	9.53	99.27	99.13±0.120	0.121
0	9.6	9.51	99.06		
80	9.6	9.51	99.06		
100	12	11.86	98.83	99.06±0.210	0.212
100	12	11.91	99.25		
100	12	11.89	99.08		
120	14.4	14.21	98.68	98.91±0.244	0.247
120	14.4	14.24	98.89		
120	14.4	14.28	99.17		

SD* -Standard Deviation,** RSD-Relative Standard Deviation

Precision

% RSD for repeatability, intraday precision and interday precision were found to be 0.319, in between 0.359 & 0.706 and in between 0.969 & 1.566 respectively. The results showed an excellent repeatability, intra-day precision and inter-day precision of the proposed method. The results obtained were presented from table 4-6.

Table 4: Repeatability studies

Concentration ($\mu\text{g/ml}$)	Absorbance	Mean $\pm$ SD* (n=6)	% RSD**
12	0.462	0.461 $\pm$ 0.001	0.319
12	0.459		
12	0.461		
12	0.463		
12	0.460		
12	0.462		

SD* -Standard Deviation, RSD** -Relative Standard Deviation

Table 5: Intra-day precision for five different concentrations of retinol acetate

Concentration ($\mu\text{g/ml}$)	Time			% RSD**
	10:30 a.m.	1:0 p.m.	4:30 p.m.	
6	0.239	0.238	0.236	0.706
9	0.350	0.348	0.346	0.551
12	0.461	0.458	0.456	0.588
15	0.567	0.566	0.563	0.359
18	0.682	0.678	0.674	0.588
21	0.786	0.781	0.777	0.562

RSD** - Relative Standard Deviation

Table 6: Inter-day precision for five different concentrations of retinol acetate

Concentration ($\mu\text{g/ml}$)	Day			% RSD**
	1	2	3	
6	0.239	0.235	0.232	1.566
9	0.350	0.345	0.340	1.425
12	0.461	0.454	0.450	1.263
15	0.567	0.561	0.556	0.969
18	0.682	0.672	0.664	1.338
21	0.786	0.774	0.768	1.165

RSD** - Relative Standard Deviation

Robustness

The robustness of this method was tested in terms of variation in wavelength change. Experimental findings proved that the change since % RSD values were found to be less than 0.4% (less than the acceptable theoretical limit of <2% RSD). The proposed UV-spectrophotometric method was found to be robust. The results obtained were presented in table 7.

Table 7: Effect of different wavelengths of light on a 12 $\mu\text{g/ml}$ solution of retinol acetate in pH 6.8 phosphate buffer

Wavelength (nm)	Concentration ($\mu\text{g/ml}$)	Absorbance	Mean $\pm$ SD* (n=3)	% RSD**
347	12	0.460	0.459 $\pm$ 0.002	0.377
		0.457		
		0.460		
348	12	0.463	0.462 $\pm$ 0.002	0.331
		0.460		
		0.462		

348	12	0.461	0.459±0.002	0.333
		0.458		
		0.459		

SD* -Standard Deviation, RSD** -Relative Standard Deviation

CONCLUSION

The obtained results and statistical parameters demonstrated that the proposed UV spectrophotometric method is simple, reliable, accurate and precise. This method was validated as per ICH guidelines and can be conveniently employed for the determination of *in vitro* drug release from the oral controlled release dosage forms and topical preparations in pH 6.8 phosphate buffer. Future studies may be conducted to develop advanced instrumental methods for the determination of retinol acetate present in pH 6.8 phosphate buffers which is used as a dissolution medium.

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AUTHOR CONTRIBUTION STATEMENT

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflict of interest.

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SPONTANEOUS REPORTING AND ANALYSIS OF ADVERSE DRUG REACTIONS IN A TEACHING HOSPITAL: A PHARMACOVIGILANCE STUDY

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ABSTRACT

The main purpose of the study is to assess the causality, severity, preventability of ADRs and factors associated with ADRs in chronic disease patients of tertiary care teaching hospital. A Prospective observational longitudinal study was conducted in a tertiary care teaching hospital at Hyderabad, India, for 3 years. Patients of age group > 18 years were included in the study. All the patients were distributed according to their gender, age, number medications used, disease condition, and socioeconomic state. The reported ADRs were analyzed by WHO-UMC causality, Hartwig's Siegel's scale and modified Shumock and Thornton criteria respectively. Statistical analysis used: Descriptive statistical analysis was used. A total of 691 patients enrolled in the study, in that 391 patients reported with 510 ADRs. Majority of the patients are female category (58.0%) and 45.8% of ADRs reported from adults (41-60 years). 65.8% patients are non-adherent to medication. Life style habits, economic status and education are found to be predictors for ADRs. WHO-UMC scale showed 42.9% of ADRs probable. Hartwig's and Siegel's severity scales shown 13.1 % ADRs are severe followed by 33.7% moderate ADRs and 40% of ADRs were preventable. Hence, our study advises that there is a need of improvement in ADR reporting from health care professionals. This study also suggests further research in India for the improvement of possible intervention strategies to reduce burden and cost of ADR.

KEYWORDS: *Prospective, ADR reporting, WHO-UMC, Hartwig's Siegel's scale, Shumock and Thornton.*

INTRODUCTION

According to WHO Pharmacovigilance (PV) is defined as the science and activities relating to the detection assessment, understanding and prevention of adverse effects or any other drug-related problem.¹ The objective of PvPI is, to monitor ADRs in Indian population, to create awareness amongst health care professionals about the importance of ADR reporting in India, to monitor benefit-risk profile of medicines, generate independent, evidence based recommendations on the safety of medicines, support the CDSCO for formulating safety related regulatory decisions for medicines, communicate findings with all key stake holders and create a national centre of excellence as par with global drug safety monitoring standards.² It is generally recommended to treat each chronic condition in accordance with disease-specific guidelines.³ According to estimates, India has the highest number of adults with diabetes reported at 50.8 millions in 2010 which is expected to rise to 87 million by 2030.⁴ The prevalence of diabetes has been reported to be rapidly increasing in both rural and urban India.⁵ The knowledge is needed to minimize and even avoid the potentially adverse effects of hypoglycemia and side effects associated with the anti-diabetic drugs.⁶ In the year 2000, it was also found that the world was estimated to have 1 billion people with hypertension and predicted to increase to 1.56 billion by 2025.⁷ Antihypertensive medications are frequently associated with Adverse Drug Reactions which may limit treatment options and reduce patient compliance, which may hinder Blood Pressure control. It was believed that different discontinuation rates for various classes of antihypertensive medications are probably related to their different rates of adverse symptoms.^{8,9} Treatment of TB requires the use of expensive and toxic anti-tubercular drugs which are given for a longer duration.^{10, 11} The Revised National Tuberculosis Control Program (RNTCP) in India follows the internationally recommended directly observed treatments (DOTS) guidelines for treatment TB from August 2007 onward.¹² The female gender, age (very young and very old), multiple medications and the co-morbid medical conditions, socioeconomic status, educational status and lifestyle habits are considered as the important risk factors for ADRs.¹³ People with diabetes have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases affecting the heart and blood vessels, eyes, kidneys, nerves and teeth. In addition, people with diabetes also have a higher

risk of developing infections.¹⁴ Globally 70% of diabetic patients are reported to be affected with hypertension and the risk of development of hypertension is twice for diabetic patients as compared to euglycemic subjects.¹⁵ A high prevalence of DRPs has been observed in T2DM patients.¹⁶

MATERIALS AND METHODS

Present study was carried out in Bhaskar Medical College and General Hospital, a tertiary care teaching hospital located in Hyderabad. The study assessed the causality, severity and preventability of ADRs in chronic diseases. Also, the study determined the prevalence of ADRs associated with their educational status, medication adherence, occupation and socioeconomic factors of patients.

Study Design: Prospective observational longitudinal study with active pharmacovigilance reporting system.

Study Period: The study was conducted over a period of 3 years from May 2015 to April 2018.

Ethics committee approval: The study protocol was reviewed and approved by Institutional Human Ethical Committee of Bhaskar Medical College and General Hospital authority prior to the commencement of study.

Study criteria

Inclusion Criteria: Patients of age from 18 years and both genders. Both inpatients and outpatient. Patients with any chronic disease and co-morbid medical condition

Exclusion Criteria: Children's and pregnant women. Patients receiving medicines other than allopathic. Patients who experienced adverse event to vaccines, blood and /or blood products. Adverse event to poisoning/ drug abuse and dependence

Source of Data: Patient case sheets including clinicians' admission notes and previous discharge summaries are used.

Assessment of ADR reports

Organ Systems Affected due to ADRs: International Statistical Classification of Diseases and Related Health Problems 10th Revision.

Medications Implicated in ADRs: Drugs that implicated in adverse reaction were coded using the WHO Anatomical,

Statistical Analysis: Descriptive statistics were used for data analysis. All values were expressed in percentages and depicted using tables and charts.

RESULTS & DISCUSSION

1. Study Population

691 patients met the study criteria were included in the study. Of which 37.0% (n=256) were inpatients and 62.9% (n=435) were outpatients.

2. Characteristics of the Study Population

Out of 691 study patients, 41.9% (n=290) and 58.0% (n=401) were male and female respectively.

a. Table 1 Demographic details of the study population

Characteristics		Inpatients (%) (n=256)	Outpatients (%) (n=435)	Total (%) (n=691)
Gender	Male	107 (41.7)	183 (42.0)	290 (41.9)
	Female	149 (58.2)	252 (57.9)	401 (58.0)
Age	Young Adult (19-39)	45 (17.5)	44 (10.1)	89 (12.8)
	Adult (40-60)	112 (43.7)	205 (47.1)	317 (45.8)

	Elderly (> 61)	99 (38.6)	186 (42.7)	285 (41.2)
No. of Drugs	1 – 2	116 (45.3)	202(46.4)	318 (46.0)
	3 – 4	89 (34.7)	169 (38.8)	258 (37.3)
	≥5	51 (19.9)	64 (14.7)	115 (16.6)
Medication adherence	Adherence	89 (34.7)	147 (33.7)	236 (34.1)
	Non	167 (65.2)	288 (66.2)	455 (65.8)
Social habits	Nil	39 (15.2)	80 (18.3)	119 (17.2)
	Alcoholic	57 (22.2)	110 (25.2)	167 (24.1)
	Smoker	59 (23.0)	93 (21.3)	152 (21.9)
	Alcoholic& Smoker	99 (38.6)	140 (32.1)	239 (34.5)
	Abuse	02 (0.7)	12 (2.7)	14 (2.0)
Education	Illiterate	141(55.0)	209 (48.0)	350 (50.6)
	Primary edu	66 (25.7)	102 (23.4)	168 (24.3)
	Secondary	32 (12.5)	75 (17.2)	107 (15.4)
	Pre university	13 (5.0)	34 (7.8)	47 (6.8)
	university	04 (1.5)	15 (3.4)	19 (2.7)
Occupation	Student	17 (6.6)	22 (5.0)	39 (5.6)
	Daily worker	55 (21.4)	87 (20.0)	142 (20.5)
	Homemaker	32 (12.5)	85 (19.5)	117 (16.9)
	Agriculture	62 (24.2)	77 (3.9)	139 (20.1)
	Salaried/ Busin	29 (11.3)	49 (11.2)	78 (11.2)
	Unemployed	61 (23.8)	115 (26.4)	176 (25.4)
Socioeconomic	Upper	03 (1.1)	18 (4.1)	21 (3.0)
	Upper middle	12 (4.6)	54 (12.4)	66 (9.5)
	Middle Class	51(19.9)	98 (22.5)	149 (21.5)
	Lower Middle	91 (35.5)	129 (29.6)	220 (31.8)
	Lower Class	99 (38.6)	136 (1.2)	235 (3.40)

b. Classification of disease condition according to ICD-10 Version: 2016

Out of 691 patients, 41.6% patients having endocrine, nutritional and metabolic diseases (E00-E90).

Table 2 Classification of disease condition according to ICD-10

ICD10 Chapter	Disease Condition	ICD-10 Code	Total (n=691)	Incidence (%)
I	Certain infectious and parasitic diseases	(A00-B99)	129	18.6
II	Neoplasms	(C00-D48)	1	0.1
III	Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	(D50-D89)	18	2.6
IV	Endocrine, nutritional and metabolic diseases	(E00-E90)	288	41.6
V	Mental and behavioural disorders	(F00-F99)	7	1.0
VI	Diseases of the nervous system	(G00-G99)	29	4.1
VII	Diseases of the eye and adnexa	(H00-H59)	2	0.2
VIII	Diseases of the ear and mastoid process	(H60-H95)	2	0.2
IX	Diseases of the circulatory system	(I00-I99)	44	6.3
X	Diseases of the respiratory system	(J00-J99)	22	3.1
XI	Diseases of the digestive system	(K00-K93)	23	3.3
XIII	Diseases of the musculoskeletal system and connective tissue	(M00-M99)	18	2.6
XIV	Diseases of the genitourinary system	(N00-N99)	10	1.4
XV	Pregnancy, childbirth and the puerperium	(O00-O99)	15	2.1

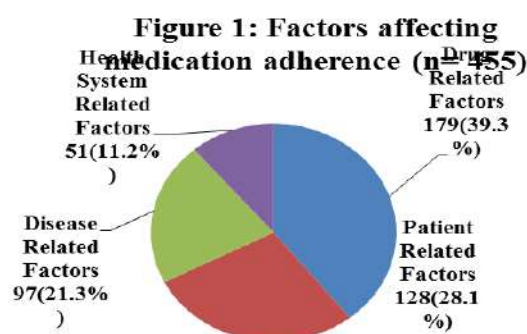
XVIII	Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	(R00-R99)	75	10.8
XIX	Injury, poisoning and certain other consequences of external causes	(S00-T98)	1	0.1
XXI	Factors influencing health status and contact with health services	(Z00-Z99)	7	1.0

3. Medication Non-Adherence

Out of 691 patients, 455 (65.8%) are non-adherent to their medication to due to following factors.

Table 3. The reasons for medication non- adherence

Factors affecting Medication adherence	Number (%) (n=455)
Drug Related Factors	179 (39.3)
Patient Related Factors	128 (28.1)
Disease Related Factors	97 (21.3)
Health System Related Factors	51 (11.2)



4. Adverse Drug Reaction

Out of 691 patients enrolled in the study, 391 patients reported with 510 ADRs during the study period.

a. Table 4. Incidence of ADRs based on patient characteristics

Characteristics	Number of patients (n=691)	Number of patients with ADR (n=391)	Incidence	Number of ADRs (n=510)	Percentage of ADRs(%)
Category					
Inpatients	256	106	41.4	143	28.0
Out patients	435	285	65.5	367	71.9
Gender					
Male	290	190	65.5	239	46.8
Female	401	201	50.1	271	53.1
Age (years)					
Young Adults	89	31	34.8	48	9.4
Adults	317	187	58.9	258	50.5
Elderly (> 61)	285	173	60.7	204	40.0
Number of Medications					
1-2	318	218	68.5	261	51.1
3-4	258	108	41.8	159	31.1
>5	115	65	56.5	90	17.6
Medication Adherence					
Adherence	236	113	47.8	169	33.1
Non Adherence	455	278	61.0	341	66.8

Disease condition ICD-10					
(A00-B99)	129	107	82.9	176	34.5
(C00-D48)	1	1	100	1	0.1
(D50-D89)	18	9	50	13	2.5
(E00-E90)	288	145	50.3	152	29.8
(F00-F99)	7	4	57.1	6	1.1
(G00-G99)	29	17	58.6	24	4.7
(H00-H59)	2	1	50	1	0.1
(H60-H95)	2	1	50	1	0.1
(I00-I99)	44	23	52.2	26	5.0
(J00-J99)	22	16	72.7	21	4.1
(K00-K93)	23	11	47.8	13	2.5
(M00-M99)	18	6	33.3	9	1.7
(N00-N99)	10	4	40	7	1.3
(O00-O99)	15	8	53.3	12	2.3
(R00-R99)	75	34	45.3	42	8.2
(S00-T98)	1	1	100	1	0.1
(Z00-Z99)	7	3	42.8	5	0.9

b. Department-wise distribution of ADRs

Table 5. Department wise distribution of ADRs

Department	No. of ADRS (n=510)	Incidence (%)
General Medicine	356	69.8
Pulmonology	78	15.2
Obstetrics and gynaecology	30	5.8
DVL (dermatology, vernerology, leprology)	16	3.1
Orthopaedics	9	1.7
Psychiatry	9	1.7
Other*	12	2.3

(* casualty, ENT, neurology & surgery)

c. Anatomical and Therapeutic Class of medication implicated in ADRs

Drug acting on alimentary tract and metabolism accounts for 34.5%.

Table 6. Anatomical and Therapeutic Class of medication implicated in ADRs

Anatomical class [Code] (Number of ADRs)	Therapeutical class [Code]	Number of ADRs n= 510(%)
Alimentary tract and metabolism [A] [n=176 (34.5)]	Drugs for acid related disorders [A02]	29
	Antiemetics and Antinauseants [A04]	23
	Drugs used in diabetes [A10]	90
	Vitamins [A11]	34
Blood and blood forming organs [B] [n=5 (0.9)]	Antithrombotic Agents [B01]	5
Cardiovascular system [C] [n=34 (6.6)]	Antihypertensive [C02]	16
	Diuretics [C03]	5
	Beta blocking agents [C07]	3
	Calcium Channel Blockers [C08]	4
	lipid modifying agents [C10]	6
Systemic hormonal preparations, corticosteroids for systemic use [H02]		27

excl. sex hormones and insulins [H] [n=31 (6.0)]	Thyroid therapy [H03]	4
Anti-infective for systemic use [J] [n=170 (33.3)]	Antimycobacterial [J04]	118
	Antibacterials for systemic use [J01]	48
	Antivirals For Systemic use [J05]	4
Musculo-Skeletal system [M] [n=19 (3.7)]	Antiinflammatory [M01]	19
Nervous system [N] [n=55 (10.7)]	Analgesic [N02]	30
	Antiepileptics [N03]	17
	Psycholeptics [N05]	4
	Other nervous system drugs [N07]	2
	Antidepressants [N06a]	2
Antiparasitic insecticides and repellents [P] [n=11 (2.1)]	product antiparasiticides, incl. Scabicides, insecticides and repellents [P03]	2
	Antiprotozoals [P01]	9
Respiratory System [R] [n=9 (1.7)]	Drugs for obstructive airway diseases [R03]	9

d. Organ system affected by ADRs.

In our study gastrointestinal system accounts for 26.2% ADRs and Skin disorders accounts for 26%, having similar reports from the study done by R.J. Lihite et al. and Rehan HS et al.¹⁹.

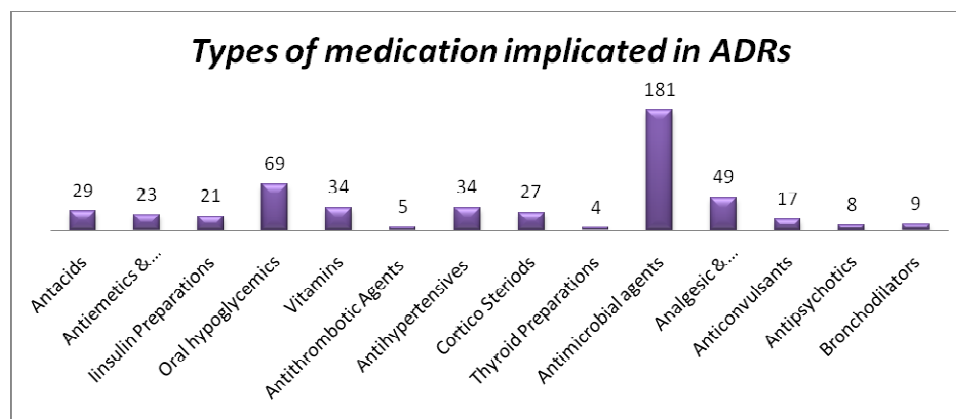
Table 7. Distribution of ADRs based on system organ class affected

System organ class (WHOART SOC code)	Number of ADRs (n=510)	Incidence (%)
Body as a whole - general disorders (1810)	59	11.5
Cardiovascular disorders (1010)	7	1.3
Central & peripheral nervous system disorders 0410	44	8.6
Gastro-intestinal system disorders (0600)	134	26.2
Hearing and vestibular disorders (0432)	01	0.1
Heart rate and rhythm disorders (1030)	4	0.7
Liver and biliary system disorders	10	1.9
Metabolic and nutritional disorders 0800	62	12.1
Musculo-skeletal system disorders 0200	12	2.3
Platelet, bleeding & clotting disorders 1230	07	1.3
Psychiatric disorders 0500	12	2.3
Red blood cell disorders (1210)	04	0.7
Respiratory system disorders 1100	09	1.7
Skin and appendages disorders (0100)	133	26.0
Urinary system disorders 1300	08	1.5
Special senses other, disorders 0433	01	0.1
Vision disorders (0431)	01	0.1
White cell disorders (1220)	02	0.3

e. Types of medication implicated in ADRs

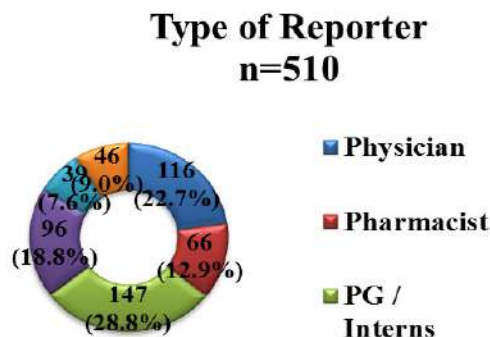
Antimicrobial agents accounts for 35.4% (n=181), which is in accordance with the result of studies done by Murphy B et. al and Lukshmy M Hettihewa et al.^{21, 22} and antidiabetic drugs accounts for 17.6% (n=90) followed by analgesic and anti-inflammatory drugs (9.6%, n= 49) of ADRs.

Figure 2: Type of medication implicated in ADRs



f. Type of Reporters

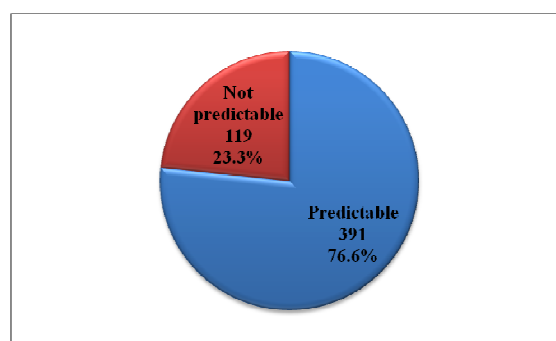
Figure 3. Type of reporters



g. Predictability of the ADRs

A total of 76.6% (n=391) of the adverse drug reactions were predictable, related with the study **Asawari L Raut et al.**²³ and 23.3% (n= 119) of the adverse drug reactions were not predictable.

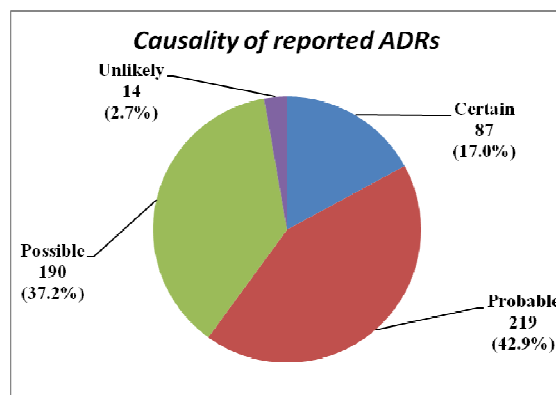
Figure 4. Predictability of the ADRs



h. Causality assessment of reported ADRs

Majority of the ADRs belonged to 'probable' in their casual relationship, as assessed by WHO probability Scale [n=219 (42.9%)], similar with study done by Rajeshreddy SGSV et al.¹⁷

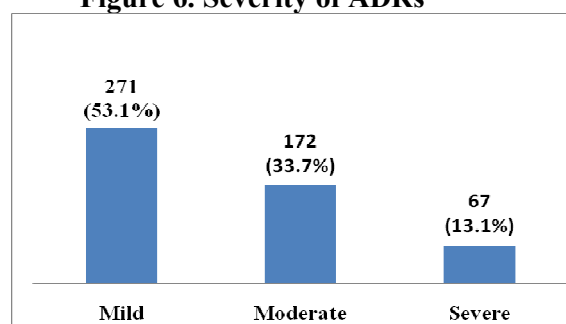
Figure 5. WHO-UMC Causality categories of reported ADRs



i. Severity assessment of ADRs

Most of the reported ADRs were of 'Mild' in their severity and hence did not require withdrawal of the suspected drug especially when the benefits outweighed the risk. This finding coincide with ponnusankar et al., Dindayal Patidar et al.^{24, 25}

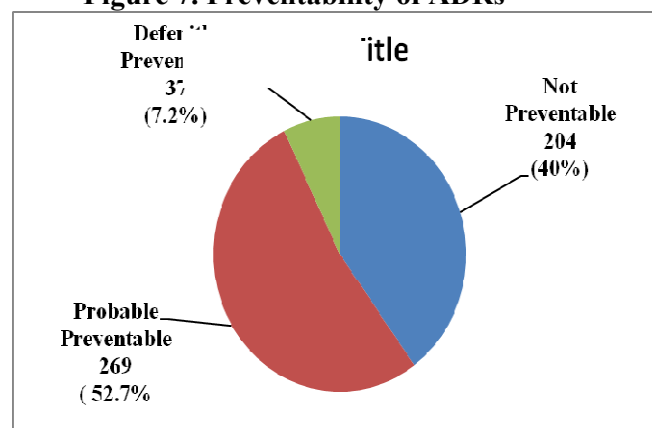
Figure 6. Severity of ADRs



j. Preventability of the ADRs

Of the 510 reported ADRs, 269 (52.7%) were classified as probable preventable, which is variance with the study done by ponnusankar *et al.*²⁴

Figure 7. Preventability of ADRs



CONCLUSION

Pharm.D interns and PG medical students as a future health-care professional should be exposed to ADR reporting during their clinical teaching posting. The present study relates to ADR profile of tubercular agents, antidiabetic drugs, cardiovascular and antibiotics, it is important to notice the physicians with latest adverse drug reactions of most commonly prescribed medicines in hospitals. Hence effective

implementation of pharmacovigilance would result in better strict vigilance use of these drugs and their safety assessment which would ultimately result in better patient care.

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ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION AND VALIDATION OF SODIUM CONTENT IN SUGAMMADEX SODIUM

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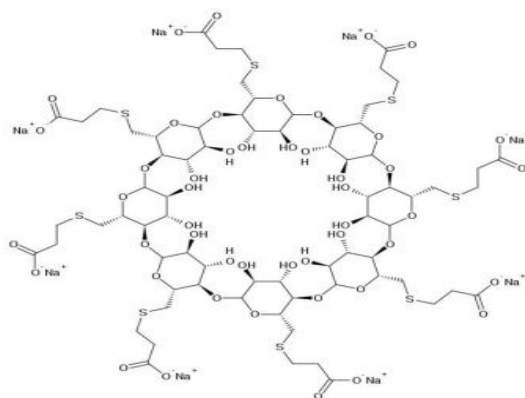
ABSTRACT

Sugammadex sodium is the generic drug name for the novel modified gamma cyclodextrin that terminates neuromuscular blockade induced by aminosteroidal neuromuscular blocking agents.¹ This paper describes the sensitive, accurate, and specific method for determination of sodium content in sugammadex sodium drug substance by using Atomic Absorption Spectroscopy method. The developed method was validated as per the ICH guidelines and USP general chapter 232 for the quantification of the sodium content in sugammadex sodium drug substance. A Suitability test was applied to various system suitability parameters and results obtained were within acceptable limits $R^2 > 0.09$. The correlation coefficient of sugammadex sodium was found to be > 0.999 . The % recovery was calculated and was found to be 99.7% to 100.4% for sodium content in sugammadex.

KEYWORDS: Sugammadex sodium, gamma-cyclodextrin, ICH guidelines, Atomic Absorption Spectroscopy, USP general chapter 232.

1. INTRODUCTION

Muscle relaxants are used during the intraoperative period to facilitate endotracheal intubation, ensure patient immobility and improve surgical exposure.² Reversal agents are used to terminating the action of muscle relaxants. The ideal reversal agents should have a fast onset, should be efficient at any time, even soon after paralyzing the patient: it should be able to provide complete reversal either for light or profound blockade; it should have a longer half-life than the muscle relaxants or it should not have any side effects. The ideal reversal agents are to be found, but sugammadex is very close to being an ideal reversal agent. Sugammadex is a synthetic cyclodextrin molecule, which acts by encapsulating of rocuronium.^{3,4,5} Sugammadex sodium is the white to off white hygroscopic powder and is chemically 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H-takes -S-(2-carboxyethyl)- 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H octathio- γ -cyclodextrin octasodium and it is freely soluble in water and insoluble in acetone nitrile and n-hexane. It acts as the neuromuscular blocking agents. There are no official and literature methods available for the sodium contents estimation in this product. Nitric acid is selected for the organic phase in the sugammadex sodium.



MATERIALS AND METHODS

Chemicals

All the chemicals used and reagents were analytical ultra-pure ICPMS. Pure standards of sodium chloride employed in the study were obtained as gift samples from Pragna generics Hyderabad. The other solvents used were nitric acid and ultrapure demineralized water from the Merck Ltd, India.

Instrumentation, digestion, and concentration optimization

For the digestion of the analytical compounds, ultra-pure mineral, the acid will be used .the mineral acid will destroy or deform all the organic contents present only the metal ions whichever will be present will leftover. The method is the line of the best fit method. Hence the concentration selections should cover 50 to 150% target concentration of sodium. The plot will draw the concentration versus standard absorbance

Instrumental Conditions

It was performed on Shimadzu Atomic Absorption Spectroscopy system equipped with Sodium Hollow Cathode lamp with the wavelength of 330nm and connected to fuel gas Acetylene /Nitrous oxide or Acetylene /Airlines with airflow of 15L/min and Acetylene flow of 1.8L/min

Blank preparation: Transferred 0.6Ml OF Nitric acid into 50Ml of volumetric flask made up to volume with demineralized water.

Sodium chloride stock solution

Accurately weighed 762mg of NaCl (M.wt-58.442), quantitatively transferred into a 200mL plastic volumetric flask, dissolve in deionized water, dilute to the mark thoroughly.

Sample preparation

Accurately weighed 0.1g of sugammadex sodium sample into a 100-Ml plastic beaker, added 12ml of nitric acid, shake well until a homogenous solution formed and made up to volume with demineralized water and mixed well.

Preparation of Linearity solutions

Pipetted out 2.0Ml, 2.3ml, 2.6ml 2.9ml and 3.2ml of sodium chloride stock solution (1000PPM) respectively into 500ml of individual plastic volumetric flask and add 0.6ml of nitric acid to volumetric flask. Made up to volume with water and mixed well.The concentration of linearity solutions are 60 µg/ml, 69µg/ml, 78µg/ml, 87µg/ml, 96µg/ml.

Bracketing method; 78µg/ml linearity solution as bracketing standard.

METHOD VALIDATION:

The proposed method was validated as per the ICH guidelines. The parameters studied for validation were system suitability, specificity, linearity, precision, accuracy, ruggedness, robustness, and solution stability.

Specificity

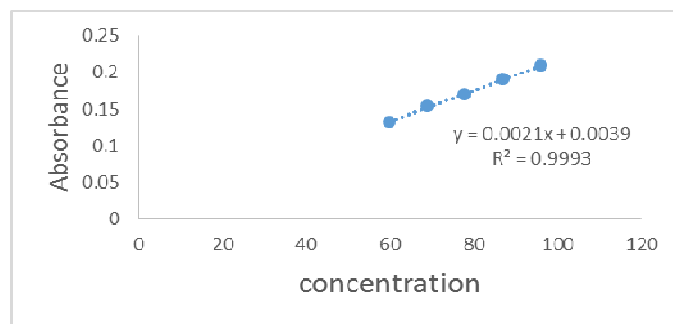
The procedure must be able to unequivocally asses each analyte element in the presence of components that may be expected to be present, including any matrix components.

Blank absorbance: Blank solution were prepared and analyzed as per the test method.

System Suitability

System suitability tests were carried out on freshly prepared linearity standard solutions from the standard stock solution of the sodium chloride. Equal volume of the standard linearity solution aspirated into the AAS flame the results obtained was used to express the system suitability of the developed method. Injected the blank (single) Lineriaty standard solution as per the method and plotted a graph between concentration and absorbance, Measure the square of the correlation coefficient.

Fig 1.0 Lineriaty of sodium content



Acceptance criteria: Square of correlation coefficient should not be less than 0.995

System precision

Injected the blank (single), linearity solutions (6 replicates of 78µg/ml solutions) as per the method. % RSD for % sodium content in six replicate injections of the standard was found to be within acceptance criteria.

Acceptance criteria: %RSD for sodium content in six replicate injection of the standard should be no more than 0.5

3.1.4 Method precision

Method precision was performed by aspirating 6 preparation of the sample. And were within the range of acceptance criteria (%RSD should be NMT 5.0) Acceptance criteria: The results should meets specification. The %RSD should be not more than 5.0%.

Intermediate precision

Intermediate precision was performed by aspirating 6 preparation of the results were in the acceptance criteria (%RSD should be NMT 5.0)

Acceptance criteria:

1. The results should meet the specification
2. The %RSD should not more than 0.5%
- 3The overall %RSD for sodium content of the method precision and intermediate precision should not be more than 5.0%.

Accuracy (recovery)

The accuracy of the method was determined by calculating recoveries of sodium content by AAS method of standard addition. A known amount of sodium content was added to a pre quantified sample solution .sodium content estimated by measuring the peak absorbance and by fitting these values to the straight-line equation of the calibration curve.

Acceptance criteria:

1. Individual recovery and mean 5 recovery of analyte should be between 95 to 105%
2. %RSD at each level for replicate test preparations should not be more than 10.0%

Robustness

Robustness was performed by altered conditions to actual conditions for sodium content was with the acceptance criteria. The % change in the results was calculated.

Acceptance criteria:

- 1.Should complies with system suitability parameters as per the test methods
- 2.The difference between the results of altered conditions to actual conditions for % sodium content NMT +-10%

Solution stability

Solution stability was determined by using the stock and standard and these were prepared and stored at room temperature and refrigerated conditions (2-8 C) for 48 hrs and percent (%) differences were calculated.

4.RESULTS AND DISCUSSION

In this Atomic absorption spectroscopic method, the condition was optimized to obtain an adequate absorption of sodium content in the Sugammadex sodium. Initially, various concentration of sodium content was tried, to get the reproducible linear graphs of standard. The developed method was validated as per the ICH guidelines and USP general chapter 232 for the quantification of sodium content in sugammadex sodium drug substance. A suitability test was applied to various systems suitability parameters and the results obtained were within acceptable limits $R^2 > 0.99$. The calibration curve was constructed with series of concentration in the range of 60-100 $\mu\text{g/ml}$. The correlation coefficient of sugammadex sodium was found to be > 0.999 . This concluded the method of linearity throughout the range selected. The precision of the method was measured in terms of repeatability, which was determined by the sufficient number of aliquots of a homogenous sample injected in AAS within the day (intraday) and next consequent three days for inter-day precision. For each case %, RSD was calculated and results were the acceptable limits. The low values of RSD indicate that the method is precise. The % recovery was calculated and was found to be 99.7% to 100.4% for sodium content in sugammadex sodium and found to be results were within acceptable limits. Hence the developed method is accurate throughout the selected range. Robustness test was carried out by small variation in the concentration of HNO_3 used for the sample digestion and % changes were calculated. The % change in the results was calculated and it was found robust as % change below 5.0% sample and standard solution are stable at ambient conditions for 36h as the % difference in the area was found to be less than 2.0%.

5.CONCLUSION

Thus the method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. Hence the developed method can be successfully applied for the estimation of sodium content in sugammadex sodium injection drug substance by AAS. Each sugammadex sodium is bonded with 8 atoms of sodium. The portion of sodium in sugammadex sodium is about 7.5 to 8.0 %. In this method, the % of sodium content is about 8.0 %. The back calculation of sugammadex with respect to sodium atoms is about 100 %. Hence each 1% of sugammadex contains about 8% of sodium atoms.

PARAMETERS	SUGAMMADEX SODIUM
Specificity (linearity solution 60 $\mu\text{g/ml}$)	0.1315 abs
System precision (%RSD)	0.46
Method precision (%RSD)	1.49
Intermediate precision (%RSD)	1.05
Robustness	8.17
Regression equation	$y = 0.0021x + 0.0039$

Tabel.1 Summary table

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BIOLOGICAL EVALUATION OF ANTI-INFECTIVE AGENTS

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ABSTRACTS

Infection: An infection is the detrimental colonization of a host organism by a foreign species. **Anti-infective:** Agents that are designed to kill, or prevent the growth of microorganisms without serious toxicity to the host. The branch of medicine that focuses on infections and pathogens is infectious disease. "When infection attacks the body, *anti-infective* drugs can help turn the tide of battle."⁽¹⁾ Anti-infective agents can be broadly classified according to the organism against which they are targeted. Effectively treating microbial infections has emerged as a great challenge to the medical fraternity especially in patients with compromised immune system brought on by cancer chemotherapy, organ transplants, surgery and afflictions with HIV/AIDS or other immune compromising events.⁽²⁾ In this study large number of compounds is synthesized and Most of the tested molecules showed bacterial growth inhibition at tested concentration. The compounds (7e, 7g, 7h, 7i and 7u) exhibited bacterial growth inhibition at concentration of less than 40.0 µg/ml against both microorganisms. Compounds (7o) showed growth of inhibition at 2.5 µg/ml against both microorganisms. Further testing for all compounds at lower concentrations is required to compare their activity with standard Streptomycin at its MIC to get exact MIC the synthesized compounds. Thus it can be concluded that designed 1, 3, 4-oxadiazole derivatives (7a-v) were synthesized successfully using designed synthetic scheme. After synthesized the molecules were characterized using spectroscopy methods and evaluated for the biological activity. All compounds exhibited antibacterial activity at tested concentration against both gram positive and gram negative organisms and one of the compound 7o exhibited activity at concentration <2.5 µg/ml. Thus, these 1, 3, 4-oxadiazole derivatives can therefore act as a potential lead for development of novel anti-infective agents.

KEYWORDS: *Detrimental, Colonization, Microorganism, Concentration, Anti-Infective Agents.*

INTRODUCTION

Anti-infective agents are substrates, inducers, and inhibitors of drug metabolism enzymes and transporters. Anti-infectives are a general term used to describe any medicine that is capable of inhibiting the spread of an infectious organism or by killing the infectious organism outright. This term encompasses antibiotics, anti-fungal, anthelmintics, anti-malarials, anti-protozoals, anti-tuberculosis agents, and anti-virals. Anti-infective An agent that is capable of acting against infection, either by inhibiting the spread of an infectious agent or by killing the infectious agent outright.

Antibacterial Agents

Antibacterial agents are used to treat infections caused by bacteria. Antibacterial agents can further be classified as antibiotics and synthetic antibacterial.³

Table 1: Major classes of synthetic antibacterial as follows⁴⁻⁷

S. No	Class	Mechanism of Action	Spectrum of Activity	Examples of Drugs	Adverse Effects
1	Sulphonamides	These are structural analogs and competitive antagonists of PABA and thus prevent normal bacterial utilization of	Sulphonamides are bacteriostatic; active against many Gram-	Sulfadiazine, Sulfisoxazole, Sulfamethoxazole, Sulfamoxole,	Crystalluria, Acute haemolytic anaemia, Agranulocytosis, Aplastic anaemia,

		PABA for the synthesis of folic acid.	+ve and Gram –ve bacteria	Sulfadoxine, etc	Hypersensitivity reactions. ⁽⁴⁾
2	Sulfones	Same as sulphonamides	Leprostatic at low conc.	Dapsone	Anaemia, Gastric intolerance, Hypersensitivity reactions. ⁽⁵⁾
3	Quinolones	Inhibits the bacterial enzyme DNA gyrase (topoisomerase II)	Highly active against enteric Gram –ve bacilli	Ciprofloxacin, Ofloxacin, Lomefloxacin, Sparfloxacin etc	GIT and CNS disturbances, Hypersensitivity reactions, Phototoxicity.
4	Oxazolidinones	Inhibition of early ribosomal protein synthesis,	<i>S. aureus</i> <i>Enterococcus faecium</i>	Linezolid (ZYVOX)	Nausea, Vomiting, Thrombocytopenia, Myelosuppression. ⁶
5	DHFR-Inhibitor	Inhibition of Bacterial enzyme of dihydrofolate reductase and Causes sequential block of bacterial folate metabolism	It has broad spectrum of antibacterial activity	Trimethoprim, Pyrimethamine	Megaloblastosis, Leucopenia, thrombocytopenia, Bone marrow toxicity in elderly. ⁷

MATERIAL AND METHODS

All synthesized molecules were evaluated for the antimicrobial activity against gram-positive and gram-negative strain using broth dilution. The anti-microbial activity of the synthesized compounds was carried out by standard procedure using broth dilution method and minimum inhibitory concentration was determined by visual comparison with the negative control tubes. ⁸

I. Antibacterial activity ⁹

The antibacterial activity would be performed by comparing of the inhibition of growth of microorganisms by measured concentration of the antibacterial agents to be examined with that produced by known concentration of a standard preparation of antibiotic having a known activity. Two general methods shall be employed; the cup-plate and turbidimetric method (broth dilution). In cylinder plate method, the sterilized agar media shall be poured into Petri-dishes and allowed to solidify. On the surface of the media, microbial suspensions shall be spread with the help of sterilized triangular loop. A stainless steel cylinder of 8mm diameter will be used to bore the cavities. All the synthesized compounds shall be placed serially diluted in the cavities with the help of micropipette and allowed to diffuse for 1 hour. These plates shall be incubated at 37°C for 24 hours and 48 hours. The zone of inhibition shall be observed around the cups after respective incubation shall be measured and % inhibition of the compounds shall be calculated.

II. Antifungal activity ¹⁰

The synthesized molecules would be tested for antifungal activity by serial dilution methods (in vitro inhibitory activity).

Procedure

Stock Solutions of Test Compounds and Standard Drug

Compounds were taken as test samples along with a standard Streptomycin sample. Weight taken in the range of 8-20 mg of each test compound and was dissolved in 1 ml of DMSO. For preparing stock solution of Streptomycin, 10 mg of Streptomycin was dissolved in 1 ml of water.

Test Organism

The organisms employed in the *in vitro* testing of the compounds were *Escherichia coli* and *S. aureus*. All the cultures were maintained on nutrient broth agar medium.

Preparation of Inoculum

Procedure for the preparation of inoculums for both the strains was same. The inoculums were prepared from a 24-hours old growth of organism on nutrient broth agar slant. To the agar slant, 10ml saline solution was added and suspension of cells was prepared. This solution was further diluted to 1 in 10 with saline.

Preparation of Medium

1.3 gms of nutrient broth was dissolved in 100 ml of sterile distilled water.

Addition of Drug, Inoculums Solution to Medium

From diluted inoculums solution prepared, 50 μ l was added to separate test tube each containing 0.9ml of medium. 50 μ l of test stock solution was added Apart from putting the controls of standard drug (Streptomycin), controls with dimethyl-sulphoxide (DMSO) was used.

RESULT AND OBSERVATIONS

At the end of incubation period, the results were interpreted by comparison with negative control. The synthesized NCEs were subjected to antimicrobial evaluation against *Escherichia coli* and *S. aureus* microorganism using broth dilution method keeping appropriate positive and negative controls simultaneously.

TABLE 3: Visual Turbidity of Evaluated Compounds Is Given In Table

Sr. No.	Mol. ID	<i>E.coli</i>	<i>S. aureus</i>	Sr. No.	Mol. ID	<i>E.coli</i>	<i>S. aureus</i>
		Visual turbidity	Visual turbidity			Visual turbidity	Visual turbidity
1	7a	–	–	13	7m	–	–
2	7b	–	–	14	7n	+	+
3	7c	–	–	15	7o	–	–
4	7d	–	–	16	7p	+	–
5	7e	–	–	17	7q	–	+
6	7f	–	–	18	7r	–	–
7	7g	–	–	19	7s	+	–
8	7h	–	–	20	7t	–	–
9	7i	–	–	21	7u	+	–
10	7j	–	–	22	7v	–	–
11	7k	+	+	23	Streptomycin	–	–
12	7l	+	–	–	Positive	++	++

=No Turbidity (No bacterial growth), + = Turbidity (Bacterial growth)

Based on the visual turbidity, the MIC of the evaluated molecules is given in Table 3 the evaluation concentration was used single therefore, the exact MIC could not determined and results are represented in less than and more than based on growth of microorganism. To get more exact MIC of the tested molecules need to be evaluated at low concentration. The evaluation results of the single concentration are tabulated in Table 4.

TABLE 4. The Evaluation Results of the Single Concentration Are Given In Table

Sr. No.	Comp-ound	<i>E. coli</i>	<i>S. aureus</i>	Sr. No.	Comp-ound	<i>E. coli</i>	<i>S. aureus</i>
		(μ g/ml)	(μ g/ml)			(μ g/ml)	(μ g/ml)

1	7a	< 60.0	< 60.0	13	7m	< 45.0	< 45.0
2	7b	< 45.0	< 45.0	14	7n	> 50.0	> 50.0
3	7c	< 50.0	< 50.0	15	7o	< 2.5	< 2.5
4	7d	< 42.5	< 42.5	16	7p	> 45.0	< 45.0
5	7e	< 35.0	< 35.0	17	7q	< 37.5	> 37.5
6	7f	< 50.0	< 50.0	18	7r	< 32.5	< 32.5
7	7g	< 32.5	< 32.5	19	7s	> 47.5	< 47.5
8	7h	< 37.5	< 50.0	20	7t	< 85.0	< 85.0
9	7i	< 37.5	< 37.5	21	7u	> 30.0	< 30.0
10	7j	< 45.0	< 45.0	22	7v	< 45.0	< 45.0
11	7k	> 75.0	> 75.0	23	Strepto- mycin	< 45.0	< 45.0
12	7l	> 45.0	< 45.0	—	—	—	—

Results of Anti-Bacterial Testing

Most of the tested molecules showed bacterial growth inhibition at tested concentration. The compounds (7e, 7g, 7h, 7i and 7u) exhibited bacterial growth inhibition at concentration of less than 40.0 µg/ml against both microorganisms. Compounds (7o) showed growth of inhibition at 2.5 µg/ml against both t microorganisms. Further testing for all compounds at lower concentrations is required to compare their activity with standard Streptomycin at its MIC to get exact MIC the synthesized compounds.

Sr. No.	Comp-ound	<i>E. coli</i>	<i>S. aureus</i>	Sr. No.	Comp-ound	<i>E. coli</i>	<i>S. aureus</i>
		(µg/ml)	(µg/ml)			(µg/ml)	(µg/ml)
1	7g	< 325	< 325	13	7s	> 475	< 475
2	7h	< 375	< 375	14	7t	< 850	< 850
3	7i	< 375	< 375	15	7u	> 300	< 300
4	7j	< 450	< 450	16	7a	< 600	< 600
5	7k	> 750	> 750	17	7b	< 450	< 450
6	7l	> 450	< 450	18	7c	< 500	< 500
7	7m	< 450	< 450	19	7d	< 425	< 425
8	7n	> 500	> 500	20	7e	< 350	< 350
9	7o	< 25	< 25	21	7f	< 500	< 500
10	7p	> 450	< 450	22	7v	< 450	< 450
11	7q	< 375	> 375	23	Strepto- mycin	< 450	< 450
12	7r	< 325	< 325	—	—	—	—

CONCLUSION

Thus it can be concluded that designed 1, 3, 4-oxadiazole derivatives (7a-v) were synthesized successfully using designed synthetic scheme. After synthesized the molecules were characterized using spectroscopy methods and evaluated for the biological activity. All compounds exhibited antibacterial activity at tested concentration against both gram positive and gram negative organisms and one of the compound 7o

exhibited activity at concentration $<2.5 \mu\text{g/ml}$. Thus, these 1, 3, 4-oxadiazole derivatives can therefore act as a potential lead for development of novel anti-infective agents.

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STANDARDIZATION: AN OVERVIEW OF PHARMACOGNOSTIC SCREENING AND PHYTOCHEMICAL SCREENING OF PLANT RAUWOLFIA SERPENTINA BENTH.

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ABSTRACT

Herbal drugs have entered the International Pharmacopoeia through the study of Ethnopharmacology and traditional medicine. The Research and Development plunge in the pharmaceutical sector is focused on progress of new innovative/indigenous plant-based drugs through exploration of leads from the established system of medicine Dr. V. E. Tyler has defined medicinal herbs as “crude drugs of vegetable beginning utilized for the treatment of disease states, often of a never-ending nature, or to attain or keep a condition of better health”. In spite of the great advances observed in current medicine in recent decades, medicinal plants have played a key role in globe health. Plant based drug can be used truthfully, i.e., they may be together, dried and used as remedial agents (crude drug), or the constituents/active morality separated by various part process, which are in employment as medicines. The active principle or compounds; that may be carbohydrate, glycosides, tannins, lipids, alkaloids, etc.; with analogous structure and activity are manufactured chemically to produce the synthetic drugs used in allopathic or contemporary system of medicine.

KEYWORDS: *Carbohydrate, Glycosides, Tannins, Lipids, Alkaloids*

1. INTRODUCTION

Herbal medicines are the oldest remedies identified to mankind. Man's belief on plants for wellbeing is as old as the survival of mankind on this planet. India is sitting on a gold mine of well-recorded and customarily well-practiced consideration of herbal medicine. There are very barely any medicinal herbs of marketable significance, which are not found in this nation. Herbal drug is peaceful the basis of about 75–80% of the earth people, principally in the increasing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects¹ (Kokate, 2000).

1.1. STANDARDIZATION

Standardization is adjusting the herbal training to a defined content of the active constituent. It refers to the process of delivering a product with a specified minimum level of one or more plant constituents.² (Mukherjee, 2002)

1.2. PLANT PROFILE³

Latin name	<i>Rauwolfia serpentina</i> Benth.
Family	Apocynaceae
Synonyms	<i>Sarpagandha</i> , <i>Snake root</i>

1.3. CHEMICAL CONSTITUENTS

Chemical constituents of *Rauwolfia serpentina* are indole alkaloids, phytosterols, fatty acids, unsaturated alcohols and sugars. These are esters derived from methyl reserpate and trimethoxy benzoic acid in the case of reserpine and trymethoxy cinnamic acid in the case of rescinnamine.

1.4. MEDICINAL AND TRADITIONAL USES

Ajmaline is a class of an anti arrhythmic agent., *Rauwolfia serpentina* alkaloids are also used as a tranquilizer and Anti hypertensives. Reserpine is used to treat high blood pressure and mental disorders including schizophrenia..

2. MATERIALS AND METHODS

2.1. Authentication

Plant was collected and the plant was authenticated from the taxonomist of Rajasthan University, Jaipur, Rajasthan.

2.2. Foreign matter analysis

2.3. Extractive Values

2.3. Successive extractive value

2.4. Loss on Drying (LOD)⁴

2.5. Ash Value

2.6. Phytochemical Screening⁵

Methanolic extract was subjected to preliminary phytochemical investigation for detection of Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Proteins and Amino acids, Saponins, Phytosterols, Acidic compounds, Resins and Reducing sugars.

2.6.1. Extract preparation:

The coarsely powder of roots of *Rauwolfia serpentina* benth were extracted with methanol. The method of preparation is described below.

2.6.1.1. Methanolic extract

2.6.1.2. Fractionation of methanolic extract:

2.6.1.3. Slurry Formation

2.6.1.4. Packing of column

2.6.1.5. Elution of the column.

2.6.2. Developing solvents used for the TLC in various proportions

1. chloroform: methanol
2. Pet ether: acetone: diethyl amine

2.6.2.1 Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

2.6.2.2 Chromatography of Basified Extract

2.6.2.3. Developing solvents used for the TLC in various proportions

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

2.6.2.4. Chromatography of Basified and centrifuged Extract⁶

Isolation of Markers

1.6.2.5. Developing solvents used for the TLC in various proportions

1.6.2.6. Visualizing agent used in TLC

2.6.2.7. Chromatography of Acid soluble Extract

Isolation of Markers

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

3. RESULTS AND DISCUSSION

In the present study, Isolation of chemical constituents and standardization of plant extracts of *Rauwolfia serpentina* was done, *Rauwolfia serpentina* is the well-known medicinal plant used from the ancient era to

till date for their medicinal values. Standardization was done on the basis of marker compounds isolated from the plant extracts. The plant was collected and analyzed.

3.1. Foreign matter analysis

Foreign matter analysis value of aerial parts powder of *Rauwolfia serpentina*

S.No.	Wt. of crude Drug (g)	Wt. of drug after removal of foreign matter (g)	Wt. of foreign matter (g)	Foreign matter (% w/w)
1.	100	99.114	0.886	0.886
2.	100	99.046	0.954	0.954
Mean				0.920

Foreign matter of aerial parts powder of *Rauwolfia serpentina* was found to be 0.920%.w/w

3.2. Loss on drying:

Loss on drying of aerial parts powder of *Rauwolfia serpentina*

S.No.	Wt. of Petri dish + Drug (Before drying) (g) A	Wt. of Petri dish + Drug (After drying) (g) B	A-B (g)	Loss on drying (% w/w)
1	18.545	18.329	0.216	10.8
2.	20.488	20.264	0.224	11.2
Mean				11.0

Moisture content of aerial parts powder of *Rauwolfia serpentina* was found to be 11.0% w/w.

3.3.Successive extractive value:

3.3.1.Petroleum ether extractive value (successive) of aerial parts of *Rauwolfia serpentina*

S.No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	□ Extractive value
1.	50.0	34.366	35.606	1.24	2.48
2.	50.0	37.484	38.644	1.16	2.32
Mean					2.40

Petroleum ether extractive value of aerial parts of *Rauwolfia serpentina*(successive extractive) was found to be 2.40%w/w

3.3.2. Ethyl acetate extractive value (successive) of aerial parts of *Rauwolfia serpentina*

S.No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	□ Extractive value
1.	50.0	34.366	35.600	1.234	2.468
2.	50.0	37.484	38.700	1.216	2.432
Mean					2.45

Ethyl acetate extractive value of aerial parts of *Rauwolfia serpentina* (successive extractive) was found to be 2.45%w/w

3.3.3. Methanolic extractive value (successive) of aerial parts of *Rauwolfia serpentine*

S.No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	□ Extractive value
1.	50.0	34.366	40.584	6.218	12.436
2.	50.0	37.484	43.766	6.282	12.564
Mean					12.5

Methanolic extractive value of aerial parts of *Rauwolfia serpentine* (Successive extractive) was found to be 12.5%w/w.

3.4. Ash values:

3.4.1. Total ash value of aerial parts of *Rauwolfia serpentine*

S.No.	Wt. of empty crucible (g)	Wt. of crucible + Wt of crude drug (before ignition) (g)	Wt. of crucible after ignition (g)	Wt. of total ash (g)	□ Total ash value
1.	26.276	28.276	26.372	0.096	4.80
2.	28.458	30.458	28.558	0.100	5.00
Mean					4.90

Total ash value of aerial parts of *Rauwolfia serpentina* was found to be 4.90%w/w

3.4.2. Acid insoluble ash value of aerial parts of *Rauwolfia serpentina*

S.No.	Wt. of drug taken (g)	Wt. of empty crucible (g)	Wt. of crucible + acid insoluble ash (g)	Wt. of acid insoluble Ash (g)	□ Acid insoluble ash value
1.	2.000	26.276	26.2893	0.0133	0.665
2.	2.000	28.458	28.4719	0.0139	0.695
Mean					0.680

Acid insoluble ash value of aerial parts of *Rauwolfia serpentina* was found to be 0.680%w/w.

3.4.3. Water soluble ash value of aerial parts of *Rauwolfia serpentina*

S.No.	Wt. of empty crucible (g)	Wt. of total ash (g)	Wt. of water insoluble ash (g)	Wt. of water soluble ash (g)	□ Water soluble ash value
1.	26.276	0.099	0.053	0.046	2.30
2.	28.458	0.097	0.047	0.050	2.50
Mean					2.40

Water soluble ash value of aerial parts of *Rauwolfia serpentina* was found to be 2.40%w/w.

3.4.4. Sulphated ash value of aerial parts of *Rauwolfia serpentina*.

S.No.	Wt. of empty crucible (g)	Wt. of crucible + Wt of crude drug (before ignition) (g)	Wt. of crucible after ignition (g)	Wt. of total ash (g)	□ Total ash value
1.	26.276	28.276	26.3344	0.0584	2.92
2.	28.458	30.458	28.5196	0.0616	3.08
Mean					3.00

Sulphated ash value of aerial parts of *Rauwolfia serpentina* was found to be 3.00%w/w

3.5. PHYTOCHEMICAL SCREENING⁶

3.5.1. Phytochemical screening of aerial parts of *Rauwolfia serpentina*

S.No.	Constituent	Observation
1.	Alkaloids	+

2.	Carbohydrates	—
3.	Glycosides	—
4.	Phenolic Compounds and Tannins	—
5.	Flavonoids	—
6.	Proteins	+
7.	Saponins	—
8.	PhytoSterol	+
9.	Amino acids	—
10.	Fixed oils	—
11.	Resin	—
12.	Reducing sugar	—

+ = Present, -- = Absent

3.5.2. ISOLATION OF PHYTOCONSTITUENTS^{7,8}

Ajmaline (RS 84- 116)

Elution of the column with chloroform: Methanol (9:1), gave cream coloured crystals of RS 84- 116, recrystallised from methanol, 6mg.

R_f: 0.6

m.p: 187⁰C

[α]_D: -3.42 (C, 0.585 in CHCl₃)

¹H NMR (CDCl₃):

M.W. :326.43



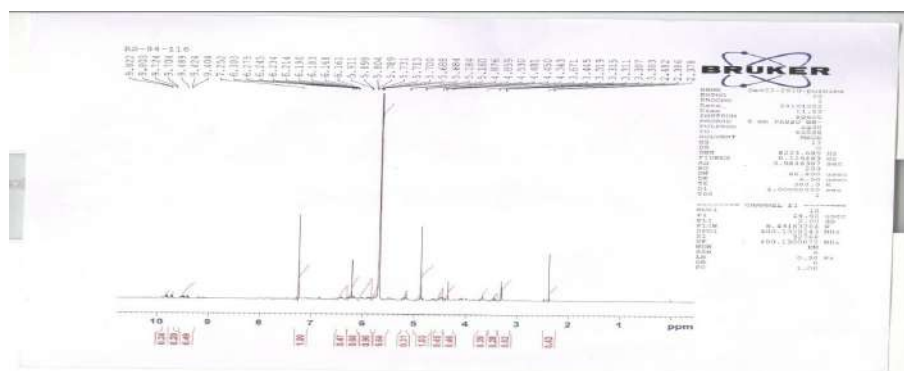
TLC Plate of Ajmaline

1. Standard

2. Mixture of standard and isolated compound

3. Isolated compound

Proton NMR spectra of Ajmaline



Beta sitosterol

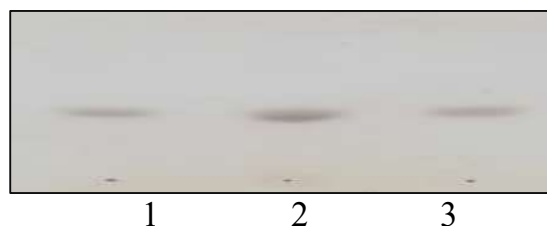
Elution of the column with chloroform: Methanol (9:1), gave cream colored crystals of RS 84- 116, recrystallised from methanol, 6mg.

R_f: 0.64

m.p: 138⁰C

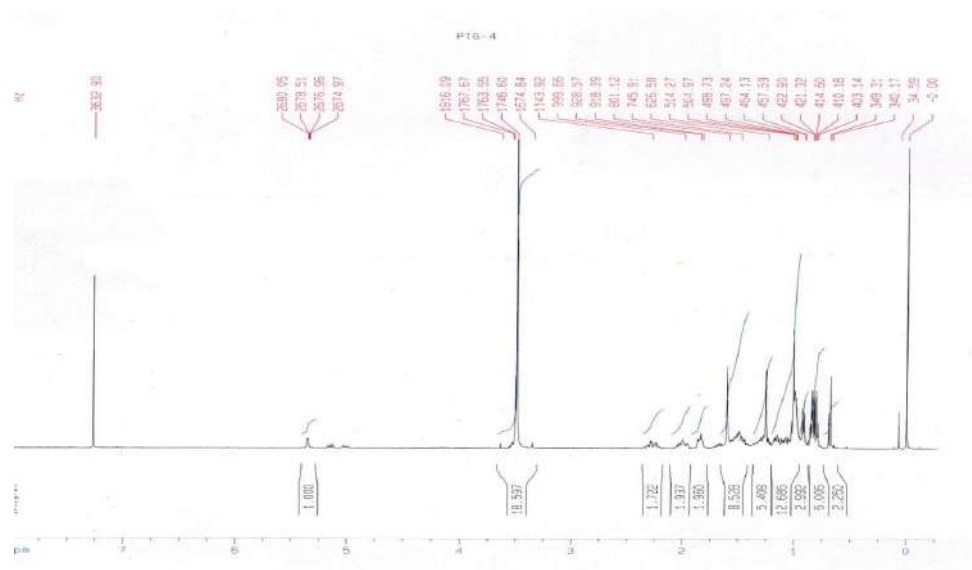
[α]_D: -3.42 (C, 0.489 in CHCl₃)

¹H NMR (CDCl₃):



TLC Plate of Sitosterol

1. Standard
2. Mixture of standard and isolated compound
3. Isolated compound



Reserpine

R_f: 0.4

m.p: 138⁰C

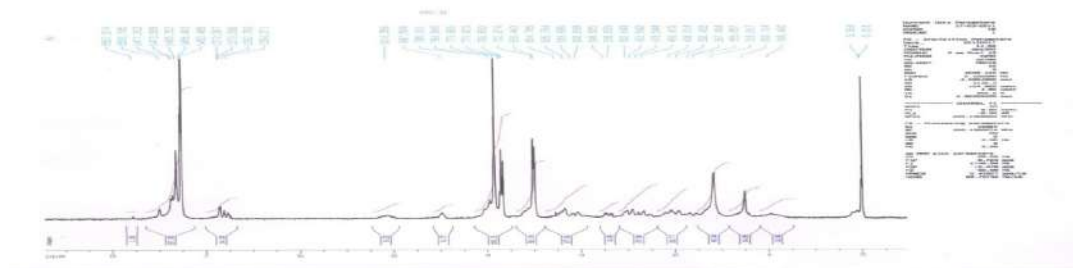
[α]_D: -3.42 (C, 0.489 in CHCl₃)

¹H NMR (CDCl₃):



TLC Plate of Reserpine

1. Standard
2. Isolated compound



Unknown

R_f: 0.43

m.p: ⁰C

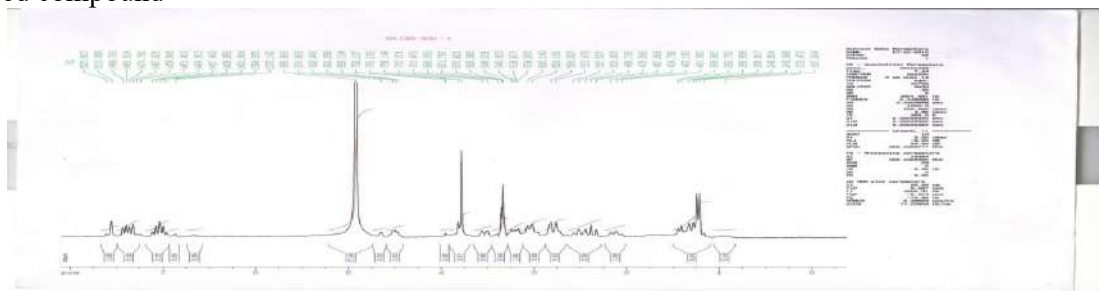
[α]_D: -3.42 (C, 0.489 in CHCl₃)

¹H NMR (CDCl₃):



1 2 3
TLC Plate of Unknown

1. Standard
2. Mixture of standard and isolated compound
3. Isolated compound



4. CONCLUSION

Thus, It can be concluded that alkaloids, protein and phytosterol are found in the plant rauwolfia serpentine 299 fraction was isolated by column chromatography in which 3 fraction was identified i.e. Beta sitasterol, Ajmaline and reserpine was found as a Phytoconstituents.

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EVALUATION OF PRESCRIBING PATTERNS OF DRUGS IN IN-PATIENT WARDS AT A TERTIARY CARE HOSPITAL

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ABSTRACT

A drug that is also called medication plays a crucial role in the treatment, prevention, and diagnosis of disease. Analyses of prescription patterns will help in the evasion of polypharmacy and improve better patient outcomes. The main objective of this study is to evaluate the prescribing patterns with respect to patient age, gender, past, and current illness along with comorbidities. To determine the patterns and patient non-compliance in order to follow the guideline of a tertiary care hospital. This is a retrospective, descriptive study in patient's ward in tertiary care multispecialty hospital which was carried out for 3 months. Several parameters were considered in evaluating the data which includes patient demographics, age, gender, comorbidities. The present study consists of sample size 1270. Out of 1270 males were high (328) in the age group of 41-60, prevalence for male is high in oncology (15.748%), the highest number of patients were observed with no social habits and patient with social habit was more in alcoholics (9.606%), number of patients were observed with one co-morbidity (30.787%), antacids were highly prescribed (26.943%), most of the medications were in oral dosage form (59.143%). Out of 9771 drugs 100 are prescribed from EDL. The average number of drugs per prescription was 7.69, only 71.5% were prescribed in generic name, percentage of antibiotics were (23.71%). The results of the study Observed that different types of prescribing errors and also patient demographics information which included gender and age were not recorded. Prescribing patterns need to be audited in order to improve the patient outcomes, safety, and efficacy of the medications and reduces the economic burden, adverse events, morbidity, and mortality.

KEYWORDS: *Prescribing pattern, WHO drug use indicators, Prescription errors, Patient safety.*

1. INTRODUCTION

The prescription has to be tailor-made for an individual patient. Prescription writing requires updated knowledge and skill. For effective treatment of a patient will depend upon the medications prescribed to the patient by the prescriber. Inappropriate prescribing may lead to morbidity, mortality and economic burden on the patients¹. Good prescribing habits will improve the effective and safe treatment, also reduces exacerbations and burden to patients. It will also improve the rational prescription patterns. The main reason behind the irrational prescribing was lack of knowledge regarding drugs and their interactions with the patient's condition.² It will lead to the development of drug interactions, bacterial resistance in case of antibiotics, adverse effects especially in elder patients because of various comorbidity conditions, use different drugs³⁻⁶ in developed and developing countries⁴. As per the World Health Organisation (WHO), more than half of irrational drug use is because of inappropriate prescribing, dispensing and selling and the remaining half is patient fails to use medications correctly⁷. The physician will prescribe appropriate medication to the patient if they have sound knowledge regarding medication and selection of individualized medication should be accurate in conditions of emergency because it will help to save the patient, if not the drug may itself leads to death of the patient⁸. People in India once they cross the age of 60 become downcast and unconfident and several alterations in the body physiology like absorption, metabolism, excretion and a proportional increase in body fat with water and muscles thereby it will cause accumulation of fat-soluble drugs by decreasing its elimination from the body. So thereby pharmacokinetics and pharmacodynamic parameters and bodyweight need to be considered before prescribing medication⁹. Paediatrics are at risk for many diseases than adults. The major factors for mortality are malnutrition and infectious disease along with diarrhoea, measles, respiratory infections, polio, pertussis, neonatal tetanus,

tuberculosis, and diphtheria. Instead of treating the condition inappropriately and reinstate polypharmacy¹⁰. In pregnancy condition several alterations in physiology will take place in the women. The appropriate medication has to be prescribed if not it will produce harm to the mother as well as infants¹¹. Irrational drug use practice is the leading problem in the health sector globally. The prescriptions have to be audited in order to reduce adverse effects and helps in the analysis of standard treatment in all health problems. This study will help to improve the prescribing patterns in tertiary care hospitals and helps to reduce the adverse effects in patients with different medical conditions and make to improve individualized prescriptions.

2. METHODOLOGY

2.1 Study design, study site, and duration of the study:

The study was conducted as a retrospective cross-sectional study in the in-patient medical wards of our tertiary care hospital in Andhra Pradesh, for period of 3 months.

2.2. Our study was followed the both criteria's

2.2.1 Inclusion Criteria

- All age group patients being admitted to the study site
- Patients of both genders being admitted to the study site

2.2.2 Exclusion Criteria:

- Outpatients departments.

2.3 Study material

All the relevant data were collected from patient records. (Medical record department)

2.4 Study procedure:

The data collection was done through chart review methods at departments for the assessment of Prescriptions that were randomly chosen to be analyzed for the WHO prescribing indicators. Inpatient case records were reviewed and all the necessary information from the case sheets of patients was collected using a data collection form. Data were collected on the demographic details of the patient such as age, gender, diagnosis, and treatment.

3. RESULTS AND DISCUSSION

3.1 Clinical characteristics of study sample

Age(years)	Male n=626	Female n=350	Total (%)
0-20	92	36	128(10.078%)
21-40	161	100	261(20.551%)
41-60	328	212	540(42.519%)
61-80	209	111	320(25.196%)
Organic system-wise distribution	Male n=626	Female n=350	Total (%) n=976
Cardiovascular system	85	38	123(9.685%)
Respiratory system	50	25	75 (5.905%)
Renal system	98	50	148(11.653%)
Hepatic system	48	15	63(4.960%)
Endocrinology	7	9	16(1.290%)
Neurology	56	45	101(7.952%)
Oncology	116	84	200(15.748%)
Gastro enterology	106	45	151(11.889%)
No.ofcomorbidites	Male	Female	Total (%)
1	277	164	391(30.787%)
2	123	117	240(18.897%)
3	51	42	93(7.322%)
More than 3	8	7	15(1.181%)

Table: 1 shows that Clinical characteristics of study sample

Table 1 describes about the clinical characteristics of study sample. In the present study, it reveals that the highest number of the patient was observed in people with age group of 41-60 years with a percentage of 42.519% more are male than female.^(13, 20) The fact that during the study period 40-60 age group people are approached to the hospital for treatment^[16]. According to organ system wise distribution highest number of patients were suffered with oncology and gastro with a percentage of 15.748% that to males were

more. According to the prevalence of disease according to organ system-wise gastroenterology cases are more because of due sedentary lifestyle, by taking excess OTC without a doctor's prescription even for a small problem. According to no. of co morbidities highest number of patients was with 1 co morbidity of percentage 30.787% in which males were more than females. The highest number of patient's was observed without any social history, patients with social habits in that more are alcoholics (122; 9.606%).

3.2 Assessment of class of drugs

Class of drugs	No. of prescriptions	Percentage
Antibiotics	2315	23.716%
Anti-hypertensives	1175	12.037%
Anti-diabetics	658	6.741%
Anti asthmatic	638	6.536%
Hormones	142	1.454%
Antiepileptic	215	2.202%
Vitamins minerals	1472	15.080%
Antimalarials	28	0.286%
Antacids	2630	26.943%
Anticancer	431	4.415%
Anticoagulants+fibrinolytics +antihyperlipidemics	779	7.980%
antidepressants	210	2.151%
NSAIDs	1972	20.202%
TT injection	50	0.512%

Table: 2 reveal that assessment of the drugs.

The percentage of antibiotics in prescriptions in the present study found to be 23.71% also revealed by the previous studies that according to WHO guidelines 15-25% antibiotics observed in the developing countries. The physician prescribing the antibiotics to reduce the burden and resistance of the patient.¹⁰ The reason for giving prophylactic antibiotics to the patients to give fast relief from the disease¹¹. There are 3 types of indicators those prescribing indicators, patient core indicators, facility indicators. A total no of 9771 drugs prescribed to 1270 patients among them 71.5% of patients prescribed the drugs with generic names in previous studies it is reported 45.0%¹⁸. In the present study report, oral formulations (59.143%) are mostly preferred by the patient based on patient condition because most of the patients are not interested to take the parenteral due to the pain or injection site reactions and there is a chance to contaminate easily when compared to orals.¹⁸

3.3 Most commonly prescribed drugs from EDL-2019

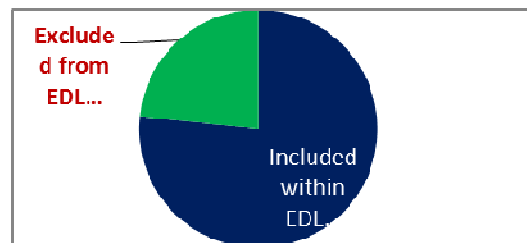


Figure 01 Reveals the most commonly prescribed drugs

In our study the percentage of the drugs that are present in the EDL list found to be 77% it is less than the previous study because of lack of awareness about WHO essential drug list so that there is a need to improve the awareness about the essential drug list through our study explain in figure 01.

3.4 Assessment of WHO drug use indicators

Drug use indicators	Findings	Optimal values
No. of prescriptions	1270	100
Total no of drugs	9771	100

Avgno.of drugs per prescription	7.69	1.6-1.8
Drugs prescribed by Generic names	71.5%	100%
Percentage of antibiotics	23.71%	20.0-26.8
Percentage of injection	40.95%	13.4-24.1
Percentage of drugs prescribed in EDL(National)	78.21%	100

Table: 3 Assessment of WHO drug use indicators.

In present study, out of 1270 prescriptions the total number of drugs found were (9771) the optimal value of the drugs (100), on an average number of the drugs per prescription (7.69) the optimal value is (1.6-1.8), and the drugs which were prescribed by generic names (71%) it was low when compared to optimal value (100 %), and average no. of drugs per prescription was (7.69) the optimal value (1.6-1.8), percentage of antibiotics were found to be (23.71%), percentage of injections prescribed (40.95%) and percentage of drugs prescribed was 78.21% from EDL. The average no of drugs per prescription¹⁴ found to be 7.69 more deviated from the optimal value, it indicates polypharmacy is also revealed by the previous studies¹¹ polypharmacy is defined as concomitant use of five or more drugs⁸. Polypharmacy indicates irrational prescribing pattern and scope for review of previous prescribing trends^{7,20}. Polypharmacy arises due to the treatment based on the symptoms rather than diagnosis⁵. The average no of drugs in the prescription is based on the no of comorbidities¹¹. WHO introduced prescribing pattern average no of drugs to prevent drug-drug interactions, to decrease the bacterial resistance and decrease hospital stay, increased prescription cost¹⁵⁻¹⁶. Polypharmacy also caused due to the pressure to prescribe the drugs by the prescriber for every symptom.¹⁷

5. CONCLUSION

One facet from our study which may be detected is that the prevalence of co-morbidities, that square measure directs consequences of multiple diseases. It absolutely was determined that almost all of the patients were diagnosed with more than one disease; they were admitted with the different types of co-morbidities. Thus, there's demand for, preventive measures, and effective informative measures within the population regarding chronic disease, and also the consequences of inappropriate management. This might cut back the associated morbidity and mortality because of unconsciousness of diseases, and additionally reduces the money burden incurred by it on the society. This study provides necessary insights into the prescribing patterns of drug use within the outpatient department of a tertiary care hospital. It's helped to spot irrational prescribing patterns of medication in medical departments. Hence, clinical caregivers should be thought-about to be an integral part of the multidisciplinary health care team. They ought to be concerned in assortment and presentation of prescribing information as a part of clinical audit and additionally direction of patients /caretakers. The correct designation of the unwellness and its management represent necessary aspects of patient care that is even a lot of necessary just in case of chronically ill patients.

6. ACKNOWLEDGMENT

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7. CONFLICT OF INTEREST

Authors do not have any conflict of interest

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SP-47

A CLINICAL CASE REPORT ON WILSON DISEASE

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ABSTRACT

Wilson's disease (WD) is defined as neurodegenerative disorder due to copper metabolism. The psychiatric manifestations of Wilson's disease include schizophrenia, psychosis, delusions. The purpose of this report is to present a short review of Wilson's disease which was diagnosed along with HP syndrome at Manipal hospital. Due to the lack of recognition about the Wilson's disease signs and symptoms leads to delays in diagnosis and management. The objectives of our case report to review the incorrect diagnosis of schizophrenia or schizophrenia-like syndrome in patients with WD and to detail one case of this mismanagement of the disease of 42 year old male patients by the diagnosis of Wilson's disease.

KEYWORDS: *Wilson's disease, Hepatopulmonary syndrome, copper metabolism*

INTRODUCTION

Wilson's disease is an autosomal recessive disorder of copper metabolism leading to the accumulation of copper metal in various organs and tissues in the body¹⁻². Dr. Wilson first described WD in 12 patients in 1912.³ Prevalence of WD is 1; 30,000⁴ is caused by mutation of the gene called ATP7B which encoding a copper transporting P-type ATPase which is essential for copper excretion into bile⁵. WD is more prevalent in younger population, with a mean age of 15–20 years and age of presentation, and rarely older than 40 years of age⁶. WD has a wide spectrum of presentations. Patients may present with primary hepatic or neurologic symptoms; a minority are diagnosed with family history screening⁷⁻⁸. Clinical findings include rigidity, tremor, drooling and impairment of liver function⁹. The variability of clinical picture of hepatic failure or bizarre neurological signs frequently slows down diagnosis in children⁹. In WD chronic liver disease leads to cirrhosis; it is called progressive lenticular degeneration.¹⁰ In WD hepatic, neurological, cardiac and renal damages occurred.¹⁰ The psychiatric alterations resulting from the accumulation of this copper metal in the basal ganglia are somehow less specific. Psychiatric features. Psychiatric symptoms were related to neurological rather than hepatic symptoms.¹⁰ Copper is absorbed from the stomach and duodenum, taken up by the liver, and secreted by the liver into the systemic circulation bound to ceruloplasmin¹¹. Wilson's disease having not only Parkinsonism symptoms but also other manifestations like cerebellar ataxia, epilepsy, and Young-onset dementia.¹³

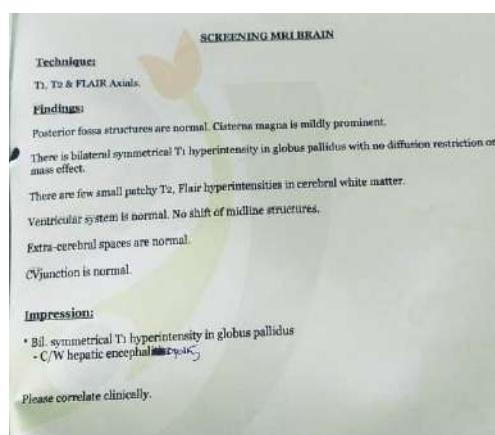
CASE PRESENTATION

A 42 yrs old male patient joined in Manipal hospital with a complaint of shortness of breath since 1month, continuous cough with sputum, altered behaviour, icterus and history of fever since 4months on and off and generalized weakness and involuntary micturition. He had history of headaches and confusion. He had past history of diabetes for 4yrs and CLD for 3yrs and history of schizophrenia for 3yrs. He had no family history. The patient's appetite was decreased, bowel irregular, micturition involuntary and disturbed sleep. The physical examination reveals that pulse 116/min, BP 110/70mmhg, pallor, icterus, and lymphadenopathy found. Systemic examination reveals all systems are normal. Figure 2 shows Endoscopic impression was portal HTN-low grade changes only, distal oesophageal ulcers-likely sources of recent GI bleed on 13/9/19. Figure 1 shows MRI brain impression was bilateral symmetrical T1 hyper intensity in Globus Pallidus on 13/8/19. 2DECHO impression was tachycardia, normal cardiac chambers, No RWMA of LV, normal LV systolic function, trivial MR/TR NO PAH, No clot/veg effusion USG impression was found to be choric liver disease mild splenomegaly mild gall bladder wall oedema mild ascites. Liver function test found to be serum creatinine 0.7mg/dl, total bilirubin 2.98 mg/dl, direct bilirubin 1.56mg/dl, indirect bilirubin 1.42mg/dl, SGOT 43 IU/L, SGPT 18IU/L, ALP 82IU/L, total protein 6.2g/dl, serum albumin 1.9g/dl, serum globulin 4.3g/dl and A/G ratio 0.44 on 15/10/19. Serum sodium 108.5mmol/L, serum potassium 4.4mmol/L, serum chloride 108.5mmol/L on 11/10/19. Thyroid values found to be T3 0.98ng/ml, T4 6.69micg/dl, and TSH 4.260mICU/ml on 9/9/19. Prothrombin time found to be 21.9sec, WBC count 5600/cumm, RBC count 3.30million/cumm, haemoglobin 11.8g/dl, PCV 35.2%, MCV 106.6fl, MCH 35.8pg, MCHC 33.6g/dl, platelet count 62000/cumm, RDW 23.9%, neutrophils 70, lymphocytes 16, monocytes 7, eosinophils 7 on 11/10/19. The diagnosis was Wilson's disease with HP syndrome. The MRI and USG investigations are shown in Fig.1

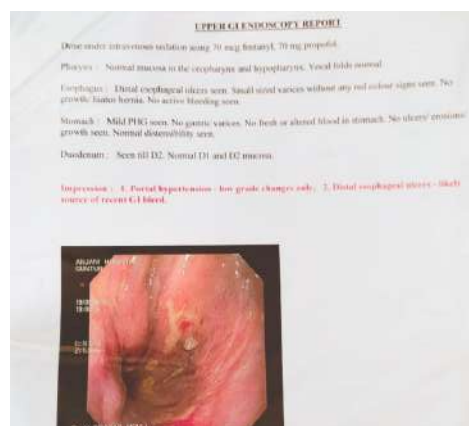
DISCUSSION

Wilson's disease is defined as a rare autosomal recessive disorder due to the excess copper accumulation. Most cases have been reported in developed countries⁶. There are three different types of Wilson disease

based on genetic variability. The juvenile type appears before 16-years of age and is predominantly a liver disease before 5 years. The saliv type appears after 16 years of age and is predominantly a neurological disease. The serum ceruloplasmin remains normal to these two types of the disease (Swaiman et al. 1982). The third type, classified as atypical Wilson disease is characterized by low serum ceruloplasmin level and clinical picture similar to those of juvenile type (Misra et al 1988, Cox et al 1972). The patient reported has atypical form of the disease with low ceruloplasmin level⁹. WD is diagnosed on the basis of several clinical and biochemical tests. The classic presentation has been characterized by age at onset between 5 and 40 years, detectable Kayser-Fleischer rings, and decreased serum ceruloplasmin.⁸ The patients with liver disease do not meet the two of the parameters in three criteria, it leads to the diagnostic unreliability.⁹ Recently, diagnostic advances have allowed for a more systematic approach to diagnosis. American Association for the Study of Liver Diseases guidelines provides a systematic approach in assisting with the diagnosis of WD⁷. In our patient, symptoms consistent with WD included cirrhosis, Schizophrenia, confusion, cough with sputum, fever, and headache. He was a known case of DM, CLD with portal hypertension. Delay in diagnosis and the start of chelation treatment adversely affect the prospects for better outcomes.¹² The standard therapy for Wilson disease is copper chelating agents (D-penicillamine or Trientine) or zinc, Dimercaprol can reduce hepatic neurologic and psychiatric findings¹⁴. Orthotropic liver transplantation is used for individuals who failed to respond to medical therapy or present with fulminant acute liver failure. The patient was a chronic schizophrenic since 15 years and using Tab. Paliperidone 3mg OD, Tab. THP (trihexyphenidyl) 2mg OD, Tab. Lorazepam 1mg SOS; after admitting hospital-physician adviced to stop Palliperidone and THP and using Inj. Lora+Serenace 1amp. As copper toxicity is due to ATB7B deficiency in Wilson disease produces liver injury So there is a need for liver transplantation, done in this patient and given supportive therapy like antibiotics such as Meropenam 500 mg.¹⁵



MRI Impression



USG Impression

Figure 1. Details of MRI and USG investigations of the patient

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MONITOR THE USE OF ANTIBIOTICS IN IN-PATIENT DEPARTMENTS AT A SOUTH INDIAN TERTIARY CARE HOSPITAL

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ABSTRACT

Antibiotics are the most commonly used medicines to treat each grievous and trivial infection. Antibiotics nowadays area unit ordinarily prescription drugs in an exceeding hospital found out. This study was undertaken to evaluate the pattern of antibiotic prescriptions in a tertiary care hospital. The main objective of this study is to promote prescribing patterns concerning patient age, gender, past, and current illness along with comorbidities. Hence this study was done to prevent polypharmacy. This is a retrospective, cross-sectional study in tertiary care multispecialty hospital, which was carried out for one year. Several parameters were considered in evaluating the data which includes patient demographics, age, gender, co-morbidities. The present study consists of sample size 1317. The highest number of patients have observed in people with an age group of 41-60 years with a percentage of 41.32% and the highest number of patients was in general medicine with a percentage of 20.86% that to males. The highest number of drugs per prescription was found to be more that is 6-10 (61.57%) and according to the classification of antibiotics, the highest frequency was found in Cephalosopriins (39.5%). In the type of formulation, most of the drugs were prescribed in oral dosage form with a percentage of 54.1% that too in males. The highest number of a patient prescribed with antacids (760: 8.32%). The results from this study say that prescribing patterns need to be audited to improve the patient outcomes, safety, efficacy of the medications and reduces the economic burden, adverse events, morbidity, and mortality.

KEYWORDS: *Antibiotic, pattern, retrospective, co-morbidities.*

1. INTRODUCTION

Antibiotics are widely used medicines to treat both life-threatening and trivial infections. Their indiscriminate use increases the risk of bacterial drug resistance. High incidences of infectious diseases, high usage of antibiotics and bacterial resistance are reported from low and middle-income countries¹. The discovery of antibiotics is a remarkable achievement of the twentieth century. Before the antibiotic era, patients who contracted common infectious diseases had significant morbidity and mortality³. Drug utilization analysis is a necessary part of Pharmacoepidemiology because it describes the extent, nature, and determinants of drug exposure. The World Health Organization (WHO) in 1997 outlined drug utilization because of the promoting, distribution, prescription and use of medication during a society, with special stress on the ensuing medical, social and economic consequences². The main aim of any DUE study is to promote rational drug use by reducing drug and health-related treatment costs, improving health-related quality of life, improving quality of medical treatment, improving coordinated healthcare, decreasing the number of medication-related problems and medication errors and improving prescriber's awareness and practice towards appropriate prescribing¹⁰. Successful use of antibiotics has brought a revolutionary change in the management of infectious diseases, but it also resulted in overuse and misuse of antibiotics. Antibiotics these days square measure ordinarily pharmaceuticals during a hospital started. Indiscriminate and inappropriate use of antibiotics not only increases treatment expenditure, causes adverse drug reaction (ADR) but also responsible for the emergence of antibiotics resistance and treatment failure. The realization of grave consequences of antibiotic resistance has forced the different stakeholders and policy makers to adopt a multi-pronged approach to combat the menace of antibiotic resistance and antibiotic stewardship constitutes an important part of that⁸. Problems with the development and spread of antibiotic resistance in hospitals have been increasing from the 1960s and are currently viewed as a major risk to clinical practice with significant mortality and healthcare costs. In this regard, many experts deemed that inappropriate and overuse of antibiotics must be reduced if we are going to lessen the prevalence of bacterial resistance [6].

Drug use pattern study from an ICU in northern India, the cost analysis of the antibiotics and the total drugs prescribed at admission revealed that patients were prescribed drugs and antibiotics value nearly Rs 2725 per patient and Rs 1995 per patient severally, antibiotic prices accounted for seventy three.2% of the entire drug expenditure and meropenem was the foremost dearliest drug ordered (accounting for 34.7% of the total antibiotic costs), while the patients spent about Rs 19,725 on total drug costs and antibiotics contributed to 51.3% of the total drug expenditure⁴. Irrational drug use practice is the leading problem in the health sector globally. The prescriptions must be audited to reduce adverse effects and helps in the analysis of standard treatment in all health problems. This study will help to improve the prescribing patterns in tertiary care hospitals and helps to reduce the adverse effects in patients with different medical conditions and make to improve individualized prescriptions.

2.METHODOLOGY

2.1 Study design, study site, and duration of the study

It is a retrospective cross-sectional study conducted at tertiary care multi-specialty hospital, Andhra Pradesh for 3 months.

2.2. Inclusion Criteria

- All age group patients being admitted to the hospital
- Patients of both genders being admitted to the hospital

2.3 Exclusion Criteria:

- Outpatients departments.
- Those do not have the antibiotics prescription

2.4 Study procedure:

The data collection was done through inpatient case records from MRD. All the necessary information from the case sheets of patients was collected using a data collection form assessment of Prescriptions that were randomly chosen to be analyzed for antibiotics patient Data were collected on the demographic details such as age, gender, diagnosis, and treatment.

2.5 Ethical Approval

The study was approved by the Institutional Ethics committee (IEC) of Nirmala College of Pharmacy, Atmakur, Guntur district, Andhra Pradesh, approved the study.

3. RESULTS

Table 3.1: Age and Gender, Department wise distribution, Number of drugs per prescription, Formulation

AGE	MALE	FEMALE	Total %
0-20	78	59	137 (12.38%)
21-40	164	115	279 (25.22%)
41-60	274	183	457 (41.32%)
61-80	165	50	215 (19.43%)
>80	10	8	18 (1.62%)

DEPARTMENT	MALE	FEMALE	(1317) %
General medicine	157	107	264 (20.86%)
Nephrology	74	41	115 (9.09%)
Gastroenterology	196	141	337 (25.96%)
Oncology	34	30	64 (5.05%)
Neurology	91	76	167 (13.2%)

Cardiology	71	76	147 (11.62%)
Ent	7	15	22 (1.73%)
Pediatrics	16	9	25 (1.18%)
Orthopedics	20	22	42 (3.32%)
Pulmonology	29	27	56 (4.42%)
Gynecology	0	12	12 (0.94%)
Endocrinology	2	1	3 (0.23%)
Plastic surgery	7	0	7 (0.55%)
Cosmetology	4	0	4 (0.31%)

NO OF DRUGS	MALE	FEMALE	TOTAL (%)
0-5	179	137	316 (25.94%)
6 - 10	430	320	750 (61.57%)
> 10	98	54	152 (12.47%)

Table:1 shows that age and gender, department wise distribution, number of drugs per prescription and formulation. It reveals that the highest number of patients were observed in people with age group of 41-60 years with a percentage of 41.32% more are male than female and the highest number of patients was in general medicine with a percentage of 20.86% that to males. The highest number of drugs per prescription were found to be more that is 6-10 (61.57%) in males and most of drugs were prescribed in oral dosage form with a percentage of 54.1% that to in males.

FORMULATION	MALE	FEMALE	N=9134(%)
Parenteral	2204	1544	3748 (45.9)
Oral	2396	1912	4308 (54.1)

TABLE 3.2: CLASSIFICATION OF ANTIBIOTICS

S.NO	Drug use indicators	Observed Values	Optimal ranges
1	Total prescriptions	1317	100
2	Avg drugs per prescription	92.4	1.6-1.8
3	Avg drugs with generic name	94.7	100
4	Drugs with Injections		13.4-24.1
5	Avg antibiotics	14.9	20.0-26.8
6	Drugs from EDL	475	100

Table:2 reveals the classification of antibiotics. The highest frequency was found in Cephalosporins (39.5%) that to in males and less frequency was found in Sulphonamides (0.6%).

Category of Antibiotics	Frequency	Percentage
BETA LACTAMS		
Penicillin's	137	10.4
Cephalosporins	521	39.5
carbapenems	52	3.9
Monobactams	11	0.8
Beta lactamase inhibitors	22	1.6
PROTEIN SYNTHESIS		
30S-Aminoglycosides	129	9.7
Tetracyclines	65	4.9
50S-Oxazolidones	53	4

Macrolides	80	6
Glycosamides	14	1
DNA TOPOISOMERASES		
Fluoroquinolones	125	9.4
FOLIC ACID SYNTHESIS		
Sulfonamides	9	0.6
DNA DAMAGE	105	7.9
m RNA SYNTHESIS	43	3.26

Table 3.4: ASSESSMENT OF CLASS OF DRUGS

S.no	Classification	Number (n=9134)	%
1	Antacids	760	8.32
2	Analgesics	605	6.62
3	Antihypertensives	535	5.85
4	Anti-inflammatory	351	3.84
5	Anti-asthmatic	332	3.63
6	Anti-histamines	305	3.33
7	Anti-diabetic	273	2.98
8	Vitamin supplements	268	2.93

Table:4 reveals that assessment of the drugs. The highest number of patients prescribed with antacids (760: 8.32%)

3. DISCUSSION

Our study reveals that the drug prescribing patterns in tertiary care hospitals do not coincide with the WHO drug prescribing pattern. Drug utilization in the hospital setting provides data to assess prescribing trends, effectiveness, and cost-effectiveness in hospital formularies. Drug utilization review used to improve the quality of the patient. This type of study is designed to review drug prescribing patterns according to the standard recommendations and guidelines for the treatment of a disease. Inappropriate antibiotic use is a major component of irrational drug prescribing in children. Our studies suggest that education about the rational use of antibiotics is required for health professionals⁸. In the present study, according to age. The highest number of patients was in general medicine⁵ with a percentage of 20.86% that to males and gender distribution 40-60 age group⁹ are mostly affected in that males are mostly affected¹¹. The highest number of drugs per prescription was found to be more that is 6-10 (61.57%) that to males were more¹²⁻¹³. According to the classification of antibiotics, the highest frequency was found in Cephalosporins (39.5%)¹⁶. In the present study report, oral formulations are mostly preferred by the patient based on patient condition because most of the patients are not interested to take the parenteral due to the pain or injection site reactions and there is a chance to contaminate easily when compared to orals. The percentage of parenteral should be less than 10% in our study percentage of parenteral found to be 45.9% the higher value leads to the rapid relief from the symptoms. In the type of formulation, most of the drugs were prescribed in oral dosage form with a percentage of 54.1% that too in males⁹. The highest number of a patient prescribed with antacids (760: 8.32%). In the present study, according to the prevalence of disease according to organ system-wise gastroenterology cases are more because of a due sedentary lifestyle, by taking excess OTC without doctor's prescription even for a small problem. There are 3 types of indicators those prescribing indicators, patient core indicators, facility indicators. A total no of 9134 drugs prescribed to 1317 patients among them 94.7 patients prescribed the drugs with generic names. Most of the drugs prescribed with generic names¹⁵ indicate rational prescribing. In previous studies the percentage of drugs that are present in the EDL list found to be 81.6%; in our study the drugs that are present in the EDL list found to be 475, it is less than the previous study because of lack of awareness about WHO essential drug list so that there is a need to improve the awareness about the essential drug list through our study. Several studies have already shown that broad-spectrum antibiotics are often indiscriminately prescribed¹⁴.

5. CONCLUSION

The study gives an overview of the prescribing pattern of antibiotics used in the study area. The study of the prescribing pattern of antibiotics seeks to monitor and evaluate if necessary and suggest modifications in prescribing patterns to make medical care rational and cost-effective. The use of antimicrobial agents, especially antibiotics has become a routine practice for the treatment of obstructive lung disease exacerbated patients.

6. ACKNOWLEDGMENT

We wish to express our gratitude to Dr. Charan Tej.K, medical superintendent, of Manipal Hospital for his assistance in collecting data.

7. CONFLICT OF INTEREST

The authors report no conflicts of interest.

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RECENT TREATMENT APPROACHES FOR MALARIA

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ABSTRACT

Malaria ranks third among the top 3 infectious diseases causing death to the tune of 243 million people, causing approximately 8,63,000 deaths annually. Most of the malarial deaths occur in children below the age of 5 years and is a major contributor to the number of deaths under the age of five. Among the five parasite species that cause malaria in humans, *P. falciparum* is most deadly and if left untreated it can progress to severe illness and death. *P. falciparum* has shown to develop resistance over the years and new anti-malarial drugs for the treatment, new treatment strategies, combination therapy and vaccine development for the same are under progress. Currently, artemisinin in combination with other anti-malarials are preferred as the first line of therapy. However, incidences of artemisinin resistance has started to appear and look out for new drug targets and strategies have gained prime importance. Gradually focus is shifting from controlling malaria to eradicating malaria; hence, more research is focused around malaria vaccine development. A lot of malaria vaccines are undergoing clinical trials and soon we can have preventive vaccines against malaria.

KEYWORDS: *Malaria, artemisinin, P.falciparum, five parasite, severe illness*

INTRODUCTION

Malaria is a life threatening disease caused by *plasmodium parasites* that are spread to people through the bites of infected *Anopheles* mosquito vector. Five species of plasmodium parasites viz. *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi* are known to cause malaria in human beings¹. Out of the five 5 parasite species that causes the malaria in humans, *P.falciparum* is most deadly. Left untreated of *P.falciparum* mediated malaria can progress to severe illness and death. Once the leading cause of the death due to infectious agents, Malaria is now ranked as the third leading cause of death due to an infectious agent after lower respiratory tract infection, diarrhoeal disease and tuberculosis. Globally, it is an estimated 3.4 billion people in 92 countries are at risk of being infected with malaria and developing disease and 11 billion are at high risk (>1 in 1000 chance of getting malaria in a year). According to the World Malaria Report 2018, there were 219 million cases of malaria globally in 2017 (uncertainly range 203-262 million) and 435000 malaria deaths, representing a malaria cases and deaths rates of 18% and 28% since 2010 respectively. The burden was heaviest in the WHO African region, where an estimated 93% of all malaria deaths occurred, and in children aged less than 5 years, who accounted for 61% of all deaths¹. Nearly 80% of global malaria deaths in 2017 were concentrated in 17 countries in the WHO African region and India; 7 of the countries accounted for 53% of all global malaria death which includes: Nigeria (19%), Democratic Republic of the Congo (11%), Burkina Faso (6%), United Republic of Tanzania (5%), Niger (4%) and India (4%). In India the majority of malaria cases are reported from eastern and central part of the country and states which have forest, hilly and tribal areas. This includes Odisha, Chhattisgarh, Jharkhand, Madhya Pradesh, Maharashtra and some north-eastern states like Tripura, Meghalaya and Mizoram. The rapid and accurate diagnosis of malaria is detrimental to provide appropriate treatment to affected individuals and in preventing the further spread of infection in the community. Following diagnosis of malaria the type of treatment depends on factors such as disease severity, the infection causing species of malaria parasite and the location from where the infection was acquired.

CURRENT TREATMENT REGIMENS²

Based on the above mentioned factors the following treatment regimen has been selected. The currently available antimalarial drugs are categorized in 3 types

1. Arylaminoalcohol compounds: Quinidine, Chloroquine, Amodiaquine, Mefloquine, and Halofantrine.
2. Antifolate compounds: Pyrimethamine, Proguanil, Chlproguanil, Trimethoprim

3. Artemisinin compounds: Arteisinin, Dihydroartemisininartemether, and Artesunate.

Atovaquone is an antimalarial in its own class with unique mode of action; combined with proguanil it is sold under the trade name Malarone³. Resistance to antimalarials is a major challenge to malaria control and there are new drug developments, new approaches to treatment strategies, combination therapy to overcome resistance and progress in vaccine development. At present, artemisinin based combination therapy is the first line therapy as the malarial parasite has developed resistance to other antimalarials.

TREATMENT OBJECTIVES²

PROPHYLAXIS: Targets the pre-erythrocytic phase

1. Primaquine
2. Proguanil

SUPPRESSIVE PROPHYLAXIS: Before travelling to an endemic area

1. Chloroquine (resistance widespread in India, no longer used as prophylactic)
2. Mefloquine 250mg 1-2 weeks before travel till 4 weeks post return
3. Doxycycline 100mg daily from day before travel to 4 weeks post return
4. Proguanil (ineffective in India so not used)

CLINICAL CURATIVE:

RADICAL CURATIVE: Targets the hypnozoites (exoerythrocytic stage)

1. Primaquine 15mg OD * 14days

GAMETOCIDAL: kills male and female gametes in patient blood

1. Primaquine single dose 45mg

ACT REGIMENS FOR UNCOMPLICATED FALCIPARUM MALARIA²

1. Artemether + Lumefantrine
Artemether 80mg BD + Lumefantrine 480mg BD * 3 days (dose reduce in children)
2. Artesunate + Mefloquine
Artesunate 100mg BD *days + Mefloquine 750mg
3. Artesunate + Amodiaquine
Artesunate 200mg + Amodiaquine 600mg * 3 days
4. Artesunate+sulphadoxine-pyrimethamine (AS+S/P)
AS 100mg BD *3 days + S 1500 mg single dose + P 75 mg single dose
5. Dihydroartemisinin – piperaquine
Dihydroartemisinin 120mg + piperaquine 960mg * 3 days

MALARIA IN PREGNANCY

Malaria during pregnancy increases the risk for adverse pregnancy outcomes including maternal anemia, prematurity, spontaneous abortion and stillbirth

FALCIPARUM MALARIA

1. Quinine 600mg TID * 7 days + clindamycin 300mg * 7 days for all trimesters
2. Artemisinin based combination therapy in 2nd 3rd trimester

VIVAX MALARIA

1. Chloroquine
2. Primaquine postponed till delivery

HISTORICAL DEVELOPMENT OF VACCINES FOR MALARIA³

The first malaria vaccine development was reported a century ago. Due to complex nature of parasites and its life cycle frivolous progress has been made. McGregor and Sydney Cohen in 1961 manifested that children could obtain protection using gamma globulin component. This vaccine was a simple component vaccine. There was certainly a need to develop multicomponent vaccine which has both cellular and humoral components glance at the complex life cycle of the parasite. The malaria vaccine technology countersigns the concept of development of multistage, multiantigen and whole organism malaria vaccine, which has resulted in the identification of a substantial number of vaccines

APPROACHES TO MALARIA VACCINE DEVELOPMENT^{3,4}**PRE-ERYTHROCYTIC STAGE VACCINE^{5,6,7,8}**

The pre-erythrocytic stage vaccine is designed to provide a level of protection that prevents any invasion of blood and hence works on clinical malaria. These are designated to target sporozoites or schizont infected liver cells and prevents the release of primary merozoites from infected hepatocytes. Vaccine candidate which has attracted many is RTS, S. This is a hybrid molecule and expressed in yeast, which consists of tandem repeat tetrapeptide ® and C – terminal T – cell epitope containing (T) regions of CSP fused to the hepatitis B surface antigen together with unfused antigen. The adjuvant ASO2 is a vital component of this vaccine. The study of RTS, S /ASO2 carried out in the children at Mozambique showed some encouraging results where the vaccine provided 30% protection against clinical malaria and more than 50% protection against severe malaria.

ASEXUAL STAGE VACCINES^{9,10}

This is another strategy in the development of malaria vaccines. Principally, it has been observed that maternal antibodies passively transferred to the fetus may provide a window of protection against clinical malaria again a majority of individuals in endemic area acquire the ability to control parasite replication after repeated attacks of malaria. Hyperimmune globulin which can be prepared from the sera of individuals chronically infected with malaria can eliminate circulating parasites from *P.falciparum* infected individuals. The main target of the current approaches for asexual stage vaccine development is merozoite, the stage that is initially released from the infected hepatocyte and rapidly invades and replicates in circulating red blood cells. Erythrocyte invasion is a rapid process and involves a number of parasite proteins that are located on the surface of the merozoites and are available to circulating antibodies. Of these antigens MSP-1, AMA-1, MSP-3 have been produced as candidate malaria vaccines and have been shown to protect nonhuman primates from uncontrolled asexual stage parasitemia with the use of complex Freund's adjuvant. Another candidate for malaria vaccines has been a surface protein, parasite present on the erythrocyte surface, such as PfEMP1 due to enormous antigenic variation these would not constitute and Var2CSA members of PfEMP1 family that are being considered for use in vaccine for pregnancy associated malaria.

TRANSMISSION BLOCKING VACCINES¹¹

In-vivo animal studies have demonstrated that it is practically possible to induce a highly effective transmission blocking activity. Many of such vaccine trails include pfs 48/45, pfs230, pfs25, pfs28 of *P.falciparum*. By using an ex vivo assay it was possible to show transmission blocking activity of sera from vaccinated humans or animals. The serum obtained from rabbits, monkeys, mice vaccinated with vaccine pfs25 from *P.falciparum* pfs 20 from *P.vivax* developed antibodies with transmission blocking activity. There was a correlation between the antibodies levels and oocyst reduction and number of mosquitoes that failed to become infected. Antibody levels remained at high levels for months after a second or third injection in mice. It was observed that the antibodies obtained from animal vaccination and phase-I clinical trials demonstrated that they could prevent the transmission of transgenic *Plasmodium bergeri* expressing the p25 antigens of either *P.falciparum* or *P.vivax*. This is an alternate approach to the membrane feeding assay for transmission blocking activity. An effective transmission reduction will be essential for eliminating malaria.

WHOLE ORGANISM VACCINE¹²**SPOROZOITES**

Studies using irradiated sporozoites have given us some valuable insights into the immune responses to the pre-erythrocytic stages of parasites and the attenuated sporozoite vaccines were found to be the most effective among malaria vaccines. The safe and protective attenuated sporozoite vaccine was first administered to humans more than 30 years ago. Reproducible data have subsequently been compiled to indicate that 92% of volunteers immunized with irradiated sporozoite were protected against parasites. Natural endemic exposure does not give rise with protective immunity.

The effectiveness of targeting liver for induction of sterile immunity has also been pursued using normal sporozoite immunizations. The generation of sporozoites has been refined, and recently it has been shown that irradiated sporozoites of *P.falciparum* can generate strong strain protection for at least 10 months in more than 90% of human recipients.

ERYTHROCYTIC STAGE

Recently it has been shown that repeated infection of human volunteers with few infected red blood cells followed by drug treatment could protect against homologous strain of *P.falciparum*. This immunity was commonly mediated through proliferative T-cell responses, nitric oxide synthase activity and interferon G production in the absence of antibodies. Advantage of this method is that immunity is generated to a range of parasite antigens in a natural setting.

CONCLUSION

Gradual development of drug resistance is one of the major hindrances for properly addressing the problem of malaria. The rate at which the drugs are developing resistance is alarming and the drug molecules are not coming out of research at the same pace. In such scenario malaria vaccines offer a better and sustainable approach for preventing the spread of Malaria. However, Malaria vaccine development has its own challenges. The biggest challenge is the complex life cycle of the malaria parasite where at each stage, the parasite transforms, its genetic profile. Stage –specific variation and large size of the parasites genome (-5500genes) have made development of vaccine more complex. An ideal malaria vaccine should be safe, highly effective and provide long term immunity besides being stable easy to administer. We hope in near future successful low expensive malaria candidate vaccine can be developed that are affordable in endemic countries

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METHOD DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

For simultaneous determination of Rosuvastatin Calcium and Fenofibrate in bulk and Pharmaceutical dosage forms an efficient and simple HPLC method has been developed. The objective of the present research work is to develop a novel, simple, accurate and precise reverse phase High Performance Liquid Chromatography (HPLC) stability indication method for rapid and simultaneous quantification of Rosuvastatin Calcium and Fenofibrate. These two drugs are separated based on HPLC separation the reverse phase mode of Luna C18 column the method is validated for parameters like accuracy, linearity, precision, ruggedness, robustness. Linearity was obtained in the concentration range of 0.5- 15 µg/ml and 8- 240 µg/ml, for Rosuvastatin Calcium and Fenofibrate. Chromatographic method is needed to develop as it is cost effective. This method is developed as it can easily analyse mixture of compound with less retention time and low solvent consumption. The proposed method is suitable for pharmaceutical analysis in analytical laboratory. This method has been successfully applied to Pharmaceutical formulations and was validated according to USP and ICH guidelines. All the parameters validated are in the acceptance range of ICH guidelines.

KEYWORDS: *Rosuvastatin calcium, Fenofibrate, HPLC, Method validation.*

INTRODUCTION

Rosuvastatin and Fenofibrate are used in treatment of hypercholesterolemia and hyperglyceridemia. Chemically it is bis [(E)-7[4-(4- fluorophenyl)-6 isopropyl-2-[methyl (methylsulphonyl)amino] pyrimidin-5-yl] (3R,5S) -3-5-dihydroxyhept-6- enoic acid]calcium salt (Fig - 1). Rosuvastatin Calcium selectively and competitively inhibits HMG-CoA reductase the rate limiting enzyme that convert 3 hydroxyl 3 methylglutaryl coenzyme A to mevalonate a precursor of cholesterol³. The typical dose of Rosuvastatin Calcium is 5 - 40 mg/day and it reduces 40 - 70% LDL levels⁴. Rat plasma is used to determine Rosuvastatin by HPLC method. Validation studies and its application to pharmacokinetics of Rosuvastatin Calcium has been reported and estimated from its formulations and biological fluids⁵⁻⁹. HPLC method is available for estimation of Rosuvastatin in an easy process. Fenofibrate, chemically propan-2-yl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanoate⁵ (Fig - 2). It is Lipid regulating drug like other fibrates reduce both low density lipoproteins and very low density lipoproteins as well as regulates triglycerides and high density lipoprotein levels⁶⁻⁷. Fenofibrate increases lipolysis and elimination of triglyceride rich particles from plasma by activating lipoproteins lipase and reducing production of apoprotein CIII³. There is a method reported for the estimation of Fenofibrate in pharmaceutical dosage forms which include HPLC method by using human plasma¹⁰.

MATERIALS AND METHODS

Apparatus

For HPLC method LC system was used consisting of pump (model shimadzu; LC-10 ATvp solvent delivery module) with Universal loop injector (Rheodyne 7725i) of injection capacity 20 microlitre detector consists of UV visible detectors SPD 10 AVP shimadzu the column used was Luna C18(5 nm, 25 cm X 4.6 mm I d) Phenomenex, USA, at ambient temperature. Chemicals and reagents: Methanol (AR grade),

Methanol (HPLC grade), water for HPLC, Acetonitrile (HPLC grade), Triethylamine (HPLC grade), Orthophosphoric acid, Pharmaceutical grade Rosuvastatin Calcium and Fenofibrate tablet of Rosuvastatin and Fenofibrate. **Drugs:** Rosuvastatin calcium, Fenofibrate.

METHODOLOGY

1. METHOD DEVELOPMENT

Method development and validation of simultaneous estimation of Rosuvastatin Calcium and Fenofibrate using RP HPLC in bulk and Pharmaceutical formulations.

Selection of wavelength: A study was performed to determine the effect of variation in temperature. Standard solution preparation as per the test method for injected into HPLC system at 35⁰C and 45⁰C temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature changes (Table-01) (Figure-03).

OPTIMIZED METHOD:

Preparation of buffer solution: 5 ml triethylamine, 1000 ml of water adjust to pH 2.5 with Orthophosphoric acid.

Mobile phase: Prepare a mixture of buffer Acetonitrile and Methanol in the ratio of (40:55:5). Filter and degas.

Chromatographic conditions:

Column	: ZODIAC C ₁₈ 150 x 4.6 mm, 5µm
Wavelength	: 243 nm
ColumnTemp	: 40 ⁰ C
Injection Volume	: 20 µl
RunTime	: 10minutes
FlowRate	: 1.5 ml/min
PumpMode	: Isocratic
RetentionTime	: About 1 to 3 minutes
Diluents	: MeOH +water

Observation: It is observed that the obtained theoretical plates, tailing, retention time and resolution was found to be within the limits. Hence proceeded to assay and validation.

Preparation of Rosuvastatin standard stock solution: Accurately about 20 mg of Rosuvastatin working standard 200 ml volumetric flask added 150 ml of valiant and volume was made up to the volume with dilute.

Preparation of Fenofibrate standard stock solution: Weight accurately about 80 mg of Fenofibrate working standard and taken into a 50 ml volumetric flask added 30 ml of diluent and sonicated to dissolve 10 cool to room temperature and volume was adjusted to 50 ml with diluent.

Preparation of standard solution: Pipette out 5 ml of standard stock solution of both Rosuvastatin and Fenofibrate into 50ml ml volumetric flask and volume was made up to mark with diluent.

Preparation of sample solution Rosuvastatin and Fenofibrate (10 mg+160 mg)

Weighed about 20 tablets and then transfer the content of 5 tablets in 250 ml volumetric flask, then added to 100 ml of the elegant sonicated for 30 minutes with intermediate shaking, cool to room temperature and made the volume with diluent, from this 5 ml of solution was taken and transferred in 200 ml volumetric flask and made up to the mark with diluent and mix. Filtrate the solution through the 0.45 micro nylon filter syringe.

Procedure injected 20µl of standard preparation 5 times and sample preparation in the chromatography record the chromatograms and measure the peak response for Rosuvastatin+ Fenofibrate the system

suitability parameters should be met from the pick response calculate the content of Rosuvastatin Calcium +Fenofibrate in the sample.

2. VALIDATION PARAMETERS OF SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE

The objective of the proposed work was to develop simultaneous methods for the estimation of Rosuvastatin and Fenofibrate. Analytical method validation is a process of performing several tests designed to verify that an analytical test method is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely. There are several parameters that are considered in the method validation process as per ICH and USP guidelines and the values for these parameters are as follows (Table-03)(Table-04).

Stability studies

Stability studies were performed as per ICH guidelines Q 1 A (R2). Stability studies define that stability of Pharmaceutical product as extent which product retains within specific limits and throughout its period of storage and use that is shelf life. The capacity of the formulation in a specific container to remain with a chemical, microbiological, theoretical, and toxicological specifications. The stability studies were performed by different type of stress conditions includes acid, alkali, peroxide, thermal, humidity, light, temp etc (Table-05,06,07,08,09)(Figure- 04,05,06).

RESULTS AND DISCUSSION

In this method for the selection of drugs Rosuvastatin Calcium and Fenofibrate the validation parameters like linearity, precision, accuracy, robustness, ruggedness, and stability studies were validated. HPLC method conditions were optimized and the separated the compounds. The accurate mobile phase must be used for the separation of the drugs. The selection of mobile phase is based on the parameters like height, tailing, theoretical plates, capacity factor, and run time. Based on these parameters Acetonitrile; Methanol; Water (50:40:10v/v) is quite robust with 0.5ml/min flow rate. The calibration curve was linear in the concentrations of 1-7 µg/ml for Rosuvastatin and 4-28 µg/ml for fenofibrate. The correlation coefficient was found to be 0.999 for both drugs. The intercept value was found to be 2009 for Rosuvastatin and 1502.1 for Fenofibrate. Sample precision were evaluated using six samples of same batch and these six separate samples were injected into the HPLC column and the peak areas were obtained and the mean and % RSD values are calculated and those are less than 2%. Accuracy was also calculated by using 3 samples of different concentrations on 3 different days and there is no difference on the assay. The proposed method shows acceptable variations of Rosuvastatin and for Fenofibrate. Robustness was determined and the %RSD was found to be in normal less than 2% flow rate, organic contents of mobile phase temperature are also established. Ruggedness was also determined by different analysts by analysis of aliquots from homogeneous lot in different laboratories by using similar operational and environmental conditions. The %RSD value was found to be less than 2%.

Table 01: Effect of variation in wavelength

System Suitability	Observed Values			Acceptance criteria
	241	243	245	
Tailing factor of ROSV peak	1.171	1.1544	1.169	NMT 2
Resolution between ROSV and FENO peak	31.524	31.524	31.525	NMT 2.5
RSD of peak areas of FENO from 5 replicate injections	0.1125	1.2479	0.3458	NMT 2

Table 02: Assay of commercial tablets

Weight (mg)	Rosuvastatin Calcium			Fenofibrate		
	Labelled amount (mg/ml)	Area count	%Assay	Labelled amount (mg/ml)	Area count	%Assay
280.8	10	362620	100.4	160	2639182	100.2
279.8	10	364717	99.8	160	2635409	99.6

Table 03: Validation report of Rosuvastatin Calcium by RP-HPLC method.

S.No	PARAMETERS			OBSERVATION	ACCEPTANCE LIMIT
1.	LINEARITY	Range($\mu\text{g/ml}$)		0.5- 15 $\mu\text{g/ml}$	
		Co-Coefficient(R)		0.999	NLT-0.999
		Slope (b)		36905	
		Intercept(a)		2009	
		Reg.Eqan($y=a+bc$)		$Y=36905x+2009$	
2.	SYSTEM PRECISION		% RSD	0.9069	% RSD NMT 2
3.	METHOD PRECISION	% RSD		1.3764	% RSD NMT 2
4.	ASSAY	% Mean Assay		100.6	95 – 105%
5.	ACCURACY	%Recovery	50%	0.4321	Recovery 98 –102 & % RSD NMT 2.0
			100%	0.3281	
			150%	0.4786	
6.	ROBUSTNESS		Flow -	1.0898	% RSD NMT 2
			Flow +	1.1076	
			Organic –	1.0283	
			Organic +	1.0323	
			λ -	1.171	
			λ +	1.1696	
			Temp –	1.1263	
			Temp +	1.1043	
7.	RUGGEDNESS	System-system		0.9069	% RSD NMT 2

Table 04: Validation report for Fenofibrate by RP-HPLC method

S.N O	PARAMETERS			OBSERVATION	ACCEPTANCE LIMIT
1.	LINEARITY	Range($\mu\text{g/ml}$)		8 – 240 $\mu\text{g/ml}$	
		Co-Coefficient(R)		0.999	NLT-0.999
		Slope (b)		16744	
		Intercept(a)		1502.1	
		Reg.Eqan($y=a+bc$)		$Y=16744x+1502$	
2.	SYSTEM PRECISION	%RSD		0.2217	%RSD NMT 2
3.	METHOD PRECISION	%RSD		1.1987	%RSD NMT 2
4.	ASSAY	%Mean Assay		100.1	95 – 105%
5.	ACCURACY	%Recovery	50%	0.4321	% Recovery 98 – 102 &% RSD NMT 2.0
			100%	0.3281	
			150%	0.4786	
6.	ROBUSTNESS		Flow -	1.0192	%RSD NMT 2
			Flow +	1.3686	
			Organic –	0.9304	
			Organic +	1.014	
			λ -	1.3176	
			λ +	1.32	

			Temp –	1.343	
			Temp +	1.355	
7.	RUGGEDNESS	System-system		0.2217	RSD NMT 2

Table: 05 – Force degradation conditions.

S.No	Stress	Condition
1	Acid	5N HCl at 60°C for 2 hours
2	Alkali	5N NaOH at 60°C for 2 hours
3	Peroxide	30% H ₂ O ₂ for 60°C for 1 hours
4	Reduction	1N sodium bisulphate for 60°C for 30 mins
5	Thermal	60°C for 1 hours
6	Photolytic	UV chamber
7	Humidity	Add water

Table 6 – Degradation conditions of FENO

S.No	Condition	% Assay	% Degradation
1	Control sample	100.6	-0.6
2	Acid	24.2	75.8
3	Base	95.5	4.5
4	Peroxide	91.8	8.2
5	Reduction	39	61
6	Thermal	103.8	-3.8
7	Photolytic	103.4	-3.4
8	Humidity	99.1	0.9

Table 7 – Degradation conditions of ROSV

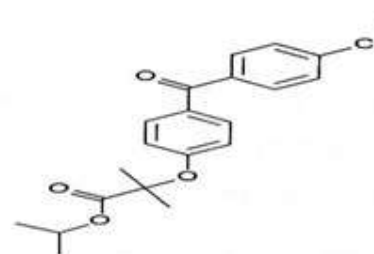
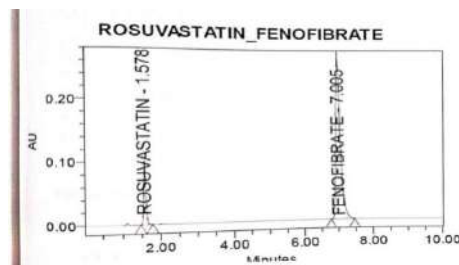
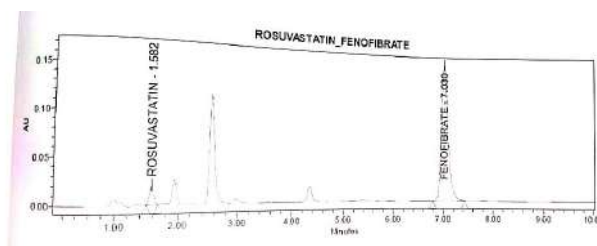
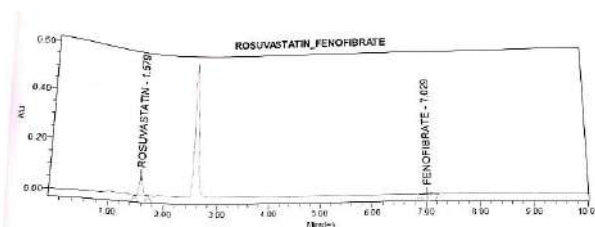
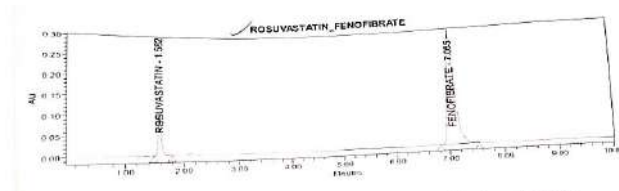
S.No	Condition	% Assay	% Degradation
1	Control sample	100.4	-----
2	Acid	57.4	42.6
3	Base	0.9	99.1
4	Peroxide	73	27
5	Reduction	87.3	12.8
6	Thermal	102.4	-2.4
7	Photolytic	101.7	-1.7
8	Humidity	97.3	2.7

Table 08: Peak Results obtained from Forced Degradation ROSV.

TYPE	AREA	USP		PURITY ANGLE	PURITY THRESHOLD	PASS/FAIL
		Count	Tailing			
Control	363669			0.29	1.117	Pass
Acid	87039	3022	1.07	---	---	Fail
Alkali	346736	3314	1.139	0.356	1.103	Fail
Peroxide	330218	3658	1.128	---	---	Fail
Reduction	140293	3276	1.127	0.524	1.234	Fail
Thermal	372274	3536	1.210	0.263	1.111	Pass
Photo	372195	3727	1.175	0.269	1.104	Pass
Humidity	355741	3782	1.145	0.307	1.1	Pass

Table 09: Peak Results obtained from Forced Degradation FENO

TYPE	RT	USP			PURITY ANGLE	PURITY THRESHOLD	PASS/ FAIL
		Area	Count	Resolution			
Control		2637296			0.123	1.014	Pass
Acid	7.030	1526025	14598	30.2208	0.056	1.019	Fail
Alkali	7.029	24404	15201	32.0185	0.756	1.904	Fail
Peroxide	7.027	1933224	14563	31.3896	0.068	1.018	Fail
Reduction	7.069	2319812	14755	31.6905	0.177	1.013	Fail
Thermal	7.065	2708422	14501	31.6905	0.196	1.013	Pass
Photo	7.057	2695040	14539	32.0015	0.178	1.012	Pass
Humidity	7.031	2590741	14674	31.8377	0.11	1.012	Pass

**Figure 01: Rosuvastatin Calcium****Figure 02: Fenofibrate****Figure 03 – Representative chromatogram for changes in wavelength****Figure 04 – Chromatogram of acid stressed sample****Figure 05 – Chromatogram of base stressed sample****Figure 06 – Chromatogram of thermally stressed sample**

CONCLUSION

Rosuvastatin Calcium and fenofibrate are quantitatively described by this method in bulk and pharmaceutical dosage formulation based on ICH guidelines. This method was accurate, simple, rapid and

selective for the estimation of drugs. This method can elute the drugs within 10 minutes. The HPLC method is simple and precise for quantification of compounds. This chromatographic method is method can be adopted for the routine quality control analysis in bulk and combined dosage forms.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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CHEMOMETRICS ASSISTED DETECTION OF ADULTERANTS IN CLARIFIED INDIAN COW GHEE USING ATR-FTIR

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ABSTRACT

Fourier Transform Infrared Spectroscopy (FTIR) is one of the most modern techniques used in the field of analytical chemistry for the identification of the constituents of the substance by determination of the functional groups. Along with the determination of active components of clarified butter (Indian cow pure ghee), the Trans fatty acids by FT-IR spectroscopy, the adulteration in ghee can also be detected by studying the spectrogram. Ghee forms as one of the major components of the diet of human beings due to its rich flavor and high nutrition. As they are high priced fat is prone to adulteration with cheaper fats. Attenuated Total Reflectance FTIR (ATR-FTIR) spectroscopy coupled with chemometrics was applied for determining the presence of goat body fat in ghee at 1%, 3%, 5%, 10%, 15% and 20% w/w levels in the laboratory made samples. The spectra of pure ghee, goat body fat and spiked samples were taken in the wavenumber range of 4000–500 cm^{-1} . The value of R^2 was found to be 0.99 for calibration and validation sets using partial least square method at all the selected wavenumber range which indicate that the model was well developed. The study revealed that the spiked samples of goat body fat could be detected even at 1% w/w level in ghee.

KEYWORDS: *Indian cow ghee, goat body fat, ATR-FTIR, principle component analysis, partial least square*

1. INTRODUCTION

Ghee is obtained by clarification of milk fat at high temperature. Ghee is almost anhydrous milk fat and there is no similar product in other countries. The composition of milk fat is unique as it contains various bioactive components like butyric acid, cis and Trans palmitoleic acid, phytanic acid, conjugated linoleic acid (CLA), alpha-linolenic acid (ALA) which is potentially identified as positive predisposing factors for human health. Ghee is reported to be the richest natural source of CLA¹. Butyric acid and linolenic acid are reported to have anti-carcinogenic properties, prevent hypertension and heart related diseases and is involved in the improvement of vision²⁻⁷. The science of chemometric solves the calibration problem for analysis of spectral data in different ways. Some are very simple to understand, while others require a strong background in linear algebra. However, they all have one thing in common: they each solve an individual problem but do not address all possible problems. The key to understand chemometrics is in not necessarily understanding the mathematics of all of the different methods; it is to know which model to use for a given analytical problem and properly applying it⁸. Unscrambler® X software version 10.2 (CAMO software AS, Nedre Vollgate 8, N-0158, Oslo, Norway) was used for all the spectra and both groups like calibration and the validations of the present study. Adulteration of ghee is more prevalent than any other fats used in food⁹. Several case studies were conducted across India for possible adulteration of ghee¹⁰⁻¹³. Hydrogenation is also performed which is again an impact on purity of ghee¹⁴. The detection of adulteration of ghee with animal body fat is one of the priority areas of research in the field of food technology as only limited methods are available for their detection. The available tests are mainly based on planar chromatography technique that involves hazardous chemicals; determination of fatty acid composition of triglycerides and 2-monoglyceride¹⁵. But, these tests suffer from repeatability problems or do not give clear cut range of determination of adulteration. The other methods are based on techniques like differential scanning calorimetry which are reported to detect the presence of foreign animal body fat in ghee only at the higher levels¹⁶⁻¹⁷. FTIR is popularly employed for determination of adulterants and fat composition in milk and milk products¹⁸⁻¹⁹ and chemometrics²⁰⁻²¹ was applied in some studies. Near infrared spectroscopy²²⁻²⁴ and ATR-FTIR²⁵⁻²⁶ were employed in some studies related to evaluation of milk, butters and margarines. There is no research found on ATR-FTIR technique for the estimation adulterants in cow butter ghee. Therefore

the present study was conducted to detect adulterants in clarified cow butter ghee using ATR-FTIR assisted with chemometrics.

2. MATERIALS AND METHODS

2.1. Collection of Samples

The cow milk was collected from locally available cow milk supplies and three branded Indian cow ghee products were purchased in the market (Durga[®], Amul[®] and Shakthi[®] manufactured in India). The goat adipose tissues were separately procured from the local slaughter house of Mangalagiri town, Guntur district, Andhra Pradesh, India.

2.2. Sample Preparation

Pure ghee from cow milk was prepared by the creaming method directly.¹⁵ The ghee gets filtered using four folds of the filter paper to get clear ghee devoid of ghee residues. The samples were stored in glass bottles under refrigerated conditions (4-6°C) until further use. Goat body fat (GBF) was prepared from the adipose tissue procured from the local slaughter house by dry rendering process at $152 \pm 2^\circ\text{C}$ till the complete extraction of fat preparation of adulterated samples, binary system comprising of PG and GBF at a level of 1%, 3%, 5%, 10%, 15% and 20% w/w to make the binary system comprising of PG and GBF. Infrared spectra were collected for each sample.

2.3. Spectra Acquisition

The fats and oils are mainly composed of triglycerides and can be directly applied in their neat form to ATR crystal. The acquired absorption spectra of the samples using Fourier transform infrared (FTIR) spectrometer at resolution of 4 cm⁻¹ and scan speed of 0.2 cm s⁻¹. Careful cleaning of IR crystal was done for each sample with isopropyl alcohol. Fat samples obtained were placed in direct contact with ZnSe crystal cell attenuated total reflectance (ATR) crystal and the reference (background) spectra using blank ATR crystal were recorded at an interval of 35 spectra of 24 scans each. The scans of each sample were maintained at $70 \pm 5^\circ\text{C}$ (to maintain the sample in completely liquid form) and were taken in the wave number of IR range (4000–500 cm⁻¹) and connected to software OPUS (7.2 Build, Bruker Optik GmbH) for the data collection.

2.4. Results Validation

The peaks and depressions of the selected spectral window (1800-1700cm⁻¹ and 1500-800cm⁻¹) for developing the model for predicting spiked GBF in PMG by PLS regression, using full cross validation option present in the Unscrambler software was performed for the developing the model for predicting spiked GBF in PMG. Based on the standard error of calibration (SEC), coefficient of determination (R^2) and standard error of prediction (SEP), the best model was selected.^{29,32}

3. RESULTS AND DISCUSSION

3.1. FTIR Spectral Analysis

Firstly, we have taken few branded ghee samples (Durga[®], Amul[®] and Shakthi[®] from India) and were examined using FTIR spectroscopy. They didn't had any adulterants based on the comparison of both reference and samples peaks. Then, we have intentionally added goat body fat in different concentrations of 1%, 3%, 5%, 10%, 15% and 20% w/w to Indian cow pure ghee. To this mixed samples, FTIR was performed by observing the differences in the peaks at different wave numbers using the chemometric study. (Figure 1)

3.2. Detection of Goat Body Fat In Indian Cow Ghee

Spectra of Indian cow ghee along with the addition of goat body fat as observed in the *Figures 2 and 3* as overlaying and overlapping of pure ghee and ghee mixed with the GBF in different concentrations (1%, 3%, 5%, 10%, 15% and 20% w/w) along with pure ghee and GBF.

3.3. Chemometric Application

Principal Component Analysis (PCA) used the combinations of absorbance at the specified wave numbers that explained most of the variance present in the data set. Selected range of wave number i.e. 1786-1680 cm⁻¹ so as to observe the samples for the control, spiked and GBF samples is represented. It can be observed from the *Figures 4-7* that PG and GBF were well separated. The results revealed R^2 closer to unity (i.e.

0.99) in all the selected spectral ranges, which further signify the better relationship between actual and FTIR predicted values of the analyte of interest (GBF in our case). Also according to International Conference on Harmonization (1994) the R^2 value higher than 0.99 is acceptable for such relationship which was observed in all the selected wavelengths. RMSEC refers to the calibration uncertainty. The difference between RMSEC and RMSEV was less, which indicates that the calibration model was accurately developed. Also, the slope was observed to be around 45, while the intercept was negligible for the scatter plots of both calibration and validation sets of sample confirming the predictability of the analyte i.e. GBF in PG (Table 1). It can also be said that all the selected wavenumber ranges ($1800-1700\text{ cm}^{-1}$ and $1500-800\text{ cm}^{-1}$) are useful for determining the presence of GBF in PG as these are associated with the specific groups present in the fat as mentioned. Infra-red spectroscopy was also used by Konevets et al.,²¹ and Gahlawat et al.,¹⁶ in the past who reported a level of detection of 10% for the animal body fats in milk fat, which is much higher than the one reported in the present study.

Table1

Effect of different spectral windows on spectral data modelling using PLS for the selected range of wave numbers for detection of adulteration of goat body fat in pure ghee

Wave number range (cm^{-1})	Calibration			Validation		
	R2	RMSEC	Bias	R2	RMSEV	Bias
1800-1700	1	2.7398	0.0	1	2.3529	-0.005
1500-800	1	1.7393	0.0	1	1.3526	-0.003

Figure 1. IR spectra of (A) Prepared pure ghee without further sample preparation and branded cowghees of (B) Durga (C) Amul and (D) Shakti

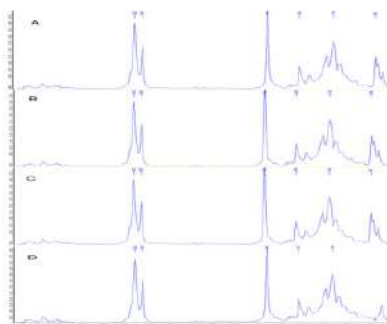


Figure 2. Overlaying Spectra of ghee along with the addition of goat body fat in the wave number range. (A) Indian cow pure ghee, (B) pure goat body fat, and (C) 1%, (D) 3%, (E) 5%, (F) 10%, (G) 15% and (H) 20% w/w of GBF

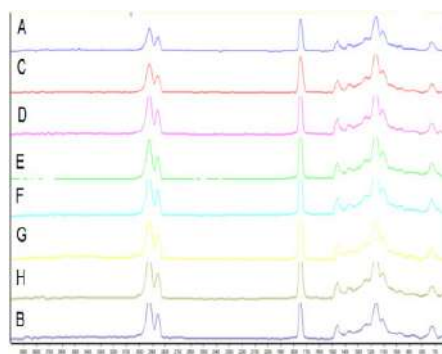


Figure 3. Spectra of ghee along with the addition of GBF in the wave number range. (1) Indian cow pure ghee, (2) pure goat body fat, and (3) 1%, (4) 3%, (5) 5%, (6) 10%, (7) 15% and (8) 20% w/w of GBF in the wave number range (A) $1800-1700\text{ cm}^{-1}$ and (B) $1500-800\text{ cm}^{-1}$ Indian cow pure ghee and GBF

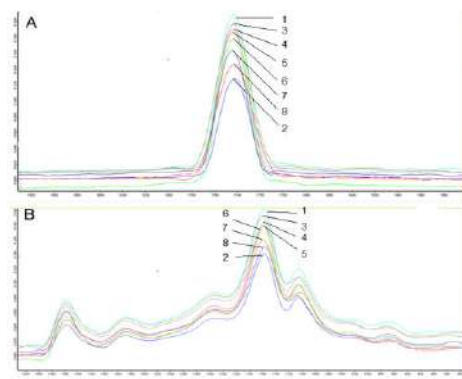


Figure 3 & 4. Principle Component Scores of PG, GBF & Ghee with different levels of GBF with wave numbers

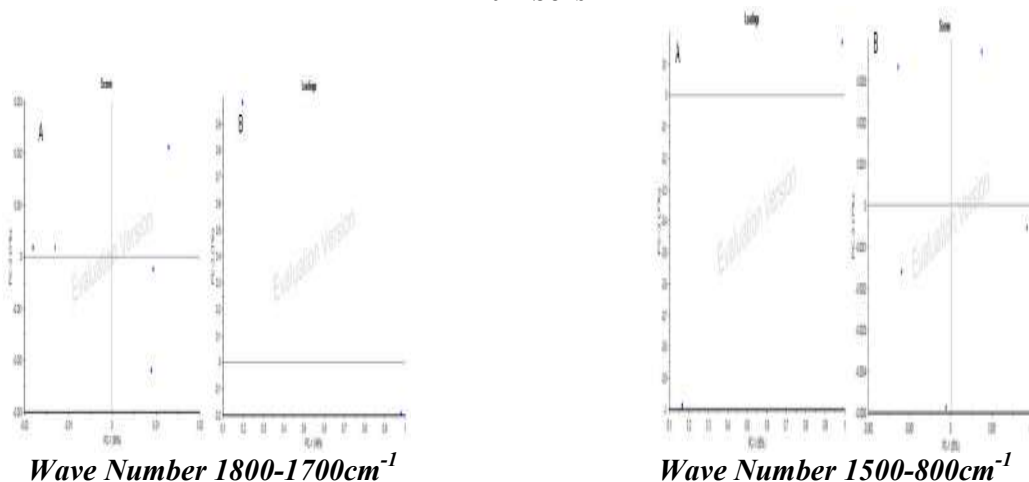
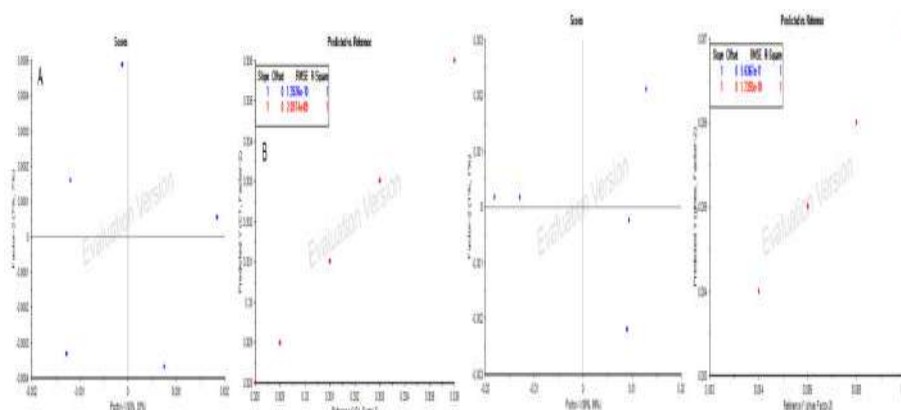


Figure 5 & 6. PLS difference graphs and predicted graphs at wave number 1800-1700cm⁻¹ & 1500-800cm⁻¹ respectively



4. CONCLUSION

The brands that which we took doesn't have any adulterated profile and the difference in the absorption values of pure ghee, goat body fat and the pure ghee spiked with goat body fat in the spectral region of 1800-1700 cm⁻¹ and 1500-800 cm⁻¹ is clearly evident from the peaks in the said regions. Using The Unscrambler® X version 10.5, almost 97% of the test samples could accurately classify themselves into their respective class in the models developed without pre-processing the data. The efficiency of the coefficient of determination using PLS for quantitatively predicting the data was also found to be high for both calibration and validation sets (0.99) at all the wavelengths studied (1800-1700 cm⁻¹ and 1500-800 cm⁻¹). The difference in the value of RMSEC and RMSEV was less, indicating that the model was well developed. The study concludes that the method is rapid, non-destructive and has great precision and

accuracy and has the potential of determining the adulteration of even 1% of goat body fat in Indian cow pure ghee.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

Conflict of interest declared none.

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A REVIEW ON NEUROLOGICAL DISORDER MIGRAINE

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ABSTRACT

Migraine is a neurological disorder and it is recurrent throbbing headache that affect one side of the head. According to the WHO nearly 303 million people of all ages, mostly adult of both gender and female predominantly affected with migraine. About 80% of families' sufferers have a family history of migraine. Although the exact cause is still unknown, but some environment and genetics and genetics are often attributed and prevalence of that condition. Common triggers of migraine are some hormonal changes, unhealth food habits, keeping awakening at nights, and deficiency in blood circulation. Imbalance in certain chemicals such as serotonin, and also some neurotransmitters may also trigger migraine. Some of the healthlinked disorders due to migraine are hypertension, seizures, hearing problems, depression, PTSD (Post traumatic disorders). Migraine has three clinical phases like pre head ache phase, head ache phase, resolution phases. NSAIDS are used for acute migraine. Some synthetic medications such as triptans, selective serotonin inhibitors and has some side effects in chronic treatment. In Ayurveda, migraine mostly be managed with some medications such as migroplex, shirashoolarivati, pankajakasthuri migraine oil and arthardhari and these medications have no side effects. In recent times there is a new approach for treatment of migraine is administration of botulinum toxins.

KEY WORDS: *Migraine, neurological disorder, post traumatic disorder, headache.*

INTRODUCTION

Migraine is a common disabling brain disorder. It is characterised by recurrent headache that are moderate to severe. The headache affects one half of the head and last from a few hours to 3 days.¹ The pain is generally worse by physical activity up to one third of people have an aura.² Migraine is believed to be due to a mixture of environment and genetic factors.³

DEFINITION

A familial, recurrent syndrome characterized usually by unilateral head pain, accompanied by various focal disturbances of the nervous system, particularly in regard to visual phenomenon, such as scintillating scotomas.⁴

TYPES OF MIGRAINE

There are two main types of migraine. This classification depends on whether the individual experiences any disturbances of the senses up to migraine.⁵

1. Migraine with aura
2. Migraine without aura
3. Chronic migraine
4. Menstrual migraine
5. Hemiplegic migraine
6. Abdominal migraine
7. Migraine with brain stem aura.

1. Migraine with aura

For many people with migraine the auras act as a warning them that a headache is soon to come. The effects of an aura can include:

- confusing thoughts or experiences⁶
- the perception of strange, sparkling or flashing lights
- zig-zagging lines in the visual field

- blind spots or blank patches in the vision
- pins and needles in an arm or leg
- difficulty speaking

2.Migraine without aura

A person will experience a migraine without any sensory disturbances leading up to the attack 70 to 90% of migraine occurs without an aura.

3.Chronic migraine

Any migraine that trigger attack on over 15 days of the month.

4.Menstrual migraine

It can attack occurs in a pattern connected to the menstrual cycle.

5.Hemiplegic migraine

It causes weakness on one side of the body for a temporary period.

6.Abdominal migraine

This is a syndrome that connects migraine attack to irregular function in the abdomen. It mainly occurs in children under 14 years of age.

7. Migraine with brainstem

This is a rare type of migraine that can trigger severe neurological symptoms.

DIFFERENCES BETWEEN MIGRAINE AND HEADACHE ⁷

HEADACHE	MIGRAINE
• An aching, dull pain in particular region on entire head. • A constricted sensation and feel of pressure across the forehead or rare portion of the head. • A feeling of tenderness in the muscle of the shoulder, scalp and neck.	• The patient is likely to suffer from depression, constipation, hyperactivity, stiffness in the neck • Temporary loss of vision, aphasia or problem in speech, visualising flashes of light sensation of pain pricking in the arms and legs. • A feeling of nausea, vomiting, light headness, which may also lead to fainting

PATHOPHYSIOLOGY

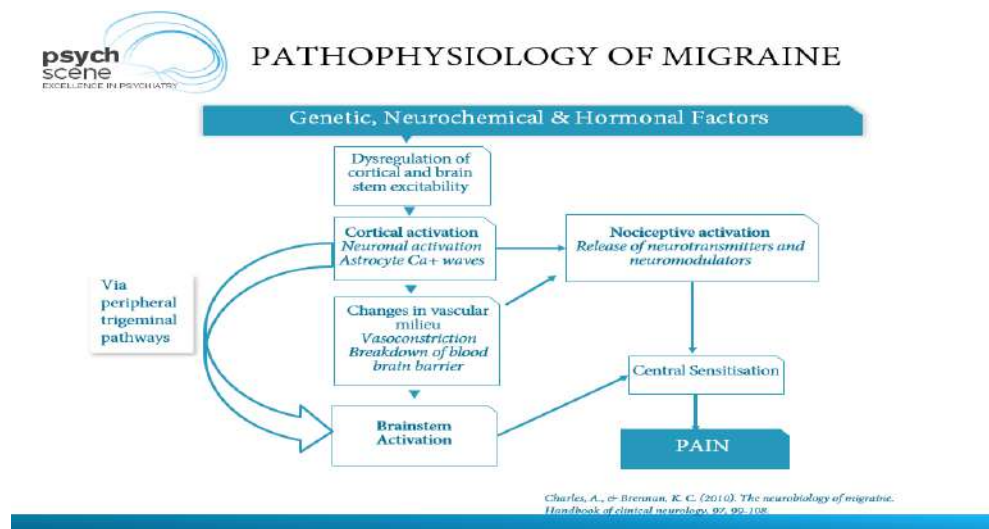
In this migraine mechanism, starting within the brain and spreading to the blood vessels ⁸. Some researchers believe neuronal mechanism play a greater role ⁹ while others believe blood vessels play the key role. ⁽¹⁰⁾ One theory is related to increased excitability of the cerebral cortex and abnormal control of pain neurons in the trigeminal nucleus of the brain system. ¹¹

AURA

Cortical spreading depression or spreading depression according to Leao, is a burst of neuronal activity followed by a period of inactivity, which is seen in migraine with an aura. Number of explanations are occurrences including activation of NMDA receptors leading to calcium entering the cell. After blood flow to the cerebral cortex in the aura affected is decreased for 2 to 6 hours. When depolarization travels down underside of the brain, nerves sense pain in the head and neck are triggered. ¹²

PAIN

The mechanism of the head pain which occurs during a migraine is unknown ¹³ some evidence supports primary role for central nervous system structure. ¹⁴ Other data support role of peripheral activation (such as the sensory nerve that surround blood vessels of the head and neck).



CAUSES OF MIGRAINE

- Allergies and allergic reactions¹³
- Physical or emotional stress
- Irregular sleep
- Exposure to smoke
- Tension headache
- Lack of food
- Teeth grinding

SIDE EFFECTS

1. Depression and anxiety¹⁴

Migraine patients are between two to five times more likely to have these symptoms than patients without migraine. About 25% of patients with migraine have depression. About 50% have anxiety.

2. Sleeping

Some people wake up with the pain even worse. Studies show that more than half of migraineurs are triggered by sleep disturbance, including anything from sleep apnea to insomnia.

3. Stress

Stress is another main trigger and cause of migraines for me. Stress, whether good or bad, does not help with migraines.

4. Stroke and seizures¹⁵

The worst part about this is that you can have stroke-like symptoms during a migraine which can make it harder to tell if you're having one. Migraines can also trigger seizures.

5. Vertigo

Vertigo has many symptoms. It can be imbalance, dizziness, nausea, feeling like you're spinning, rocking or falling, pressure in your ears and several other things. You can be lying down and you continue to still have this feeling.

MEDICATION

SYNTHETIC DRUGS

FROVA

Frova is a headache medicine that narrows blood vessels around the brain. This medicine also reduces substances in the body that can trigger headache pain, nausea, sensitivity to light and sound, and other migraine symptoms. Frova is used to treat migraine headaches.¹⁶

SIDE EFFECT

Changes in vision, chest pain, increased sweating, irregular heartbeat, Stomach pain, agitation.

NARATRIPTAN

Naratriptan is used for the treatment of the acute migraine attacks and the symptoms of migraine, including severe, throbbing headaches that sometimes are accompanied by nausea and sensitivity to sound or light.¹⁷

SIDE EFFECT

Drowsiness, tingling of fingers and toes, dizziness, bloody diarrhoea, stomach pain.

ZOLMITRIPTAN

Zolmitriptan is a headache medicine that narrows blood vessels around the brain. Zolmitriptan also reduces substances in the body that can trigger headache pain, nausea, sensitivity to light and sound, and other migraine symptoms. Zolmitriptan is used to treat migraine headaches in adults.¹⁸

SIDE EFFECTS

Chest pain, tightness, stomach pain, cough, diarrhoea, sleepiness, drowsiness.

ALMOTRIPTAN

Almotriptan is a medicine for headache that vasoconstricts blood vessels around the brain. Almotriptan is used for the treatment of migraine headaches in adults.

SIDE EFFECTS

Dizziness, headache, sleepiness, dry mouth, back pain, sore throat, vomiting, muscle ache.

HERBAL DRUGS USED TO MIGRAINE TREATMENT

GINGER

A case study published in 1990 found that a patient who consumed 500 to 600 mg of powdered ginger mixed with plain water was able to stop migraine attacks (study). The patient consumed the ginger water at the first sign of a migraine and was able to stop migraines every time.¹⁹



PEPPERMINT

Peppermint has been used as a natural remedy and herbal treatment for headaches, nausea, gas, irritable bowel syndrome (IBS), and other conditions. Some people use peppermint leaf or peppermint oil as a dietary supplement, and others use peppermint essential oil applied directly to the skin. (Essential oils are distilled and concentrated oils from the plant.)²⁰



APPLE CIDER VINEGAR

Has been used for centuries as a natural antiseptic ingredient to disinfect the wounds, apple cider vinegar also helps calm sunburn, relieve heartburn or acid reflux, eliminate foot fungus, and possibly cure a headache. When your head hurts, you want relief fast.²¹

COFFEE

Whether it's a run-of-the-mill tension headache or a migraine, caffeine can help. That's why it's an ingredient in a lot of popular pain relievers. It can make them as much as 40% more effective. Sometimes you can stop the pain in its tracks just by having caffeine alone²².



CONCLUSION

By using all the above herbal products, we can treat migraine without any side effects like. Because herbal products have more therapeutic effect and no side effects and people who cannot effort to by medicines by using these herbal products migraines can be treated.

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ESTIMATION OF ANTI-ULCER ACTIVITY OF AQUEOUS EXTRACTS FROM THE LEAVES OF *Nyctanthes arbortristis* ON ALBINO RATS

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ABSTRACT

Peptic ulcer disease (PUD) is one of the prevalent pathogenic conditions. Helicobacter pylori and the use of Non Steroidal Anti Inflammatory Drugs (NSAIDS) are two of the most common etiological causes in the peptic ulcer disease. Recent years have shown significant increase in NSAIDS consumption though they have side effects. To reduce some of these effects and protect gastro intestinal tract, herbal treatment can be given instead. The objective of the present study is to investigate and determine the gastro protective (anti – ulcer) activity of aqueous and hydro-alcoholic extract of leaves of *Nyctanthes arbortristis* in NSAID induced ulcerated rats. The extracts were studied in two dose levels (200 mg / 400 mg) in rats against Aspirin (NSAID – 500 mg /Kg body weight). Ranitidine (50mg / Kg body weight) was used as a standard drug. The therapeutic efficacy of the extracts of leaves in two doses was compared with standard. The leaf extracts has shown promising results by decreasing the aspirin induced ulcers in rat models. This shows that the leaf extracts have significant anti ulcer activity. This study can help in further studies for their anti-ulcer efficacy and in future works for the development of new formulations.

KEYWORDS: *Anti-ulcer, Nyctanthes arbortristis, Aspirin, Ranitidine & Aqueous extract*

1. INTRODUCTION

1.1. Ulcer

An inflamed breach in the skin or the mucous membrane of the alimentary track lining is considered as an ulcer¹. Peptic ulcer disease is one of the most prevalent gastro intestinal disorders ². In homeostatic conditions, a physiologic equilibrium is maintained between gastric acid secretion and gastric & mucosal defence systems³. Gastric ulcer is an imbalance between the offensive and defensive factors⁴. The development of ulcers is also enhanced by poor digestion, decreased excretion, improper metabolism and physical stress etc⁵. In Ayurvedha, gastric disorders are classified as Sula, Parinamasula which corresponds to the clinical conditions called peptic ulcer and functional dyspepsia respectively^{6& 7}. In current allopathic medicine, Proton pump inhibitors or cytoprotective agents like sucralfate, H2 receptor antagonists are used for the treatment of ulcer⁸. These drugs show high incidence of relapse and side effects on clinical evaluation. Based on the review of the literature, the medicinal plants are more effective to prevent ulcer with no side effects. Such medicinal plants that show anti-ulcer activity include *Ocimum sanctum*, *Azadirachta indica*, *Asparagus recemosus*, *Centella asiatica*, *Bacopa monnieri*, *Bidens pilosa*, etc⁵.

1.2. *Nyctanthes arbortristis*

Nyctanthes arbortristis is a medicinal plant. It belongs to the family Oleaceae. It is also known as Coral Jasmine, Night Jasmine, Parijata, Shefali, Harshringar and kannika. This plant is native to India, widely distributed from Sub Himalayan regions to Godavari⁹. In ancient days *Nyctanthes arbortristis* is used to treat various ailments like fever, cough, malaria, bloody dysentery and gastritis. Juice of leaves of this plant is used as an antidote and reptile venom¹⁰. The leaves of the plant contains D-mannitol, Flavone glycosides, Tannic acid, Ascorbic acid, Glucose, Fructose, Carotene and other important constituents¹¹. Some pharmacological activities carried on this plant revealed that it has various therapeutic activities like CNS, Antihistaminic activity, Anti-inflammatory activity, Anti-depressant activity, Immuno-modulatory activity etc,¹². It shows significant Anti bacterial activity against Staphylococcus aureus, Staphylococcus epidermis, Salmonella typhi and Salmonella para typhi A¹³. It also exhibits Antioxidant property¹⁴, Antifungal activity¹⁵, Anti cancer property¹⁶ and Hepato-protective activity¹⁷. The study was designed to investigate the

therapeutic efficacy of the aqueous and hydro-alcoholic extracts of *Nyctanthes arbortristis*. This research paper presents the data of anti-ulcer activity of *Nyctanthes arbortristis*

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Nyctanthes arbortristis* were collected from Mangalagiri, Guntur district, Andhra Pradesh, India in the morning by hand picking method in the month of December. The leaves were shade dried. The dried leaves were made into powder with the help of electrical grinder. The obtained powder was then subjected to soxhlation to get the aqueous and hydro-alcoholic extracts with water and ethanol as solvents respectively.

2.2. Phytochemical Screening

The extracts of *Nyctanthes arbortristis* leaves were subjected to various chemical tests in order to identify the chemical constituents. Alkaloids, Tannins, Glycosides, Carbohydrates & Flavonoids

2.3. Experimental Animals

The wistar albino rats, weighing 150 – 200 gm were selected to determine the anti-ulcer activity of the extracts of *Nyctanthes arbortristis*. All animals were maintained under standard laboratory conditions (Temperature 25±2°C, relative humidity 55±10% and 12 hr light: 12 hr dark cycle) before the start of the experiment. Animals were fed with standard laboratory feed and ad libitum. The experiment was carried out after the prior approval of all experimental protocols by the institutional animal ethical committee (Approval No: 003/IAEC/NCPA).

2.4. Experimental Design

The animals were divided into 7 groups and each group consists of 6 animals.

Table 2 : Grouping of animals based on their treatment

S.NO	GROUPS	TREATMENT	ROUTE OF ADMINISTRATION
1	GROUP I	Normal Saline	Oral
2	GROUP II	Aspirin 500mg/kg.Bw	Oral
3	GROUP III	Standard (Ranitidine 50 mg / kg.Bw)	Oral
4	GROUP IV	Hydro-alcoholic extract 200mg/kg.Bw	Oral
5	GROUP V	Aqueous extract 200mg/kg.Bw	Oral
6	GROUP VI	Hydro-alcoholic extract 400mg/kgBw	Oral
7	GROUP VII	Aqueous extract 400mg/kg.Bw	Oral

2.5. Method

Aspirin induced ulcer model method was selected for estimation of anti-ulcer activity. This method is also called NSAID induced ulcer model. The animals were fasted (with free access to water) for a period of 24 hrs to ensure complete gastric emptying. Aspirin was administered in a dose of 500 mg / kg .Bw orally to GROUPS II, III, IV, V, VI and VII. The animals were allowed to fast for 4 more hours and then were sacrificed. The stomach was dissected and the mucosa was examined under the microscope.

2.6. Parameters

2.6.1. Ulcer Index

$$\text{Ulcer Index} = \frac{\text{Total number of ulcers}}{\text{Number of ulcerated animals}} \times 100$$

2.6.2. Percentage Ulcer Inhibition

$$\text{Percentage of ulcer index} = \frac{\text{Control mean ulcer index} - \text{Test ulcer index}}{\text{Control mean ulcer index}} \times 100$$

2.6.3. Percentage Protection

Percentage protection= Ulcer Index of Treated group / Ulcer Index of Control group ×100

2.7. Statistical Analysis

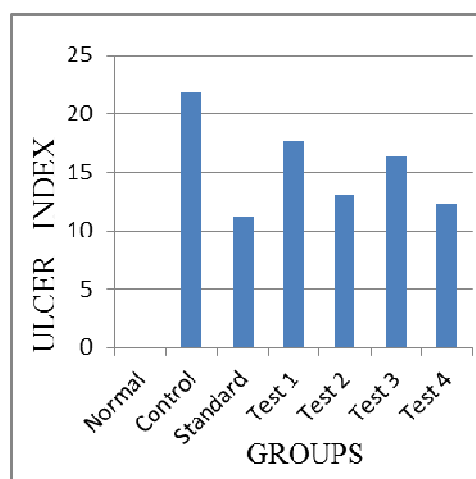
The statistical analysis of all anti-ulcer activity values are tabulated and it is expressed as Mean $\pm$ Standard Error Mean (SEM)

3. RESULTS

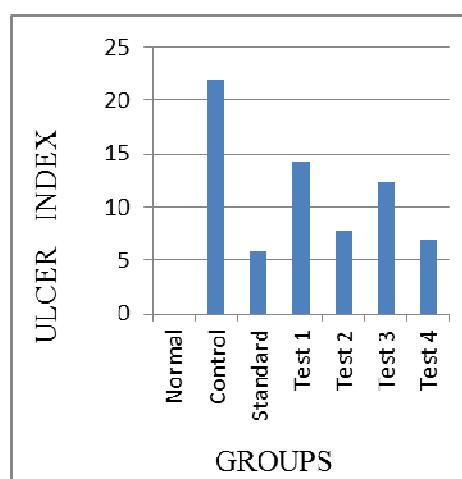
3.1. Ulcer Index And Percentage Of Ulcer Inhibition

Table 3 : The ulcer index of all the groups before treatment (pre), after treatment (post) and percentage of ulcer inhibition was given in the table.

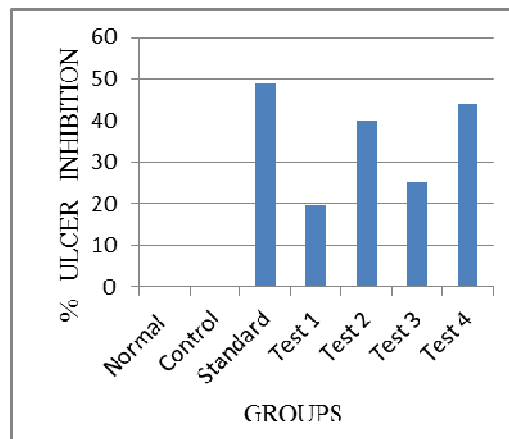
S.NO	GROUP TREATMENT	ULCER INDEX (PRE)	ULCER INDEX (POST)	% OF ULCER INHIBITION
1	Normal	0	0	0
2	Control	21.86	21.86	0
3	Standard (Ranitidine)	11.20	5.9	48.76
4	Hydro-alcoholic extract 200mg/kg.Bw	17.60	14.18	19.48
5	Aqueous extract 200mg/kg.Bw	13.06	7.81	40.2
6	Hydro-alcoholic extract 400mg/kg.Bw	16.40	12.31	24.97
7	Aqueous extract 400mg/kg.Bw	12.26	6.88	43.9



1. Ulcer index before treatment



2. Ulcer index after treatment



3. Percentage of ulcer inhibition after treatment

3.2. Comparison Of Mean $\pm$ SEM

GROUPS	MEAN $\pm$ SEM	% ULCER INHIBITION
Control	7.39	0
Standard	3.93	48.76
Hydro-alcoholic extract 200mg	7.13	19.48
Aqueous extract 200mg	4.86	40.2

Hydro-alcoholic extract 400mg	6.53	24.97
Aqueous extract 400mg	4.46	43.9

4. DISCUSSION

The study discussed that *Nyctanthes arbortristis* exhibits Ulcer healing properties. NSAIDs like Aspirin induced gastric damage is possibly mediated through Leukotrienes and 5 –Lipoxygenase pathway¹⁸. There are number of drugs available for gastric ulcer, in the market. Those drugs produce some side effects such as Hematopoietic changes¹⁹, acute interstitial nephritis²⁰, Thrombocytopenia²¹, Anaphylaxis reactions²², Nephrotoxicity & Hepato-toxicity²³. In the model, the aqueous extract of leaves of *Nyctanthes arbortristis* showed promising results for anti-ulcer activity and has less side effects when compared to marketed drugs and hydro-alcoholic extract of the same leaves.

5. CONCLUSION

This study revealed the therapeutic efficacy of the aqueous extract of leaves of *Nyctanthes arbortristis* in rats for the treatment of peptic ulcer disease. The 400 mg dose of aqueous extract significantly protected the mucous from being damaged than the hydro-alcoholic extract. Due to presence of compounds like Flavanoids, Tannins and Alkaloids there are possibilities of development of herbal formulation using this aqueous extract. Thus, signifying that this study serves as a forward step for future works on peptic ulcer.

6. ACKNOWLEDGEMENT

We sincerely thank our institution for allowing us to carry out this work and constant support for the completion of this work.

7. CONFLICT OF INTREST

We are interested in carrying out further work for the separation and isolation of compounds from the leaves of same plant (*Nyctanthes arbortristis*)

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ASSESSMENT OF PREVALENCE AND PRESCRIBING PATTERN IN HYPERTENSIVE PATIENTS AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Drug utilization review is a powerful tool for evaluating the prescribing patterns, distribution of drugs and appropriateness of the prescription. In this study, we have collected 558 cases of hypertensive patients. HTN is a major risk factor for the development of cardiovascular morbidity and mortality. The major objective of this study was to evaluate the disease prevalence and drug utilization pattern in hypertensive patients. It is retrospective descriptive study carried out in a tertiary hospital for 3 months durations. In age and gender distribution, 41-60 years of age group and females are mostly affected. Patients with one co morbidity along with HTN and females are mostly affected. Patients with stage 1 HTN are more .endocrine disorders are mostly observed in the patients. In majority of the people there is no social history. There was no family history in majority of people. Rural areas people have mostly affected among them males are in high number with HTN. Most of the patients are having mixed diet. There was no physical activity in most of the patients out of which females are in high numbers. Patients with no tension and stress are more in number out of the females are in highest number. Patients given with monotherapy are in highest number out of which males are more. Antihypertensives are the major class of drugs given in these patients. In our study which can observe the prevalence of hypertension and its co-morbidities, which are direct consequences to cardiovascular diseases like coronary artery disease and stroke. Thus, there is requirement for, preventive measures, and effective educative measures in the population about hypertension and the cost of inappropriate control of hypertension.

KEYWORDS: *Drug utilization review, Hypertension, Prevalence, Risk factors, drug use indicators*

1.INTRODUCTION

Drug utilization review is the major factor for the evaluation of quality, prescribing patterns, distribution of drug¹ The major global burden for cardiovascular morbidity and mortality in hypertension² WHO defines hypertension as Systolic Blood Pressure (SBP) ≥ 140 mmHg and/ or Diastolic Blood Pressure (DBP) ≥ 90 mmHg³ HTN is the third most risk factor for different diseases in South Asia⁴ The greater part of the cardiovascular sicknesses are additionally averted by tending to the movement of hazard factors like smoking, utilization of liquor and tobacco use, negative eating routine, physical latency abuse populace wide manners by which People with upset or WHO office unit at high vessel chance variables, which early recognition and the executives abuse substance and meds, as relevant. Crucial modifiable hazard variables of CVDs are an unfortunate eating routine, physical latency, tobacco use and in this way the impacts hint strange blood super particle profile and fat. So scientists reputed that diverse preventive moves had been made by western nations that decreased the peril related with the frequency of CVDs It effectsgreater than one billion persons worldwide and is considered as the major risk factor for cardiovascular mortality. In nearly 75% of adults with cardiovascular disease, hypertension is the most common cause of morbidity⁵ According to the 2018 report of Indian Council of Medical Research (ICMR), Hypertension contributes to approximately 10.8% of all deaths⁶. It is predicted to get increased to 1.56 billion adults in 2025⁷. Most of the cardiovascular diseases can be prevented by eliminating risk factors like tobacco use, unhealthy diet, obesity, physical inactivity, and harmful use of alcoho⁸. In this drug utilization study, we follow WHO prescribing indicators which are essential for promoting rational drug use. Control of the cardiovascular maladies will require alteration of hazard factors that have two qualities. At first the hazard factors have high

hazard and high pervasiveness or both, and also, most or the entirety of the dangers must be reversible expense viably on treatment. Computer aided design and hypertension were expressly aligned with dangers of cardiovascular ailments and the relationship of BP with a huge degree of most populaces having non-ideal pulse esteems. Previous investigations supposed diverse preventive techniques related with the discerning utilization of medicine are taken by created nations that decreased the peril identified with the rate of CVDs, conjointly reputed that populace based endorsing patterns significantly affect persistent wellbeing. Notwithstanding, the examinations in creating nations are close to nothing and inadequate, prominently in tertiary consideration arrangements. Therefore, that was the tactic that we tend to adopt for this study.

2. METHODOLOGY

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Acharya Nagarjuna University Institute Review Board (PD010/IRB/NRML/2016-2017)

2.1 Study design, study site, and duration of the study

It is a retrospective observational study conducted at tertiary care Super specialty hospital, Vijayawada, Andhra Pradesh for a period of 3 months from august 2019 to October 2019. All the procedures were performed according to the ICMR guidelines.

2.2. Our study was followed the both criteria's

2.2.1 Inclusion Criteria

- All age group patients being admitted to the cardiology department into the hospital
- Patients of both genders.

2.2.2 Exclusion Criteria

- Out patients departments.

2.3 Study procedure and analysis

The data collection was done through chart review methods at departments for the assessment. Inpatient case records were reviewed and were followed from the date of admission until the date of discharge. All the necessary information from the case sheets of patients was collected using a data collection form. The patients, prescriptions were monitored for the identification of risk factors and prevalence and prescribing pattern, which occur in our study setting, were listed. The root cause analysis will be carried out for such errors and they will be categorized into different types of variable in hypertensive patients.

3. RESULTS AND DISCUSSION

This retrospective-observational study was carried out in a tertiary care hospital, rural area of Andhra Pradesh. In our nation, different types of research studies on cardiovascular disease are relatively outlandish. For the most part, cardiovascular sickness patients were rarely had odds of readmission in medical clinics and number of days in hospitalization is seen as additional in our nation inside and out examination of the wellbeing related issues of cardiovascular infections various situations are clarified for determination and helpful decision⁹⁻⁸ Better clinical administrations will positively affect the social insurance framework. Numerous investigations were directed on the assessment of medication use design on various sorts of foundational maladies and clarify the now solid proof from huge clinical preliminaries that viably forestall these conditions.^{8,9,10}

3.1 STAGES OF HYPERTENSION

According to our study, person having abnormal systolic blood pressure was found to be highest out of which stage 1 HTN is most commonly observed.

3.2 CLINICAL CHARACTERISTICS OF HYPERTENSIVE PATIENTS

According to our study, people suffering from HTN with the age group of 41- 60 years are having highest percentage of 61.6 %, among them males are having highest number of 162.our study concludes that persons suffering from HTN along with 1 co morbidity are having the highest percentage of 48%.among them females are found to have the highest number of 136.

Table 1 Clinical characteristic among the people suffering from HTN.

Age in years	Male	Female	Total (%)
0-20 Yrs	1	0	1 (0.17)
21-40	74	54	128 (22.9)
41-60	162	81	234(43.5)
61-80	49	134	183(32.7)
>80	3	0	3(0.53)
Comorbidities			
HTN+ co morbidities			
1 Co morbidities	132	136	268(48)
2 co morbidities	101	76	177(31.7)
3 co morbidities	40	44	84(15.09)
>3 co morbidities	16	13	29(5.19)
Organ system			
CVS	11	8	19(3.4)
Endocrine	230	252	482(86.3)
Renal	50	14	64(11.4)
CNS	51	29	80(14.3)
Respiratory	19	9	28(5.01)
Musculo skeletal	8	6	14(2.5)
Hepatic	6	3	9(1.6)
Others	45	40	85(15.2)
Risk factors			
Smoking	125	4	129(23.1)
Alcohol	103	3	106(18.99)
No social history	58	260	318(56.98)
CVD family history			
Yes	111	102	213(38.1)
No	178	167	345(61.9)
Locality			
Urban	110	94	204(36.55)
Rural	179	175	354(63.44)
Food habits			
Veg	14	36	50(8.96)
Mixed	275	233	508(91.03)
Physical activity			
Yes	84	57	141(25.26)
No	204	206	410(73.47)

Our study shows that the majority of the populations of 86.3% are having endocrine diseases revealed coronary artery diseases along with other cardiovascular co-morbidities were a high proportion. Out of these females are in higher number of 252. The majority of the population of 59.6% are having no social history out of which females are in high number of 260. Highest percentage of people having no family history that leads to HTN of 61.9% out of these men are found to be in highest number of 178. According to our study, the majority of populations living in rural areas are mostly affected with HTN with percentage of 63.44% out of which males are having high number of 179. 91.03% population are vegetarians out of which males are more in number, 275 and females of no 233. According to our study majority of the population are having no physical activity, 73.47% out of which females are in high number 206 Our study shows that majority of the population are having no tension or stress out of which females are of high number 101.

3.3 ANTIHYPERTENSIVE THERAPY

Table 02 Antihypertensive therapy

ANTIHYPERTENSIVE THERAPY	MALE	FEMALE	TOTAL %
Monotherapy	171	127	298(53.40)
Dual therapy	119	128	247(44.26)
Triple therapy	1	0	1(0.17)

According to our study patients using monotherapy are in highest percentage, 53.40%, out of which males are having highest no of 171. Our examination investigates that the vast majority of the patients with CAD ought to recommend with standard and prophylactic operators. To dodge this present, doctor's endorsed world's generally recommended and for the most part distributed meds. As determination with anti hypertensive medications can decrease dismalness and mortality.

3.4 OTHER CLASS OF DRUGS

Table 03 other classes of drugs

OTHER CLASS OF DRUGS	MALE	FEMALE	TOTAL %
Antibiotics	35	28	63(11.2)
Antidiabetics	121	142	402(71.8)
Antihypertensives	317	278	842(150.8)
Antiplatelet	23	17	40(7.16)
Iron supplement	11	11	22 3.94
Hypolipidomics drugs	19	12	31(5.55)
Antithyroid	2	6	8(1.43)
Anti asthmatic	7	11	18(3.22)
Anti angina	9	1	10(0.17)
Others	31	27	60(10.3)

According to our study majority of the population 150.9% are using anti hypertensives out of which males are majorly effected 317 and females are less affected 278, and fewer people are using antiangina drugs out of which males are most affected 9 and females of less no 1. Additionally, most of patients were come to visit the centres from provincial zones. In this manner generally speaking, our theory likely brought about an underestimate of the genuine pervasiveness of cardiovascular illnesses extent will increment to 29% roughly 1.56 billion by 2025. That conforms to the past report on ways of life nourishment propensity, less exercise, unexpected frailty cleanliness, that upgrading the cardiovascular hazard. ⁽¹⁰⁾ Recent investigations delights According to India Council of Medical Research, in 2020 CVDs will be the essential driver of bleakness and mortality where 2.6 million are anticipated to bite the dust because of coronary illness which establishes 54.1% of all CVD passings in India

4. CONCLUSION

One aspect from our study which can be noticed is the prevalence of co-morbidities, which are direct consequences of hypertension. It was observed that most of the patient's past history was hypertension when they were admitted with the co-morbidities like stroke, coronary artery disease. This may reduce the associated morbidity and mortality due to unawareness of hypertension, and also reduces the financial burden incurred by it on the society. The increasing burden of hypertension, cardiovascular diseases, and other non-communicable diseases must be addressed and effective control measures should be implemented. Our study shows that the prescriptions were in accordance with JNC VIII guidelines. Calcium channel blockers were the most commonly prescribed drug class followed by Beta Blockers and from the study it can also be said that hypertensive nephropathy needs combination of three or four drugs along. Therefore, we enumerate the risk factors of CVD in our study participants as have the different types of modifiable and un modifiable risk factors.

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6. CONFLICT OF INTREST

Conflict of interest declared none

7. REFERENCES

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AWOMEN WHO GAINED PERMANENT HYPOPARATHYROIDISM AND HYPOTHYROIDISM FOR NOTHING: A TOTAL THYROIDECTOMY CASE REPORT

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ABSTRACT

The term non-invasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP) was recently introduced to recognize the indolent behavior of thyroid neoplasms, previously classified as non-invasive follicular variant of papillary thyroid carcinoma. Total thyroidectomy is a surgical procedure that involves the removal of all or a part of thyroid gland. The thyroidectomy is usually performed in the conditions of thyroid cancer, toxic thyroid nodule, multi nodular goiter and grave's disease. Papillary carcinoma appears as an irregular solid or cystic mass or nodule in a normal thyroid parenchyma. These papillary carcinoma tumors are well differentiating despite spread easily to the other organs. The present case study reports how a 29years old female patient who had been misdiagnosed as papillary carcinoma initially and underwent total thyroidectomy and later diagnosed with NIFTP which is benign, had developed permanent hypoparathyroidism and hypothyroidism. This study also depicts the importance of endocrinologist referral, appropriate and clear reporting of histopathological studies by the pathologists before opting total thyroidectomy as the treatment approach.

KEYWORDS: *Papillary carcinoma, NIFTP (Non Invasive Follicular Thyroid neoplasm with Papillary like nuclear features), total thyroidectomy, hypoparathyroidism.*

1. INTRODUCTION

The term non-invasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP) was recently introduced to recognize the indolent behavior of thyroid neoplasms, previously classified as non-invasive follicular variant of papillary thyroid carcinoma. A non-invasive neoplasm that arises from thyroid follicular cells showing a predominantly follicular growth pattern and with nuclear features of papillary thyroid carcinoma.¹ The clinical manifestations of the patient is similar to other thyroid tumors; usually include pain less, asymptomatic, mobile thyroid gland nodule or enlargement. Additional symptoms of difficulty in swallowing, hoarseness may be present. The clinical evaluation for NIFTP is usually done with Ultrasound studies and fine needle aspiration cytology (FNAC).² General treatment options include Lobectomy, thyroidectomy and radiation therapy (radioactive iodine). Total thyroidectomy is a surgical procedure that involves the removal of all or a part of thyroid gland. The thyroidectomy is usually performed in the conditions of thyroid cancer, toxic thyroid nodule, multi nodular goiter and grave's disease. ³ Papillary carcinoma appears as an irregular solid or cystic mass or nodule in a normal thyroid parenchyma. These papillary carcinoma tumors are well differentiating despite spread easily to the other organs. ² NIFTP cytology was more likely to be micro-follicular pattern, lack pseudo- inclusion and present with a nuclear score of two. NIFTP lesions were less likely to have nuclear grooves and more likely to have smaller nuclear sizes.⁴

2. CASE STUDY

A 29 yrs old female patient presented with complaints of medial cervical swelling with no history of weight loss/ weight gain/ profuse sweating/ fever. Her Ultrasonography report of neck FNAC revealed +ve diagnosis of multinodular goiter and total thyroid profile were found to be abnormal (increasing T3 1.89ng/ml and border line thyroid stimulating hormone (TSH) 11.4 microgram/dl). Left hemithyroidectomy was done under general anesthesia. Later as biopsy report revealed encapsulated follicular variant of papillary carcinoma, completion thyroidectomy was done under GA along with central compartment neck

dissection. Microscopic examination section studied showed histological appearance of nodular thyroid goiter and no residual papillary carcinoma. Since the post operative period serum calcium levels were low (7.4mg/dl). Calcium gluconate IV injections given and supplemented with oral calcium tablets. Patient was not given any thyroxine supplements in an attempt to increase the cravings for thyroxine and make the patient suitable for radioactive iodine therapy (RAI). The TSH level reached 54.4 IU/L. The endocrinologist suggested for the review of left lobe hemithyroidectomy slides report before starting RAI. The review of slides by the other 3 pathologists revealed the diagnosis of nodular hyperplasia / goiter with features suggestive of Non-invasive follicular thyroid neoplasm with papillary like nuclear feature (NIFTP). In order to confirm the same, the third opinion was also taken which also confirmed NIFTP but not papillary carcinoma. The patient initially treated with oral calcium tablets like tablet CCM(Calcium Citrate Malate 250mg Calcium + Vitamin D3100IU + Folic Acid 50mcg 2tablets four times daily), Tab. Rolcalcitriol (Calcitriol 0.5mcg 1tablet twice daily). Later upon follow up tab Admax (calcitriol 0.25mcg + calcium carbonate 400mg+ elemental zinc 7.5mg) and tab Shelcal (500mg elemental calcium + cholecalciferol Vitamin D3 1000 IU) along with levothyroxine 150mcg were given based on the serum calcium and thyroid profile results.

Table1. Pre and post surgical Laboratory Investigations and corresponding therapy

DATES	T3 (ng/ml)	T4 (mic.g/dl)	TSH (IU/ml)	CALCIUM (mg/dl)	Corresponding Therapy
Pre Surgical					nil
28/10/2016	1.89	11.4	2.05	-	
10/05/2017	1.52	12.9	1.12		
Post Surgical					
22/05/2017				7.4	IV Calcium Gluconate
23/05/2017				8.8	1.Tab.CCM P/O 2tabQID, 2.Tab.RolCalcitriol 1tab BD
24/05/2017				8.1	
02/06/2017				6.8	
31/10/2017			54.80	5.6	Started Levo Thyroxine 125mcg& on same calcium supplements as before but BD dose
18/11/2017				6.6	
05/01/2018	1.11	10.28	42.69	6.0	
07/02/2018	1.12	8.68			Levo Thyroxine 150mcg
13/04/2018		1.22	3.67	5.5	1.Tab.shelcal 1tab BD 2.Tab.Admax 1tab BD
20/09/2018		0.866	13.16	8.8	
04/03/2019		1.15	4.02	9.4	
31/10/2019			3.87	8.4	

3. DISCUSSION

In the present case study the women was initially diagnosed with nodular goiter for which left hemithyroidectomy was done and the biopsy report revealed papillary carcinoma. Due to the risk of metastasis and malignancy suspection in the right lobe, completion thyroidectomy was performed for which biopsy reports of right lobe showed no evidence of malignancy. Then the patient was advised to go for Radio Active Iodine (RAI) therapy. Later up on advice from the endocrinologist during the counseling session of RAI therapy, the left thyroid lobe post surgical slides were again sent for histopathology studies

which revealed the diagnosis of NIFTP but not papillary carcinoma. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is an encapsulated or clearly delimited, noninvasive neoplasm with a follicular growth pattern and nuclear features of papillary thyroid carcinoma (PTC), but without well-formed papillae or psammoma bodies and without typical findings of the aggressive subtypes of PTC or poorly differentiated carcinoma (Nikiforov *et al.* 2016, 2018, Lloyd *et al.* 2017). It is considered a 'borderline' or 'pre-malignant' lesion.⁵ The NIFTPs had a preceding cytologic interpretation of benign (31%), atypia of undetermined significance (34%), follicular neoplasm (9%), suspicious for malignancy (12%), or malignant (14%).⁶ The patient developed hypocalcemia which was a consequence of hypoparathyroidism, and hypothyroidism since the thyroid gland was completely removed. Hypoparathyroidism is an uncommon disorder which is characterized by hypocalcemia and hyperphosphatemia due to decreased or inappropriately normal serum levels of parathyroid hormone (PTH). During the neck surgery (75% of cases) parathyroid glands are inadvertently injured, removed or deprived of their blood supply and hence neck surgery is the most common cause of hypoparathyroidism.⁷ The patient had been managed with the calcium, vitamin D3 and thyroid supplements. Conventional therapy of hypoparathyroidism consists of the use of calcium supplements, and active vitamin D which addresses the hypocalcemia of hypoparathyroidism, but fails to provide a physiologic replacement for the lack of PTH.⁷ Hence the patient should take the calcium and active vitamin D3 throughout her entire life. Studies conducted by different researchers showed that the temporary hypocalcemia is one of the most common complications after total thyroidectomy with a reported incidence of 2–32% of cases.^{8,9,10} Permanent hypoparathyroidism, although not as common, has also been reported in approximately 0.2–10% of cases, depending on the experience of the center.¹¹ The treatment approach faced a step wise failure in this case. There would be an endocrinologist referral before going for completion thyroidectomy surgery which was lacking in this case. And also the patient would have been taken the second opinion from the other pathologists before going for completion thyroidectomy which was also missing in this case. The consequences might be still more severe and different if the patient had been treated with RAI which is not necessary here. But luckily the advice from the endocrinologist before going for RAI therapy to review the left lobe biopsy reports by the other pathologists saved the patient from taking expensive RAI therapy thus lowering treatment cost. The renaming of a malignant entity as a nonmalignant (albeit not benign) neoplasm has contributed to optimizing patient care by decreasing medical expense and complications possibly caused by further treatment including radioactive iodine, and reassuring patients with this diagnosis.¹² Though the patient was prevented from RAI therapy, total thyroidectomy had been performed by then and the patient was suffering from hypocalcemia along with the hypothyroidism. The calcium levels were altered during all follow up visits in the two years post surgical period despite with the oral calcium and vitamin D supplements. Finally the calcium levels are now being maintained only with the calcium supplements and active vitamin D3 revealing the permanent hypoparathyroidism. The patient's final diagnosis after the review from the other pathologists (second and third opinions) was found to be a nodular goiter and NIFTP which is benign.

4. CONCLUSION

This case report strongly depicts the need for the endocrinologist referral and also the appropriate and clear reporting of histopathology and cytological studies by the pathologists before going for total thyroidectomy. Inappropriate diagnosis may be responsible for the increased treatment costs, psychological stress and also irreversible effects like permanent hypoparathyroidism for nothing. Careful attention need to be provided during the confirmation of Papillary Carcinoma since it is often mislabeled with NIFTP.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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A REVIEW ON EFFECT OF GLUCOCORTICOIDS ON C-REACTIVE PROTEIN

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ABSTRACT

C - Reactive Protein is an acute phase reactant. It is increased in the conditions such as inflammation and infection which is synthesized by stimulation of Hepatocytes. CRP plays a major role in apoptosis, Nitric Oxide release and activation of complement pathways. Its release is mediated by inflammatory mediators such as IL-6, TNF- α , IL-8 and monocyte chemo attractant protein. Increase in CRP may also lead to certain complications because CRP activates inflammatory pathways and thus causes the local tissue damage. Hence it is necessary to reduce the CRP. Glucocorticoids such as Beclomethasone, Betamethasone, Budesonide, Cortisone, and Dexamethasone. Hydrocortisone, Methylprednisolone, Prednisolone are used in treatment of several inflammatory diseases which include auto-immune diseases also. They have the ability to reduce the CRP by suppressing the inflammatory and pro inflammatory mediators. This is achieved through downregulation of the inflammatory pathways. Many advances have been developed to elucidate various mechanisms such as gene transcription and cell signalling in inflammation involved in the reduction of CRP by Glucocorticoids. Glucocorticoids produce their actions by binding to the Glucocorticoid Receptor present in the cytoplasm of cells. A negative regulatory mechanism called Transpression is responsible for the anti-inflammatory actions of Glucocorticoids. Glucocorticoids reduce CRP levels by downregulating the inflammatory cytokines. Thus they can be used to prevent the complications caused by increased CRP levels. This review paper mainly focuses on the role of CRP in inflammation and infections, Glucocorticoid receptor, Genomic actions of Glucocorticoids as well as the role of Glucocorticoids in reducing the CRP.

KEYWORDS: *CRP, Glucocorticoids, Inflammation, Infection.*

1. INTRODUCTION

C-reactive protein (CRP) increases up to 1,000-fold at sites of infection or inflammation as it is an acute phase reactant. Its increase is usually seen in the active form of immune disorders¹. Corticosteroids (also known as glucocorticosteroids, glucocorticoids or just steroids) are among the most widely used drugs in the world and are effective in many inflammatory and immune diseases. Many advances have been developed to understand the molecular mechanisms (gene transcription and cell signalling in inflammation) in anti-inflammatory actions of Glucocorticoids in asthma and other inflammatory disease². Glucocorticoids have the potential to decrease CRP without modifying disease progression. Low doses of steroids suppress CRP in patients with moderate disease activity³.

2. C-reactive protein (CRP)

ESR a marker of inflammation, infection and tissue damage has been widely replaced by C - reactive protein because of its enhanced responsiveness and specificity. C-reactive protein levels rise more quickly than ESR (increasing within 2 hours and peaking at 48 hours) and are less affected by anemia, pregnancy, and elevated protein level⁴. Hepatocytes play a major role in synthesizing the CRP. CRP is used as a marker of infection and in several cardiovascular events as it plays an important role in complement pathway; apoptosis, phagocytosis, Nitric oxide release and production of cytokines. Hence studies are conducted to understand the basic mechanism behind these reactions.¹ There are studies that show the CRP levels increase in infections and inflammatory processes, the data that show the role of CRP isoforms is unknown. Thus, studies are necessary to describe the specific cellular processes of each CRP isoform.

2.1 Role of CRP in inflammation

CRP is mainly synthesized by IL-6 dependent hepatic biosynthesis and mediates acute-phase response after an inflammatory reaction. CRP activates the C1q molecule in the complement pathway which further leads to the opsonisation of pathogens. CRP can also initiate cell-mediated pathways of host defense by binding to Fc receptors of IgG[8]. Interaction of CRP to Fc receptors releases pro-inflammatory cytokines by binding to Fc.IgG lacks the ability to recognize self and foreign molecules because they only recognize distinct antigenic epitopes, whereas the CRP can recognize⁹. In atherosclerotic lesions, inflammatory response is achieved by association of CRP with terminal complement complexes¹⁰ and also binds to damaged cell membranes¹¹. Damage to heart tissue by local complement activation is mainly due to CRP¹². In lesions of vasculitis and allergic encephalomyelitis, there exists correlation between the localization of CRP in neutrophil infiltrates. CRP is present as a major part in the fluid phase rather than becoming deposited in tissues at sites of inflammation or injury¹³⁻¹⁴.

2.2 Role of CRP in Infection

C - reactive protein levels are increased in bacterial infection¹⁵. This is mainly due to monocytic mediators IL-1 and IL-6 decay¹⁶. CRP binds to PCh and activates the classical complement¹⁷. This reaction is seen in mice against the Gram-positive pathogen *Streptococcus pneumoniae* where it binds to the cell. The pneumococcal polysaccharide C present in the bacterial cell wall binds to the CRP¹⁸. CRP has protective role against bacterial infections as well as the marker of infection and inflammation which is achieved through activation of complement and opsonisation of pathogens.

2.3 Role of CRP in activation of Complement

The major defences of the human system are the Complement which clears the foreign particles and organism's after an antibody recognizes them. Many investigations have been made to understand the role of CRP in activating the complement system. The ability of CRP to activate the Complement was first described by Kaplan and Volkanis in 1974 by using C-polysaccharide and phospholipid ligands¹⁹. The crucial step is the activation of complement by CRP since when complement was depleted, and the effects of CRP were abrogated²⁰. The competition between the initiator (C1q) and the inhibitor (C4bp) of the classic complement pathway for mCRP binding controls the local balance of inhibition and activation of the pathway in tissues. A high degree of control over the classic complement pathway is achieved when mCRP binds to the C4bp inhibitor²¹.

2.4 Role of CRP in Apoptosis

CRP can exert its anti-apoptotic property when its cyclic pentameric structure is lost. This may suggest that the native isoform is responsible for the anti-apoptotic property. Binding of native CRP (nCRP) to low-affinity IgG FcγRIIa (CD32) and IgG FcγRI (CD64) leads to depressed functional activities, degranulation, and the generation of superoxide by inducible respiratory burst. mCRP binds to low-affinity IgG FcγRIIIb (CD16) that can delay apoptosis by triggering the cell survival pathway in neutrophils, even at low concentrations²². The nCRP isoform has the ability to opsonize apoptotic cells and induce the phagocytosis of damaged cells. Removal of nCRP-bound apoptotic monocytes and macrophages may be via FcγR-mediated phagocytosis²³. CRP binds to apoptotic cells, inhibits the assembly of terminal complement components, and promotes the opsonization of apoptotic cells^{22,24}.

2.5. Role of CRP in Nitric Oxide production

C-reactive protein at concentrations which are known to cause cardiovascular risk reduces the angiogenesis, cell migration and capillary-like tube formation which further attenuate the NO production.²⁵ The role of CRP in upregulating the expression of adhesion molecules and inhibition of Endothelial nitric oxide synthase (eNOS) was shown by Eisenhardt et al.²⁵ showed that CRP upregulated the expression of adhesion molecules and inhibited endothelial nitric oxide synthase (eNOS) expression, indicating a role for CRP in the production of NO. It is suggested that CRP inhibits the angiogenesis and promotes the pathogenesis of atherosclerotic vascular disease by vasoconstriction, leukocyte adherence and inflammation by downregulation of eNOS which further inhibits NO production. In contrast, mCRP upregulates eNOS in neutrophils by reverse transcription polymerase chain reaction which shows the amplification of eNOS mRNA, but not iNOS or nNOS mRNA which further enhances the NO production. Thus mCRP and nCRP have the opposite effects where one increases the production of CRP where as the other isoform inhibits it. It is also highlighted in this study that induction of NO formation in neutrophils by mCRP is by initiation of

Calcium (Ca^{2+}) mobilization and activation of calmodulin and PI3 kinase²⁶. The effect of CRP isoforms on other inflammatory cells, such as monocytes or macrophages is not yet known.

3. Molecular mechanism of Glucocorticoids

Glucocorticosteroids suppress inflammation in a very large choice of diseases, together with allergic diseases, arthritis, inflammatory gut sickness and reaction diseases. There are vital advances in our understanding of how glucocorticoids suppress inflammation and this could purpose the thanks to the event of improved glucocorticoids and a lot of specific therapies within the future²⁷.

3.1 Glucocorticoid receptors

Antiinflammatory actions of Glucocorticoids are created after they bind to the endocrine receptor (GR) gift within the protoplasm of target cells. GR-p, a splice variant of GR has been known that probably interfere with the actions of Glucocorticoids by binding to deoxyribonucleic acid²⁸. Associate Nterminal domain (t₁) is concerned in transcriptional trans-activation of genes once binding to desoxyribonucleic acid has occurred and this region may additionally be concerned in binding to different transcription factors. In human GR there's another trans-activating domain (r2) adjacent to the endocrine binding domain and this region is additionally vital for the nuclear translocation of the receptor. The inactivated GR is guaranteed to a macromolecule complicated (approx. three hundred kDa) which incorporates 2 molecules of 90 kDa heat shock macromolecule (hsp90), a 59 kDa immunophilin macromolecule and numerous different repressive proteins. The hsp90 molecules act as a 'molecular chaperone', preventing the unoccupied GR localizing to the nuclear compartment. Once the endocrine binds to GR, hsp90 dissociates, therefore exposing 2 nuclear localization signals and permitting the activated GRglucocorticoid complicated to quickly go into the nucleus and bind to DNA²⁹.

3.2 Genomic actions of Glucocorticoids

The main actions of endocrines are created once the steroid nucleus of the drug binds to the Glucocorticoid receptor that is followed by the translocation which additionally causes changes within the sequence structure. After binding to the glucocorticoid receptor, a steroid-GR complicated is made. After translocation, steroid- GR dimerizes then regulates the endocrine-sensitive genes which leads to the formation of glucocorticoid response elements (GRE). This stimulates the transcription of sensitive genes, which leads to the changes in synthesis of ribonucleic acid. This is later followed by changes in macromolecule synthesis. This results in the reduction within the synthesis of inflammatory cytokines that plays a crucial role in medicinal drug and immunomodulatory effects of glucocorticoids.³⁰

4. Role of Glucocorticoids in reducing the CRP

Oral corticosteroids have a potent antiinflammatory action, though it's not sure however they'll specifically scale back liquid body substance serum globulin levels. Oral corticosteroids have a potent antiinflammatory action, though it's not sure however they'll specifically scale back liquid body substance serum globulin levels. Don D Sin expressed that inhaled corticosteroids might have an effect on serum globulin production indirectly by downregulating the expression of sure cytokines, that have a crucial restrictive role in serum globulin production. Previous studies indicate that IL-6, once referred to as hepatocyte-stimulating issue, could be a major communication protein for serum globulin expression by hepatocytes and is thought for its ability to stimulate the assembly of different acute-phase proteins. IL-6 is created by a range of cells, together with T lymphocytes, macrophages, B cells, fibroblasts, epithelium cells, and airway animal tissue cells. In COPD, IL-6 expression within the airways is increased³¹. Fluticasone downregulates IL-6 production within the airways, that then reduces serum globulin expression by the liver. Oral corticosteroids cause a major reduction in serum globulin levels occurred within the absence of great changes in IL-6 levels³². it's attainable that general corticosteroids might have had a drug impact on serum globulin through pathways that were freelance of IL-6. It's additionally attainable that the powerful actions of general corticosteroids may have altered the assembly and/or metabolism of IL-6 or different cytokines, influencing the expression or regulation of IL-6³³. A recent study found that Glucocorticoids will scale back the serum globulin and different circulating inflammatory protein levels in acute inflammatory states by downregulating some inflammatory cells and protein levels within the airways. They can suppress the high serum globulin levels that additionally reduces the severity of flow of air obstruction and therefore the risk of viscus injury³⁴. Glucocorticoids like Budesonide {can additionally|also can|can even|may also|may} induce WBC death and also a decrease in IL-8 levels that additionally reduces the discharge of serum globulin.

Treatment with Fluticasone showed a decrease in CD8:CD4 ratios and bated subendothelial mast cells that additional reduces the discharge of pro-inflammatory mediators³⁵. One plausible rationalization has showed that Glucocorticoids will down-regulate the discharge of pro-inflammatory cytokines. In case of arthritis, Prednisolone has the power to scale back the serum globulin in patients with moderate sickness. Steroids might scale back the serum globulin and therefore prevents the incidence of fibrillation as a result of fibrillation is related to the increased liquid body substance levels of serum globulin that additional activates the complement system and promotes additional cardiac muscle damage³⁶. A concomitant medical aid of Corticosteroids along side antibiotics will induce the serum globulin levels in infection patients as a result of steroids will suppress the inflammatory responses whereas antibiotics will either kill or stop the microorganism growth. In case of Community noninheritable respiratory disorder, short doses of alkyl anti-inflammatory might have a plus of reducing serum globulin levels similarly because the bated probabilities of treatment failure³⁷. A daily dose of Methylprednisolone in kids with cavum assist Device support has shown to scale back the serum globulin levels in SIRS³⁸.

5. CONCLUSION

Glucocorticoids have the potential to scale back the serum globulin that is elevated principally throughout infections and inflammatory conditions and therefore helps within the assessment of those sickness conditions. Increase in serum globulin levels additionally results in sure sickness conditions as a result of they activate the complement systems. This results in the native tissue harm and additionally causes death in some cases like cardiac muscle tissue harm. Increased serum globulin may additionally results in the event of fibrillation that is one the foremost serious vas diseases. Glucocorticoids scale back the serum globulin by downregulating the inflammatory cytokines. Thus glucocorticoids will scale back additional tissue harm and might forestall complications caused by increased serum globulin. They can modify the serum globulin once given through oral, parenteral and nasal routes. Glucocorticoids also can be used along side antibiotics in treatment of significant infections like infection, Community noninheritable respiratory disorder. Systemic actions of Glucocorticoids are through downregulation of IL-6 wherever as nasal actions ar through downregulating the protein levels within the airways.

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SMOKELESS TOBACCO AND THE RISK OF STROKE

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ABSTRACT

Cerebrovascular stroke is one of the most leading causes of death and disability worldwide. Stroke affects 13.7 million people per year and is the second leading cause of death, with 5.5 million deaths per year. In 2016, the global impact of ischaemic stroke events was 9.5 million and the incidence and prevalence of stroke have increased rapidly over time. Smoking tobacco is associated as a risk factor for stroke with nicotine being the major contributor among other constituents present in the smoke of tobacco. Nicotine is also present in chewing tobacco, and thought as a contributor to stroke, however due to limited number of studies on the association of chewing tobacco and stroke, the results are inconclusive. A variety of smokeless tobacco (SLT) products such as snuff, tobacco gum, tobacco toothpastes, herbal smokeless tobacco, tobacco water, betel quid, khaini, gutkha etc. are widely used across the world and are specific to certain low and middle income countries in Asia. The detrimental cardiovascular effects of smoking tobacco are well documented but the same is not true for consumption of SLT. Hence, the systematic review is aimed at exploring the association of smokeless tobacco with stroke.

KEYWORDS: Stroke, Disease burden, smokeless tobacco, Prevention.

INTRODUCTION

Cerebrovascular stroke is one of the most leading causes of death and disability worldwide. It can divide into ischaemic Stroke and haemorrhagic Stroke. The latter of which includes intracerebral haemorrhage and subarachnoid haemorrhage¹. Significant health burden in low income and middle-income countries² there is little knowledge on ischaemic Stroke in India³⁻⁶. Ischaemic stroke described as an infarction of the brain, spinal cord, or retina⁷ and it represents ~71% of all strokes⁸. In the 1970s, pioneering experiments identified that much of the initial clinical deficit in patients with that much of the initial clinical gap in patients with stroke because of a hypo-perfused, hibernating, non-functional part of the brain termed the ischaemic penumbra⁹. The landmark discovery formed the rationale for the reperfusion therapies that have transformed outcomes for patients with ischaemic stroke since the first positive trial of stroke thrombolysis, published in 1995¹⁰. Estimating for total year-end stroke-related medical costs increasing from US\$71.55 billion in 2012 to \$183.13 billion by 2030¹¹, Stroke affects 13.7 million people per year and is the second leading cause of death with 5.5 million deaths per year, the incidence and prevalence of ischaemic stroke have evolved. In 2017, the global impact of ischaemic stroke events was 9.5 million (see Fig.1)¹².

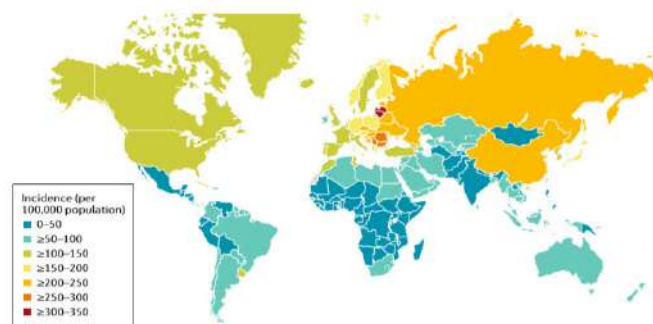


Figure 1 Epidemiology of Ischaemic Stroke. The global distribution of ischaemic stroke incidence by country. Data on the Global Burden of Disease Study 2017.

The warning signs of stroke may include^{13,14}

- Numbness of the face, arm, or leg on one side of the body.

- Confusion, trouble speaking or understanding;
- difficulty seeing in one or both eyes;
- struggle to walk, dizziness, loss of stability or coordination
- severe headache with no known cause.

Pathophysiology of Stroke

Most ischaemic strokes are thromboembolic, with conventional sources of embolism being significant artery atherosclerosis and cardiac disease, particularly atrial fibrillation. Other causes of ischaemic stroke include small vessel disease, which is associated with elevated blood pressure and diabetes mellitus and is common in Asia. Less frequent overall, but proportionally more prevalent in younger patients, are arterial dissection, vasculitis, patent foramen ovale (PFO) with paradoxical embolism and haematological disorders (Fig.2)

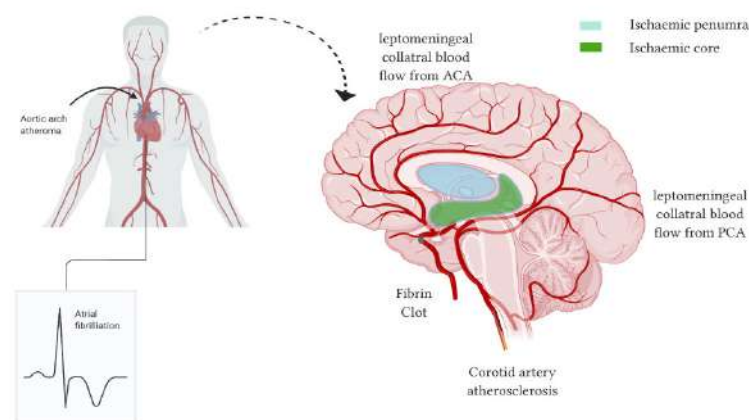


Figure. 2 Ischaemic stroke mechanisms.

The heart and cerebrovascular tree representing cardioembolic stroke. From the left atrial appendage in atrial fibrillation. Carotid artery atherosclerosis and small vessel disease due to lipohyalinosis and parent vessel atherosclerosis. When a thrombus occludes the middle cerebral artery distal to the circle of Willis (that is, the circulatory anastomosis that supplies blood to the brain and surrounding structures). Leptomeningeal anastomoses with the anterior cerebral artery (ACA) and posterior cerebral artery (PCA) branches will provide retrograde blood flow to a variable proportion of the middle cerebral artery territory. This collateral flow might be enough to sustain metabolic viability but not electrical activity in the ischaemic penumbra. The penumbra will contribute to the clinical deficit, but this is reversible with rapid reperfusion. Regions without adequate collateral blood flow will become irreversibly injured, termed the ischaemic core, and the extent of irreversible injury expands with time.

Diagnostic tests and imaging

Primary diagnostic studies approved by current guidelines for patients suspect a stroke. Figure.3 provides the algorithm of the stroke diagnosis. A critical piece of information is the time of onset. This value does not assist in diagnosing stroke, but it determines whether the patient meets the 3-4.5hour eligibility windows for thrombolysis among persons with a diagnosis of ischaemic stroke. All patients with stroke symptoms should undergo urgent neuroimaging with non-contrast computed tomography (CT) or MRI^{15,16}.

Smokeless Tobacco

The term Smokeless tobacco (SLT) refers to the consumption of unburned Tobacco, in the form of chewing, spitting, dipping, and snuff, people of many regions, including India, Pakistan, other Asian countries, and North America, have a long history of smokeless tobacco use. Approximately 28 chemical constituents present in SLT. Those are carcinogenic nature, among which nitrosamine is the most prominent¹⁷. SLT products used either orally or through the nostrils are consuming in about 140 nations among adults worldwide¹⁸. SLT smokeless forms in India, which carry betel quid chewing, mishri, khaini, gutka, snuff, and as a constituent of pan masala.

Betel quid

Betel quid is a combination of betel leaf, areca nut, slaked lime, Tobacco, catechu, and spices according to individual preferences. Tobacco is widely available in India; it is prevalent all over India.

Khaini

It comprises roasted tobacco flakes mixed with soaked in this mixture prepared by the user keeping the ingredients on the left palm and rubbing it with the right thumb. They keep the prepared pinch in the lower labial or buccal sulcus. Its use is every day in eastern India.

Mawa

It is a mixture of areca nut, tobacco and slaked lime and chewed. It's used commonly in rural areas of Gujarat province. It is popular among the young population of ages ¹⁹⁻²².

Snuff

It is a black-brown powder got from Tobacco through roasting and pulverisation. They use snuff via nasal insufflation and are prevalent in eastern parts of the country. They also apply it on the gum by a finger in western India, wherever it identifies as Bajar and mishri.

Gutka

It is a made smokeless tobacco product (MSTP), a mix of areca nut, Tobacco and some spices marketed in various flavours in colourful containers.

Pan Masala

Pan Masala is a betel quid mixture, which includes areca nut and some seasonings, but may or may not include Tobacco the combination is chewed and sucked. Unlike cigarettes, a price levied on pan masala is cheap. Low cost and not being compared with smoke have led to a tremendous increase in using many areca nuts and smokeless Tobacco among the Indian population, including adolescents. They have also promoted it as a "post-meal mouth freshener, making it accessible. Initially, it was more prevalent in Northern India, but with substantial publicity, it is now being used all over the country. Pan Masala is as unhealthy as smoking, although the type of harmful effects is different. We have associated its use with a high risk of oral cancer and submucous fibrosis in mouth, which also has a high potential for cancer development. They make it using waste tobacco, mid-ribs of tobacco leaves and floor sweepings from cigarette factories. It is available as small packets and cans, sold at affordable prices with attractive, shiny coloured wrappings ^{17,18,19,20}. Smokeless tobacco associates with many health obstacles. Using smokeless Tobacco can influence nicotine addiction ^{21,22} is associated with the condition of the mouth, may enhance the risk for death from heart disease and stroke ²³.

Pathophysiology of tobacco use in stroke

There is no exact pathophysiology of smokeless Tobacco contributes to stroke through various pathways. But similar compounds in both Tobacco and Smokeless Tobacco. Nicotine, carbon monoxide and oxidant gases are the main components of smokeless Tobacco that can cause a stroke ²⁴. The toxic substance in tobacco products damages the blood vessels, including inflammation and endothelial cell damage ^{25,26}. The dominant factor in the pathogenesis of smoking-induced cardiovascular events. Although less known about the cerebrovascular effects of smokeless Tobacco, SLT contains over 2000 chemical compounds, including nicotine. SLT may cause a stroke by elevating the blood pressure temporarily and contributing to chronic hypertension ²⁷.

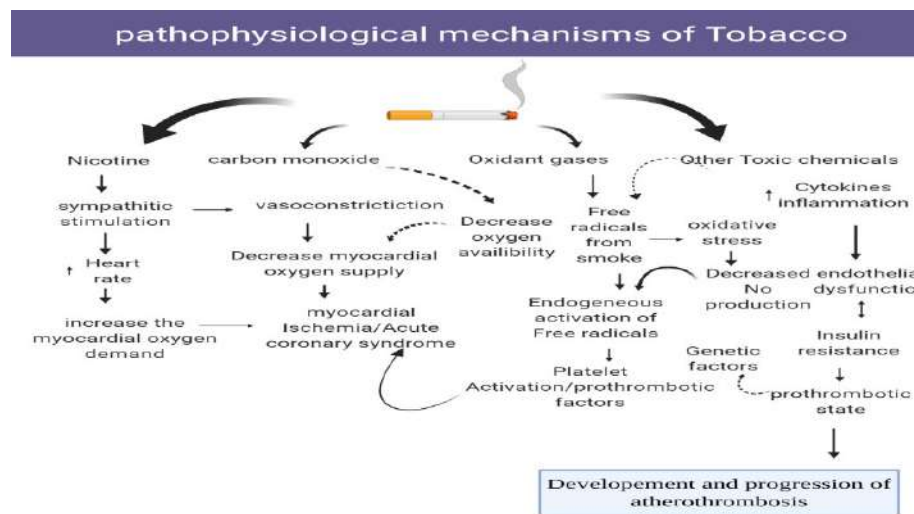


Figure-3: Overview of the various pathophysiological mechanisms of Tobacco in the development of cardiovascular disease, BP; Blood pressure, PR; pulse rate.

Preventions measures²⁸

As per the American cancer society prevention of smokeless Tobacco at our home. Some steps are adopted from ACS those have given below.

- Choose the date and register it on your calendar.
- Explain your friends and family about quit day.
- Acquire quit of all the Tobacco in your home, car, and at work.
- Keep maintaining Stock up on oral substituents-sugarless gum, carrot sticks, chocolates, and cinnamon sticks.
- Consider discussing your doctor or dentist. Possess them to check the mouth, and communicate to them about your plan for stopping.
- Make a schedule of your triggers – situations, places, or feelings – that make you more likely to use Tobacco. Being conscious of these can help you withdraw them or at least ready for them.
- Think about your past attempts to quit.

CONCLUSION

Stroke is one of the most leading causes of death and disability worldwide, and it can classify into two types, which is Ischaemic Stroke, Haemorrhagic Stroke. The risk factors of the stroke might be influencing the several things is there; however, the stroke is developing due to the accumulation of the plaque in the vascular system. The diagnostic procedures are CT, MRI, and some significant laboratory parameters. The management of stroke is antiplatelet therapy, Refusion therapies, endovascular thrombectomy. Smokeless Tobacco also may induce ischaemic stroke, and Smokeless Tobacco contains unburned nicotine along with 2000 chemical compounds which are causing Chronic hypertension may lead the inflammation and thrombus formation. SLT may also cause oral cancers, dentifrices, heart diseases and sometimes may lead to death. However, the smokeless tobacco modifiable risk factor, those who are practising the neurology / general medicine physicians must remind it SLT also may cause a stroke.

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IN VITRO ANTI OXIDANT POTENTIAL SCREENING OF DIFFERENT LEAF EXTRACTS OF *CRATEVA RELIGIOSA* LINN

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ABSTRACT

Free radicals are toxic byproducts of natural cell metabolism and are responsible for causing a wide number of health problems. In this study petroleum ether, chloroform, ethanol extracts of *A* Linn leaves were evaluated *In vitro* by experimental parameters such as Nitric oxide radical scavenging capacity. *Crateva religiosa* Linnis used as an antioxidant, antidiabetics, hepatoprotective, cytotoxic activity, genotoxicity, antitumour activity, and antilice agent. It is related to contain alkaloids, carbohydrates, fixed oils, tannins & phenolic.

KEYWORDS: *Crateva religiosa*, antioxidant activity, Nitric oxide.

INTRODUCTION

According to the WHO survey 80% populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. The use and search of drugs and dietary supplements from plants have been intensified in recent years¹. Medicinal plants are a source of great economic value in the Indian sub continent. For most of the disease, plant materials are used as drugs because of its active compounds. In many disorders the free radical mediated damage may play an important role. Free radicals are responsible for causing a wide number of health problems which include cancer, aging, heart diseases and gastric problems etc. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age². Antioxidant compounds can decrease oxidative stress and minimize the incidence of these diseases. The mechanism of the action of these antioxidant compounds include suppression of reactive oxygen species formation either by inhibition of the enzymes or by chelating of trace elements involved in free radical production, scavenging of reactive species and up- regulating or protecting antioxidant defense³. *Crateva religiosa* L. is referred as bullock's heart belongs to Capparidaceae family having a smooth skin fruit that becomes dull red when ripe. Less volatile substances such as alkaloids, diterpenoids, and acetogenins⁴ have been identified so far from oil of various parts of the plant. However, the antioxidant potential of *Crateva religiosa* leaf is not yet been explored properly. With this in mind, the present work was an attempt to perform anti oxidant studies on the leaf extracts of *Crateva religiosa* Linn.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Crateva religiosa* Linn were collected from guntur district in march 2017 and were authenticated by prof. D. Ramakanth raju retired botanist and a voucher specimen (c.r.v.s-010, 12/03 /2017) has been deposited in the university..

Preparation of Plant Extracts

Collected plant material has been dried under shade and made into coarse powder passed through sieve# 20 and has been successively soxhletated using solvents of increasing polarity petroleum ether, chloroform, ethanol obtained extracts were made solvent free using Rota evaporator and stored in dessicator until further use.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of extracts was measured according to method⁵ Griess reagent was prepared accordingly: Solution A: 1% Sulphanilamide in 5% ortho Phosphoric acid or 25% v/v Hydrochloric acid. Solution B: 0.01% Naphthyl ethylene diamine in distilled water. Solution A and Solution B were in mixed equal volumes within 12 hrs of use. Sodium nitropruside 5mM was prepared in phosphate buffer PH 7.4 (0.0373g in 25ml). To 1ml of various concentrations (100-500µg/ml) of the extracts, 0.3 ml of sodium nitropruside was added in the test tubes. The test tubes were incubated at 25°C for 5hr. 0.5ml of Griess reagent was added. The absorbance was measured at 546 nm.

STATISTICAL ANALYSIS⁶

All the experiments were performed in triplicate and results were averaged. Linear regression was used to calculate the IC₅₀ values.

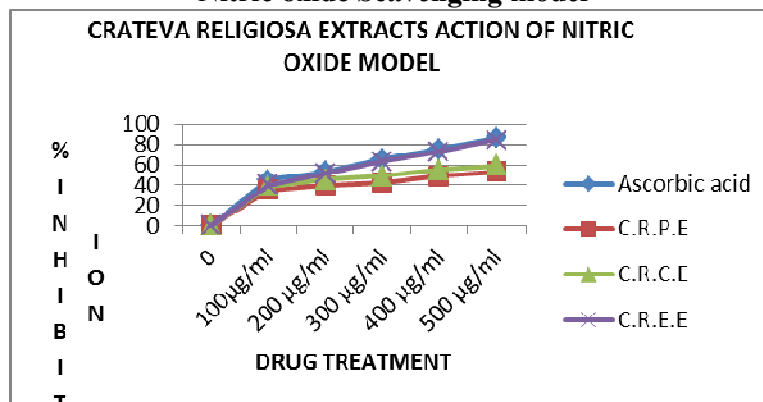
RESULTS

Several concentrations ranging from 100-500µg/ml of the petroleum ether, chloroform, ethanol *extracts of Crateva religiosa leaves* were tested for their antioxidant activity in nitric oxide. *In vitro* model. It was observed that free radicals were scavenged by the test compound in a concentration dependent manner in the nitric oxide scavenging model.

Table: 1 *Crateva religiosa* Linn leaf extracts in Nitric oxide Scavenging model

Concentration	100µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Ascorbic acid	45.56	53.22	65.69	75.23	86.46
C.R.P.E	35.23	39.45	42.45	49.33	53.21
C.R.C.E	38.91	45.44	49.22	54.89	59.06
C.R.E.E	40.05	51.16	63.43	72.91	84.73

Graph :2 Graphical representation of *Crateva religiosa* Linn leaf extracts in Nitric oxide Scavenging model



From the results obtained from the *in vitro* reaction of extracts with reagents in Nitric oxide model ascorbic acid has shown highest percentage inhibition at higher concentration of 86.46%, in the nitric oxide antioxidant model *Crateva religiosa* has shown significant antioxidant activity.

DISCUSSION

In the present study, the nitrite produced by the incubation of solution of sodium Nitroprusside in phosphate buffer was reduced by both the extracts of both species⁷. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with nitric oxide and thus inhibit the generation of nitrite⁸.

CONCLUSION

Oxidative stress has been implicated in the pathology of many diseases and condition including diabetes, cardiovascular diseases⁹, inflammatory conditions¹⁰, cancer¹¹ and aging¹². From the present research work we conclude that *Crateva religiosa* ethanolic extract has got maximum antioxidant activity than compare to other extracts in a dose dependent manner. This may be attributed to the presence of acetogenins¹³, phenols¹⁴ which probably play a role as an effective free radical scavenger¹⁵.

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A REVIEW ON BIO-PHARMACEUTICAL CLASSIFICATION SYSTEM CLASS IV DRUG: ACETAZOLAMIDE

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ABSTRACT

Biopharmaceutical classification system provides the concept of bio-availability, permeability and solubility of the drugs and it also reduces the study of bioequivalence by utilisation of in-vitro dissolution tests. Class IV drugs according to BCS (amphotericin B, acetazolamide, furosemide, retonavir and paclitaxel) exhibits low permeability and low solubility. Thus these drugs want more compatibility and effective delivery. Acetazolamide is a carbonic anhydrase inhibitor used for high altitude sickness, diuretics and as adjunct treatment of glaucoma and epilepsy. It is approved by FDA on 16/1/2019(ORIG-1) and it has been using from 1952 by WHO. This review article is focused mainly on treatment of central serous retinopathy by acetazolamide and its pharmacokinetic and pharmacodynamic information.

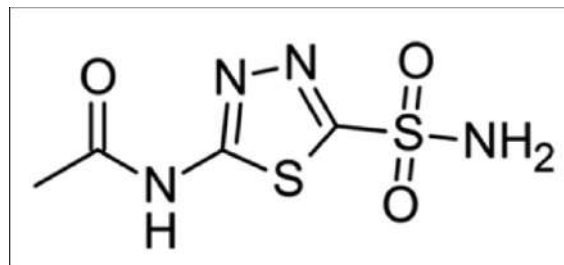
KEYWORDS: *BCS classification parameters, mechanism of action, pharmacokinetics, acetazolamide*

1. INTRODUCTION

1.1. Definitions

Drugs are classified in BCS on the basis of permeability, solubility and dissolution. BCS class IV drugs exhibits many problems characteristically for the effective oral and per oral delivery. Low solubility and low permeability, poor absorption and low aqueous soluble drugs are classified under class IV. Almost all the class IV drugs are substrate for P- Glycoprotein (low permeability) and substrate for CYP3A4 (extensive presystemic metabolism) which further potencies poor therapeutics of these drugs. For these drugs the formulation and the development of an efficacious delivery system for BCS class IV drugs very difficult tasks for any formulary dosage form. There is a lot of formulation importance on composition and design for successful drug development for both oral and per oral delivery. Some techniques used for formulation are lipid based delivery systems, polymer based nanocarriers, crystal engineering liquid-solid technology and miscellaneous techniques. However due to lack of basic physical chemistry under these formulations development is still driven by trial and error methods¹⁻⁴.

1.2. Drug Profile of Acetazolamide



IUPAC name: N-5[5-Sulfomyl-1, 3, 4-thiadiazole-2yl] acetamide

Synonyms: Acetazolamide, 2 Acetylamino-1,3,4-Thiadiazole-5-Sulfonamide

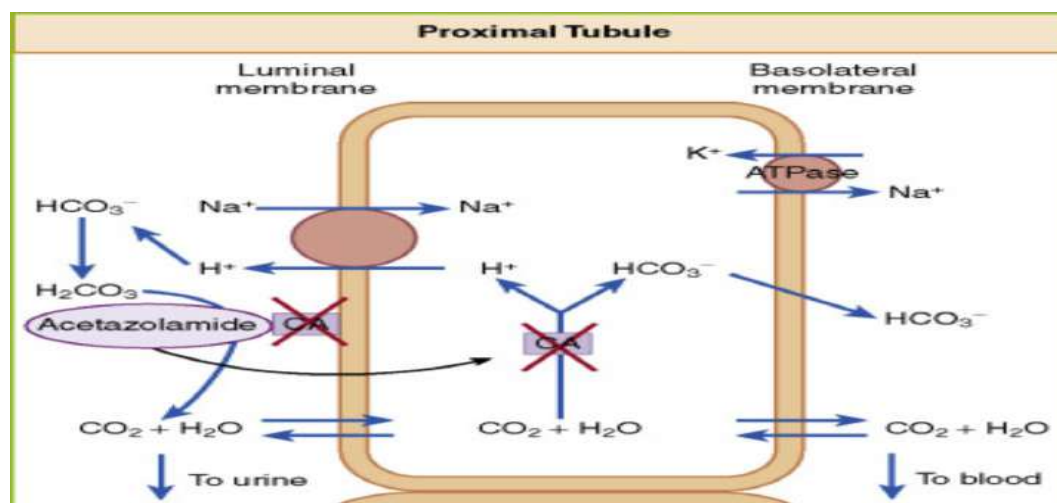
Molecular formula: C₄H₆N₄O₃S₂

Molar mass: 22.24g/mol

Melting point: 258 to 259°C

1.3.Mechanism of Action

The enzyme carbonic anhydrase is present in the kidney, gastric mucosa, pancreas, eyes CNS and RBC. The reaction that undergone by catalyses as follows



The mechanism of diuresis involves the proximal tubule of the kidney. This enzyme allows the reabsorption of bicarbonate, sodium, and chloride. Inhibition of this enzyme leads to excretion of the above mentioned ions are eliminated along with the intracranial pressure, excess of water, intraocular pressure and lowering of B.P. By the elimination bicarbonate, the blood gets acidic, causing compensatory hyperventilation with deep respiration decrease in levels of CO_2 and increase in levels of O_2 in the blood. It acts by increasing the p^{H} of the urine and releasing bicarbonate in urine. It is used as a diuretic in instances of abnormal fluid retention to decrease CSF production in increased intracranial tension and to make urine alkaline in renal tubular acidosis, also used for glaucoma and certain convulsive disorders (e.g., epilepsy)^{5,6&7}.

1.4. Pharmacokinetics

Protein binding – 70-90%

Metabolism – non metabolised (directly excreted through urine)

Elimination half- life – 2-4 hrs

Excretion - Urine (90%) via both active tubular secretion and passive reabsorption

1.5. Onset of Action

Mainly through oral and intravenous routes as described below

1.5.1. Oral Route

Acetazolamide rapidly gets absorbed in the GIT, and peak concentrations of serum for the tablets and extended release capsules are in 2-4 hrs and 8-12 hrs respectively. Onset of action in the form of tablets for glaucoma is about 2hours. The half-life of this formulation is 10-15hours. Approximately 47% of an extended release dosage form eliminated through urine within 24 hours³.

1.5.2. IV Route

Onset of action is about 2 minutes, reaching a peak effect in 15 minutes and remains for 4-5 hours. Acetazolamide is excreted through urine when given orally. 90-100% of druggets excreted through kidneys within 24 hours.

2. Dosage and administration

2.1. Epilepsy

Beneficial effects is not known in epilepsy, by direct inhibition of carbonic anhydrase in the central nervous system or by the slight degree of acidosis produced by the divided dosage. The best results upto date has been seen in petit mal in children. However, good results have been observed in patients, in other types of seizures such as myoclonic jerk patterns, grand mal and mixed seizure patterns. The recommended total daily dose is 8 to 30 mg per kg in divided doses. Even-though some of the patients respond to a low dose, the optimum range appears to be from 375 to 1000 mg daily. When Acetazolamide is administered in

combination with other anti-seizures, it is recommended that the starting dose is 250 mg once daily with the existing medications. This can be increased to levels as observed above. The change from other medications to the drug should be moderate and in accordance with regular practice in epilepsy therapy⁸.

2.2. Congestive Heart Failure

In congestive heart failure as diuretic, the initial dose is 250 to 375 mg once daily in the morning (5 mg/kg). If after an initial response, the patient fails to continue to lose inflammation fluid, skipping medication for a day does not increase the dose but allow for kidney recovery. Acetazolamide yields best diuretic results⁹.

2.3. Acute Mountain Sickness

Daily dosage is 500 mg to 1000 mg, in divided doses using tablets or sustained-release capsules as appropriate. It is preferable to initiate a dose 24 to 28 hours before ascent and to continue for 48 hours at high altitude, or longer as necessary to control symptoms. In circumstances of rapid ascent, as in rescue or military operations, the higher dose range of 1000 mg is recommended¹⁰.

3. Dosing

3.1. For Prevention of Altitude Sickness

Less than 12 years: efficacy and safety were not established.

Greater than 12 years: orally before every 8-12 hours 500-1000mg/day in divided doses. To start 24-48 hours before the ascent and continued during the ascent and at least 48 hours after arrival at high altitude if necessary, to control symptoms¹¹.

3.2. Seizures

Less than 12 years: not established

Greater than 12 years: 8-30mg/kg/day orally for every 6-12 hours in divided doses, dose to not exceed 30mg/kg/day or 1g/day (tablet)¹²

3.3 Diuretics (PO,IV)

Child: 5mg/kg/dose once daily or every other day.

Adult: 250 -375 mg/dose once daily or every other day¹³.

3.4. Glaucoma

Child: less than 12 years: (IM/IV): 20-40mg/kg/day divided in 6 hourly doses. PO: 8-30 mg/kg/day divided in 6-8 hours. Maximum dose: 1000mg/24 hours.

Child: for 12 years: 15 to 30mg/kg/day in divided doses every 6- 8 hours. Maximum dose: 1000mg/24 hours.

Adult: PO (simple chronic: open angle) 1000mg/24 hours in divided doses every 6-8 hours. IV (acute secondary; closed angle): 500MG/dose IV for rapid reduction in intra ocular pressure¹⁴.

3.5. Urine alkalinisation

Adult: 5mg/kg/dose PO repeated 2-3 times over 24 hours¹⁵.

3.6. Pseudo tumour cerebri

Child: start with 25mg/kg/day divided 1-4 times daily, increase by 25mg/kg/day until clinical response or as tolerated up to maximum of 100mg/kg/day.

Adult: start with 1g/day divided 1-4 times daily, increase by 250mg/day until clinical response or as tolerated up to maximum of 4g/day^{16, 17}.

4. Adverse effects

There is a broad range of general and specific ADRs that are induced by the use of acetazolamide like the above side effects and some may experience tinnitus, taste alterations, blood stools and paraesthesia, aplastic anaemia, hypo-natremia and hypokalemia. Flushing, drowsiness, ataxia, confusion, fatigue, vertigo, fever, seizures, dizziness, depression, malaise, headache, excitement, rash, erythema, multiforme, photosensitivity, stevens-johnson syndrome, urticaria, toxic epidermal necrolysis, tinnitus, myopia, muscle weakness, pain at injection site, paresthesia, jaundice, glycosuria, hyperglycemia, hypoglycaemia, metallic taste, development of renal calculi, nausea, vomiting, polyuria, metabolic acidosis, melena, growth retardation¹⁸.

5. CONCLUSION

Acetazolamide the BCS class IV drug needs various doses, exhibit different pharmacokinetic and pharmacodynamic properties for different conditions.

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A COMPREHENSIVE REVIEW ON GENE MUTATIONS LEADING TO DIFFERENT TYPES OF CANCERS

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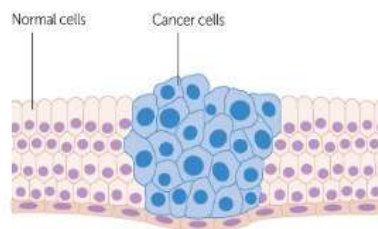
ABSTRACT

Cancer is the uncontrolled growth of abnormal cells in the body. Cancer develops when the body's normal control mechanism stops working. Old cells do not die and instead grow out of control, forming new, abnormal cells. In skin cancer CDKN2A, located on chromosome 9p21, encodes a low-molecular-weight protein, p16, that inhibits the activity of the cyclin D1-cyclin-dependent kinase 4 (CDK4) complexes. This complex phosphorylates the retinoblastoma protein, allowing the cell to progress through the G₁ cell-cycle checkpoint. Thus, p16 acts as a tumor suppressor and negatively regulates cell growth by arresting cells at G₁. In lung cancer, Mutant p53 is inactive for transcriptional transactivation. Cancer cells therefore accumulate mutant p53 (in the absence of MDM2) and fail to induce p53-dependent growth arrest, DNA repair or apoptosis. The mutant protein is a target for rescue by drugs that bind to the core domain to reverse the effects of mutation. The prostate cancer- HOXB13 suppresses the growth of AR-negative PC3 prostate cancer cells by the down-regulation of T-cell factor 4 (TCF-4). HOXB13-expressing PC3 cells exhibited significant inhibition of *in vitro* and *in vivo* cell growth with G₁ cell cycle arrest mediated by the suppression of cyclin D1 expression. *BRCA1* may normally serve as a negative regulator of mammary epithelial cell growth whose function is compromised in breast cancer either by direct mutation or alterations in gene expression can cause breast cancer. Colorectal cancer is caused by Mutations of mismatch repair proteins (i.e., MSH2, MSH6, MLH1, or PMS2) contribute to microsatellite instability and increased mutation rate in cancer cells. Kidney cancer is caused by mutations in the tumor suppressor gene *VHL*, MET, FLCN. VHL it is the substrate recognition component of the E3 ligase complex, it is composed of Cullin-2, Rbx1, Elongin B, Elongin C, that participates in the oxygen-sensing system that drives Hypoxia inducible factors alpha (HIF) degradation.

KEYWORD: *Cancer, CDKN2A, retinoblastoma protein, G₁ cell-cycle, PC3 prostate cancer*

INTRODUCTION

Cancer is the uncontrolled growth of abnormal cells in the body. Cancer develops when the body's normal control mechanism stops working. Old cells do not die but instead grow out of control, forming new abnormal cells. These extra cells may form a mass of tissue called a tumor.



Causes of cancer: A number of reasons can cause gene mutations such as smoking, radiation, viruses, cancer causing chemicals, obesity, hormones, chronic inflammation and lack of exercise.

Related to work and living environments

- Asbestos fibers
- Tar and pitch

- Some metal compounds
- Some plastic chemicals(vinyl chloride)

Bacteria and viruses can cause cancer

- Helicobacterpyroli
- HBV, HCV
- HP, EBV etc

Radiations can cause cancer

- Ionising radiation-X-ray radiation, soil radiation
- Non-ionising radiation-sun ultraviolet radiation

Some drugs may increases the risk of cancer:

Certain neoplastic agents, certain harmones , medicines that cause immune deficiency.]

Symptoms of cancer

- Blood in the urine
- Hoarseness
- Persistent lumps or swollen glands
- Change in a wart or mole
- Usual vaginal bleeding
- Un expected weight loss, night sweats, fever
- Itching in the anal or genita area

TYPES OF CANCER

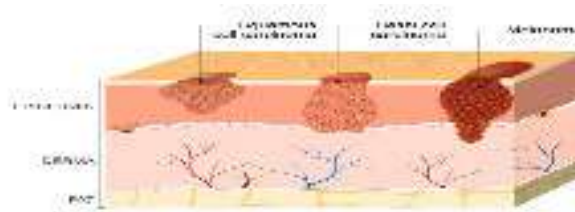
1. Skin cancer
2. lung cancer
3. Prostate cancer
4. Breast cancer
5. Colorectal cancer
6. Kidney/Renal cancer
7. Bladder cancer
8. Non-hodgkins cancer

Gene mutations in skin cancer

Skin cancers are locally destructive cancerous malignant growth of the skin. They originate from the cells of the epidermis, the superficial layer of the skin and majority of skin cancers rarely spread to other parts of the body and life become life threatening. Three major types of skin cancers are

1. Basal cell carcinoma
2. Squamous cell carcinoma
3. Melanoma

The major tumor suppressor gene associated with melanoma is CDKN2A .It is caused by mutations in the CDKN2A gene on chromosome9. Cyclin dependent kinase inhibitor 2A, It is a gene which in humans located chromosome9, band p21.3.It is ubiquitously express in many tissues and cell types. The gene codes for two proteins, including INK4 family member p16 and p14 both acts as tumorsupressors by regulating the cell cycle. P16 it inhibits cyclin dependent kinase 4 and 6 (CDK4 and CDK6) and there by activates the retinoblastoma family of proteins which block traversal from GT to S-phase.p14 activates the p53 tumor suppressor. The CDKN2A gene also contains one of 27 SNPS associated with increased riskof coronary artery disease.



Gene mutations in Lung cancer

Two major types of lung cancers are:-

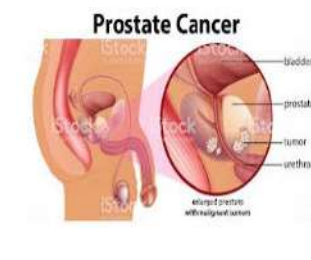
1. Non-small cell lung cancer
2. Small cell lung cancer

Causes of lung cancer include smoking, exposure to certain toxins and family history. Somatic mutations in the TP53, EGFR and KRAS genes are common in lung cancers. The TP53 gene provides instructions for making a protein called p53 i.e., located in the nucleus of cells throughout the body where it attaches to DNA.



Gene mutations in Prostate cancer

It is a form of cancer that begins in the gland cells of the prostate, which is found only in males. Pathogenic variants in genes of high and moderate penetrance, such as BRCA1, BRCA2, the mismatch repair genes and HOXB13 confer modest to high life time risk of prostate cancer. BRCA1 is a human tumor suppressor gene and is responsible for preparing DNA. HOXB13 gene it provides instructions for producing a protein that attaches to specific regions of DNA and regulates the activity of other genes.



Gene mutations in Breast cancer

Breast cancer is a cancer that forms in the cells of the breast. After skin cancer, breast cancer can occur in both men and women, cancer forms in either the lobules or the ducts of breast. BRCA1 is a human tumor suppressor gene and is responsible for preparing DNA.

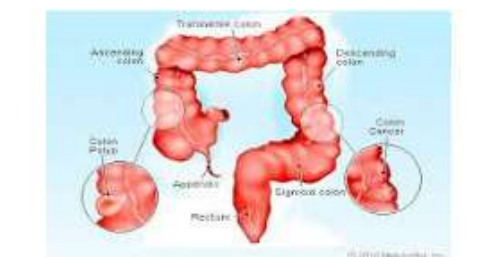


Gene mutations in colorectal cancer

Colorectal cancer also known as bowel cancer, colon cancer or rectal cancer, is any cancer that affects the colon and the rectum. Two most common inherited colorectal cancer syndromes are hereditary

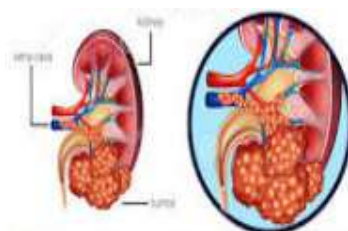
- 1.HNPCC-hereditary non poly posis colorectal cancer
- 2.FAP-familial adenomatous poly posis

MSH2 and MSH6 act on chromosome 2 and 3. It provides instructions for making a protein that that plays on essential role in DNA repair. It helps fix errors that are made when DNA is copied in preparation for cellular division. MSH2 and MSH6 to form a protein complex. This complex identifies location on the DNA where errors during DNA replication .



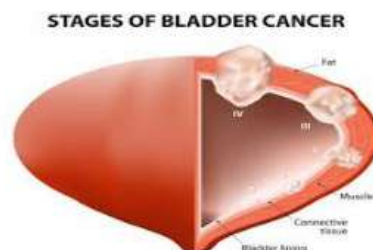
Gene mutations in kidney or rectal cancer

VHL, MET, FLCN, TSC1, TSC2. FH. Von hippel syndrome is an inherited disorder characterized by the formation of tumors and fluid. Familial hypercholesterolaemia is a dominantly inherited disorder present from birth that markedly elevates plasma low density lipoprotein cholesterol causes premature coronary heart disease.



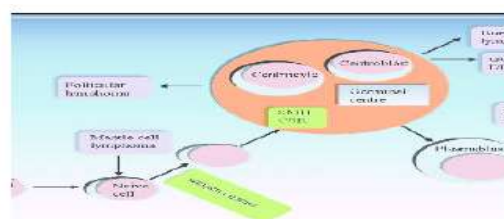
Gene mutations in Bladder cancer

Telomerase reverse transcriptase it is a catalytic subunit of the enzyme called telomerase. It is a ribonucleo protein enzyme essential for the replication of chromosome termini in most eukaryotes active in progenitor and cancer cells .catalyses the RNA – dependent extension of 3-chromosomal termini with the 6-nucleotide telomeric repeat unit.



Gene mutations in Non-hodgkins lymphoma

It is a cancer that starts in the lymphocytes. B-lymphocytes they function in the humoral immunity component of the adaptive immune system by secreting antibodies. T-lymphocytes which develop in the thymus gland and place a control role in the immune response.



CONCLUSION

This review article discussed about different types of cancers that are becoming major cause of death in human population. This article covered types of cancers and their pathogenic factors. Gene mutations in different types of DNA (Tumour suppressor) will lead to cancer and same have been elaborately discussed in the above article.

ACKNOWLEDGEMENT

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A REVIEW ON CURCUMA ANGUSTIFOLIA ROXIB

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ABSTRACT

Green plants bio-synthesizes and stores a variety of chemical products, which can be extracted for various scientific investigations. Many secondary metabolites of plant are having pharmacological importance. *Curcumaangustifolia* is also known as Arrow root or White turmeric. It belongs to the family Zingiberaceae. *Curcuma* genera (Zingiberaceae) are present in around 80 species. *Curcumaangustifolia* grows well in dark red soil, grey soil, and red soil. The tuberous of the plant can be harvested in January month consists of various phytochemical constituents such as genins, Eucalyptol, Camphene, Flavonoids, Curcumanoids, oils and amylase also present. This review will help for researchers to carryout scientific validation of folkore uses of *Curcumaangustifolia*.

KEYWORDS: *Curcumaangustifolia*, Phytochemical, Pharmacological activities.

INTRODUCTION

*Curcumaangustifolia*Roxib is also called the *tikhur* and mostly known as the East Indian arrowroot or white turmeric (Paikra et al., 2013)¹ It is a perennial flowering plant, with modest and small spiked inflorescences of 3 to 4 yellow, funnel-shaped flowers within tufts of pink terminal bracts.² The blossoms are typically observed toward the start of the blustery season July to August. The leaves may develop going to 36-37cm long and 8-10cm wide. Stems are typically short, supplanted by pseudo stems shaped by leaf sheaths. The leaves additionally smell and taste like turmeric. The incredible criticalness of the tikhur plant is its solid rhizome which can grow up to 1.5m long. The flowers are swinger, epigenous, zygomorphic. Calyx is normally cylindrical, dainty, split on 1 side, in some cases spathe like Apex 3-toothed or lobed. Corolla proximally rounded, distally 3-lobed; projections shifting fit as a fiddle, Stamens or staminodes 6, into whorls. Sidelong 2 staminodes of external whorl petaloid, or framing little teeth at the base of labellum, ovary substandard, 3-locule at first, 1-or 3-locule when adults; ovules ± various per locule. Created style 1, slim, set in a wrinkle in fiber and between anther locules.³The fruits are a capsule, fleshy or dry, dehiscent or indehiscent, sometimes berrylike⁴The Seeds are typically small, arillite.⁵

Table:1 Scientific classification of curcuma angustifolia⁶⁻⁸

Kingdom	Plantae
Subkingdom	Viridiplantae
Super division	Embryophyta
Division	Tracheophyta
Class	Magnoliopsida
Subclass	Commelinidae
Order	Zingiberales
Family	Zingiberaceae
Genus	<i>Curcuma</i>
Species	<i>Angustifolia</i>

Botanical description

The sort *Curcuma* of thefamily Zingiberaceae involves around 80 species, generally conveyed in the tropic of Asia, Africa, Southeast Asia, Australia and other warm pieces of the world 1. *Curcuma angustifolia*

Roxib. Normally known as East Indian Arrowroot, is a significant plant ², having gigantic fare an incentive in national and global market.⁶This plant is most commonly found in India especially in North East and West coastal plains and hills.

Vernacular names of *curcuma angustifolia*⁹⁻¹²

Sanskrit- Tavakshira, Tavakshira, Payaksheera, Tavakshiri, Vamsalocana. Telugu-Gaddalu, Ararut. Hindi- Tekhur, Tikhur, Theksura, Thavsasheera, Thikora, Thavakheera. Tamil- Kisangu, Araukizhangu, Kooa, Artimavu, Kookai, Kua. English- East Indian Arrowroot, Curcuma starch. Kannada- Kaadu Arrowroot Kovegida, Kove hitting gida, Thavakeela. Malayalam-Koova, Kuva-Kizhanna. Bengali- Tikkur, Keturihalodhi. Gujarat-Tavkhir, Tikhur. Tulu-Koove. Marathi-Tavakira, Thavakheera, Thavali. Manipuri-Yaipan.

Climatic conditions required for growing of *curcuma angustifolia*¹³

The suitable climatic conditions for germination of seeds at 20°C.

Soli requirement for *curcuma angustifolia*¹⁴

The yield and quality *Curcuma angustifolia* mainly based on soil, climatic conditions like pH etc., and process. The suitable pH is dark-red soil (pH 5.2), gray soil (pH 7.4) and red soil (pH 4.4). The dark red soil has the capacity to produce highest plant height, root biomass and shoot biomass than other soil plants and contain high protein, and fat contents 5.2% and 71% are respectively.

Harvesting of *curcuma angustifolia*^{15,16}

The wet and cool circumstances at altitudes of 450m are reasonable for the yield. Planted in late harvest time and watered incidentally during the dry time frame. It's a seasonal product and the seeds Rhizomes will be available only during harvesting season. Collecting done in January month.

Yield of *curcuma angustifolia*

The yield of *Curcuma Angustifolia* starch is 125-140g from 1kg rhizomes suggesting the tremendous loss of starch together with time and work prerequisite. (Das et al., 2015; Tiwari and Patel, 2013).^{17,18}

Chemical constituents

The *Curcuma angustifolia*, rhizome is a brownish yellow colour, contains-Carbohydrates: 23.44%, Proteins: 1.5%, Fat: 0.17%, Ash: 0.88, Moisture: 75 % (wb) ¹⁹In Starch- Carbohydrates: 83%, Proteins: 1.6%, Fats: 0.9%, Ash: 1.0%¹⁹. In other investigations, Carbohydrates: 97%, Proteins: 0.89%, Fats: 0.56%, Ash: 0.68%²⁰. Its minerals ranges (µg/ gram weight) are: Phosphorous-536, Calcium-916, Magnesium-196, Iron-138.5, Sodium-55.6, Potassium-70.1, Copper-6.19, Zinc-42.2, and Manganese-19.9 ²⁰. The total amylase content is 31.4% in the *curcuma angustifolia*.¹⁹

Phytochemicals

The *Curcuma Angustifolia* contains are Stem: Glycosides tenacissosidas A-E, Feronic acid-polyoxytregnanes, Marstenacigenins A & dresgenin.²⁰ Seed-Drebogenin, polyhydroxy, pregnanecissogenin, tenasogenin²⁰. Root: D-cymarose, B-D-glucose 1-L-thebetose, cinnamic acid, and acetic acid²⁰ Leaf-The major components are 8,9-dihydro-9-cycloisolongifolene, Xanthorrhizol isomer and a few amounts of Curzerenone, Eucalyptol, Camphor, Germacrone, ar-tumerone, Curdione, and Camphen.²¹ The rhizomes of *C. angustifolia* contains the secondary metabolites like alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, curcumins, steroids, glycoside, and oils.^{22,23} The leaf and rhizomes contains Curzerenone (32-72.6%), 14-hydroxy-d-cadinene (18.6%), gamma-eudesmol acetate (7.3%), camphor (3.3%) and germacrone (3.3%).²⁴

Quantitative analysis of phytochemical constituents of *curcuma angustifolia*

The quantitative analysis is soaking of rhizome in cold temperature (10-12h), to produce pulp and followed by different steps. The results are Carbohydrates: 23.4%, Proteins: 1.7%, Fat: 0.17%, Ash: 0.88%, Moisture: 76% and also contains the minerals, mainly phosphorous: 536µg/g, calcium: 916µg/g. They also contain the polysaccharide is amylase: 31.4 %.²⁰

Pharmacological activities

Antimicrobial, anti ulceration.^{25, 26} Antioxidant²⁴, Antifungal and Anti biotics.²⁷ .

Astringent

The *Curcuma angustifolia* acts as the astringent in the treatment of Diarrhoea, Dysentery and Colitis.⁶ In Raktapitta, it mitigates the pitta dosha and has astringent property, captures dying⁶

Diuretic

It produces the diuretic action, edema induced by the cardiac problems.

Anti ulcer

Amlapitta is a hyperacidity disease, like gastritis and peptic ulcer diseases. *Curcuma angustifolia* decreases acid production and reduces adverse effects than other drugs.⁶

Stomatitis

The powder of rhizomes with nectar applied to the mucous layer of the oral depression in stomatitis. It additionally advances the mending of stomach ulcers.⁶

Anti cancerous

Sangamitra Nayak *et al.*,²⁸ has worked the methanolic rhizome extract of *Curcuma angustifolia*, on the cervical cancer line (HeLa). It reveals that it has the antioxidant, antimicrobial and potentially anti cancerous activity.

Hepatoprotective activity

Lisona Elias *et al.* screened the hepatoprotective activity of *Curcuma angustifolia* on paracetamol-induced hepatotoxicity in albino rats. It reveals that the plant has a moderate to good protection and cytoprotection activity.

Antioxidant

Sudipta Jena, Asit Ray *et al.* Screened the antioxidant activity of essential oil extracted from the rhizomes and leaf. This assay reveals that it can decrease the levels of stable free radicals as potential than other drugs.²⁹

Uses

The Nutraceuticals are inserted into food systems for the development of functional foods. Aside from rhizomes, different parts, for example, inflorescence, tuberous roots, and rootstocks of *Curcuma angustifolia* have been accounted for to be a rich wellspring of proteins and starches and are utilized in culinary arrangements as vegetables and nourishment starters.³⁰⁻³⁹ These products are easily digestible and also contain minerals mainly phosphorous. So given to the children. It is nutritive and is utilized as a pleasant, no irritating diet in certain incessant illness during improvement structure fevers.⁴⁰

Improved commercial varieties of *curcuma angustifolia*

In India and other countries peoples are mostly like to prefer the *Curcuma angustifolia* kept in gardens, houses and offices because of its appearance like an ornamental plant with long leaves, also produces good fragrance and have high nutritional value⁴¹. Based on the levels like nutritional, mineral and easily availability. It uses from cereal, tuberous starches to new starch sources like mango ginger rhizome (Policegoudra and Aradhya, 2008)⁴², harms tuber starch (Malumba *et al.*., 2016)⁴³, Lotus rhizome (sukhija *et al.*, 2016, and Zhu, 2017)⁴⁴, kudzu root (Chen *et al.*,)⁴⁵, kiwifruit starch (Li and Zu, 2017).^{46,47} and Parota starch (Estrada-Leon *et al.*, 2016)⁴⁸.

CONCLUSION

This Review will help the researchers to carry out further scientific validation of folklore usages of this *Curcuma angustifolia* actions.

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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL RP-HPLC METHOD FOR THE ESTIMATION OF RIVASTIGMINE TARTRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Rivastigmine is a cholinesterase inhibitor used for treatment of mild to moderate Alzheimer's disease and Parkinson's. Rivastigmine tartrate improves the function of nerve cells in the brain. It works by preventing the breakdown of a chemical called acetylcholine people with dementia usually have lower levels of this chemical, which is important for the processes of memory, thinking and reasoning. The objective of the present work is to develop simple, accurate, economic, selective and precise High performance Liquid Chromatography (HPLC) method has been developed and validated for rapid and simultaneous quantification of Rivastigmine Tartrate in capsule dosage form. Mobile phase contained a mixture of OPA buffer and Acetonitrile in the ratio of 75:25v/v, flow rate 1.0ml/min and UV detection at 198nm and it shows a good linearity in the concentration range of 2-75µg/ml. The developed method has been validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD less than 2. So, the proposed method was found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for routine analysis of Rivastigmine tartrate in bulk drug and the pharmaceutical formulations.

KEY WORDS: *RP-HPLC, Rivastigmine tartrate, Validation.*

INTRODUCTION

Dementia is a progressive brain dysfunction, which ends up in a very restriction of daily activities and in most cases leads within the longterm to the necessity for care. Alzheimer's is the most frequent kind of insanity in adulthood. Suffered from principally impaired memory and orientation ⁽¹⁾ Rivastigmine is could be a enzyme substance used for treatment of gentle to moderate Alzheimer's disease and Parkinson's. Rivastigmine has incontestible treatment effects on the psychological feature practical & activity normally related to Alzheimer's and Parkinson's disease dementias ⁽²⁾. This act to boost the cholinergic function is presently used as normal treatment choices options for patients with Alzheimer's malady ⁽³⁾. Rivastigmine salt has a formula of $C_{14}H_{22}N_2O_2.C_4H_6O_6$ and molecular weight of 400.43g/mol. It is having off-white in color and is soluble in following solvents, soluble in water, ethanol, and acetonitrile. And its storage condition is 2-8°C ⁽⁴⁾. Within the gift investigation, a simple, sensitive, precise & correct. It absolutely was developed for the quantitative estimation of the Rivastigmine salt in bulk and pharmaceutical indefinite quantity forms. The mechanism is unknown; it's postulated to exert its therapeutic impact by enhancing cholinergic operate. This can be accomplished by increasing the concentration of neurotransmitter through reversible inhibition of its chemical reaction by enzyme. In additionally to positive needs for analytical methodology, the hanging advantage of this methodology is that it's economical.

MATERIALS AND METHOD

Optimised Methodology

Buffer: 1ml oxyacid in 1 litre water.

Mobile phase: Mixture of buffer, Acetonitrile in the ratio of (75:25)

Diluent: Use mobile part as a agent

Column: Agilent C₁₈, 250 nm × 4.6mm, 5µm

Wave length: 198nm

Injection volume: 20 μ l

Column Temperature: Ambient

Flow rate: 1.0 ml/min

Retention time of Rivastigmine is concerning 4-5min

Chemicals

H₃PO₄, KH₂PO₄, HCL, FeCl₃, CH₃CN,

CH₃OH and H₂O were purchased from Merck Ltd. NaOH is purchased from Finar Reagents. And MBTH is purchased from Loba Chemie Ltd.

Equipment

Working normal of Rivastigmine was obtained from well purported analysis laboratories ⁽⁵⁾. Water HPLC with EMPOWER2 package victimization electronic device detector, cyber labs sonicator, datla instruments pH meter Model DI-45P were used.

Selection of wavelength

A solution of Rivastigmine salt (20 μ g/ml) was scanned within the UV region victimization ACN: OPA buffer within the ration of 25:75% v/v. The absorbance most was found at 198nm. Rivastigmine salt resolution in several mobile parts ratios and different flow rate value additionally scanned within the UV region victimization PDA detector. Thus 198nm was elect as detection wavelength for estimation of Rivastigmine salt by HPLC methodology.

Preparation of Standard Solution

Weigh accurately concerning 10 mg of Rivastigmine salt operating normal into a 100ml meter flask. Add 70 ml of agent, sonicated to dissolve and dilute to meter agent 5ml to 25ml with the agent. Further dilute 5ml to 25ml with the agent (Fig-1) Preparation of Sample Solution Weighed 14 capsules and weigh powder then take 14 equivalent of sample into 100 ml volumetric flask. Add 70 ml of agent, sonicated to dissolve and dilute to volume agent. Further dilute 5ml to 50 ml with the agent. Filter through 0.45 μ Nylon syringe filter. Graph shows in (Fig-2)

System quality

A standard Rivastigmine resolution (160 ppm) was ready and injected into the HPLC system. The system quality parameters were evaluated from normal chromatograms by conniving the half RSD from 6 replicate injections of Rivastigmine retention time's peak areas. The number as per the test methodology, the average % recovery was calculated

Linearity

Dimensionality of detector response for Rivastigmine was established for 8 concentrations starting from 2 μ g/ml to 75 μ g/ml for the target concentration i.e 20.8 μ g/ml. The ultimate concentration of every resolution in μ g/ml was calculated and plotted against space response. The dimensionality data was given within the following table no: 1 and also the graph in fig no: 3

Accuracy

The accuracy of the strategy were examined by best-known quantity of rivastigmine to pool ⁽⁶⁾. A study of accuracy was conducted for Rivastigmine Assay in Triplicate (50%, 100%, & 150%) and also the results was in table no; 2

Precision

The exactitude of the analytical methodology was studied by analysis of multiple sampling of homogenous sample & results was in table no; 3

Limit of Quantification

The LOQ resolve by victimization the formulae $LOQ = 10 \sigma / S$. Value was found to be 0.708775 ppm & results in table no:4

Limit of Detection

The LOD resolve by victimization formulae $LOD = 3.3 \sigma / S$. The LOD value of Rivastigmine was found to be 0.233896 ppm and also the results was in table no: 4

Robustness

The robustness of the projected 'method resolve by analysis of aliquots from homogenous tons by differing physical parameters like rate, mobile part composition, wavelength & the results was in tableno:5

Forced degradation studies

The specificity of the strategy was incontestable through forced degradation studies conducted on the sample victimization acid, alkaline, oxidative, reductive thermal and photolytic degradations & the results was in table no:6

RESULTS & DISCUSSION

Method development & Validation The development of an analytical methodology for the determination of medication by RP-HPLC has received appreciable attention in recent years as a result of their importance in internal control of medication and drugproduct. The target of the study was to develop an, easy, rapid, precise, correct and sensitive RP-HPLC methodology for the analysis of Rivastigmine salt in bulk and its pharmaceutical indefinite quantity kind by victimization solvent system of ACN: OPA within the ratio 25:75 and AGILENT C₁₈, 250 nm × 4.6nm, 5µm stationary part. The activity condition is ready at flow rate of 1ml/min with electronic device detector at 198 nm. As per ICH requirements validation studies are dispensed by victimization freshly ready solutions. Optical characteristics got in following Table 7.

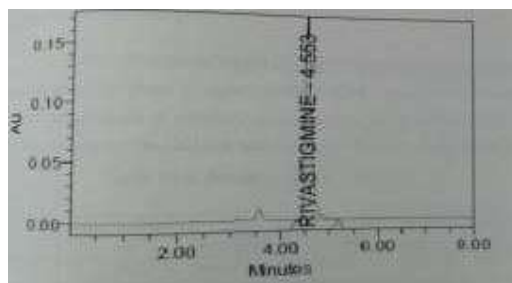


Fig-1 aRepresentativeChromatogram of sample

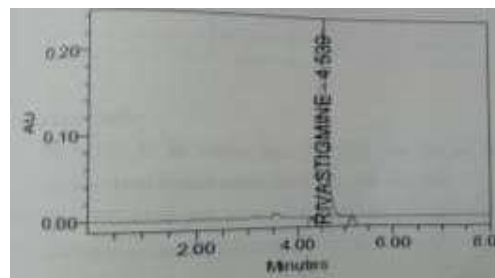


Fig-2 a representative chromatogram of Standard

S NO.	Conc	% Linearity	Peak area
1	0.5	2	124367
2	1.02	5	201554
3	2.55	12	501118
4	5.10	25	990751
5	7.65	37	1472450
6	10.20	49	2032026

Table 1. Linearityvalues

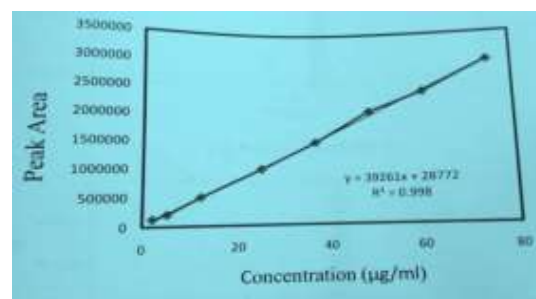


Fig-3 Linear graph ofRivastigmine

Table 2.Accuracy results of Rivastigmine by HPLC

S.NO	Accuracy	Conc. (ppm)	Area	Amount Recovered	% Recovery	Results
1	50%	10	9712888	9.96	99.6	Mean=100.46 SD=0.9016 %RSD=0.8977
2	50%	10	998706	10.24	101.4	
3	50%	10	989595	10.14	100.4	
1	100%	21	2109941	21.63	101.5	Mean=100.5 SD=0.8717 %RSD=0.8673
2	100%	21	2091009	21.43	100.1	
3	100%	21	2153600	22.07	99.9	
1	150%	28.5	2820247	28.91	100.6	Mean=100.93

2	150%	28.5	2796406	28.66	100.8	SD=0.41633 %RSD=0.4124
3	150%	28.5	2880458	29.52	101.4 Mean=100.6 SD=0.231 %RSD=0.230	

Table 3. System Precision

S.no	RT	Area	USP Plate count	USP tailing
1	4.591	2011934	6414	1.52
2	4.595	2016033	6442	1.50
3	4.591	2026170	6437	1.51
4	4.578	2037466	6420	1.51
5	4.595	2043551	6429	1.51
6	4.588	2041303	6426	1.52
	Mean=4.363 %RSD=0.0236	Mean=2206102 %RSD=0.260	Mean=8154.3 %RSD=0.5630	Mean=1.469 %RSD=0.2455

Table 4. LOD & LOQ values of Rivastigmine

LOD	LOQ
0.23389	0.708775

Table 5. Robustness data of Rivastigmine

1	1.2	3.757	1714954	6262	1.58	99.5
2	1.2	3.762	1666756	5939	1.56	100.1
3	1.2	3.763	1678040	6036	1.55	100.4
		%RSD 0.08549	%RSD 1.49460	%RSD 0.23528	%RSD 0.97709	%RSD 0.458258
1	1.0	4.591	2011934	6414	1.52	100.20
2	1.0	4.595	2016033	6442	1.50	100.45
3	1.0	4.591	2026170	6437	1.51	100.47
		%RSD 0.050279	%RSD 0.36313	%RSD 0.232206	%RSD 0.662251	%RSD 0.149884
1	0.8	5.785	2633181	7214	1.69	99.4
2	0.8	5.781	2693396	7251	1.66	99.5
3	0.8	5.787	2616744	7232	1.70	99.1
		%RSD 0.039922	%RSD 1.52414	%RSD 0.255826	%RSD 1.236655	%RSD 0.209564

Table : 6. Results of forced degradation

S.no	Stress conditions	R _t	Peak area	Plate count	USP tailing	% degraded	Purity angle	Purity Threshold
1	STD	4.361	2105106	8686	1.432	-0.2	0.249	1.266
2	Acid	4.405	1467424	10831	1.303	17.4	0.48	1465
3	Base	4.414	1435482	11164	1.270	19.9	0.322	1.383
4	Peroxide	4.391	1151919	10378	1.310	19.7	7.209	17.299
5	Reduction	4.391	1453163	9605	1.347	19	0.654	1.456
6	Thermal	4.369	1931141	8603	1.449	18.4	0.229	1.291
7	Photo	4.366	2100841	8454	1.492	18.9	0.235	1.292
8	Humidity	4.370	1863784	8669	1.441	19.8	0.269	1.326
9	Hydrolysis	4.367	2041027	8381	1.446	18.9	0.26	1.313

Table 7. Validation Parameters of Rivastigmine tartrate by HPLC

Parameter	Acceptance Criteria	Rivastigmine Tartarate
Linearity range	Correlation coefficient	2-75µg/ml
Correlation coefficient	r ² >0.999	
LOD	S/N > 2 or 3	0.233896µg/ml
LOQ	S/N > 10	0.708775µg/ml
System Precision	RSD< 2%	%RSD = 0.456
Intermediate Precision	RSD< 2%	%RSD = 0.385
Method Precision	RSD< 2%	%RDD = 0.298
Accuracy	Recovery 98-102%	% recovery = 98.08-101.9
Specificity	No interference from blank, placebo & other degradation products with the main peak Purity angle < threshold angle	No interference and Peak pure
Solution Stability	>12 hour	Stable up to 24 hour % RSD = 0.55
Robustness	RSD NMT 2% in modified Condition Flow minus Flow plus Organic plus Organic minus Wavelength plus Wavelength minus	Complies %RSD=0.085 %RSD=0.039 %RSD=0.12 %RSD=0.202 %RSD=0.026 %RSD=0.026

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Rivastigmine tartrate in bulk and pharmaceutical dosage forms. The results expressed in Tables: for the HPLC is promising. In addition to positive requirements for analytical methods, the striking advantage of all percentage developed methods is that they are economical. And method is validated is validated in terms of accuracy, precision, linearity, and limit of detection, limit of quantization & robustness. This method can be used for the routine determination of Rivastigmine

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A SAFE APPROACH FOR IMMUNIZATION-EDIBLE VACCINES

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ABSTRACT

Edible vaccines hold nice promise as a cheap, easy-to-administer, easy-to-store, fail-safe and socioculturally promptly acceptable vaccine delivery system, particularly for the poor developing countries. It involves introduction of hand-picked desired genes into plants and so inducement these altered plants to manufacture the encoded proteins. Introduced as an idea concerning a decade ago, it's become a reality nowadays. a range of delivery systems are developed. At first thought to be helpful only for preventing infectious diseases, it's additionally found application in hindrance of response diseases, contraception, cancer medical aid, etc. Edible vaccines are presently being developed for variety of human and animal diseases. There is growing acceptance of transgenic crops in each industrial and developing countries. Resistance to genetically modify dysfunction foods could have an effect on the long run of edible vaccines. They need passed the most important hurdles within the path of associate degree rising vaccinum technology. Numerous technical obstacles, restrictive and non-scientific challenges, although all appear surmountable, need to be overcome. This review tries to debate the present standing and way forward for this new preventive modality.

KEYWORDS: *Chimeric viruses, Norwalk virus, transgenic.*

INTRODUCTION

Edible vaccines are nothing however transgenic plant associate in nursing animal based mostly production of or those who contain agents that trigger an animal's reaction. In straightforward terms, edible vaccines are plant or animal created prescribed drugs. Edible vaccines hold nice promise as a cost- effective, simple to administer, simple to store, fail-safe and socioculturally without delay acceptable vaccinum delivery system, particularly for the poor developing countries. It involves introduction of elect desired genes into plants so causing these altered plants to manufacture the encoded proteins. Vaccines are revolutionary for the hindrance of infectious diseases. Despite worldwide protection of youngsters against the six devastating diseases, two hundredth of infants are still left unimmunized; responsible for about 2 million extra deaths per annum, especially within the remote and impoverished elements of the globe¹. This is as a result of the constraints on vaccinum production delivery and distribution. Immunization through polymer vaccines is another however is a chic approach, with unsatisfactory immune response². As medical man aforesaid that, "let thy food be the thy medicine", scientists recommend that plants and plant viruses will be genetically designed to supply vaccines against diseases like dental caries; and serious infections like diarrhoea, AIDS, etc³. Native white corpuscle populations, generating liquid body substance immunoglobulin G, IgE responses, native immune gamma globulin response and memory cells, which might promptly neutralize the attack by the important infectious agent⁴.

Preparation of edible vaccines

Introduction of foreign deoxyribonucleic acid into plants ordering will either be done by bombarding embryonic suspension cell cultures mistreatment gene-gun or a lot of usually through *Agrobacterium tumifaciens*, a present soil microorganism, that has the power to induce into plants through some reasonably wound (scratch, etc). It possesses a circular Ti cellular inclusion (tumor inducing), wherever it will live. This ability may be exploited to insert foreign DNA into plants ordering. However before this, the cellular inclusion has to be disarmed by deleting the genes for growth regulator and plant hormone synthesis, so it dies not manufacture neoplasm. Genes for antibiotic resistance are accustomed choose out the transformer's cells and whole plants, that contain the foreign factor, and for expressing the required product, which might then be regenerated from them⁵. The deoxyribonucleic acid integrates indiscriminately into plants ordering,

leading to a special substance expression level for every freelance line⁶, so 50-100 plants are transformers along at a time, from that one will opt for the plant expressing the very best levels of substance and least range of adverse effects. Production of transgenic plants is species dependent and takes 3-9 months. Reducing now to 6-8. Weeks are presently below investigation. Some antigens, like infectious agent capsid parts, have to be compelled to self-assemble into VLPs (virus like particles). VLPs mimic the virus while not carrying deoxyribonucleic acid or ribonucleic acid and thus don't seem to be infectious. Every single substance expressed in plants should be tested for its correct assembly and might be varied by animals studies, Western blot, and quantal by enzymes-links immunosorbent assay (ELISA)⁷. Successful expression of foreign genes in plants cells and or its edible parts have given a possible to explore more and expand the likelihood of developing plants expressing quite one matter macromolecule. Multicomponent vaccines may be obtained by crossing 2 plants lines harboring totally different antigens. Adjuvants might also be co-expresses at the side of the antigens within the same plant. B monetary unit of eubacterium cholerae poison (VC-B) tends to related to copies of it, forming a rounded pve-member ring with a hole within the middle⁸. This feature will bring many totally different antigens to M cells at just one occasion – for instance, a power edible vaccinum against Indian cholera, ETEC (Enterotoxigenic E. coli) and reovirus might with success elicit significance cant response to any or all 3-12⁹ world alliance for vaccines and immunization (GAVI) accords terribly priority to such combination vaccines for developing countries.

Various strategies

Approaches to membrane vaccine formulation embody (i) gene fusion technology making non-toxic derivatives of mucosal adjuvants; (ii) cistrontically inactivating substances by deleting an important gene; (iii) co-expression of antigen and a protein, which modulates and controls membrane immune reaction; and (iv) genetic material itself, permits DNA/ ribonucleic acid uptake and it's endogenous expression within the host cell various membrane delivery system embody perishable micro- and nanoparticle liposomes, live microorganism/viral vectors and membrane adjuvants¹⁰. Prime boost strategy combines completely different routes of administration and vaccinum sorts, especially wherever multiple antigens or doses are needed¹¹. For example, one duct dose of MV-H DNA morbilli virus haemagglutinin) followed by multiple oral MV-H Boosters may embody kitchen utensil qualities of MV neutralizing Antibodies than with either vaccine alone¹². Certain viruses is redesigned to precise fragments of antigenic proteins on their surface, like CPMV (cowpea mosaic virus), alfalfa mosaic virus, TMV (tobacco mosaic virus), CaMV (cauliflower mosaic virus), potato virus X and tomato bushy stunt virus. Technologies concerned are overcoat and epicoat technology¹³.

Challenges

There are several queries which require to be answered before developing a plant-based vaccine. Three successful human clinical trials have shown that adequate doses of matter are achieved with plant-based vaccines¹⁴⁻¹⁶. As an example, oral hepatitis-B dose is 10-100 times the epithelial duct dose; and one hundred gram potato expressing B monetary unit of labile poisonous substance of ETEC (LT-B) is needed in 3 totally different doses, to be immunogenic¹⁷. Makes an attempt at boosting the quantity of antigens typically cause scrubby growth of plants and reduced tuber/fruit formation, as an excessive amount of m-RNA from the transgene causes gene-silencing in plant ordination¹⁸. a number of the techniques to overcome these limitations are (i) optimisation of writing sequence of bacterial/viral genes for expression as plant nuclear genes, (ii) expression in plastids¹⁹, (iii) plant viruses expressing foreign genes²⁰, (iv) coat-protein fusions²¹, (v) viral-assisted expression in transgenic plants²² and (vi) promoter components of bean plant disease virus with communicator genes GUS (β -glucuronidase) and GFP (green β uorescent protein), substituted later with target substance genes¹⁸. Antigengenes could also be joined with restrictive components that switch on the genes additional readily; or do therefore solely at elite times (after the plant is almost absolutely grown); or solely in its edible regions. Exposure to some outside matter molecule might also be tried²³.

Clinical Trials

Antigen expression in plants has been with success shown in past, like LT-B (ETEC) in tobacco and potato, lyssa virus-G supermolecule in tomato²⁴, HBsAg in tobacco²⁵ and potato, norwalk virus in tobacco and potato, CT-B (vibriocholerae) in potato(1). Moral issues typically preclude clinical trials from directly assessing protection, except in an exceedingly few cases²⁶. In distinction, veterinary researchers will assess immune protection additional directly.

ETEC

Charles Arntzenat Boyce Thompson Institute, USA, accomplished the primary printed productive human trial in 1997²⁷. Eleven volunteers were fed raw transgenic potatoes expressing LTB. Ten(91%) of those people developed neutralizing antibodies, and six (55%) developed a membrane response.

Norwalk virus

Nineteen out of twenty folks fed with transgenic potato expressing Norwalk virus matter showed seroconversion²⁸. Try are current to engineer bananas and pulverised tomatoes expressing Norwalk virus.

Cholera

Transgenic potato with CT-B factor of vibrio cholera was shown to be efficacious in mice. Intake one potato every week for a month with periodic boosters was a fore mentioned to supply immunity²⁹. Co-expression of mutant Asiatic cholera poison monetary unit A (mCT-A) and LT-B in crop seed has been shown to be effective by nasal Administration and is extraordinarily practical³⁰.

Measles

Mice fed with tobacco expressing MV-H (measles virus hemagglutinin from Edmonston strain) might attain protein 5 times the amount thought of protecting for humans and that they conjointly incontestible liquid body substance immune serum globulin in their faeces³¹. Prime boost strategy by combining parental subsequent oral MV-H boosters might induce titers twenty times human protecting levels. These titers were considerably bigger than with either immunizing agent administered alone. MV-H edible immunizing agent dose not cause atypical contagious disease which can be often seen with current vaccine³² for achieving its destruction. This success in mice has prompted similar experiment in primates. Once given with CT-B 35-50 gram me MV,-T lettuce is enough, but an inflated dose would run alone.

Hepatitis B

For hepatitis B virusparentalsVI, ps might invoke specific antibodies in mice³³. 1st human trials of potato based mostly immunizing agent against hepatitis B have according encouraging results the quantity of HBsAg required dose might achieve single potato. Levels of specific antibodies considerably the protecting level of 10mlU/mL in humans. When cloned into Cauliflower mosaic virus inclusion HBsAg subtype showed higher expression in roots as compared to leaf tissue of transgenic potato. The assembly in f matter by completely different promoters, likepatatin promoter. Prime boost strategy in mice with one sub – immunogenic parental dose of yeast derived recombinant HBsAg and subsequent oral transgenic potato semiconductor diode to development of antibodies with a single sub immunogenic parental dose yeast derived recombinantHBsAg oral transgenic potato led to development of antibodies that peak at > 1000mlU/ml and were maintained at > 200 ml U/ml. this might helpful in protection strategy for developing countries³⁴. Enough matter for four, 000 immunizing agent doses were obtained from simply thirty tomato plants. Transgeniclettuce is additionally being developed.

Rabies

Tomato plants expressing madness matter might induce antibodies in mice³⁵. As an alternative, TMV may additionally be used³⁶. Reworked tomato plants mistreatment CMV with the conjugated protein factor of madness virus was shown to be immunogenic in animals³⁷.

HIV

Initial success in splice HIV into CPMV has achieved. Two' HIV macromolecule factor and CaMV as promoter were with success injected into tomatoes with a needle and expressed protien was incontestible by enzyme chain reaction in numerous components of the plant, together with the ripe fruit as in second generation plant.

STD's

Human nonmalignant tumour virus type-11 (HPV-11) recombinants VLPs are produced in insect cells are specifically immunogenic when given orally to BALB/c mice. The response observed is dose and conformationally-dependent and possess a restricted genotype.

CONCLUSION

Edible plant-derived vaccine might result in a way forward for safer and more practical protection. They overcome some of the difficulties related to ancient vaccines, like production, distribution and delivery, and that they will be incorporated into the immunization plans. They have passed the key hurdles within the path of rising vaccine technology. Before turning into a reality, the technical obstacles, though all appear surmountable, ought to be overcome. However, with restricted access to essential health care in abundant of the world and with the scientific community still combating complex diseases like HIV, malaria, etc., an economical, safe and efficacious delivery system within the variety of edible vaccines will become a vital element in our disease-prevention arsenal.

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A SYSTEMIC REVIEW ON PHYTOSOMES IN PHARMACEUTICAL INDUSTRY

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ABSTRACT

Lipid solubility and molecular size are the foremost limiting factors for molecules to move from the biological membrane to be absorbed consistently following oral or topical administration. Many plant extracts and phytoconstituents, despite having glorious bio-activity in vitro demonstrate less or no in vivo actions thanks to their poor macromolecule solubility or improper molecular size or each, ensuing poor absorption and poor bioavailability. It's typically found that, once individual constituents are isolated from the plant extract there's loss of specific bio-activity. Typically some constituents of the multi-constituent plant extract are destroyed in viscous environment once taken orally. Phytosomes are advanced forms of flavourer formulations that are higher absorbed, and as a result to produce a higher bioavailability and actions than the conventional flavourer extracts. They're made by a patented method whereby the standardized plant extract or its constituents are absolute to phospholipids, mainly phosphatidylcholine, manufacturing a macromolecule compatible molecular complex. This phyto-phospholipid complicated (phytosome) resembles a bit cell. Phytosomes exhibit higher pharmacokinetic and pharmacodynamic profile than conventional flavorer extracts. Phytosome technology has been effectively accustomed increased the bioavailability of the many popular flavourer extracts as well as milk weed, ginkgo, grape seed, green tea, hawthorn, ginseng etc and may be developed for varied therapeutic uses or dietary supplements.

KEY WORDS: *Phytosomes, phosphatidylcholine, phytoconstituents, phyto-phospholipid.*

INTRODUCTION

Phytosomes, conjointly referred to as herbosomes, are cellular lipid structures ("phyto" suggests that plant, "some" suggests that cell-like). A phytosome could be a advanced of a natural active ingredient and a lipid - principally phospholipid. its claimed that phytosome will increase absorption of "conventional flavorer extracts" or isolated active principles each locally also as orally.

ADVANTAGES

1. Lipid, i.e., phosphatidylcholine one of the valuable components of phytosome includes a bifunctional activity by acting as a vehicle moreover as health profit like hepatoprotective activity.
2. The absorption of hydrophilic active constituents is exaggerated that also increase the effectiveness.
3. Phytosomes have higher stability.
4. Phytosome has the ability to permeate through skin due to its lipid layer around the phytoconstituent and so enhance the effectiveness.
5. By increasing the solubility of gall to flavourer origin phytoconstituents,
6. Phytosomes enhance the liver targeting ¹.
7. Phytosome increase the solubility of digestive juice to flavourer constituents²
8. Period of action is inflated³⁻⁵
9. They permeate the non-lipophilic biology extract to permit higher absorption from the enteric lumen, that is otherwise unattainable ⁶
10. The formulation of phytosome is safe and also the elements have all been approved for pharmaceutical and cosmetic use.
11. They need been wont to deliver liver protective flavonoids as a result of they'll be created simply bioavailable by phytosomes. additionally to the present, phosphatidylcholine is additionally hepatoprotective then provides a synergistic result for liver protection⁷

12. This technology offers value effective delivery of phytoconstituents and synergistic edges once used as useful cosmetics to shield the skin against exogenous or endogenous hazards in traditional still as trying environmental conditions⁸
13. They'll be additionally used for increased permeation of drug through skin for transdermic and dermal delivery⁹
14. The sac system is passive, non-invasive and is obtainable for immediate commercialisation¹⁰
15. Phosphatidylcholine, a vital a part of the cytomembrane utilized in phytosome technology, acts as a carrier and additionally nourishes the skin
16. There's no drawback with drug demurrer throughout formulation preparation.
17. The dose demand is reduced because of improved absorption of the most constituent. they'll even be given in smaller quantities to attain the specified results¹¹
18. Low risk profile: this technology has no large-scale drug development risk since the pharmacological medicine profiles of the phytosomal elements are well documented within the scientific literatures¹¹
19. Phytosomes are safe to use for transdermic drug delivery.

DISADVANTAGES

- Despites of many benefits of phytosomes some fatal disadvantages like phospholipids (lecithin) will induce proliferation on MCF-7 carcinoma cell line has been reported¹².
- Phytoconstituent is apace eliminated from phytosome. The period of action is brief.

METHOD OF PREPARATION

1. **Solvent evaporation method:-**A set amount of drug, compound and lipid (phosphatidylcholine) is taken during a spherical bottom flask (RBF). It's refluxed with specific solvent at 50-600C for two hours. Then the mixture is targeted to 5-10 min to induce the precipitate that is filtered and picked up. The dried precipitate is phytosome loaded with drug¹³.

2. **Rotary evaporation technique:** Amount of drug, polymer, and solvent is taken during a rotary flask and stirred for three hours below 400C. As a result, skinny film of sample is obtained to that n- alkane is supplementary and unendingly stirred exploitation magnetic stirrer. As a result, phytosomes loaded with drug are obtained, that is collected and holds on at temperature¹⁴.

3. **Antisolvent precipitation technique:** Better-known amount of drug, phospholipids and compound is taken in spherical bottom flask (RBF) and refluxed with specific solvent not surpassing 600C for two hours. The mixture is targeted to 5-10ml and n -Hexane is fastidiously supplementary to that with continuous stirring to induce the precipitate that is filtered and unbroken in vacuum dessicator nightlong. The dried precipitate is crushed in mortar and sieved through 100- mesh size. As a result, phytosomes loaded with drug are obtained, that are placed in amber colored glass bottles at temperature¹⁴⁻¹⁶.

Selection of dose type for delivery of phytosomes:

Phytosome will be developed for each oral further as topical use. Following are few instructed dose forms for phytosome delivery:

1. **Soft gelatin capsules:** The phytosome will be distributed in oily vehicles (vegetable or semi-synthetic oil) to get suspension to be stuffed in soft gelatin capsules¹⁷
2. **Hard gelatin capsules:** Phytosomes will be stuffed in to onerous gelatin capsules. an instantaneous volumetrical filling method while not precompression (precompression would possibly have an effect on the disintegration time) will be applied. The tenuity of phytosome advanced appears to limit the utmost quantity of powder (usually less than three hundred mg for size capsule) that may be stuffed into capsule. With the utilization of piston pump capsule filling method we will increase quantity of powder to be stuffed in capsule¹⁸.
3. **Tablets:** Thanks to restricted flow ability, viscousness and low apparent density of phytosome advanced, an instantaneous compression method will be applied just for lower unitary doses. The phytosome advanced ought to be diluted with 60-70% of excipients to get tablets with applicable

characteristics. Wet granulation ought to be avoided thanks to the negative impact of water and warmth on stability of the phyto-phospholipid advanced¹⁹.

4. **Topical dose form:** foremost prepare emulsion at temperature (not above 40°C) at the moment incorporates the phytosome advanced into it. The phyto-phospholipid complexes are dispersible within the main lipid solvents used in topical formulation²⁰⁻²¹.

PHYTOSOMAL HERBAL PRODUCTS

PLANT/CONSTITUENTS	BIOLOGICAL ACTIVITY	APPLICATION OF PHYTOSOMAL TECHNOLOGY.
Curcumin	Anti cancer	Improved anti -oxidant activity and bioavailability
Embelin	Anti fertility	Solubility enhancement
Epigallocatechins	Anti cancer	Absorption enhancement
Ginkobiloba	Anti asthmatic	Improved efficacy
Ginsenosides	Immunomodulator	Absorption enhancement
Hawthorn	Cardio protective and hypertensive	Improved efficacy and absorption
Marsupium	Anti viral	Bioavailability enhancement

PATENTS

TITLE OF THE PATENT	INNOVATION	PATENT NO	REFERENCE
Phospholipid complexes of olive fruits or leaves extracts having improved bioavailability	Phospholipid complexes of olive fruits or leaves extracts or compositions containing it having improved bioavailability	EP/1844785	22
Compositions comprising Ginkgo biloba derivatives for the treatment of asthmatic and allergic conditions	Compositions containing fractions deriving from <i>Ginkgo biloba</i> , useful for the treatment of asthmatic and allergic conditions	EP1813280	23
Fatty acid monoesters of sorbityl furfural and compositions for cosmetic and dermatological use	Fatty acid monoesters of sorbityl furfural selected from two different series of compounds in which side chain is a linear or branched C3-C19 alkyl radical optionally containing at least one ethylenic unsaturation least one ethylenic unsaturation	EP1690862	24
Cosmetic and dermatological composition for the treatment of aging or photo damaged skin	Composition for topical treatment of the skin comprises a substance that stimulates collagen synthesis and a substance that enhances the interaction between extracellular matrix and fibroblasts Cosmetic or dermatological composition for topical treatment	EP1640041	25
Treatment of skin, and wound repair, with thymosin β 4	Compositions and methods for treatment of skin utilizing thymosin β 4.	US/2007/0015698	26
Soluble isoflavone compositions	Isoflavone compositions exhibiting improved solubility (e.g., light transmittance), taste, colour, and texture	WO/2004/045541	27

	characteristics, and methods for making the same		
An anti-oxidant preparation based on plant extracts for the treatment of circulation and adiposity problems	Preparation based on plant extracts which has an anti-oxidant effect and is particularly useful in treatment of circulation problems such as phlebitis, varicose vein, arteriosclerosis, haemorrhoid and high blood pressure.	EP1214084	28

CONCLUSION

Phytosome are advanced sort of flavoured extract that are absorbed higher than typical herbal extract. The article therefore reviews the advantages, physical characteristics, chemical properties, and methodology of preparation of phytosomes. These are novel complexes showing far better absorption profile following oral administration thanks to improved lipid solubility that modify them to cross the biological membrane, leading to increased bioavailability i.e. additional quantity of active principle within the circulation. Phytosomes are therapeutically used for hepatoprotective and liver diseases.

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ARTEMISIA NILAGIRICA

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ABSTRACT

A medicinal plant is a plant that has similar properties to conventional pharmaceutical drugs. Major population in countries of Africa and Asia depends on traditional herbal treatments for their primary health care needs. *Artemisia nilagirica* is a potent medicinal plant widely found in India and has been used to treat human disease for centuries. The review article gives information about the different chemical constituents and medicinal uses of *Artemisia nilagirica*. *Artemisia nilagirica* which is locally called as Indian worm wood belongs to Asteraceae family. The plant has been used since centuries in Anti- microbial, Anti-bacterial, Anti-asthmatic, Anti-filarial, insecticidal, Anti-cancer, Anti-oxidant activity. This plant contains various chemical constituents like alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenol, phlobatannins, quinines, tannins, saponins, terpenoids, volatile oils. Presence of wide range of chemical compounds imply that the plant may want to function as a potent material for the improvement of novel Sellers having good efficacy in diverse disorders inside the coming year.

KEY WORDS: *Medicinal plants, Indian wormwood, diseases, Phytophthoracapsici, Sesquiterpenoids, bronchial congestion.*

INTRODUCTION

Among all living organisms that are considered as an important group, as they support human life in many ways. Plants contain active chemical compounds which support human life⁽¹⁾. *Artemisia nilagirica* is provincially called as 'Indian wormwood'. It is used in the treatment of various diseases like malaria, inflammation, diabetes, stress, depression, diabetes and many other microbial diseases⁽²⁾. *Artemisia nilagirica* is shown to have some active chemical components which show effective action against the diseases like parasitic and helminthic diseases. It also shows efficient action on neurological disorders, dermal infection and antifungal, antimicrobial, larvicidal, inflammatory activities⁽³⁾.

PLANT DESCRIPTION

Kingdom	Plantae ⁽⁴⁾
Phylum	Tracheophyta ⁽⁴⁾
Class	Magnoliopsida ⁽⁴⁾
Order	Asterales ⁽⁴⁾
Family	Asteraceae ⁽⁴⁾
Genus	<i>Artemisia</i> ⁽⁴⁾
Species	<i>Artemisia nilagirica</i> ⁽⁴⁾

COMMON NAMES Damanak, Nagadaman

Assamese	Nilum, Nilum
Bengali	Nagadana
English	Fleabane, Indian Wormwood, Mugwort
Hindi	Nagdona, Davana
Kannada	Manjepatre, Urigattige
Konkani	Surpin
Malayalam	Masipatri, Makkippuvu
Manipuri	Leibakngou

Marathi	Dhordavana, Gondhomaro
Oriya	Dayona
Others	Maasipathri, Mugwort, Fleabane, Indian Wormwood
Sanskrit	Damanak, Nagadaman
Tamil	Makkippu
Tangkhul	Harana
Telugu	Masipatri

MORPHOLOGY

The plant breeds throughout India. It is tall aromatic shrub. The stems are leafy and branched. Flowers are small ⁽⁵⁾. Leaves and flowering tops are bitter, astringent and aromatic ⁽⁶⁾. Shrubs are tall, aromatic, perennial, fruticose. Stems paniculately branched, incanus, pubescent or tomentose. Achenes minute, oblong-ellipsoid, blackish; pappus absent.

CHEMICAL CONSTITUENTS

Test	Result	Reference
Alkaloids	++	6,7
Amino acids	++	7
Carbohydrates	+	7
Flavonoids	++	6-9
Glycosides	+	6
Phenol	++	7
Phlobatannins	-	7
Quinines	+	7
Tannins	++	6,7
Saponins	+	7-9
Terpinoid	++	6-11
Volatile oil	+	7

USES

Antibacterial activity of *Artemisia nilagirica*

Artemisia is one of the important genera of asteraceae family. It has many therapeutically important essential oils and secondary metabolites. *Artemisia* is found to possess insecticidal activity ⁽¹⁰⁾. Overall 59 compounds were discovered in essential oils of *Artemisia* which shows inhibitory action on *Phytophthora capsici* ⁽¹¹⁾. The antibacterial activity was determined by disk diffusion method and minimum inhibitory concentration (MIC) test ⁽¹²⁾.

Essential Oil Composition and Antifungal Activity

It has been shown that the plant contained monoterpenoids (79.91%) and sesquiterpenoids (18.25%). The major constituents of this oil were α -Thujone (36.35%), β -thujone (9.37%), germacrene D (6.32%), 4-terpineol (6.31%), β -caryophyllene (5.43%), camphene (5.47%) and borneol (4.12%). The study was performed by using Gas Chromatography-Mass Spectrometry. It shows antifungal activity against *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* ⁽¹³⁾.

Antiulcer activity

Limonene is used as flavoring agent in foods and beverages also it is considered as a low toxic component ⁽¹⁴⁾. Hence, studies have shown the gastroprotective activity of this essential oil which is a major constituent of *A. nilagirica* ^(15,16).

Antioxidant and Anticancer Activity

A. nilagirica has a significant effect on anticancer and antioxidant activity, the anticancer and antioxidant effect of Ethanolic extract of *A. nilagirica* may be due to free radical quenching properties of sesquiterpene lactones and flavanoid present in ethanolic extract ⁽¹⁷⁾.

Antiasthmatic activity

Nilagirica was prescribed as anti-asthmatic drug. It has decreased the production of nitrate ion thus decreasing the lung inflammation and relaxing the bronchial congestion⁽¹⁸⁾.

CONCLUSION

A Nilagirica is widely distributed through outasia especially in India, china, japan, and Afghanistan. In India it is found in hilly regions of Sikkim, western ghatsetc. The plant has many evidences from past for its therapeutic uses in parasitic infections. It is reported to contain essential oil, sesquiterpine lactone, coumarins and other including many phytochemicals. It is an important source of various types of compounds with diverse chemical structure.

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ROLE OF MICROBES IN DNA AMPLIFICATION

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ABSTRACT

DNA enzyme is Associate in nursing protein that synthesizes polymer molecules from deoxyribonucleotides, the building blocks of polymer. These enzymes are essential for polymer replication and frequently add pairs to form 2 identical DNA strands from one original DNA molecule. Throughout this method, polymer enzyme & quot; reads & quot; the present DNA strands to form 2 new strands that match the existing ones. Polymerase chain reaction (PCR) could be a methodology wide utilized in biological science to form many copies of a selected DNA section. Using PCR, copies of DNA sequences are exponentially amplified to get thousands too voluminous a lot of copies of that individual DNA section. PCR is currently a standard and infrequently indispensable technique utilized in medical laboratory and clinical laboratory analysis for a broad type of applications together with medical specialty research and criminal forensics. PCR was developed by the yank chemist Kary Mullis in 1983. PCR needs a deoxyribonucleic acid enzyme protein that creates new strands of DNA, victimization existing strands as templates. The deoxyribonucleic acid enzyme usually utilized in PCR is termed Taq polymerase, once the heat tolerant bacteria from that it absolutely was isolated (*Thermusaquaticus*). Most DNA polymerases may be active even at temperature, the mix of reaction elements throughout PCR setup will result in nonspecific primers hardening to every alternative or to the guide.

KEY WORDS: *DNA polymerase, Thermos aquaticus, Polymerase Chain Reaction, Topoisomerase.*

1. INTRODUCTION

DNA could be a molecule composed of 2 chains that coil around one another to make a helix carrying genetic directions for the event, functioning, growth and replica of all well-known organisms and plenty of viruses. The 2 DNA strands are referred to as polynucleotides as they're composed of easier monomeric units referred to as nucleotides. The two DNA strands are composed of simpler monomeric units called nucleotides^{1,2}.

2. DNA REPLICATION

DNA synthesis occurs during the S phase of the cell cycle and is ensured by the replisome, a molecular machine made of a large number of proteins acting in a coordinated manner to synthesize DNA at many genomic locations, the replication origins³.

3. ENZYMES INVOLVED IN DNA REPLICATION

S.No.	Enzyme	Function in DNA replication
1	DNA helicase	Seperates the 2 strands of DNA at Replication fork behind the topoisomerase
2	DNA polymerase	Responsible for catalyzing the addition of nucleotide substrates to DNA in the 5'to 3' direction
3	DNA clamp	A macromolecule that prevents elongating deoxyribonucleic acid polymerases from dissociating from the DNA parent strand.
4	ssDNA binding protein	Bind to ssDNA and stop the deoxyribonucleic acid spiral from re-annealing when DNA helicase unwinds it, so maintaining the strand separation, and facilitating the synthesis of the aborning strand.

5	Topoisomerase	Relaxes the DNA from its super-coiled nature.
6	DNA gyrase	Relieves strain of unwinding by DNA helicases
7	DNA ligase	Re-anneals the semi-conservative strands and joins Okazaki Fragments of the insulant strand.
8	Primase	Provides a starting point of RNA for DNA polymerase to begin synthesis of new DNA strand
9	Telomerase	Lenhtenstelomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes ⁴ .

DNA POLYMERASE

DNA enzyme is associate protein that synthesizes polymer molecules from deoxyribonucleotides, the building blocks of polymer. These enzymes are essential for polymer replication and frequently add pairs to make 2 identical DNA strands from one original DNA molecule. Throughout this method, polymer enzyme "reads" the present DNA strands to make 2 new strands that match the existing ones^{5,6,7}.

ROLE OF POLYMERASE IN PCR

Typically, PCR consists of a series of 20–40 continual temperature changes, known as thermal cycles, with every cycle ordinarily consisting of 2 or 3 separate temperature steps (see figure below). The athletics is commonly preceded by one temperature step at a really hot temperature (& amp; gt; 90 °C (194 °F)), and followed by one hold at the top for final product extension or temporary storage. The temperatures used and therefore the length of your time they're applied in every cycle rely on a range of parameters, as well as the accelerator used for DNA synthesis, the concentration of bivalent ions and dNTPs within the reaction, and therefore the melting temperature (Tm) of the primers.

DNA POLYMERASE ENZYME CONTAINING ORANISMS

DNA polymerases are central players in DNA repair and replication. Five polymerases are now recognized in *Escherichia coli*, nine in *Saccharomyces cerevisiae*, and 16 in humans. Based on differences in the primary structure of their catalytic subunits, DNA polymerases are classified into several distinct families⁸.

ECOLI

The microorganism is fully grown and civilised simply and inexpensively during a laboratory setting, and has been intensively investigated for over sixty years⁹. There are 5 polymer polymerases in *escherichia*. The most replicative enzyme is politico III. The chemical action monetary unit, selected politico III*, is encoded by *dnaE*. Pol I, encoded by *polA*, plays roles in process Okazaki fragments and conjointly in gap-filling throughout excision-repair processes. The opposite 3 polymer polymerases are elicited to higher levels of expression by the SOS DNA-damage response^{10,11}. These low-processivity erring polymerases play major roles in bypassing otherwise replication-blocking example lesionsvia trans-lesion synthesis and conjointly replicate undamaged polymer¹².

SACCHAROMYCES CEREVISIAE

Pol ϵ is one in every of four essential nuclear DNA polymerases (Pol α , δ , ϵ , and σ) found in yeast^{13,14,15}. Most models of replication propose that politician α initiates replication, that politician δ is accountable for more synthesis of the insulant strand, which politician ϵ is accountable for leading strand synthesis. However, this role for politician ϵ is way from verified, and this accelerator remains the foremost enigmatic of all the polymerases^{16,17}.

PYROCOCCUS FURIOSUS

A polymer enzyme was discovered in *P. furiosus* that was thought to be unrelated to alternative illustrations polymer polymerases, as no vital sequence similarity was found between its 2 protenism and people of alternative illjustrations polymer polymerases¹⁸.

METHANOCALDOCOCCLUS JANNASCHII:

Methanocaldococcusjannaschii(formerly*Methanococcusjannaschii*) may be a thermophilicmethanoenicarchean within the category *Methanococci*¹⁹.

THERMUS AQUATICUS

Thermusaquaticus has well-tried to be quite an helpful organism within the field of Biotechnology, as its catalyst Taq enzyme is harvested to be used in enzyme chain reactions (PCR) Taq polymerase's resistance to heat is Associate in Nursing adaptation to its setting, however isn't the sole reason it's the selection to be used in PCR²⁰.

ADVANTAGES OF POLYMERASE

PCR contains a variety of benefits. It's fairly easy to know and to use, and produces results quickly. The technique is extremely sensitive with the potential to supply millions to billions of copies of a selected product for sequencing, cloning, and analysis. QRT-PCR shares identical benefits because the PCR, with an extra advantage of quantification of the synthesized product. Therefore, research alterations of organic phenomenon levels in tumors, microbes, or different sickness states. PCR may be a terribly powerful and sensible analysis tool. The sequencing of unknown etiologies of the many diseases is being puzzled out by the PCR. The technique will facilitate determine the sequence of antecedently unknown viruses associated with those already glorious and so provide us a much better understanding of the sickness itself.

CONCLUSION

DNA polymerase is an crucial enzyme for each essential residing phenomena (DNA replication/repair) in cells and applications to genetic engineering in vitro. Therefore, numerous structural and useful investigations of DNA polymerase had been said to date. In this take a look at, we superior PCR enzymes that offer a superior extension reaction compared to Taq polymerase, the equal antique enzyme for PCR. As compared to the PCR usual performance of Taq polymerase, those enzymes executed the amplification of either the same duration of DNA in a shorter time or a longer DNA in the identical response time.

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A VALIDATED REVERSE PHASE-HPLC-PDA METHOD FOR ESTIMATION OF ZOLMITRIPTAN IN BULK AND DOSAGE FORMS

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ABSTRACT

The aim of the present work is to develop and validate a rapid, efficient and economical RP-HPLC method for the analysis of Zolmitriptan (ZMT) in bulk and dosage forms like tablets and mouth dissolving films (MDFs). ZMT was separated on Agilent Eclipse C₁₈ column (150 x 4.6mm, 5µm) with a mobile phase composed of 0.02%v/v formic acid: methanol (75:25% v/v) in isocratic mode and eluents were monitored at 221 nm. ZMT was eluted at 3.3min and showed a good linearity in the concentration range of 2-10µg/mL with a correlation coefficient >0.999. The method was validated as per regulatory requirements. The developed HPLC method was successfully used for the analysis of ZMT in bulk and dosage forms like tablets and mouth dissolving films.

KEYWORDS: *Zolmitriptan, PDA detection, Mouth dissolving films, Dissolution studies, Method validation.*

INTRODUCTION

Zolmitriptan is a second generation triptan used in the treatment of acute migraine attacks with or without aura and cluster headaches. Chemically, it is (S)-4-({3-[2-(dimethylamino) ethyl]-1H-Indol-5-yl} methyl)-1, 3-oxazolidin-2-one¹. ZMT is a selective serotonin receptor agonist of the 1B and 1D subtype². Various analytical methods have been reported in the literature for quantitative estimation of ZMT in several dosage forms like tablets by HPLC³⁻⁶, spectrofluorimetry^{7, 8} and stability indicating methods⁹ and no validated methods were reported for the estimation of ZMT in MDFs. However the mobile phases in most of the methods consisted of phosphate buffer and ACN with longer retention times. Hence, the present investigation was aimed at developing a rapid and economic RP-HPLC-PDA method for the analysis of ZMT in tablet dosage forms and in mouth dissolving films.

MATERIAL AND METHODS

Chemicals

ZMT was a gift sample from Mylan Laboratories Ltd, Hyderabad, India. Formic acid, water and methanol were purchased from E. Merck, Mumbai, India.

Equipment

A RP-HPLC system (Shimadzu) comprising of Degasser (DGU-20A3), Binary pump (LC-20AD), an Auto sampler (SIL 20- AC HT), and PDA detector (SPD M20A) was used for the analysis of ZMT in different samples. Shimadzu LC solution software was used to collect and process the data. Separation was achieved on Agilent Eclipse C₁₈ column (150 × 4.6mm, 5 µm).

Chromatographic Conditions

Mobile phase consisting of 0.02% v/v formic acid: methanol (78:22 v/v) was used in isocratic mode and filtered through membrane filter of 0.45µm (Millipore) and pumped at a flow rate of 1 mL/min. The injection volume was 10µL and eluents were monitored at 221nm.

Preparation of Stock and Standard Solutions

The stock solution of ZMT was prepared by accurately weighing 10 mg of ZMT, transfer it into 10 mL volumetric flask and dissolved completely by adding 5mL of methanol and make up the volume up to the mark with methanol. From the stock solution subsequent dilutions were made to prepare a concentration range of 2-10 µg/mL solutions.

Preparation of ZMT Tablets

In the present experiment, ZMT tablets (5 mg) were prepared by direct compression method, due to the unavailability of marketed tablet formulation for ZMT in India. Sufficient quantities of povidone, croscarmellose sodium, and talc and magnesium stearate were used. Microcrystalline cellulose was used to raise the bulk volume of the tablets to a targeted weight of 50mg for each tablet. All the ingredients were passed through sieve # 80 before mixing and mixed for 5 min in a poly bag. The mixture was then compressed on single punch tablet machine (Cadmach, India) using 4mm punches and maintained a hardness of 4-5 Kg/cm².

Preparation of ZMT MDFs

ZMT (100mg) was dissolved in a beaker containing mixture of solvents (water & methanol) and other ingredients like; PEG 400, pineapple flavor and aspartame were added one by one. Finally the polymer HPMC E3 was added and mixed thoroughly. The mixture was sonicated for 5 minutes and casted on a glass plate with a wet film applicator set at 30 (750µm) mil thicknesses. Films were dried at 45°C for 60min in hot air oven. Then the dried films were peeled off from the glass plate, cut into appropriate sizes, and stored in desiccator until use.

Validation of the HPLC Method

The method was validated according to the ICH guidelines¹⁰.

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that of formulations. The overlay of diluent, placebo, standard and samples were presented in Figure-2 and 3 D plots were shown in Figure 3.

2.7.2 Linearity

A series of standard dilutions of ZMT were prepared over a concentration range of 2-10µg/mL (2, 4, 6, 8, 10µg/mL) from stock solution and injected in triplicate. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the data was subjected to regression analysis and was given in Table-1.

Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions by using a minimum of six determinations at 100% of the test concentration. The standard deviation and the relative standard deviation were reported for precision and the data was given in Table-1.

Accuracy

To ascertain the accuracy of the proposed method recovery studies were performed by the by spiking 80, 100, 120% of the known quantities of standard solutions were analyzed by developed method in triplicate. The data was given in Table-1.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on calibration curves. They were expressed as $LOD = (3.3 \times \sigma)/m$; $LOQ = (10.0 \times \sigma)/m$ (Where, σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Robustness

In order to show the robustness of the method developed, the the flow rate was changed by 5% and the effect of wavelength was studied by changing wavelength by ± 2 nm. Whereas, the effect of mobile phase composition was studied by changing $\pm 2\%$ and the data obtained was given in Table-2.

System Suitability

System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50 μ L. The system suitability test parameters like retention time, tailing factor and theoretical plate number were noted and % RSD was calculated.

Assay for Tablets

Twenty tablets were weighed and finely powdered. A powder equivalent to 10 mg of ZMT was accurately weighed and transferred to a 10 mL volumetric flask and 5 mL of methanol was added to the same and volume was made up to the mark with methanol. The solution was filtered and the filtrate was diluted with diluent (water) to obtain a concentration in the range of 2-10 μ g/mL. The amount present in the each tablet was calculated.

Assay for MDFs

One cm^2 film was taken from the ZMT MDFs, placed in a 10mL volumetric flask and dissolved in 5mL of methanol and volume was made up to the mark with methanol and analyzed by HPLC method. The estimations were carried out in triplicate.

Dissolution Analysis for Tablets

The prepared ZMT tablets were subjected to in vitro drug release studies in 900 mL of distilled water for 30 minutes in eight stage dissolution test apparatus (LAB INDIA DS 8000, Mumbai, India) maintained at $37 \pm 0.5^\circ\text{C}$ and the paddles were adjusted at 50 rpm speed. 5mL of the samples were withdrawn at each time interval (2, 5, 10, 15, 20 and 30 minutes) and replaced with 5 mL of fresh medium. The samples were filtered and analyzed using the above developed RP-HPLC method. The % release of ZMT in the test samples was calculated.

Dissolution Analysis for MDFs

The in-vitro dissolution studies of ZMT MDFs were performed using 500 mL of artificial saliva as dissolution medium using USP Type V as Dissolution Rate Testing apparatus. A Temperature of 37°C and 50 rpm were maintained. Each film of appropriate size ($3 \times 204 \text{ cm}^2$) equivalent to 6.25 mg dose was cut and placed on a watch glass covered with nylon wire mesh and placed in dissolution flask. At predetermined time intervals 10, 20, 30, 40, 50, 60, 80, 100, 120, and 180 sec 5mL of aliquots were withdrawn and replaced with same volume of fresh dissolution medium and the samples were analyzed by above RP-HPLC method. The dissolution was conducted in triplicate.

Filter Compatibility Study

The compatibility of dissolution samples with both the filters (nylon (0.45 μ m), and polyvinylidene difluoride (0.45 μ m)) were checked. ZMT standards were prepared using dissolution medium and filtered through the filters. Both the unfiltered and the filtered samples were injected and the chromatograms were observed.

RESULTS AND DISCUSSION

Method Development

Initially, mobile phase optimization was carried with Agilent Zorbax (150x4.6 mm, 5 μ) column using 10mM ammonium acetate: acetonitrile as mobile phase at a flow rate of 0.8mL/min, ZMT was eluted as a broad peak with tailing. The further trial was carried out with Agilent Eclipse plus (150x4.6mm, 5 μ) column, mobile phase (10mM ammonium acetate and acetonitrile (75:25% v/v)) at a flow rate of 0.8mL/min, ZMT peak was merged with the solvent front under these conditions. Further trials were performed on Agilent Eclipse plus (150x4.6mm, 5 μ) column with 0.02% formic acid: methanol as mobile phase combinations and finally 75:25% v/v of 0.02% formic acid: methanol was selected at flow rate of 1.0mL/min at 221nm. Under these conditions a sharp peak of ZMT was eluted at 3.318min (Figure 1) and tailing factor, peak symmetry, theoretical plate number were all within the limits. The peak purity index was found to be greater than 0.9999 and this indicating purity of the drug sample used in the analysis and shown in Figure-1 along with UV spectra.

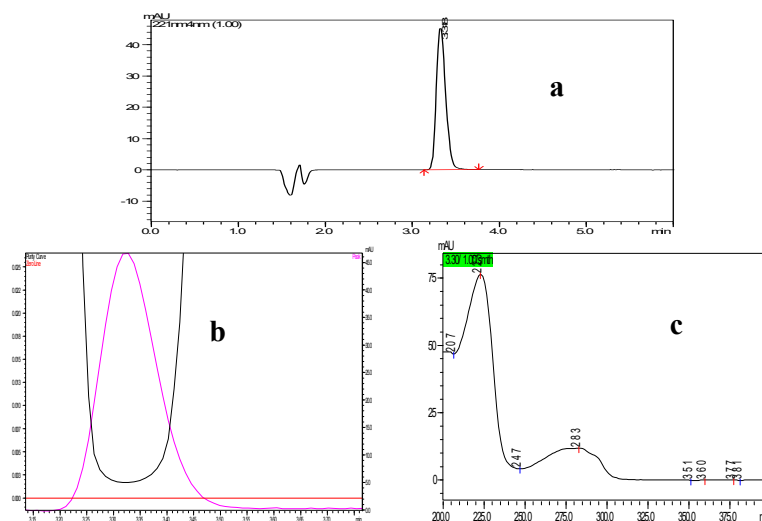


Figure 1: *a - Chromatogram of ZMT (6 µg/mL), b - Peak purity index of ZMT and c – UV Spectrum of ZMT*

Method validation

The method has been validated as per ICH-Guidelines for the following parameters.

Specificity

The specificity of the method was confirmed by determining if any interference is there from the excipients by injecting diluents, placebo, standard and samples (tablet and film) individually. The results revealed that there were no co-eluting peaks in chromatograms at the retention time of ZMT, indicating the purity of the peak without any interferences and the peak purity indices for sample and standard were greater than 0.999 confirming the specificity of the method.

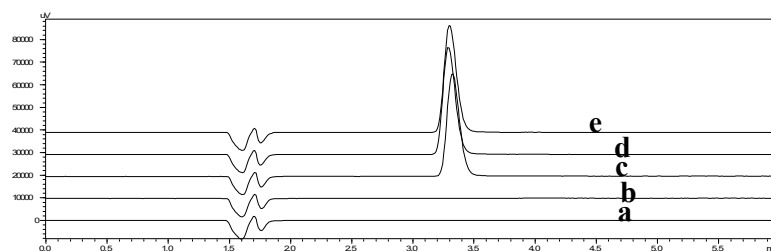


Figure 2: *Overlay of the diluent (a), placebo (b), standard (c), ZMT tablet sample (d) and ZMT mouth dissolving film (e)*

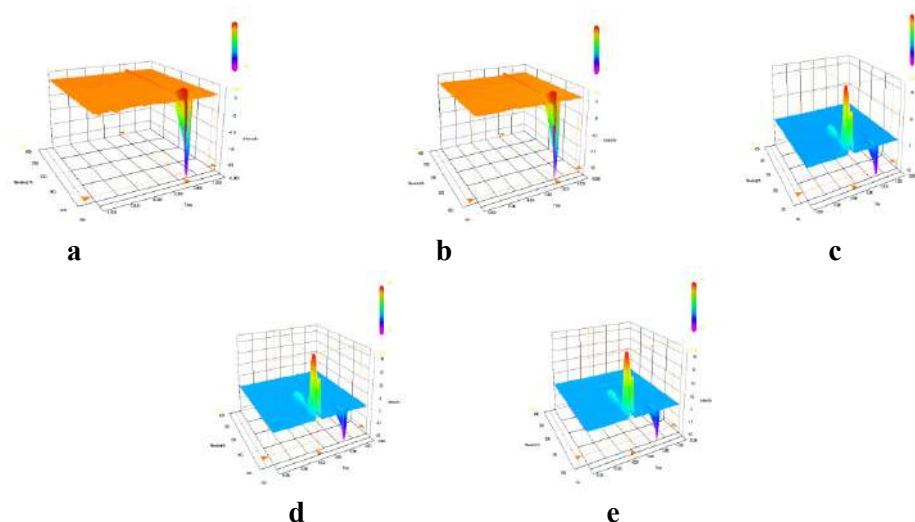


Figure 3: *plots of diluent (a), placebo (b), standard (c), ZMT tablet sample (d) and ZMT mouth dissolving film (e)*

Linearity

The range of reliable quantification was set at the concentrations of 2-10 μ g/mL of ZMT. Peak areas and concentrations were subjected to least square regression analysis. The correlation coefficient (R) was found to be 0.999 indicating a linear response over the concentration range selected.

Precision

Precision studies were carried out by using six replicates of concentration at 6 μ g/mL level and the data was given in Table-1. The % RSD was found to be below 2 for peak areas and this shows the closeness of the data values to each other, indicating the precision of the method.

Accuracy

Accuracy of the proposed method was ascertained by spiking the known quantities of standard at 80, 100, 120% to the drug product solution and were analyzed in triplicate. The % RSD and the % recovery were within the acceptable limit indicating that the proposed method is accurate. The data from the calibration curve was given in Table-1.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of ZMT determined from the calibration curves was found to be 0.0061 μ g/mL and 0.018 μ g/mL respectively indicating that ZMT peak could be determined at these concentrations without any base line disturbances.

Robustness

As part of robustness, change in retention time was observed due to change in flow rate and mobile phase, whereas the change in wavelength doesnot change the retention time and the parameters like theoritical plate number, tailing factor remain unchanged and were within the limits. The results were shown in Table-2.

Table 1: Linearity, Precision, Accuracy and Assay data of ZMT

Validation data of ZMT	
Linearity(n=3)	
Range	2-10 μ g/mL
Regression equation	Y = 59660x -24945
Regression coefficient (R ²)	0.999
Correlation coefficient (R)	0.998
Accuracy(n=3) (% level of addition)	Mean Recovery (% RSD)
80	100.16 (0.86)
100	101.49 (0.16)
120	100.38 (0.97)
Precision(n=6)	Average Peak Area (% RSD)
	325855 (0.50)
% Assay (n=3)	Mean $\pm$ SD (% RSD)
Tablets	99.98 $\pm$ 0.22 (0.22)
MDFs	100.27 $\pm$ 0.35 (0.34)

Table 2: Robustness data

Chromatographic parameter	Retention time (min)	Theoretical plates (#)	Tailing factor (T _f)
Flow rate (mL/min)			
0.95	3.456	4123.52	1.328
1.0	3.319	4031.88	1.317
1.05	3.306	3929.43	1.298
Wave length (nm)			
219	3.318	4035.01	1.318
221	3.319	4031.88	1.317
223	3.319	4031.92	1.319
Composition of mobile phase			

76:24	3.246	3902.42	1.314
78:22	3.319	4031.88	1.317
80:20	3.387	4169.62	1.330

System Suitability

System suitability was carried out by different injection volumes (10 μ L - 50 μ L) of 6 μ g/mL. The system suitability test parameters (like retention time (0.509%), tailing factor (0.986%) and theoretical plate number (1.479%)) exhibited %RSD values less than 2% indicating that the analytical conditions maintained were suitable for the estimation of ZMT in formulations.

Stability of the Analytical Solution

The stability of the stock solution was determined by analyzing the samples stored under refrigeration (8 $\pm$ 1 $^{\circ}$ C) conditions at different time intervals up to 1 month. The variation in assay values at different time intervals were found to be less than 2% of the initial time interval and these results indicating that the solutions were stable for a period of 1 month when stored at 8 $^{\circ}$ C.

Assay for Tablets and MDFs

The percent assay of ZMT in tablets and MDFs were found to be about 99.98 $\pm$ 0.22 and 100.27 $\pm$ 0.35 respectively indicating that the proposed RP-HPLC method can be successfully used for the estimation of ZMT in bulk, tablet dosage forms, and MDFs.

Dissolution analysis for Tablets and MDFs

The above validated method was used for the *in vitro* dissolution analysis of ZMT in tablets and MDFs. A rapid ZMT dissolution was observed with the tablet and a complete dissolution was observed within 30 min for tablets and within 300sec for MDFs. The dissolution profile was presented in Figure-4.

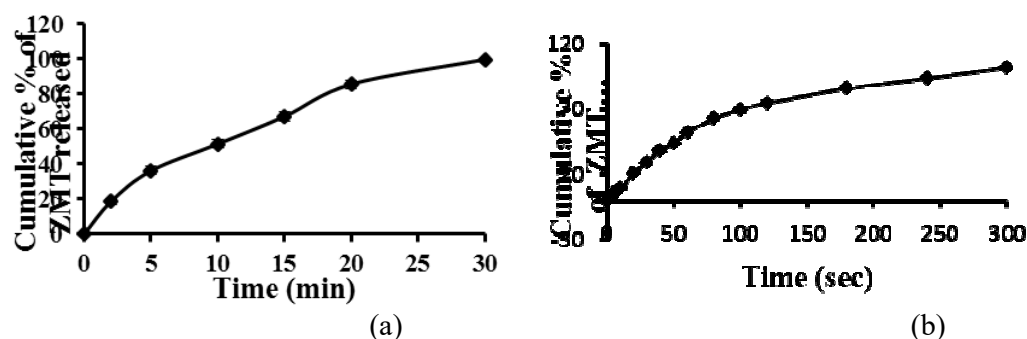


Figure. 4: Dissolution profile of (a) ZMT tablets (b) ZMT MDFs

Filter compatibility study

Compatibility of 0.45 μ m nylon filter and Polyvinylidene Difluoride (PVDF) filters with ZMT was studied. Standard, sample solutions i.e. assay and dissolution samples were filtered, analysed and peak areas for ZMT were noted. The % difference in ZMT peak area was found to be greater for PVDF filters when compared to nylon filters and based on the results nylon filters of 0.45 μ m were used in this investigation. The data was given in Table-3.

Table 3: Filter compatibility study

Sample name	Peak area of ZMT	% Difference
Standard sample	325855	-
Samples filtered through 0.45 μ m nylon filter	325442	0.126
Samples filtered through PVDF filter	322754	0.951

CONCLUSION

In this work, rapid and efficient RP-HPLC-PDA method was developed for the analysis of ZMT in bulk and dosage forms like tablets and mouth dissolving films. The method was validated as per ICH guidelines, and found to be applicable for the estimation of ZMT in formulations and dissolution samples using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity were found to be within

the limits. Also the method provides selective quantification of ZMT without interferences from diluent and placebo samples. Therefore, this method can be selectively employed to quantify ZMT not only in formulations like tablets and mouth dissolving films but also in dissolution studies.

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DEVELOPMENT AND VALIDATION OF RP-HPLC-PDA METHOD FOR THE ANALYSIS OF RIZATRIPTAN IN BULK AND DIFFERENT DOSAGE FORMS AND IN DISSOLUTION SAMPLES

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ABSTRACT

The aim of the present work is to develop and validate a rapid, efficient and economical RP-HPLC-PDA method for the analysis of Rizatriptan (RZT) in bulk and dosage forms like tablets and mouth dissolving films (MDFs). RZT was separated on Agilent Eclipse C₁₈ column (150 x 4.6mm, 5µm) with a mobile phase composed of 0.02%v/v formic acid: methanol (78:22% v/v) in isocratic mode and eluents were monitored at 221 nm. RZT was eluted at 3.01min and showed a good linearity in the concentration range of 2-10µg/mL with a correlation coefficient >0.999. The validation parameters like specificity, linearity, accuracy and limit of detection, limit of quantification, precision, robustness are all fulfilled as per regulatory requirements. The developed HPLC method was successfully used for the analysis of RZT in bulk and dosage forms like tablets and mouth dissolving films.

KEYWORDS: *Rizatriptan, PDA detection, Mouth dissolving films, Dissolution studies, Method validation.*

INTRODUCTION

Rizatriptan benzoate (RZT) is an antimigraine drug used to treat migraine. Chemically, it is N, N dimethyl-5-(1H-1, 2, 4-triazol-1-ylmethyl)-1H-indole-3-ethanaminemonobenzoate ¹. It is a selective 5-hydroxytryptamine 1B/1D (5-HT_{1B/1D}) receptor agonist. It stimulates the 5-HT_{1B/1D} receptor blocking neuronal transmission of vasoactive neuropeptides and reverse dilation of cranial vessels associated with migraine ². Various analytical methods have been reported in the literature for quantitative determination of RZT individually in several dosage forms like tablets by HPLC³⁻⁵ UV spectrophotometry^{6, 7}, fluorimetry⁸ and no validated method was reported for the estimation of RZT in MDFs. However the mobile phases in most of the methods consisted of phosphate buffer and ACN with longer retention times. Hence, the present investigation was aimed at developing a rapid and economic RP-HPLC-PDA method for the analysis of RZT in tablet dosage forms and in mouth dissolving films.

MATERIALS AND METHODS

Chemicals

RZT was a gift sample from Mylan Laboratories Ltd, Hyderabad, India. Formic acid, water and methanol were purchased from E. Merck, Mumbai, India. RZT was available as tablets with brand name RITZA (manufactured by NATCO Pharma Limited, Kothur) with a labeled claim of 5mg.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. All the data was processed using LC solutions software. The chromatographic analysis was performed on Agilent Eclipse C₁₈ column (150 × 4.6mm, 5µm).

Chromatographic Conditions

Mobile phase consisting of 0.02% v/v formic acid: methanol (78:22 v/v) was used in isocratic mode and was filtered through membrane filter of 0.45 μ m (Millipore) and pumped at a flow rate of 1 mL/min. The injection volume was 10 μ L and eEluents were monitored at 221nm.

Preparation of stock and standard solutions

The stock solution of strength 1 mg/mL was prepared by dissolving 14.75 mg of Rizatriptan benzoate equivalent to 10 mg of Rizatriptan base in 5 mL of methanol and volume was adjusted to the mark with methanol in a 10mL volumetric flask. The stock solution was further diluted to get standard solutions with in a concentration range of 2-10 μ g/mL.

Preparation of RZT MDFs

RZT (100mg) was dissolved in a beaker containing mixture of solvents (water & methanol) and other ingredients like; PEG 400, pineapple flavor and aspartame were added one by one. Finally the polymer HPMC E3 was added and mixed thoroughly. The mixture was sonicated for 5 minutes and casted on a glass plate with a wet film applicator set at 30 (750 μ m) mil thicknesses. Films were dried at 45°C for 60min in hot air oven. Then the dried films were peeled off from the glass plate, cut into appropriate sizes, and stored in desiccator until use.

Validation of the HPLC Method

The method was validated according to the ICH guidelines⁹ for the following parameters

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that of formulations. The overlay of diluent, placebo, standard and samples were presented in Figure-1 and 3 D plots were shown in Figure 2.

Linearity

A series of standard dilutions of RZT were prepared over a concentration range of 2-10 μ g/mL (2, 4, 6, 8, 10 μ g/mL) from stock solution and injected in triplicate. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the data was given in Table-1.

Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions by using a minimum of six determinations at 100% of the test concentration. The data was given in Table-1.

Accuracy

To ascertain the accuracy of the proposed method recovery studies were performed by spiking 80, 100, 120% of the known quantities of standard within the range of linearity to the drug product solution and were analyzed by developed method in triplicate. The data was given in Table-1.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on calibration curves. They were expressed as $LOD = (3.3 \times \sigma)/m$; $LOQ = (10.0 \times \sigma)/m$ (Where, σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Robustness

In order to show the robustness of the method developed, the flow rate was changed by 5% and the effect of wavelength was studied by changing wavelength by ± 2 nm. Whereas, the effect of mobile phase composition was studied by changing $\pm 2\%$ and the data obtained was given in Table-2.

System Suitability

System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50 μ L. The system suitability test parameters like retention time, tailing factor and theoretical plate number were noted and % RSD was calculated.

Assay for tablets

Twenty tablets were weighed and finely powdered. A powder equivalent to 10 mg of RZT was accurately weighed and transferred to a 10 mL volumetric flask and 5 mL of methanol was added to the same and made up to the mark with methanol. The solution was filtered and the filtrate was diluted with diluent (water) to obtain a concentration in the range of linearity and the amount present in the each tablet was calculated by using regression equation.

Assay for MDFs

One cm² film was taken from the RZT MDFs, placed in a 10mL volumetric flask and dissolved in 5mL of methanol and volume was made up to the mark with methanol. Samples were suitably diluted with water and analyzed by HPLC method. The estimations were carried out in triplicate.

In vitro Drug Release Studies for Tablets

Marketed tablets were subjected to *in vitro* drug release studies in distilled water for 30 min in eight stage dissolution test apparatus (LAB INDIA DS 8000, Mumbai, India) using specified volume of dissolution media (900 mL) maintained at 37±0.5°C and the paddles were adjusted at 50 rpm speed. 5mL of the samples were withdrawn at each time interval (2, 5, 10, 15, 20 and 30 minutes) and replaced with 5 mL of fresh medium. The samples were filtered and analyzed using the above developed RP-HPLC method. The % release of RZT in the test samples was calculated.

In vitro Drug Release Studies for MDFs

The *in vitro* dissolution studies were conducted using 500mL of artificial saliva as dissolution medium with modified Type 5 dissolution apparatus. A temperature of 37°C and 50 rpm were maintained. 1×1 cm² film equivalent to 5mg dose was cut and placed on a watch glass covered with nylon wire mesh. The watch glass was then dropped into dissolution flask. 5mL samples were withdrawn at predetermined time intervals 10, 20, 30, 40, 50, 60, 80, 100, 120, 180, 240, 300, 360 sec and replaced with 5mL of fresh dissolution medium. The samples were analyzed by above HPLC method. The dissolution experiments were conducted in triplicate.

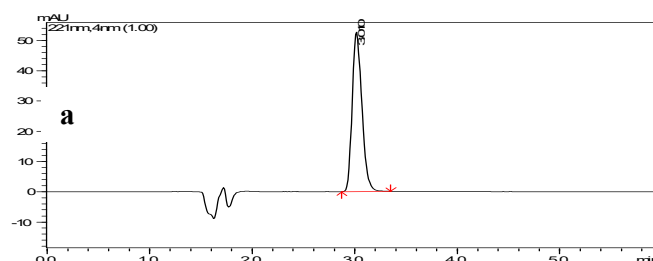
Filter Compatibility Study

Dissolution samples were checked for compatibility with both the nylon filter (0.45µm) and Polyvinylidene Difluoride (PVDF) filters (0.45µm). RZT standard in dissolution medium was prepared and the solution was filtered through the filters. Both the filtered and unfiltered standard samples were injected and chromatograms were observed.

RESULTS AND DISCUSSION

Method Development

Initially, mobile phase optimization was carried with Agilent Zorbax (150x4.6 mm, 5µ) column using 10mM ammonium acetate: acetonitrile as mobile phase at a flow rate of 0.8mL/min, RZT was eluted as a broad peak with tailing. In other trial Agilent Eclipse plus (150x4.6mm, 5µ) column with 10mM ammonium acetate and acetonitrile (75:25% v/v) as mobile phase was used at a flow rate of 0.8mL/min and under these conditions the RZT peak was merged with solvent front. Further trials were performed on Agilent Eclipse plus (150x4.6mm, 5µ) column with 0.02% formic acid: methanol as mobile phase combinations and finally 78:22% v/v of 0.02% formic acid: methanol was selected at flow rate of 1.0mL/min at 221nm. Under these conditions a sharp peak of RZT was eluted at 3.01min (Figure 1) and tailing factor, peak symmetry, theoretical plate number were all within the limits. The peak purity index was found to be greater than 0.9999 and this indicating purity of the drug sample used in the analysis and shown in Figure-3 along with UV spectra.



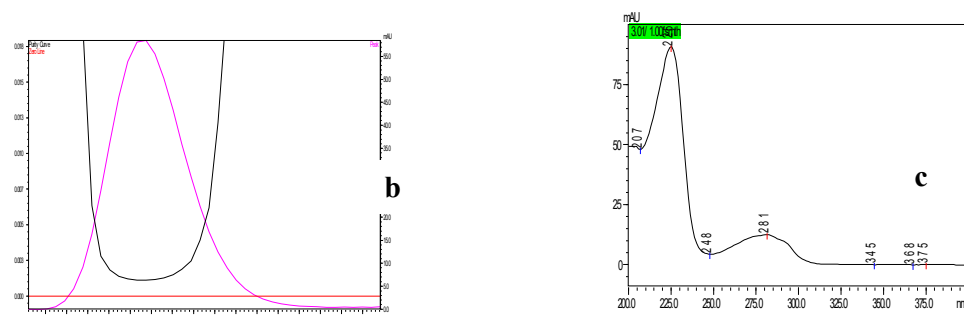


Figure 3: a - Chromatogram of RZT (6 µg/mL) b - Peak purity index of RZT and c – UV Spectrum of RZT

Method validation

The method has been validated as per ICH-Guidelines for the following parameters.

Specificity

The specificity of the method was established by injecting the solutions of diluent, placebo, standard and samples (tablet and film) individually to examine any interferences from the excipients, from the overlay of chromatograms as shown in (Figure-2) and 3D plots in Figure 3, it can be inferred that there were no co-eluting peaks at the retention time of RZT, this shows that peak of analyte was pure without any interferences and the peak purity indices for sample and standard was found to be greater than 0.999 and this confirms specificity of the method.

Linearity

The range of reliable quantification was set at the concentrations of 2-10 µg/mL of RZT. Peak areas and concentrations were subjected to least square regression analysis. The correlation coefficient (R) was found to be 0.999 indicating a linear response over the concentration range selected. The data from the calibration curve was given in Table-1.

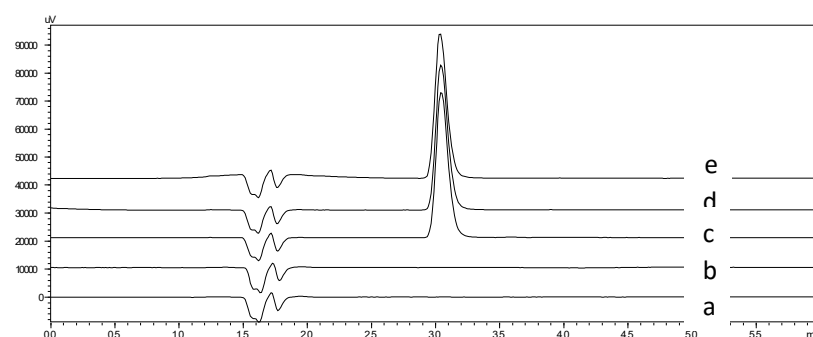
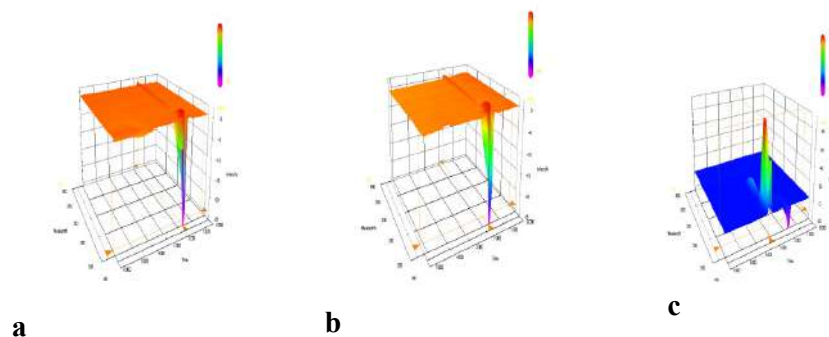


Figure 1: Overlay of the diluent a, placebo b, standard c, RZT tablet sample d and RZT mouth dissolving film e



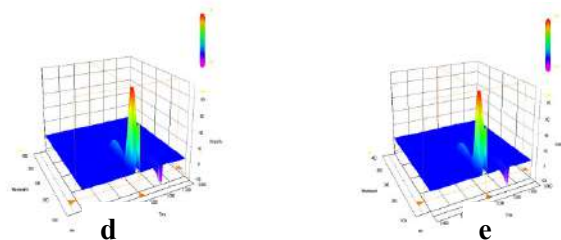


Figure 2: 3D Plots of diluent (a), 2bo (b), standard (c), RZT tablet sample (d) and RZT mouth dissolving film (e)

Table 1: Linearity, Precision, Accuracy and Assay data of RZT

Validation data of RZT	
Linearity(n=3)	
Range	2-10 μ g/mL
Regression equation	$Y = 59081x - 1818$
Regression coefficient (R^2)	0.999
Correlation coefficient (R)	0.999
Accuracy(n=3)	
% level of addition	Mean Recovery (% RSD)
80	100.82(0.28)
100	100.28(0.86)
120	100.92(0.26)
Precision(n=6)	Average Peak area of the standard sample (% RSD)
	349438 (0.35)
% assay (n=3)	Mean $\pm$ SD(% RSD)
Tablets	100.16 $\pm$ 1.33(1.32)
MDFs	100.27 $\pm$ 0.35(0.34)

Precision

Precision studies were carried out by using six replicates of concentration at 6 μ g/mL level and the data was given in Table-1. The % RSD was found to be below 2 for peak areas and this shows the closeness of the data values to each other, indicating the precision of the method.

Accuracy

Accuracy of the proposed method was ascertained by spiking the known quantities of standard at 80, 100, 120% to the drug product solution and were analyzed in triplicate. The % RSD and the % recovery were within the acceptable limit indicating that the proposed method is accurate.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined from the calibration curves. The LOD for RZT was found to be 0.0307 μ g/mL, the drug peak could be detected without any base line disturbances at this concentration. The LOQ for RZT was found to be 0.0931 μ g/mL.

Robustness

As part of the robustness, retention times were significantly changed with flow rate, mobile phase and no change in the retention time was observed in wavelength change. The parameters like theoretical plate number, tailing factor were not changed and were within the limits. These results indicated that the method is robust.

Table 2: Robustness data for RZT

Chromatographic parameter	Retention time (min)	Theoretical plates (#)	Tailing factor (T_f)
Flow rate (mL/min)			
0.95	3.04	3903.52	1.375
1.0	3.00	3832.88	1.338
1.05	2.89	3814.43	1.352

Wave length (nm)			
219	3.00	3835.01	1.337
221	3.00	3832.88	1.338
223	3.00	3841.92	1.338
Composition of mobile phase			
76:24	2.98	3802.42	1.314
78:22	3.00	3832.88	1.338
80:20	3.08	3869.62	1.350

System Suitability

System suitability studies were carried out by different injection volumes (10 μ L - 50 μ L) of 6 μ g/mL. The % RSD values for system suitability test parameters like retention time (0.184%), tailing factor (0.954%) and theoretical plate number (1.334%) were less than 2% indicating the present conditions were suitable for the analysis of RZT in formulations.

Stability of the Analytical Solution

The stability of the stock solution was determined by analyzing the samples stored under refrigeration (8 $\pm$ 1 $^{\circ}$ C) conditions at different time intervals up to 1 month. The variation in assay values at different time intervals were found to be less than 2% of the initial time interval and these results indicating that the solutions were stable for a period of 1 month when stored at 8 $^{\circ}$ C.

Assay for Tablets and MDFs

Assay of RZT tablets and MDFs were performed by the proposed method and the percent assay of the formulation was calculated as an average of three determinations, which was about 100.16 $\pm$ 1.33 in tablets and 100.27 $\pm$ 0.35 in MDFs. These results indicate that the present RP-HPLC-PDA method can be successfully used for the assay of RZT in bulk, tablet dosage forms and MDFs.

Dissolution Analysis for Tablets and MDFs

The above validated method was used for the *in vitro* dissolution analysis of RZT in tablets and MDFs. A rapid RZT dissolution was observed with tablets and MDFs prepared and a complete dissolution was observed within 30min for tablets and within 300sec for MDFs. The dissolution profile was presented in Figure-4.

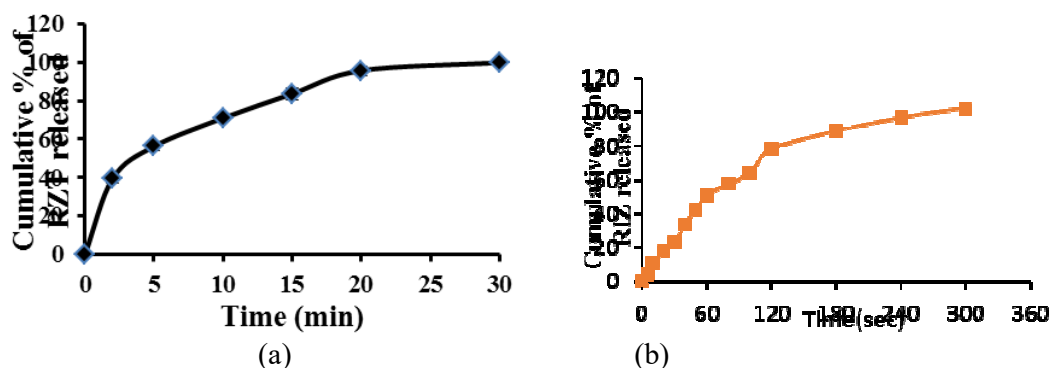


Figure. 4: Dissolution profile of (a) RZT tablets and (b) RZT MDFs

Filter Compatibility Study

Compatibility of 0.45 μ m nylon filter and Polyvinylidene Difluoride (PVDF) filters (0.45 μ m) were studied. Standard, sample solution and dissolution samples were filtered and analysed and the variation in the assay value was calculated. After the analysis it was found that nylon filters were suitable for filtration. The data was given in Table-3.

Table 3: Filter compatibility study

Sample name	Peak area of RZT	% Difference
Standard sample	349438	-

Samples filtered through 0.45µm nylon filter	349142	0.084
Samples filtered through PVDF filter	348754	0.195

CONCLUSION

In this work, rapid and efficient RP-HPLC-PDA method was developed for the analysis of RZT in bulk and formulations like tablets and mouth dissolving films. The method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for the estimation of RZT in formulations and in dissolution samples using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of RZT without interferences from diluent and placebo. Therefore, this method can be selectively employed to quantify RZT not only in formulations like tablets and mouth dissolving films but also in dissolution studies.

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DEVELOPMENT OF VALIDATED RP-HPLC-PDA METHOD FOR THE ANALYSIS OF ALMOTRIPTAN IN BULK AND DIFFERENT DOSAGE FORMS AND IN DISSOLUTION SAMPLES

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ABSTRACT

The aim of the present work is to develop and validate a rapid, efficient and economical RP-HPLC-PDA method for the analysis of Almotriptan (AMT) in bulk and dosage forms like tablets and mouth dissolving films (MDFs). AMT was separated on Agilent Eclipse C₁₈ column (150 x 4.6mm, 5µm) with a mobile phase composed of 0.02%v/v formic acid: methanol (70:30% v/v) in isocratic mode and eluents were monitored at 227 nm. AMT was eluted at 3.73min and showed a good linearity in the concentration range of 2-10µg/mL with a correlation coefficient >0.999. The validation parameters like specificity, linearity, accuracy and limit of detection, limit of quantification, precision, robustness are all fulfilled as per regulatory requirements. The developed HPLC method was successfully used for the analysis of AMT in bulk and dosage forms like tablets and mouth dissolving films.

KEYWORDS: *Almotriptan, PDA detection, Mouth dissolving films, Dissolution studies, Method validation.*

INTRODUCTION

Almotriptan malate is a serotonin receptor agonist used in the acute treatment of migraine headache with or without aura, in adults and adolescents aged 12 to 17 years. Chemically, AMT is known as 1-[[[3-[2-(Dimethylamino) ethyl]-1H-indol-5-yl] methyl] sulfonyl] pyrrolidine¹. AMT stimulates specific serotonin receptors in intracranial blood vessels and sensory trigeminal nerves thereby promoting vascular constriction and providing relief from migraine². Various analytical methods have been reported in the literature for quantitative determination of AMT individually in several dosage forms by HPLC³⁻⁵, HPTLC⁶, fluorimetry⁷, UV spectrophotometry^{8,9} and by LC-MS^{10,11} and no validated method was reported for the estimation of AMT in mouth dissolving films. However the mobile phases in most of the methods consisted of phosphate buffer and ACN with longer retention times. Hence, the present investigation was aimed at developing a rapid and economic RP-HPLC-PDA method for the analysis of AMT in tablet dosage forms and in mouth dissolving films.

MATERIALS AND METHODS

Chemicals

Almotriptan was a gift sample from Mylan Laboratories Ltd, Hyderabad, India. Formic acid, water and methanol were purchased from E. Merck, Mumbai, India.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. All the data was processed using LC solutions software. The chromatographic analysis was performed on Agilent Eclipse C₁₈ column (150 × 4.6mm, 5µm).

Chromatographic Conditions

Mobile phase consisting of 0.02% v/v formic acid: methanol (70:30 v/v) was used in isocratic mode and was filtered through membrane filter of 0.45 μ m (Millipore) and pumped at a flow rate of 1 mL/min. The injection volume was 10 μ L and eluents were monitored at 227nm.

Preparation of Stock and Standard Solutions

The stock solution of strength 1 mg/mL was prepared by dissolving 14 mg of Almotriptan malate equivalent to 10 mg of Almotriptan base in 5 mL of methanol and finally volume was adjusted to the mark with methanol in a 10mL volumetric flask. The stock solution was further diluted with water to get standard solutions within a concentration range of 2-10 μ g/mL.

Preparation of AMT Tablets

In the present experiment, AMT tablets (6.25mg) were prepared by direct compression method, due to the unavailability of marketed tablet formulation for AMT in India. Sufficient quantities of povidone, croscarmellose sodium, and talc and magnesium stearate were used. Microcrystalline cellulose was used to raise the bulk volume of the tablets to a targeted weight of 50mg for each tablet. All the ingredients were passed through sieve # 80 before mixing and mixed thoroughly for 5 min in a poly bag. The resultant powder blend was compressed on a single punch tablet press (Cadmach, India) using 4mm punches (round shape) maintained a hardness range of 4-5 kg/cm².

Preparation of AMT MDFs

AMT (100mg) was dissolved in a beaker containing mixture of solvents (water & methanol) and other ingredients like PEG 400, pineapple flavor and aspartame were added one by one. Finally the polymer HPMC E3 was added and mixed thoroughly. The mixture was sonicated for 5 minutes and casted on a glass plate with a wet film applicator set at 30 (750 μ m) mil thicknesses. Films were dried at 45°C for 60min in hot air oven. Then the dried films were peeled off from the glass plate, cut into appropriate sizes, and stored in desiccator until use.

Validation of the HPLC Method

The method was validated according to the ICH guidelines ¹².

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that of formulations. The overlay of diluent, placebo, standard and samples were presented in Figure-1 and 3 D plots were shown in Figure 2.

Linearity

A series of standard dilutions of AMT were prepared over a concentration range of 2-10 μ g/mL (2, 4, 6, 8, 10 μ g/mL) from stock solution and injected in triplicate. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the data was subjected to regression analysis and was given in Table-1.

Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions by using a minimum of six determinations at 100% of the test concentration. The data was given in Table-1.

Accuracy

To ascertain the accuracy of the proposed method recovery studies were performed by spiking 80, 100, 120% of the known quantities of standard to the drug product solution and were analyzed by developed method in triplicate. The data was given in Table-1.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on calibration curves. They were expressed as $LOD = (3.3 \times \sigma)/m$; $LOQ = (10.0 \times \sigma)/m$ (Where, σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Robustness

In order to show the robustness of the method developed, the flow rate was changed by 5% and the effect of wavelength was studied by changing wavelength by $\pm 2\text{nm}$. Whereas, the effect of mobile phase composition was studied by changing $\pm 2\%$ and the data obtained was given in Table-2.

System Suitability

System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50 μL . The system suitability test parameters like retention time, tailing factor and theoretical plate number were noted and % RSD was calculated.

Assay for tablets

Twenty tablets were weighed and finely powdered. A powder equivalent to 10 mg of AMT was accurately weighed and transferred to a 10 mL volumetric flask and 5 mL of methanol was added to the same and made up to the mark with methanol. The solution was filtered and the filtrate was diluted with diluent (water) to obtain a concentration in the range of 2-10 $\mu\text{g/mL}$. The amount present in the each tablet was calculated.

Assay for MDFs

One cm^2 film was taken from the AMT MDFs, placed in a 10mL volumetric flask and dissolved in 5mL of methanol and made up to the mark with methanol and analyzed by HPLC method. The estimations were carried out in triplicate.

Dissolution analysis for Tablets

The prepared AMT tablets were subjected to in vitro drug release studies in 900 mL of distilled water for 30 minutes in eight stage dissolution test apparatus (LAB INDIA DS 8000, Mumbai, India) maintained at $37\pm 0.5^\circ\text{C}$ and the paddles were adjusted at 50 rpm speed. 5mL of the samples were withdrawn at each time interval (2, 5, 10, 15, 20 and 30 minutes) and replaced with 5 mL of fresh medium. The samples were filtered and analyzed using the above developed RP-HPLC method. The % release of AMT in the test samples was calculated.

Dissolution analysis for MDFs

The *in vitro* dissolution studies of MDFs were conducted using 500mL of artificial saliva as dissolution medium with modified Type 5 dissolution apparatus. A temperature of 37°C and paddle was rotated at 50 rpm. $1\times 1\text{ cm}^2$ film equivalent to 6.25mg dose was placed on a watch glass covered with nylon wire mesh and then dropped into dissolution flask. 5mL samples were withdrawn at predetermined time intervals 10, 20, 30, 40, 50, 60, 80, 100, 120, 180sec and replaced with 5mL of dissolution medium. The samples were analyzed by above HPLC method. The dissolution experiments were conducted in triplicate.

Filter Compatibility Study

Dissolution samples were checked for compatibility with both the nylon filter (0.45 μm) and Polyvinylidene Difluoride (PVDF) filters (0.45 μm). AMT standard in dissolution medium was prepared and the solution was filtered through the filters. Both the filtered and unfiltered samples were injected and chromatograms were observed. The data was shown in Table 3.

RESULTS AND DISCUSSION

Method Development

Initially, mobile phase optimization was carried with Agilent Zorbax (150x4.6 mm, 5 μ) column using 10mM ammonium acetate: acetonitrile as mobile phase at a flow rate of 0.8mL/min, AMT was eluted as a broad peak with tailing. In other trial Agilent Eclipse plus (150x4.6mm, 5 μ) column with 10mM ammonium acetate and acetonitrile (75:25% v/v) as mobile phase was used at a flow rate of 0.8mL/min and under these conditions the AMT peak was merged with solvent front. Further trials were performed with 0.02% formic acid: methanol as mobile phase combinations and finally 70:30% v/v of 0.02% formic acid: methanol was selected at a flow rate of 1.0mL/min at 227nm. Under these conditions a sharp peak of AMT was eluted at 3.73min (Figure 1) and tailing factor, peak symmetry, theoretical plate number were all within the limits. The peak purity index was found to be greater than 0.9999 and this indicating purity of the drug sample used in the analysis and shown in Figure-1 along with UV spectra.

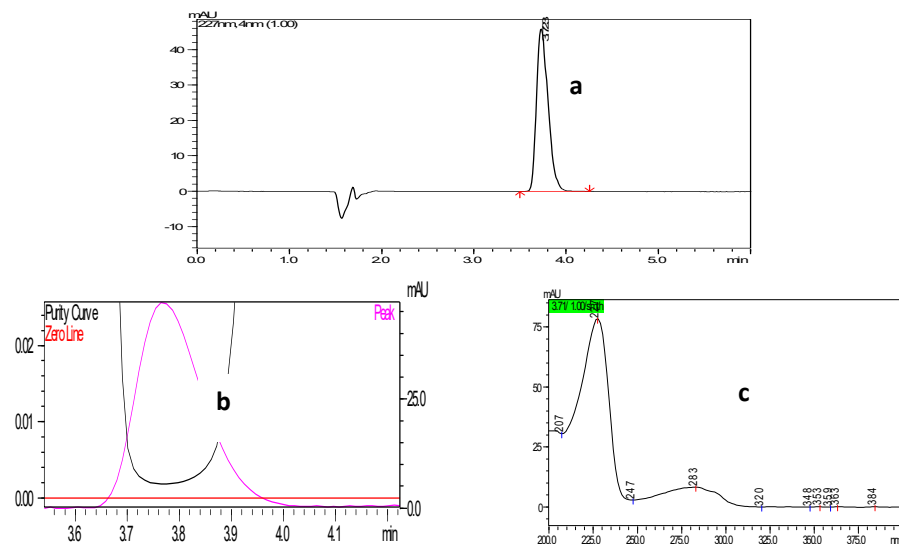


Figure 1: a - Chromatogram of AMT (6 µg/mL) b - Peak purity index of AMT and c – UV Spectrum of AMT

Method validation

The method has been validated as per ICH-Guidelines for the following parameters.

Specificity

The specificity of the method was established by injecting the solutions of diluent, placebo, standard and samples (tablet and film) individually to examine any interference from the excipients. From the overlay of chromatograms as shown in Figure-2 and 3D plots of chromatograms shown in Figure 3, it can be inferred that there were no co-eluting peaks at the retention time of AMT, this shows that peak of analyte was pure without any interferences and the peak purity indices was found to be greater than 0.999 and this confirms specificity of the method.

Linearity

The range of reliable quantification was set at the concentrations of 2-10µg/mL of AMT. Peak areas and concentrations were subjected to least square regression analysis. The correlation coefficient (R) was found to be 0.999 indicating a linear response over the concentration range selected. The data from the calibration curve was given in Table-1.

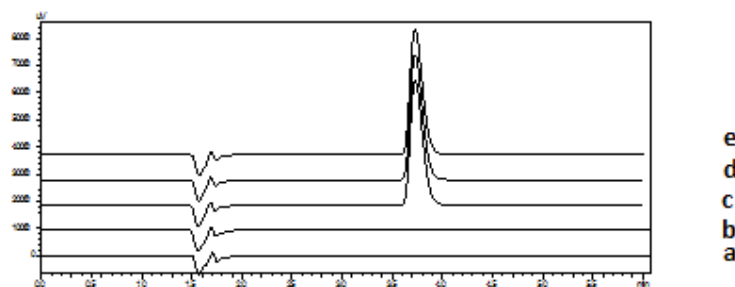


Figure 2: Overlay of the diluent-(a), placebo-(b), standard-(c), AMT tablet sample-(d) and AMT Mouth dissolving film-(e) chromatograms

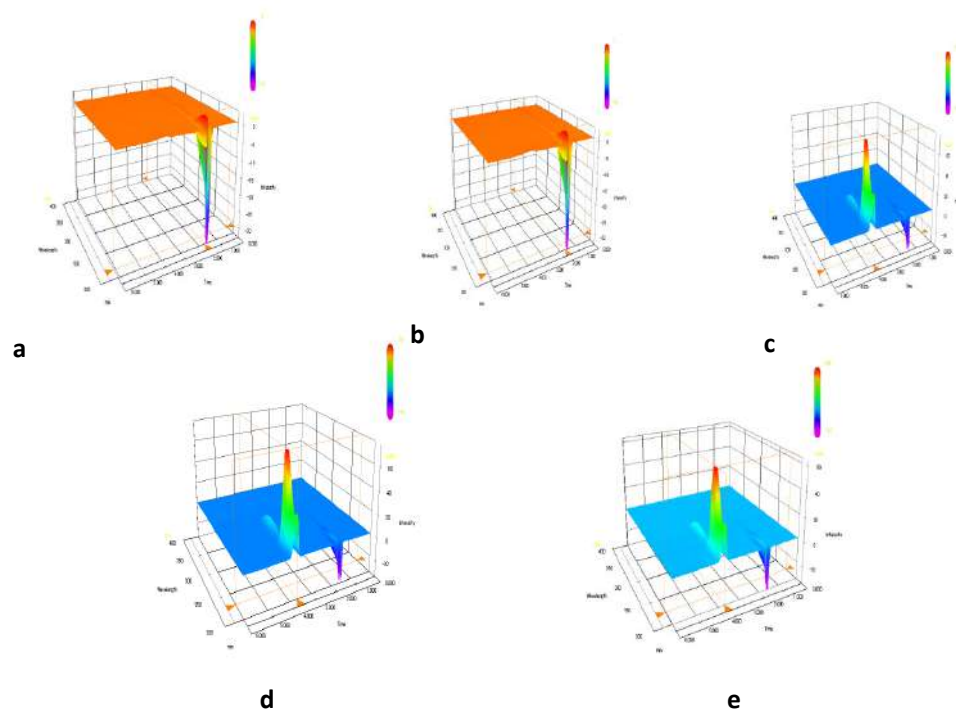


Figure 3: 3D Plots of diluent-(a), placebo-(b), standard-(c), AMT tablet sample-(d) and AMT Mouth dissolving film-(e)

Precision

Precision studies were carried out using six replicates of concentration at $6\mu\text{g/mL}$ level and the data was given in Table-1. The % RSD was found to be below 2 for peak areas and this shows the closeness of the data values to each other, indicating the precision of the method.

Accuracy

Accuracy of the method was ascertained by spiking the known quantities of standard at 80, 100, 120% to the drug product solution and were analyzed in triplicate. The % RSD and the % recovery were within the acceptable limit indicating that the proposed method is accurate.

Table 1: Linearity, Precision and Accuracy data of AMT

Validation data of AMT	
Linearity(n=3)	
Range	2-10 $\mu\text{g/mL}$
Regression equation	$Y = 66205x - 32111$
Regression coefficient (R^2)	0.999
Correlation coefficient (R)	0.998
Accuracy(n=3)	
% level of addition	Mean Percent Recovery(%RSD)
80	101.43(0.27)
100	100.79(0.05)
120	100.66(0.14)
Precision(n=6)	
	Average Peak area of the standard sample(%RSD)
	406442 (0.37)
Percent assay (n=3)	
Tablets	Mean $\pm$ SD(%RSD)
	100.68 $\pm$ 0.57(0.56)
MDFs	100.92 $\pm$ 0.52(0.51)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on statistical calculation from the calibration curves, The LOD for AMT was found to be 0.0712 μ g /mL, and LOQ was found to be 0.2158 μ g/mL.

Robustness

As part of the robustness, retention times were significantly changed with flow rate, mobile phase and no change in the retention time was observed in wavelength change. The parameters like theoretical plate number, tailing factor were not changed and were within the limits. These results indicated that the method is robust.

System suitability

System suitability studies were carried out by different injection volumes (10 μ L - 50 μ L) of 6 μ g/mL. The % RSD values for system suitability test parameters like retention time (0.749%), tailing factor (0.45%) and theoretical plate number (1.63%) were less than 2% indicating the present conditions were suitable for the analysis of AMT in formulations.

Stability of the Analytical Solution

The stability of the stock solution was determined by analyzing the samples stored under refrigeration (8 $\pm$ 1 $^{\circ}$ C) conditions at different time intervals up to 1 month. The variation in assay values at different time intervals were found to be less than 2% of the initial time interval and these results indicating that the solutions were stable for a period of 1 month when stored at 8 $^{\circ}$ C.

Assay for Tablets and MDFs

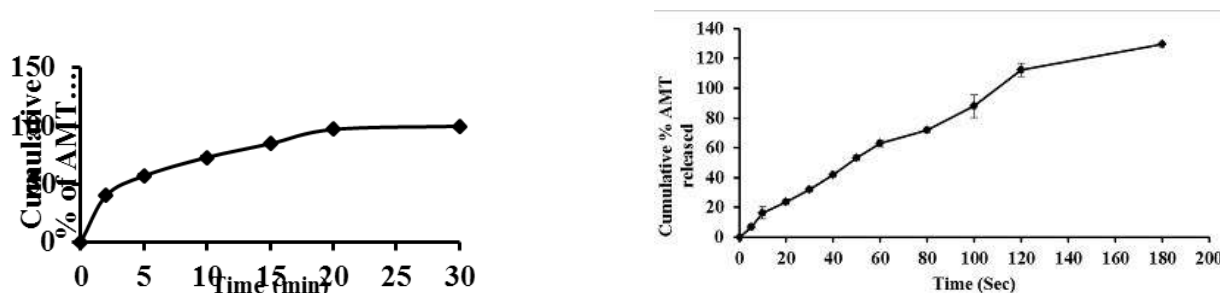
Assay of AMT in tablets and MDFs were performed by the proposed method and the percent assay of the formulation was calculated as an average of three determinations, which was about 100.68 $\pm$ 0.22 and 100.92 $\pm$ 0.52 respectively. These results indicate that the present RP-HPLC-PDA method can be successfully used for the assay of AMT in bulk, tablet dosage forms, and MDFs.

Table 2: Robustness data

Chromatographic parameter	Retention time (min)	Theoretical plates (#)	Tailing factor (T _f)
Flow rate (mL/min)			
0.95	3.77	3877.52	1.491
1.0	3.72	3632.88	1.475
1.05	3.69	3544.43	1.454
Wave length (nm)			
225	3.72	3657.01	1.469
227	3.72	3632.88	1.475
229	3.72	3669.62	1.474
Composition of mobile phase			
68:32	3.63	3602.42	1.443
70:30	3.72	3632.88	1.475
72:28	3.79	3669.62	1.481

3.5 Dissolution analysis for Tablets and MDFs

The above validated method was used for the *in vitro* dissolution analysis of AMT in tablets and MDFs. A rapid AMT dissolution was observed in both the dosage forms and a complete dissolution was observed within 30 min for tablets whereas 180sec for MDFs. The dissolution profile was presented in Figure-4.



(a) (b)
Figure.4: Dissolution profile of AMT (a) tablets, (b) mouth dissolving film

Filter compatibility study

Compatibility of 0.45µm nylon filter and Polyvinylidene Difluoride (PVDF) filters (0.45µm) were studied. Standard, sample solution and dissolution samples were filtered and analysed and the variation in the assay value was calculated. After analysis it was found that nylon filters were suitable for filtration. The data was given in Table-3.

Table 3: Filter compatibility study

Sample name	Peak area of AMT	% Difference
Standard sample	406442	-
Samples filtered through 0.45µm nylon filter	405442	0.246
Samples filtered through PVDF filter	402954	0.858

CONCLUSION

In this work, rapid and efficient RP-HPLC-PDA method was developed for the analysis of AMT in bulk and formulations like tablets and mouth dissolving films. The method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for the estimation of AMT in formulations using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. Therefore, this method can be selectively employed to quantify AMT not only in formulations like tablets and mouth dissolving films but also in dissolution studies.

ACKNOWLEDGEMENTS

The authors are thankful to Mylan Laboratories Ltd, Hyderabad, India for providing gift sample of AMT and also to the Siddhartha Academy of General and Technical Education, Vijayawada, for providing necessary facilities to carry out this research work.

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ENHANCEMENT OF DISSOLUTION PROPERTIES OF VALSARTAN BY SOLVENT DEPOSITED SYSTEMS

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ABSTRACT

The aim of the present investigation is to prepare and evaluate solvent deposited systems (SDS) of valsartan (VAL) and to find out the effect of various formulation variables like polymers and solubilizing agents on drug release properties of VAL. Microcrystalline cellulose (MCC) and sucrose were used as polymeric carriers and sodium lauryl sulfate (SLS) and polyvinyl pyrrolidone (PVP) as solubilizing agents. The prepared SDS were characterized for hardness, weight variation, disintegration time, FTIR and drug release behavior for tablets. FTIR studies confirm the compatibility of VAL with excipients used in the formulation. In-vitro drug release studies indicate that the formulations containing MCC as carrier showed slower VAL release compared to formulations containing sucrose as carrier. Addition of SLS and PVP brought a significant increase in VAL release compared to formulations without them. The formulation F6 showed quicker disintegration (within 32 sec) and VAL release rates (complete release was obtained within 180 sec) along with good physico-mechanical properties.

KEYWORDS: *Valsartan, solvent deposited systems, formulation variables, and compatibility.*

1. INTRODUCTION

Recent developments in combinatorial chemistry and high throughput screening used in drug discovery resulted in increased number of drugs with poor aqueous solubility. Approximately 90% of the new chemical entities are considered poorly soluble with either high or low permeability. BCS class II drugs are highly permeable and low soluble in nature¹. Thereby their absorption or bioavailability is limited by dissolution rate. Hence dissolution enhancement is the key factor in formulating BCS class II drugs². Valsartan (VAL), N-(1-oxopentyl)-N [[2¹-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-L-valine, a potent and selective AT₁ receptor antagonist used in the treatment of hypertension. VAL is a BCS class II drug and is highly hydrophobic in nature. The poor aqueous solubility of drug is associated with slow drug dissolution and slow erratic absorption leading to low oral bioavailability³. Till date several approaches have been developed to enhance the dissolution properties of poorly soluble drugs like complexation, solid dispersions, and soft gels⁴⁻⁷. Solvent deposited systems (SDS) based on powder solution technology shows promising potential in improving the dissolution rate of poorly soluble drugs like VAL. SDS not only enhances the drug dissolution but can be commercially viable and has industrial scale-up feasibility due to low cost and ease of handling^{8,9}. So, the present investigation was aimed to formulate VAL SDS and to evaluate its efficacy.

2. MATERIALS AND METHODS

VAL is obtained as a gift sample from Hetero Drugs Pvt Ltd, Hyderabad. Ethanol, micro crystalline cellulose (MCC), sucrose, and sodium lauryl sulfate (SLS) purchased from Qualigens fine chemicals, Mumbai. All other materials used in the study were of analytical grade.

2.1 Preparation of VAL SDS

The VAL SDS was prepared by Spraying Technique. The required quantities of drug and carrier as given in Table 1 were taken. Initially carrier was taken in a mortar and VAL was sprayed on carrier (MCC or sucrose) which was dissolved in ethanol with intermittent mixing and dried at 40°C for 10 min. 40 mg equivalent of VAL was accurately weighed and compressed into tablets by direct compression method.

Table 1: Formula for the Preparation of VAL SDS

S.No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6
1.	VAL	40	40	40	40	40	40
2.	MCC	150	200	-	-	-	-
3.	Sucrose	-	-	150	200	200	200
4.	SLS	-	-	-	-	30	-
5.	PVP	-	-	-	-	-	30

2.2 FTIR Studies

FTIR studies were carried out using ATR-FTIR spectrometer (Bruker, Germany). ATR spectra were measured over the wave number range of 4000-500 cm^{-1} at a resolution of 1.0 cm^{-1} . The powder sample was simply placed onto the ATR crystal and the sample spectrum was collected.

2.3 VAL content

The VAL content in tablets was determined by dissolving one tablet in 20ml ethanol and 20 ml of pH 6.8 phosphate buffer was added and the contents were sonicated for 10min and made up to the mark with buffer in 100 ml volumetric flask. This solution was suitably diluted with pH 6.8 phosphate buffer and was assayed at 250nm for VAL¹⁰.

2.4 Hardness

The hardness of the prepared tablets was measured by using the Pfizer tablet hardness tester and the hardness of the each tablet was recorded.

2.5 Weight Variation

Weight variation was calculated as per method described in USP. Twenty tablets were selected at random and their average weight was determined using an electronic balance. The tablets were weighed individually and compared with average weight.

2.6 In-vitro Disintegration test

The disintegration time was determined by using USP tablet disintegration test apparatus using 900 ml of pH 6.8 phosphate buffer and maintained at $37 \pm 0.5^\circ\text{C}$.

2.7 In-vitro Dissolution

The dissolution rate testing of VAL formulations was studied using USP XXII dissolution rate testing apparatus, (paddle type) (LAB INDIA DISSO 2000). The paddle was rotated at a speed of 50 rpm and the dissolution medium (900 ml pH 6.8 Phosphate buffer) was maintained at a temperature of $37.5^\circ \pm 0.5^\circ\text{C}$. At specific time intervals a 5 ml aliquot was collected and was replaced with fresh quantity of dissolution medium. The samples were assayed for VAL content by measuring the absorbance at 250 nm using U.V Spectrophotometer (ELICO SL 159). The percent of VAL dissolved at various time intervals was calculated and plotted against time³.

3. RESULTS AND DISCUSSION

3.1 Preparation of VAL SDS

VAL SDS prepared by spraying technique on carrier gave porous powder due evaporation of ethanol leaving the drug on the surface of the carrier which resulted in faster drying rates. This porous powder equivalent to 40 mg VAL (250 mg) was weighed and then compressed into tablets by direct compression method. The VAL SDS thus prepared were stable and porous indicating the easiness of the method compared to other methods used for the enhancement of solubility.

3.2 FTIR Studies

The FTIR spectra of VAL and VAL SDS F6 were shown in Figure 1. VAL showed characteristic peaks at 3393 cm^{-1} (N-H stretching), 2981 cm^{-1} (C-H stretching), 2931 cm^{-1} (O-H stretching), 1695 cm^{-1} (C-O stretching), 1653 cm^{-1} (C-N stretching), and 753 cm^{-1} (C-H bending) respectively. The VAL SDS also

showed the same characteristic peaks as of pure VAL indicating that there is compatibility between the VAL and the excipients used in the formulation.

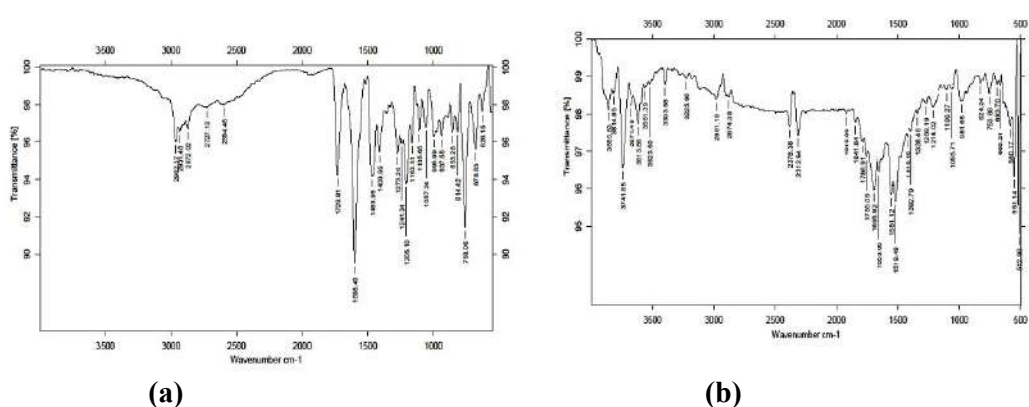


Figure 1: (a) FTIR spectra of pure VAL, (b) FTIR spectra of VAL SDS F6

3.3 Hardness

Generally the tablets should be sufficiently hard to resist the breaking during normal handling and yet soft enough to disintegrate properly after swallowing. All the formulations showed acceptable hardness (3.8-4.4 kg/cm²). The results were shown in Table 2.

3.4 Weight Variation

The weight of the individual tablet will be implying the VAL dose for effective therapy. The prepared VAL tablets were checked for weight variation and from the results it is observed that all the formulations showed uniform weight and the percent of error is found to be in the range of -1.49 to +2.1. The results were shown in Table 2.

3.5 Disintegration

The disintegration time of VAL SDS were shown in Table 5. From the results it is observed that formulation F6 disintegrates within 3 min; other formulations took around 3-10 min, whereas the formulation F4 showed a higher disintegration of about 13 min. The longer disintegration time may retard the release rate of the drug. The results were shown in Table 2.

Table 2: Evaluation Parameters of VAL SDS

S.No.	Formulation	Hardness kg/cm ²	% Error of Weight Variation	% Drug content	Disintegration time (min)
1	F	3.8	-1.49	99.13	10
2	F2	4.2	0.07	104.1	8
3	F3	4.1	-0.58	96.5	8
4	F4	4.4	1.68	100.5	6
5	F5	4.0	2.1	101.5	4
6	F6	4.2	-1.2	99.6	3

3.6 In-vitro Dissolution Studies

In the present investigation, dissolution of VAL SDS were carried out using Type II dissolution apparatus and 900 ml of pH 6.8 phosphate buffer was used as dissolution medium. The effect of various formulation variables like carriers and solubilizing agents on VAL release from SDS was studied and the comparative release profiles were shown in Figure 2. Totally 6 different formulations were prepared using MCC and sucrose as carriers and SLS and PVP as solubilizing agents. F1 and F2 formulations were prepared with MCC as carrier. The cumulative percentage of VAL release at the end of 5 min is 48.3±0.23 and 52.1±1.03 for F1 and F2 respectively. Complete VAL release was obtained at the end of 45 min and 30 min for F1 and F2 respectively and the comparative release profiles are shown in Figure 2a. The VAL release from F2 is significantly higher than formulation F1 indicating that increase in concentration of MCC increased the dissolution of VAL. The cumulative percent of VAL released at the end of 5 min is 64.31±1.21 and 70.43±0.89 for F3 and F4 respectively. Complete VAL release was obtained at end of 30 and 20 min

respectively for F3 and F4 and the comparative profile was shown in Figure 2b. The VAL release from F4 is higher than F3. Overall, the VAL release is higher for F4 (containing sucrose as carrier) than F2 (containing sucrose as carrier). Effect of solubilizing agents on VAL release was also observed. Both SLS and PVP were added to the formulations. The cumulative percent of VAL released at the end of 5 min is 86.47 ± 0.26 and 90.31 ± 1.3 respectively for F5 and F6. Complete release of VAL was observed at the end of 10 min and 7 min respectively for F5 and F6 respectively indicating that the addition of solubilizing agents showed significant effect on VAL release from the formulation, which might be due to effective decrease in surface tension. Overall sucrose was selected as carrier because F4 shows higher dissolution properties than F2. VAL SDS with PVP and SLS showed superior dissolution properties compared to formulations without PVP and SLS. SDS with PVP (F6) gave higher dissolution properties when compared to SLS formulation (F5). The comparative release profiles for SDS with and without PVP and SLS are given in Figure 2d.

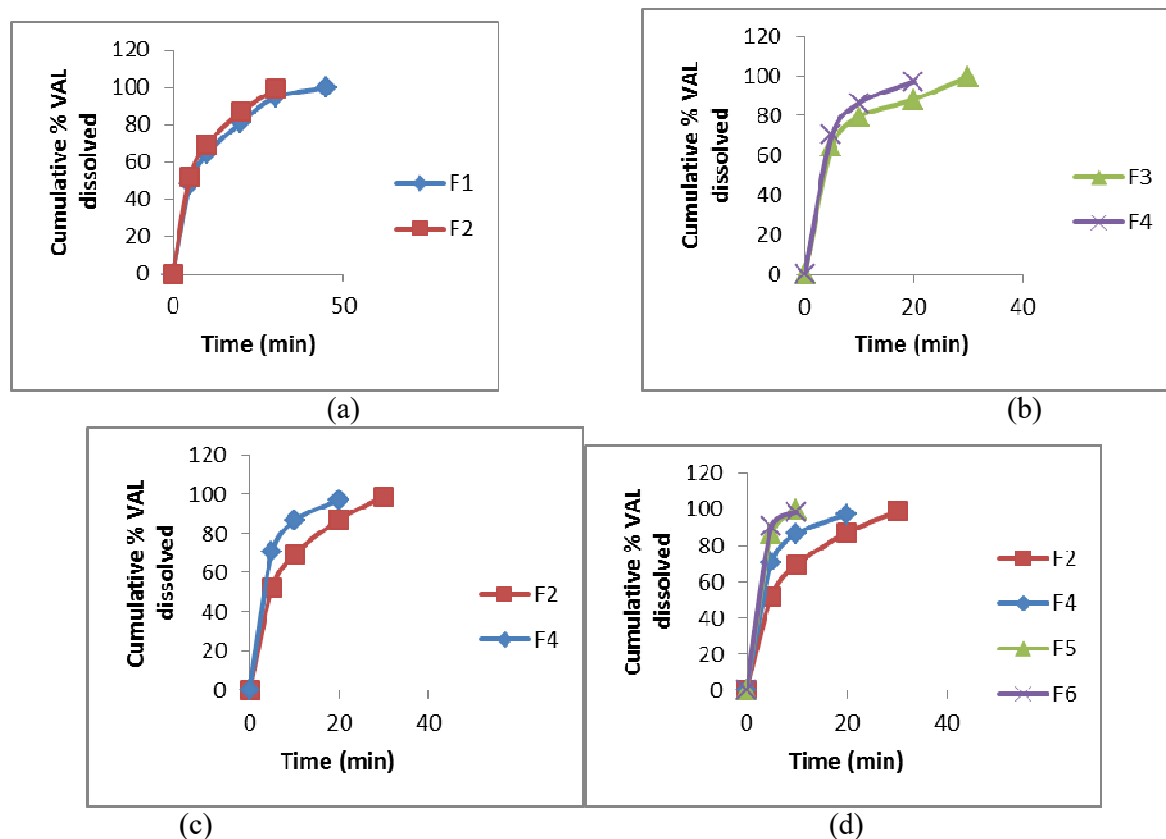


Figure 3: Comparative in-vitro dissolution profile of VAL

4. CONCLUSION

In the present investigation VAL SDS were successfully formulated. The effect of carriers on dissolution rate of VAL was studied and found that sucrose is a better carrier than MCC and the effect of solubilizing agents also studied which enhanced the dissolution profile of VAL from SDS. Overall formulation F6 containing sucrose as carrier and PVP as solubilizing agent showed better dissolution properties.

5. ACKNOWLEDGEMENTS

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ENHANCEMENT OF DISSOLUTION PROPERTIES OF IBUPROFEN BY LIQUISOLID COMPACT TECHNIQUE

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ABSTRACT

The present investigation was aimed to prepare and evaluate ibuprofen (IBU) liquid solid compacts (LSC) to enhance the dissolution properties of IBU and thereby its bioavailability. IBU LSC were prepared by using microcrystalline cellulose (MCC) and lactose as carriers in different ratios, aerosol 200 as coating material, propylene glycol (PG) as vehicle and sodium starch glycolate (SSG) and croscarmellose sodium (CCS) as superdisintegrants. The LSC prepared were evaluated for flow properties and then compressed into tablets by direct compression method. The tablets prepared were evaluated for weight variation, friability, hardness and in-vitro dissolution. The FTIR studies reveal that there is no interaction between IBU and the excipients used in the formulation. The flow properties of the prepared LSC indicate a good flow property and the post compressed parameters of IBU LSC were all within the acceptable limits. The in-vitro dissolution studies showed that a 1.3 fold increase in dissolution was observed in IBU LSC containing MCC as carrier than the formulations containing lactose as carrier. The addition of SSG, CCS, and inulin resulted in 1.47, 1.25, and 1.19 fold increase in IBU release than the formulations without superdisintegrants. Overall the formulation F6 showed faster dissolution of IBU along with good physico mechanical and flow properties.

KEYWORDS: *Ibuprofen, dissolution properties, LSC, and superdisintegrants.*

1. INTRODUCTION

Among the different routes of drug administration oral route is the most preferred and widely accepted route of drug administration due to low cost factor and ease of drug administration. About 40% of the drugs administered through oral route exhibit low aqueous solubility and low bioavailability due to less absorption¹. Over past few years, many techniques have been developed to increase the dissolution characteristics of water insoluble drugs and among them liquisolid compact technique is effective to improve the dissolution characteristics of water insoluble drugs². Liquisolid compacts (LSC) are considered as acceptably flowing and compressible powdered forms of liquid medications that imply oily liquid drugs and solutions or suspensions of water insoluble solid drugs carried in suitable nonvolatile solvent systems. In this technique the drug was dissolved in a liquid vehicle and was incorporated in to a carrier material having porous nature and adsorption properties. Then coating materials were added to the wet preparation to give the desired flow properties. LSC of poorly soluble drugs containing a drug molecularly dispersed in solubilizing vehicle show enhanced drug dissolution due to an increased surface area of drug, and an improved wettability of the drug particle³. Accordingly, this improved drug dissolution may result in higher drug absorption and thus, an improved oral bioavailability^{4, 5}. Ibuprofen (IBU) is one of the NSAID's which belongs to BCS class II drug having low aqueous solubility or practically insoluble in water. It is a propionic acid derivative having pharmacological effect as a anti-inflammatory, analgesic and antipyretic. IBU works by inhibiting the production of prostaglandins by decreasing the activity of enzyme cyclooxygenase. The present work focuses on the preparation and evaluation of IBU by LSC to improve the solubility and dissolution properties of the drug.

2. MATERIALS AND METHODS

2.1 Materials

Ibuprofen (IBU) was a gift sample obtained from Aurobindo Pharma Pvt Ltd, Hyderabad, Aerosil 200 and Propylene glycol purchased from thermo Fischer scientific, India Pvt Ltd, Microcrystalline cellulose (MCC), Lactose, croscarmellose, sodium starch glycolate, and inulin were obtained from Loba Chemie Pvt.Ltd.

2.2 Method

2.2.1 Preparation of LSC

IBU LSC was prepared as per the table 1. Required amount of IBU (100 mg) was weighed and transferred to a mortar and dispersed in propylene glycol (PG). The calculated amount of carrier and coating material was added to the dispersion and blended in the mortar. To this the remaining ingredients were added and blended well. The final mixture was compressed in to tablets manually using multi station rotary tablet punching machine.

Table.1. Composition of Ibuprofen tablets by LSC technique:

S.No	Formulation	Liquid vehicle	Carrier	Disintegrant (3%)	Liquid load factor (L_f)	Aerosil (mg)	Unit dose Weight (mg)
1	F 1	PG	MCC	-	0.11	19	400
2	F 2	PG	MCC	-	0.14	20	400
3	F 3	PG	MCC	-	0.24	21.5	400
4	F 4	PG	Lactose	-	0.11	19	400
5	F 5	PG	Lactose	-	0.14	20	400
6	F 6	PG	Lactose	-	0.24	21.5	400
7	F 7	PG	MCC	Sodium starch glycolate	0.14	20	400
8	F 8	PG	MCC	Croscarmellose sodium	0.14	20	400
9	F 9	PG	MCC	Inulin	0.14	20	400
Liquid load factor $L_f = W/Q$ Final tablet weight includes 1 % of lubricant							

2.3 EVALUATION of LSC

2.3.1 Flow behavior

Before compressing in to the tablet the powder mixture was subjected to different flow properties such as angle of repose, Carr's index and Hausner's ratio to reduce the dose variation in the production of dosage forms⁶.

2.3.2 Angle of Repose

Fixed funnel method was used to determine the angle of repose for powder mixture. Accurately weighed powder mixture was taken in a funnel and was allowed to flow freely through the funnel on to the surface⁶. The procedure was carried in triplicate and the results were given in Table2. The height and diameter of the powder cone was measured angle of repose was calculated using the following formula.

$$\tan \theta = h/r$$

Where, θ is the angle of repose, h is the height in cm r is the radius in cm.

2.3.3 Bulk Density

The bulk density was determined by using bulk density apparatus. The powder blend was poured in to a graduated cylinder and the weight and bulk volume of the powder was determined⁶. The bulk density was calculated using the formula:

$$BD = M / V_b$$

Where, M is the mass of powder, V_b is bulk volume of powder.

2.3.4 Tapped Density

A measured mass of powder was filled in a cylinder and tapped continuously up to a fixed time. The volume that was occupied by the powder blend and weight of the powder was noted and tapped density was calculated using the following formula⁶:

$$Dt = M/Vt$$

Where, M is the mass of powder, Vt is tapped volume of powder

2.3.5 Carr's Index (%)

Carr's index is the simplest way of measuring the flow properties of the powder and it was an indirect method to determine the bulk density, size and shape of the particles⁶. Values were given in table 2. Carr's index was calculated using the following formula:

$$CI (\%) = [(Tapped\ density - Bulk\ density) / Tapped\ density] \times 100$$

2.3.6 Hausner's Ratio

The ease of powder flow can be indirectly determined through Hausner's ratio and the formula to determine Hausner's ratio was as follows⁶:

$$\text{Hausner's Ratio} = \text{Tapped density (pt)} / \text{Bulk density (pb)}$$

Hausner's ratio values were mentioned in table 2.

2.3.7 FTIR studies

The compatibility between IBU and excipients used for the preparation of LSC were determined by FTIR³. Samples were analyzed by using an ATR-FTIR spectrometer (Bruker, Germany) over the wavenumber range of 4000-600 cm^{-1} at a resolution of 1.0 cm^{-1} . The FTIR spectra of both IBU and IBU LSC were recorded to evaluate the compatibility of IBU with excipients used in the formulation

2.3.8 IBU Content

The prepared LSC mixtures were assayed for IBU content. 10 mg of LSC mixture was weighed and dissolved in sufficient amount of methanol⁸. The above solution was suitably diluted with pH 7.2 buffer and the IBU content was determined using UV spectrophotometer at 222nm.

2.3.9 Hardness

The hardness of the IBU LSC compact tablets was evaluated by using Pfizer hardness tester and the values were expressed in kg/cm^2 and given in Table 3. Hardness of the tablets was checked by taking a set of three tablets randomly from each formulation and the mean values were noted³.

2.3.10 Friability:

Roche friabilator is the equipment which is used for the determination of friability. A set of pre weighed tablets were placed in a friability chamber which revolves at 25rpm and they are subjected to fall from a height of 6 inches for about 100 revolutions⁹. The weight of the tablets before and after subjecting to friability test was noted and the percentage of friability is calculated by using the following formula and the values were given in Table 3.

$$F = \{(W\ initial) - (W\ final) / (W\ initial)\} \times 100$$

2.3.11 In-vitro Dissolution studies

The in vitro drug dissolution study was performed by using USP dissolution type II apparatus (LABINDIA DS-8000) at 50 rpm using phosphate buffer pH 7.2 phosphate buffer (900 ml) as dissolution medium maintained at 37 ± 0.5 °C. 5ml of aliquots were withdrawn at respective time intervals and filtered through 0.45 μm filter paper and replaced with 5ml of fresh dissolution medium³. The samples were then analyzed at 222nm by UV-visible spectrophotometer. The cumulative amount of drug released was calculated as a function of time to construct release profile graphs. The release data was fitted to different kinetic models.

3. RESULTS AND DISCUSSION

In the present investigation LSC were prepared using various carriers like MCC and lactose, and superdisintegrants like SSG, CCS and inulin. The tablets were prepared by direct compression method and were evaluated for various physico mechanical properties and invitro drug release profile.

3.1 Evaluation of LSC

3.2.1 Flow Properties

The prepared mixtures of LSC flow properties were determined by angle of repose, Carr's index and Hausner's ratio. The results were given in table 2. From the results it is observed that there is 1.17 and 1.30 fold increase in angle of repose and Carr's index for the formulation containing MCC as carrier compared to formulations containing lactose as carrier. The addition of superdisintegrants didn't show any significant effect on the flow properties of LSC. In overall all the formulations showed good flow ability.

Table.2. Flow Properties of LSC Physical Mixtures

Formulation	Angle of repose	Carr's index	Hausner's ratio
F1	27.85±0.50	16.35±0.26	1.12±0.12
F2	31.26±0.18	14.65±0.3	1.14±0.06
F3	29.84±0.25	18.33±0.52	1.10±0.62
F4	32.23±0.63	21.22±0.68	1.12±0.88
F5	30.22±0.38	24.56±0.12	1.11±0.28
F6	32.61±0.41	22.03±0.44	1.15±0.03
F7	32.16±0.22	20.98±0.12	1.3±0.08
F8	31.63±0.20	22.68±0.08	1.03±0.64
F9	33.53±0.19	21.63±0.13	1.14±0.24

3.1.2 FTIR studies

The FTIR spectrum of pure IBU and IBU LSC were shown in Fig 1. The FTIR spectra of IBU exhibited characteristic peaks at 2918.50cm⁻¹ due to alkane stretching, (C-H stretching), 1698.84cm⁻¹ (C=O stretching of the carboxyl ion). All the peaks were retained in IBU LSC indicating that there is no interaction of IBU and excipients within the formulations.

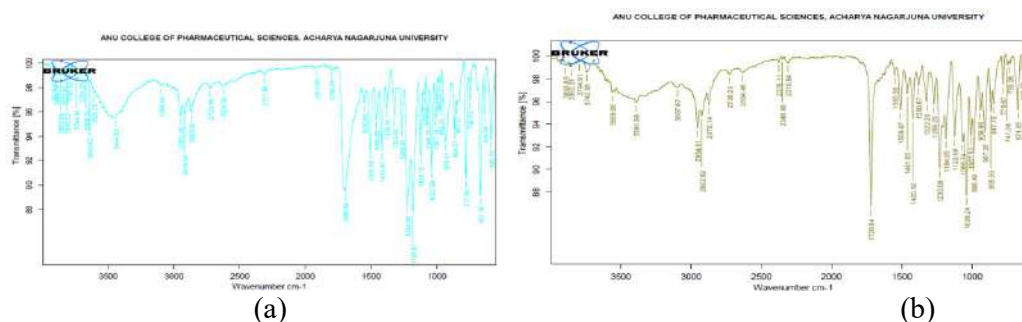


Fig.1. FTIR Spectra of (a) Pure IBU, (b) IBU LSC F9.

3.1.3 Drug Content

The drug content was within the acceptable range for all formulations indicating uniform distribution of IBU in all the formulations. IBU content was found to be 97% to 101% in all the prepared formulations.

3.1.4 Hardness

The hardness of the prepared LSC tablets is measured using Pfizer hardness tester and was found to be in the range of 4-5 kg/cm². The carrier doesn't show any significant effect on the hardness of the tablets. However, the concentration of MCC showed a slight effect on the hardness of the tablets i.e as the concentration of MCC increases the hardness of the tablet increased.. The results are shown in table.3.

3.1.5 Friability

The friability of the tablets was within the limits as per I.P i.e. <1% and values were given in table 3. Test results indicate that tablet can overcome shock and wear without being destroyed during the production process, packing and transporting of the dosage form.

Table.3. Evaluation of IBU LSC Tablets

Formulation	Hardness	Friability	Drug content
F1	4.2±0.3	0.86±0.02	96.42±1.86
F2	4.3±0.2	0.79±0.05	95.33±0.88
F3	4.1±0.6	0.84±0.02	98.63±2.23
F4	4.4±0.1	0.81±0.06	97.21±1.27
F5	4.2±0.4	0.86±0.01	95.41±0.59
F6	4.4±0.1	0.85±0.09	96.81±2.21
F7	4.2±0.8	0.82±0.03	97.71±1.63
F8	4.1±0.3	0.80±0.02	98.26±1.45
F9	4.3±0.8	0.84±0.10	96.83±0.76

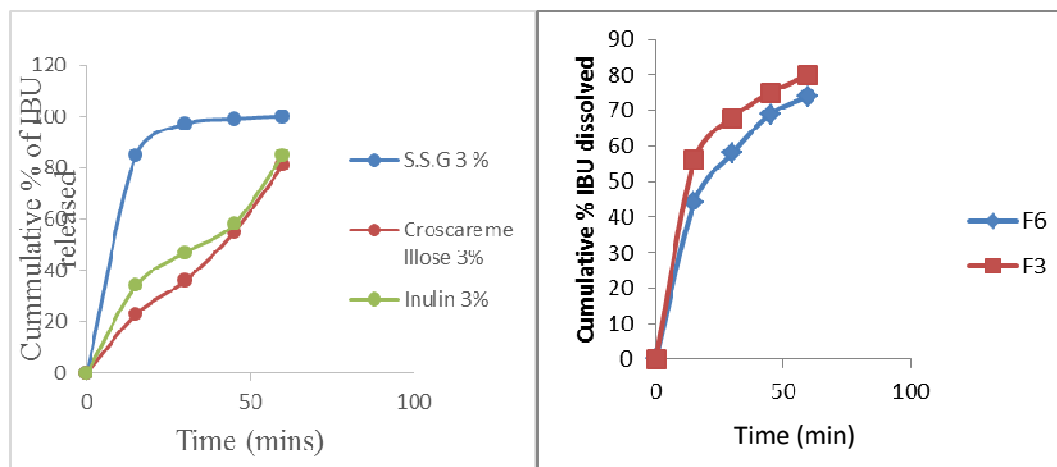


Fig.3 Comparative in-Vitro dissolution profiles of (a) IBU LSC (containing superdisintegrants) and (b) IBU LSC (containing carriers)

3.1.6 Dissolution

The dissolution profiles of IBU LSC tablets were shown in Fig.2. From the results it is observed that as the concentration of carrier increases the dissolution of IBU increased. A 1.23 fold increase in IBU release was observed in formulation F3 containing MCC as carrier compared to formulation F6 containing lactose as carrier. Further trials were carried out to study the effect of superdisintegrants like Sodium starch glycolate, croscarmellose sodium and inulin on the release of IBU from the formulations; it is observed that a faster drug release was observed in the formulations containing superdisintegrants than the formulations without superdisintegrants. A 1.78, 1.51, and 1.44 folds increase in IBU release was observed in formulation F7, F8, and F9 respectively containing SSG, CCS, and inulin than formulation F3 without super disintegrants. Among the three superdisintegrants used formulation F (7) containing S.S.G showed faster dissolution than CCS and inulin.

4. CONCLUSION

From this investigation, it can be concluded that IBU can be successfully formulated as LSC. From the results it is observed that the nature of carrier had shown significant effect on dissolution properties of IBU. In addition, superdisintegrants was found to play a crucial role on the IBU release rates. Faster drug release rates from LSCs may provide quick onset of action with enhanced oral bioavailability and therapeutic efficacy.

5. ACKNOWLEDGEMENTS

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IDENTIFICATION AND DETERMINATION OF FLAVONOID IN CARICA PAPAYA, PUNARNAVA AND ABUTILON INDICUM BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Flavonoids (Rotenoids) present in carica papaya, punarnava (*Boerhaavia diffusa*) and abutilon indicum. It is known for its completely different biological activities such as antioxidant, anti-diabetic, anti-stress, anticonvulsant, antibacterial, hypoglycemic, anti-nociceptive, hepato-protective, anti-proliferative and anti-estrogenic, anti-inflammatory. The following work constitutes the enrichment of iso-flavonoids present in the methanolic extract of carica papaya, punarnava (*Boerhaavia diffusa*) and abutilon indicum by spike technique using various solvents such as acetone, water, Butanol, ethyl acetate, hexane, chloroform and methanol. Enrichment was determined by uplc, Column Luna C18, 250mm x 4.6mm, 5µm. Buffer: Dissolve 1ml Ortho Phosphoric acid in 1000ml of water. Mobile phase: Mixed Buffer and Acetonitrile in the ratio of 60:40% v/v. Flow rate 1.0 ml/ min. Wave length 255 nm. Retention time of Quercetin is about 5.6 min. The development method was validated according to the ICH guidelines for specificity, precision, accuracy and linearity. The method showed good reproducibility and recovery with %RSD less than 2. So the proposed method was found to be simple, specific, precise, accurate and linear. Hence it can be applied for the analysis of carica papaya, punarnava and abutilon indicum.

KEYWORDS: *Flavonoids, carica papaya, punarnava, abutilon indicum, uplc*

INTRODUCTION

Papaya skin, pulp and seeds contain a variety of phytochemicals, including carotenoids and polyphenols as well as benzyl isothiocyanates and benzyl glucosinates, with skin and pulp levels that increase during ripening. The unripe fruit is used traditionally among the Yoruba tribe of Nigeria for the treatment of various human and veterinary diseases including malaria, hypertension, diabetes mellitus, hypercholesterolemia, jaundice, intestinal helminthiasis and for the management of sickle cell anaemia.¹ Punarnava Himalaya Herb is used to treat jaundice,² general fever and obesity. It is also used as anti-inflammatory and diuretic agent. The juice of Punarnava root is useful for the people having night blindness.³ Abutilon indicum its various parts of the plant are used as a demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic, and analgesic and to treat leprosy, ulcers, headaches, gonorrhea, and bladder infection.⁴ The leaf extracts of Abutilon were used to evaluate the enzyme inhibiting activity of protease, phosphomonoesterase, phosphodiesterase, acetylcholinesterase, phospholipase A2, and hyaluronidase and Lamina acid oxidase toxic enzymes present in snake venom. The extract showed promising leads to inhibiting enzymes and would be further confirmed by in vivo and pharmacological studies. Herbal medicines⁵ are natural products and their phytoconstituents vary counting on time and region, processing and storage. Variations in the collection, processing or storage of an herb may impact its efficacy profile. The present study is aimed to carry out research work to evaluate Pharmacognostic investigation on Root & Leaf extract of Abutilon indicum. From the literature study it is found that a very little work is done on root & leaf part of plant for setting standardization limits.⁶ The wound healing activity of Abutilon indicum Linn in Wistar Albino Rats using two different models Viz., excision and incision.⁷ Eudesmic acid, ferulic acid and caffeic acid have been isolated from the methanol extract of leaves of the plant Abutilon indicum. The concerted use of IR, ¹H-NMR, ¹³C-NMR, mass spectroscopy and chemical methods allowed the identification of these compounds.⁸ The antibacterial and antifungal activity has also been studied for these isolated compounds.⁹ Total phenolic and flavonoid contents were

determined by Folin-Ciocalteu and aluminum chloride colorimetric methods respectively. Bioactive fractions were then characterized and analyzed by silica thin layer chromatography.¹⁰

MATERIALS AND METHODS

Reagents and chemicals

Punarnava, caricapapaya and abutilon indicum crude drugs were extracted from plants. Quercetin API sample. Ortho Phosphoric Acid (HPLC grade), Acetonitrile (HPLC grade), HPLC grade Water (Milli Q or equivalent) was used in the buffer preparation.

Instrumentation

Chromatography was performed with waters acquity uplc I class plus system provided with a quaternary pump, high-speed autosampler, column oven, degasser and PDA detector to provide a compact and with class Empower-2 software.

Chromatographic conditions

The separation was achieved by using Luna C18, 250mm x 4.6mm, 5 μ m. column with a mobile phase consisting of Buffer and Acetonitrile (60:40%v/v), and flow rate was maintained at 1.0mL/min, detection wave length was at 255nm. The sample dilution was carried by using buffer: acetonitrile (60:40%v/v) ratio as diluents.

METHOD DEVELOPMENT

Preparation of Standard stock solution

Weighed accurately about 10 mg of Quercetin working standard into a 10 ml volumetric flask. Added 7 ml of diluent, sonicated to dissolve and diluted to volume with diluent. Further diluted 1ml of the above solution to 100ml with diluent.

Preparation of punarnava, carica papaya, Abutilon Indicum Standard solution

0.9 ml standard punarnava stock solution in to 100 ml volumetric flask and make up with diluent. 1.8 ml standard caricapapaya stock solution in to 100 ml volumetric flask and make up with diluent and 1.7 ml standard abutilon indicum stock solution into 100 ml volumetric flask and make up with diluent..

Preparation of Sample Solution

Weighed accurately about 1800 mg of sample Punarnava, Carica Papaya, and Albutilon into a 100 ml volumetric flask. 70 ml of diluent was added and sonicated to dissolve and diluted to volume with diluent. Filtered through 0.45 μ nylon syringe filter. Further diluted 5ml of the above solution to 50ml with diluent.

METHOD VALIDATION

System suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits

Assay

The ability to assess unequivocally the analyte in the presence of components that may be expected to present, such as impurities, degradation products and matrix components.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. Percentage recovery and related standard deviation were calculated.

Precision

Precision of an analytical technique is that the degree of agreement among individual take a look at results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations.

Linearity and range

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated.

LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined based on the calibration curves.

Method Validation

In this method system suitability, assay, linearity, precision, accuracy, LOD, LOQ, are validated for the selected drugs. The proposed method having standard solution and sample solution chromatograms are shown in Fig. 1,2 and 3.

System suitability

Injected 20 μ L of Standard preparation and Sample preparation. Recorded the chromatograms and measure the peak responses for punarnava, carica papaya and abutilon indicum. Relative Standard Deviation of five replicate injections of Standard preparation for punarnava, carica papaya and abutilon indicum peaks is not be more than 2.0%.

Assay

Recorded the chromatograms and measured the peak responses for punarnava, carica papaya and abutilon indicum. Calculated the content of punarnava, carica papaya and abutilon indicum in the sample. The calculated % Assay was found to be 100%.

Linearity and range Linearity of detector response for Carica papaya, punarnava and abutilon indicum were established for 6 concentrations ranging from 0 to 0.27 μ g/ml respectively. The slope, y-intercept, correlation coefficient (R^2) were calculated. The linearity results were given in the figure 4,5,6.

System precision (repeatability) For injection repeatability, six injections from the same standard preparations were made and the relative standard deviation for the replicate injections was calculated. The readings of system precision were given in Table no 1.

Accuracy

The pre analyzed sample was spiked with 50%, 100%, and 150% of mixed standard solution. The average % recoveries of both the drugs were calculated and the results and chromatograms were shown below in Table no 2.

Limit of detection and limit of quantification

LOD and LOQ values can be calculated with the help of linearity studies. The linearity study was performed three times and calibration graph was plotted.

RESULTS

Figure-1 chromatogram of carica papaya with spiked solution of quercetin

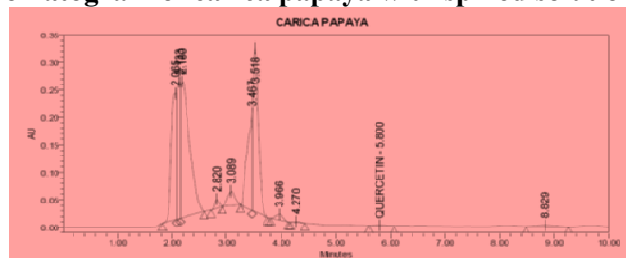


Figure- 2 chromatogram of Abutilon with spiked solution of quercetin

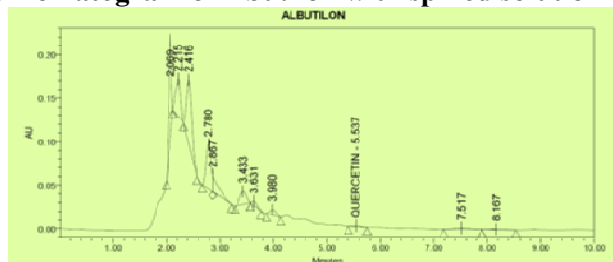
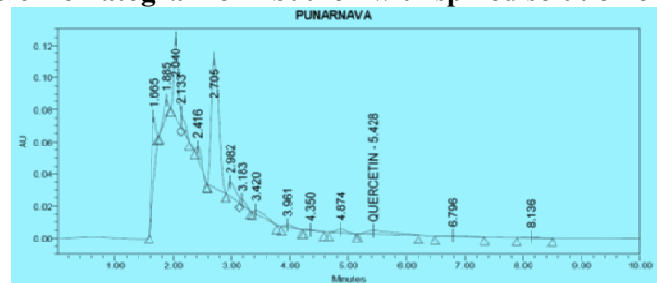


Figure- 3 chromatogram of Abutilon with spiked solution of quercetin



		MEAN:12113 %RSD:0.441	MEAN: 12290 %RSD: 0.6288	MEAN: 62484 %RSD:0.3498

Table -2 Accuracy results of caricapapaya, Albutilon indicum, punarnava

S.NO	ACCURACY%	% RECOVERY CARICAPAPAYA	%RECOVERY ABUTILON INDICUM	%RECOVERY PUNARNAVA
1	50	100.0	100.9	100.4
	50	101.0	100.8	100.2
	50	100.0	100.6	100.9
2	100	100.5	100.7	100.2
	100	100.0	100.9	99.9
	100	100.5	100.7	100.5
3	150	100.3	100.6	100.6
	150	100.7	100.7	100.8
	150	100.4	100.5	100.3
		MEAN:100.4 %RSD:0.1	MEAN:100.7 %RSD:0.1	MEAN:100.4 %RSD:0.21

CONCLUSION

The developed method was validated according to the ICH guidelines for specificity, precision, accuracy and linearity. So the proposed UPLC method was found to be simple, specific, precise, accurate and linear. The developed chromatographic method can be effectively applied for routine analysis in drug research.

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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF BENZOIC ACID, SALICYLIC ACID, TRIAMCINOLONE ACETONIDE, ICHTHAMOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A Simple, accurate, rapid and precise Ultra performance liquid chromatographic method has been developed and validated for the determination of Benzoic acid, Salicylic acid, Triamcinolone acetonide and Ichthammol. The chromatographic separation was done on isocratic elution using pentafluorophenyl column (150x4.6mm, 3 μ). Flow rate is 1mL/min and UV detection was carried out at 278nm. The mobile phase consisted by Octane Sulphonic acid with addition of 1ml triethyl amine and adjusted pH 7.0 with ortho phosphoric acid: Acetonitrile in 70:30 % V/V. The retention time for Benzoic acid, Salicylic acid, Triamcinolone acetonide and Ichthammol were found to be 2.54, 5.24, 8.62, 7.77 min respectively. The detector showed linear response over the concentration range of 60-900 μ g/ml of Benzoic acid, 30-450 μ g/ml of Salicylic acid, 20-300 μ g/ml of Ichthammol and 0.1-1.5 μ g/ml of Triamcinolone acetonide for the drugs by showing a good correlation coefficient of 0.999. This proposed method is highly sensitive, precise and accurate which reduces cost of analysis, hence recommended for routine quality analysis in laboratories.

KEYWORDS: *Benzoic acid, Salicylic acid, Triamcinolone acetonide, Ichthammol, UPLC.*

INTRODUCTION

Benzoic acid is a colourless crystalline solid and a simple aromatic carboxylic acid. The name is derived from gum benzoin, which was for a long time its only known source. Benzoic acid occurs naturally in many plants and serves as an intermediate in the biosynthesis¹ of many secondary metabolites. Salts of benzoic acid are used as food preservatives² and benzoic acid is an important precursor for the industrial synthesis of many other organic substances. The salts and esters of benzoic acid are known as benzoates. Benzoic acid is a constituent of Whitfield's ointment^{3,4} which is used for the treatment of fungal skin diseases⁵ such as tinea, ringworm, and athlete's foot⁶. As the principal component of gum benzoin, benzoic acid is also a major ingredient in both tincture of benzoin and Friar's balsam⁷. Such products have a long history of use as topical antiseptics⁸ and inhalant decongestants. Benzoic acid was used as an expectorant, analgesic⁹, and antiseptic¹⁰ in the early 20th century. Salicylic acid is a lipophilic mono hydroxyl benzoic acid, a type of phenolic acid¹¹, and a beta hydroxy acid. It has the formula C₇H₆O₃. This colourless crystalline organic acid is widely used in organic synthesis and functions as a plant hormone. It is derived from the metabolism of salicin. In addition to serving as an important active metabolite of aspirin, which acts in part as a prodrug to salicylic acid, it is probably best known for its use as a key ingredient in topical anti-acne¹² products. The salts and esters of salicylic acid are known as salicylates. It is on the World Health Organisation¹³ model list of essential medicines, the most important medications needed in a basic health system. Salicylic acid as a medication is used most commonly to help remove the outer layer of the skin. As such, it is used to treat warts¹⁴, psoriasis¹⁵, acne, ringworm¹⁶, dandruff¹⁷ and ichthyosis¹⁸.

MATERIALS AND METHODS

Materials and Reagents

Waters alliance-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used. Acetonitrile, Ortho Phosphoric Acid (OPA), Octane Sulphonic acid and Tri ethyl amine and water (HPLC grade) were purchased from Merck (India) Ltd. Worli, Mumbai, India. All API's of Benzoic acid, Salicylic acid, Triamcinolone acetonide and Ichthammol as reference standards were procured from Spectrum Pharma research solutions pvt ltd, Hyderabad. Octane Sulphonic Acid + Acetonitrile (70+30) are used as mobile phase. Water + Acetonitrile (50+50) are used as diluents.

Preparation of Buffer

Octane sulphonic acid with addition of 1ml triethyl amine and adjust pH=7.0 with OPA.

Preparation of Standard Stock Solution

Accurately weighed 60mg of Benzoic acid, 30mg of Salicylic acid, 20mg of Ichthammol and 0.1mg of Triamcinolone acetonide and transferred into a 10ml volumetric flask. The compounds were then dissolved in buffer, sonicate in 15min to dissolve the compounds and the volumes are made up to the mark with solvent. From the above solution pipette out 1ml into one separate 10ml volumetric flask and made upto the mark with solvent.

Analysis of formulation

Weigh 200mg of ointment and then taken into a 20ml volumetric flask. Add 15ml diluent, sonicate to dissolve and centrifuse for 30min. and dilute to volume with diluent. Filter through 0.45 μ Nylon syringe filter.

RESULTS AND DISCUSSION

Method Validation

In this method system suitability, linearity, precision, accuracy, LOD, LOQ, robustness, forced degradation and stability studies are validated for the selected drugs Benzoic acid, Salicylic acid, Ichthammol and Triamcinolone acetonide drugs. The proposed method having standard solution and sample solution chromatograms are shown in Fig. 1.

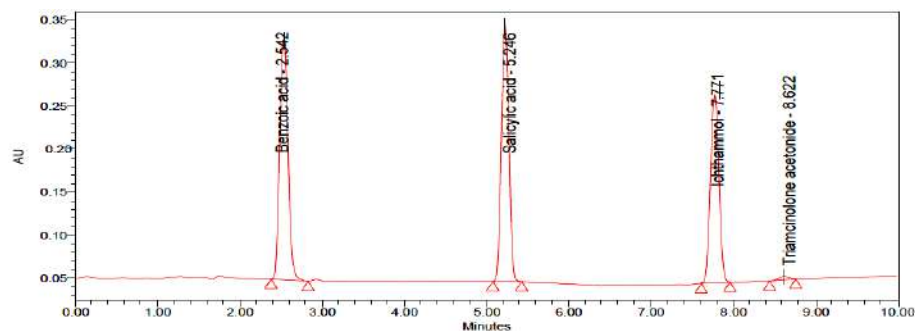


Fig. 1: Chromatogram of Standard

System suitability

The HPLC system was stabilized for 60min to get a stable base line. Six replicate injections of the standard solution containing 600 μ g/ml of Benzoic acid, 300 μ g/ml of Salicylic acid, 200 μ g/ml of Ichthammol and 1 μ g/ml of Triamcinolone acetonide were assessed to check the system suitability. The number of theoretical plate count for Benzoic acid, Salicylic acid, Ichthammol and Triamcinolone acetonide were 3038, 14504, 25887 and 22050 respectively. Tailing factor is 1.15, 1.14, 1.11 and 0.96 respectively.

Linearity

The linearity of the HPLC method was demonstrated for Benzoic acid, Salicylic acid, Ichthammol and Triamcinolone acetonide ranging from 60-900 μ g/ml of Benzoic acid, 30-450 μ g/ml of Salicylic acid, 20-300 μ g/ml of Ichthammol and 0.1-1.5 μ g/ml of Triamcinolone acetonide, which is equivalent to 10% to 150% of the working strength. Six standard solutions at the concentrations within the mentioned range were prepared and analyzed as per the method. The linearity results obtained are shown in Table 1.

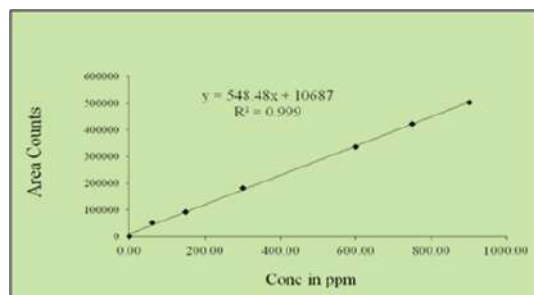


Fig. 2: Linearity plot for Benzoic acid

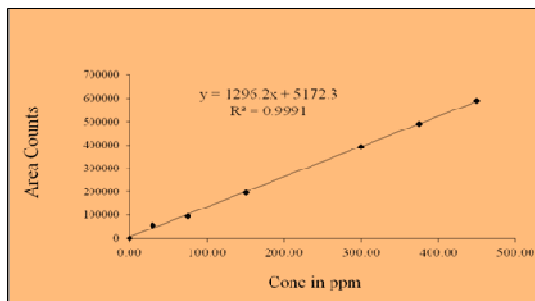


Fig. 3: Linearity plot for Salicylic acid

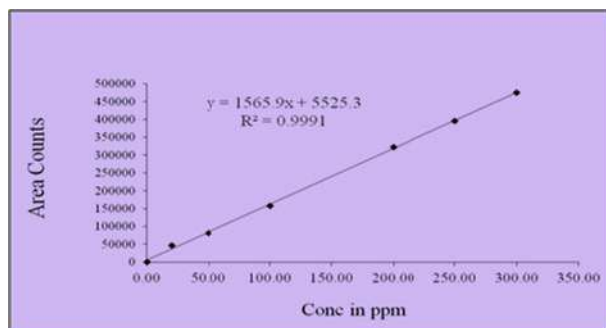


Fig. 4: Linearity plot for Ichthammol

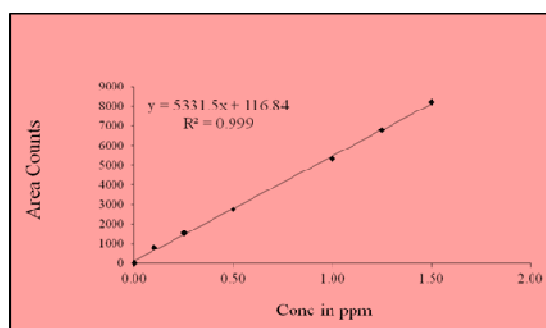


Fig.5: Linearity plot for Triamcinolone acetonide

Limit of detectin and quantification

LOD values for Benzoic acid, Salicylic acid, and Ichthammol and Triamcinolone acetonide were 0.6 μ g/ml, 0.3 μ g/ml, and 0.2 μ g/ml and 0.005 μ g/ml. LOQ values for Benzoic acid, Salicylic acid, Ichthammol and Triamcinolone acetonide were 6 μ g/ml, 3 μ g/ml, 2 μ g/ml and 0.01 μ g/ml.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch. From these six separate samples solution was injected and the peak areas obtained used to calculate mean and percentage RSD values. The present method was found to be precise as %RSD of the less than 2.0%. The results are given in Table 2.

Analyte	Amount present	%RSD
Benzoic acid	600	0.81
Salicylic acid	300	0.10
Ichthammol	200	0.28
Triamcinolone acetonide	1	0.56

Table 1: Method Precision results

Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentration levels (50%, 100% and 150%). APIs with concentration 300, 600 and 900 μ g/ml of Benzoic acid, 150, 300 and 450 μ g/ml of Salicylic acid, 100, 200 and 300 μ g/ml of Ichthammol and 0.5, 1 and 1.5 μ g/ml of Triamcinolone acetonide were prepared. As per test method the test solution was injected to three preparations each spike level and the assay was performed. The percentage recovery values were found to be in the range of 98-101%. The results are given in table 3.

% of target concentration	Benzoic acid (% recovery)	Salicylic acid (% recovery)	Ichthammol (%recovery)	Triamcinolone acetonide (%recovery)
50	100.4	100.25	99.9	100.45
100	99.8	100.30	100.2	100.3
150	100.1	100.55	100.45	100.7
Mean(% recovery)	100.1	100.37	100.18	100.48

Table 2: Accuracy (recovery) study results**Ruggedness**

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method.

Forced Degradation

Forced degradation conditions such as acidic, basic, peroxide, reduction and thermal stress were attempted as per ICH Q1A (R2). The effect of assay on their results is shown below table 5.

Degradation	Benzoic acid (% Degradation)	Salicylic acid (% Degradation)	Ichthammol (% Degradation)	Triamcinolone acetone (% Degradation)
Acid	16.3	14.9	14.6	11.6
Alkali	15.1	14.5	16.5	16.2
Peroxide	15.8	16.6	14.9	11.1
Reduction	13.7	15.3	13.8	12.7
Thermal	14.4	15.1	15.5	15.8

Table 3: Forced degradation results**CONCLUSION**

All these factors lead to the conclusion that the proposed method is simple, specific, accurate, precise and reproducible. Statistical analysis proves that the method is suitable for the analysis of Benzoic acid, Salicylic acid, Ichthammol and Triamcinolone acetone.

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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC METHOD FOR GRAMICIDIN, NEOMYCIN AND TRIAMCINOLONE ACETONIDE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, rapid, economical, precise and accurate reverse phase high performance liquid chromatography method for estimation of Gramicidin, Neomycin and Triamcinolone acetonide has been developed. Chromatographic conditions used are stationary phase waters symmetry C₁₈ column with dimensions 150x4.6mm, 3.5μ. Use buffer as 2.5ml Hexane Sulphonic acid in 1lt water and adjust pH = 2.5 with OPA. The mobile phase is Hexane sulphonic acid: Acetonitrile in 40:60. The flow rate was 1ml/min., and the detection wavelength 232nm. Retention time of Gramicidin, Neomycin and Triamcinolone acetonide was 2.49, 5.15 and 6.22 min respectively. The method has been validated for linearity, accuracy, precision, stability studies and forced degradation studies including acid, base, hydrolysis, peroxide and thermal degradation were successfully applied for the estimation of Gramicidin, Neomycin and Triamcinolone acetonide.

KEYWORDS: *Gramicidin, Neomycin, Triamcinolone acetonide and RP-HPLC.*

INTRODUCTION

Gramicidin is a heterogeneous mixture of three antibiotic¹ compounds, Gramicidins A, B and C, making up 80%, 6% and 14% respectively², all of which are obtained from the soil bacterial species *Bacillus brevis*³ and called collectively gramicidin D. Gramicidin D contains linear pentadeca peptides⁴ that are chains made up of 15 amino acids⁵. This is in contrast to gramicidin S, which is a cyclic peptide chain. Gramicidin is active against Gram-positive bacteria⁶, except for the Gram-positive bacilli, and against select Gram-negative organisms⁷, such as *Neisseria* bacteria⁸. Its therapeutic use is limited to topical application⁹, as it induces hemolysis in lower concentrations than bacteria cell death, so it cannot be administered internally. Since the exterior epidermis¹⁰ is composed of dead cells, applying it to the surface of the skin will not cause harm. It is used primarily as a topical antibiotic and is one of the three constituents of condurmer antibiotic polysporin ophthalmic solution. Neomycin is an amino glycoside¹¹ antibiotic found in many topical medications such as creams, ointments and eyedrops. Neomycin belongs to aminoglycoside class of antibiotics that contain two or more amino sugars connected by glycosidic bonds¹². Neomycin is typically used as a topical preparation, such as Neosporin (neomycin/polymyxin B/ bacitracin)¹³. It can also be given orally, where it is usually combined with other antibiotics. Neomycin is not absorbed from the gastrointestinal tract¹⁴ and has been used as a preventive measure for hepatic encephalopathy¹⁵ and hypercholesterolemia¹⁶. By killing bacteria in the intestinal tract, it keeps ammonia levels low and prevents hepatic encephalopathy, especially prior to GI surgery. It works as an antibiotic that is active against streptomycin¹⁷ resistant bacteria, including in the case of tuberculosis organisms¹⁸. It has also been used to treat small intestinal bacterial over growth¹⁹. It is not given via injection, as neomycin is extremely nephrotoxic²⁰ even when compared to other aminoglycosides.

MATERIALS AND METHODS

Waters alliance-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used. Acetonitrile, Ortho phosphoric acid (OPA), Hexane Sulphonic acid and water (HPLC grade) were purchased from Merck (India) Ltd. Worli, Mumbai, India. All API's of Gramicidin, Neomycin and Triamcinolone acetonide as reference standards were procured from

Spectrum Pharma research pvt. Ltd., Hyderabad. Buffer: Acetonitrile in the ratio of 40:60 are used as mobile phase and diluent.

Preparation of Buffer

Dissolve 2.5gms of Hexane Sulphonic acid in 1lt of water and adjust pH=2.5 with OPA. Filter the solution through 0.45µ filter paper.

Preparation of Standard Stock solution

Weigh accurately about 25mg of Gramicidin, 250mg of Neomycin and 100mg of Triamcinolone acetone and transferred into a 100ml volumetric flask. Add 70ml of diluent, sonicate to dissolve and dilute to volume with the diluent. Further dilute 5ml of the above solution to 50ml with diluent.

Preparation of Sample solution

Accurately weigh 1gm of the sample and transferred into 10ml volumetric flask. Add 7ml of diluent and centrifuge for 30min. to dissolve the ointment. Filter through 0.45µ filter paper.

Chromatographic Conditions

Chromatographic separation was carried out in isocratic mode at room temperature using a Symmetry C₁₈ (150x4.6mm, 3.5µ) Column. The mixture of HAS: Acetonitrile 40:60v/v at a flow rate 1.0 ml/min was used as a mobile phase. The injection volume was 10µl and eluents was monitored at 232nm using PDA detector. The run time was 8min.

Method Validation

The analytical method was validated as per ICH Q2(R1)[28] guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), Limit of quantification (LOQ) and forced degradation.

System suitability

The HPLC system was stabilized for 60min to get a stable base line. Six replicate injections of the standard solution containing 25µg/ml of Gramicidin, 250µg/ml of Neomycin and 100µg/ml of Triamcinolone acetone were assessed to check the system suitability. The number of theoretical plate count for Gramicidin, Neomycin and Triamcinolone acetone were 2936, 8930 and 10719 respectively. Tailing factor for Gramicidin, Neomycin and Triamcinolone acetone were 1.07, 1.09 and 1.12 respectively. All these parameters were found to be within the limit. Chromatogram of standard was given in fig 1.

Linearity

Linearity of the method was evaluated by preparing a standard solution containing 25µg/ml of Gramicidin, 250µg/ml of Neomycin and 100µg/ml of Triamcinolone acetone (100% of targeted level of the assay concentration). Sequential dilutions were performed to the given solutions at 10, 25, 50, 100, 125 and 150% of the target concentrations. These were injected and the peak areas are used to plot calibration curves against the concentration. Linearity graphs was given in fig 2,3,4.

Limit of detection and quantification

LOD values for Gramicidin, Neomycin and Triamcinolone acetone were 0.025µg/ml, 0.25µg/ml and 0.1µg/ml. LOQ values for Gramicidin, Neomycin and Triamcinolone acetone were 0.25µg/ml, 2.5µg/ml and 1.0µg/ml respectively.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch. From these six separate sample solutions were injected and the peak areas obtained used to calculate mean and percentage RSD values. The present method was found to be precise as %RSD of the less than 2.0%.

Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentration levels (50%, 100% and 150%). APIs with concentration 12.5, 25 and 37.5µg/ml of Gramicidin, 125, 250 and

375µg/ml of Neomycin and 50, 100 and 150µg/ml of Triamcinolone acetonide were prepared. As per the test method the test solution was injected to three preparations each spike level and the assay was performed. The percentage recovery values were found to be in the range of 98-101%.

Ruggedness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method.

Robustness

Robustness of the method was found to be %RSD should be less than 2%. Slightly variations were done in the optimized method parameters like flow rate ($\pm 20\%$), Organic content in mobile phase ($\pm 10\%$).

Forced Degradation

Forced degradation conditions such as acidic, basic, peroxide, thermal, hydrolysis stress were attempted as per ICH Q1A (R₂). The effect of assay on their results are shown below table 1

RESULTS

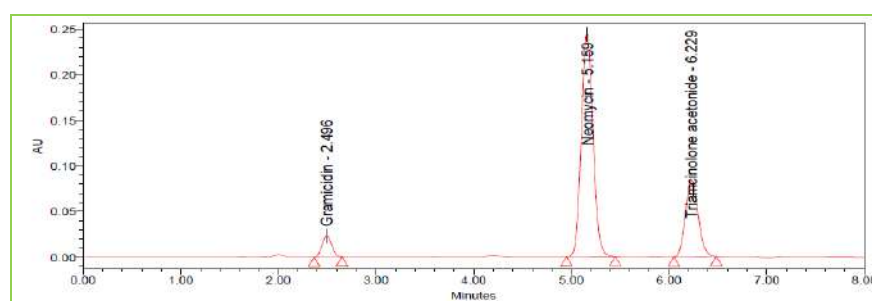


Figure 1: Chromatogram of standard

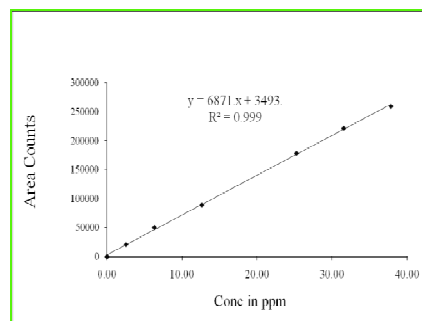


Figure 2: Linearity plot for Gramicidine

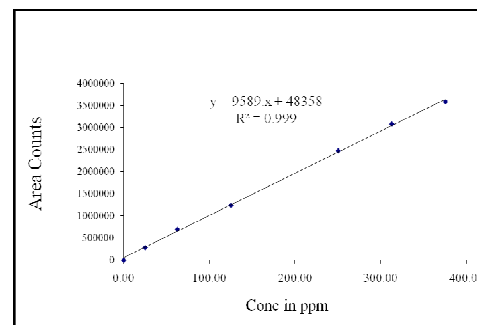


Figure 3: Linearity plot for Neomycin

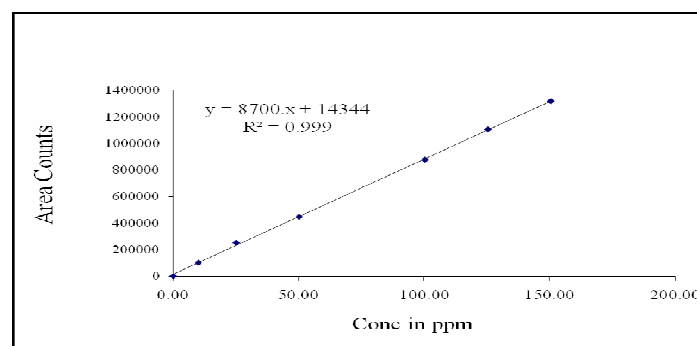


Figure 4: Linearity plot for Triamcinolone acetonide

Degradation	Gramicidin (% of Degradation)	Neomycin (% of Degradation)	Triamcinolone acetonide (% of Degradation)
Acid	15.3	14.5	17.6
Alkali	13.1	15.5	16.2

Peroxide	12.8	14.9	11.1
Thermal	15.6	11.8	12.7
Hydrolysis	16.6	15.5	15.8

Table -1 Forced Degradation results

CONCLUSION

This method described the quantification of Gramicidin, Neomycin and Triamcinolone acetonide in bulk and pharmaceutical formulation as per ICH guidelines. The developed method was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of sample preparation and the less expensive reagents were used. In addition four compounds are eluted within 10mins. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility data are satisfactory. The developed chromatographic method can be effectively applied for routine analysis in drug research.

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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF EPALRESTAT AND PREGABALIN IN RAT PLASMA AND ITS APPLICATION TO PHARMACOKINETICS

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ABSTRACT

A novel, simple, rapid and accurate RP-HPLC Bioanalytical method was developed for the simultaneous estimation of Epalrestat and Pregabalin in bulk and dosage form. The chromatographic separation was performed on Discovery column (250 x4.6 mm, 5 μ particle size) the optimized mobile phase consists of 0.01M potassium dihydrogenphosphatebuffer: Methanol (25:75v/v) with a flow rate of 1.0 ml/min and UV detection at 226nm. The Retention time was 2.2min for pregabalin, 2.8min for Epalrestat. Linearity range was 0.125, 0.250, 0.500, 1, 2, 4 and 8 mcg/mL. Accuracy was in the range of 98.14-100.43% for both the drugs. Limit of detection and Limit of quantification are 0.21mcg and 0.65mcg for epalrestat and 0.08mcg and 0.25mcg for pregabalin. The method was validated by determining its accuracy, precision, and system suitability.

KEYWORDS: *RP-HPLC, Bio analytical method, Epalrestat, Pregabalin, Validation.*

INTRODUCTION

Epalrestat is a carboxylic acid derivative and a noncompetitive and reversible aldose reductase inhibitor used for the treatment of diabetic neuropathy, which is one of the most common long-term complications in patients with diabetes mellitus¹. Chemical name is 5-[(z, e)- β -metylcinnamylidene]-4-oxo-2-tioxo-3-tiazolidineacetic acid². Pregabalin is used for treating pain caused by neurologic diseases such as post herpetic neuralgia as well as seizures. It is also used for treating fibromyalgia.³ Chemical name is (3s)-3-(amino methyl)-5-methylhexanoic acid⁴. Pregabalin works in different ways in epilepsy it stops seizures by reducing the abnormal electrical activity in the brain with nerve pain it blocks pain by interfering with pain messages travelling through the brain and down the spine in anxiety it stops your brain from releasing the chemicals that make you feel anxious⁵. Epalrestat suppresses accumulation of Sorbital in nerve by inhibiting aldose reductase, and relieves numbness or pain of the hands and feet and leg cramp, which induced by diabetic peripheral neuropathy.⁶ Peripheral Neuropathy is one of the most common and disabling long-term complications of diabetes mellitus. Diabetic neuropathy is the condition, either clinically evident or subclinical, that occurs in diabetes mellitus cases in the absence of other causes of peripheral neuropathy. Diabetes mellitus (DM) is a chronic metabolic disorder manifested in the form of hyperglycemia as a consequence of defect in insulin secretion and/or insulin action along with an imbalance in the metabolism of carbohydrates, fats and proteins.⁷ Diabetic nephropathy will affect approximately 30% of all patients with diabetes. Diabetic nephropathy (DN) is one of the most common micro vascular complications of diabetes defined as rise in urinary albumin excretion rate, often associated with an increase in blood pressure, but without evidence of other causes of renal disease.⁸ Literature survey revealed that RP-HPLC have been reported for the estimation of Epalrestat and pregabalin. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Epalrestat and pregabalin in Bulk and pharmaceutical dosage form.⁹ Development and Validation of Stability Indicating Hplc Method for Simultaneous Estimation of Epalrestat and Methylcobalamin in Tablet Dosage Form.¹⁰

MATERIALS AND METHODS

Chemicals

The reagents used in this work were methanol (HPLC grade –Merck Life sciences pvt, Ltd., Mumbai, India), orthophosphoric acid, (ThermoFisher scientific, pvt, Ltd., Mumbai, India), HCl (AR), NaOH (AR), Hydrogen peroxide (85% w/v) (AR), which were procured from Merck Life Sciences, pvt, Ltd., Mumbai, India. HPLC grade water (Lichrosolv, Merck Life Sciences, pvt, Ltd., Mumbai, India). The working standards of epalrestat and pregabalin were procured from Standard Laboratories Hyderabad, India. Prealdonil (Zydus) Tablet containing Epalrestat 150mg and pregabalin 75mg were procured from local market.

Instrumentation

The instruments used in the study were electronic balance (Sigma 200), digital pH meter (Systronics), sonicator (Cyberlabs), UV-visible spectrophotometer (ThermoScientific) HPLC (Waters Alliance, Empower2 software) Detector (PDA)

METHOD DEVELOPMENT

Preparation of Standard solution

10mg of Pregabalin and Epalrestat were dissolved in 10mL of HPLC grade acetonitrile.

Preparation of calibration Standard solutions

Suitable calibration standards were prepared in plasma to get final concentrations of 0.125, 0.250, 0.500, 1, 2, 4 and 8 mcg/mL. Sample preparation: 0.2ml of plasma was transferred to polypropylene tubes (5ml capacity) and 2ml of diethyl ether was added. Mixture was vortexed for 15min and centrifuged up to 20min. Supernatant layer was separated to clean polypropylene tubes and evaporated at 50°C at nitrogen atmosphere up to 20min. The dried residue was reconstituted in 0.5ml of mobile phase and 10µL sample was injected to RP-HPLC.

METHOD VALIDATION

System Suitability

Six replicate injections of aqueous mid QC were injected. The %CV (%RSD) for the analyte and internal standard should not be more than 5%.

Selectivity (Matrix Screening) and Specificity

Ten lots of blank plasma samples were analyzed out of which six lots free from interference were selected for assessing the selectivity, specificity. Endogenous/potential interfering peak areas for blank samples must be less than 20% of the LLOQ peak area of analyte retention time.

Limit of Quantification (LOQ) / Sensitivity

Six LLOQ standards were prepared in screened plasma lot and signal to noise ratio (S/N) was calculated using analyst software. The mean S/N ratio of LLOQ should be ≥ 5 .

Linearity

Suitable calibration standards were prepared in plasma to get final concentrations of 0.125, 0.250, 0.500, 1, 2, 4 and 8 µg/mL.

RESULT AND DISCUSSION

Stability (Freeze- thaw, Auto sampler, Room temperature, Long term)

Stability studies in plasma were performed at the LQC and HQC concentration level using six replicates at each level. Analyte was considered stable if the % Change is less than 15% as per US FDA guidelines. The stability of spiked human plasma samples stored at room temperature (bench top stability) was evaluated for 61 h. The stability of spiked human plasma samples stored at -30 °C in autosampler (autosampler stability) was evaluated for 70.0 h. The autosampler sample stability was evaluated by comparing the extracted plasma samples that were injected immediately (time 0 h), with the samples that were reinjected after storing in the autosampler at 20°C for 70.0 h. The reinjection reproducibility was evaluated by comparing the

extracted plasma samples that were injected immediately (time 0 h), with the samples that were re-injected after storing in the autosampler at 20°C for 70 h.

Pharmacokinetic studies

Pharmacokinetic studies were done in albino wister rats weighing 180-200 g (n=6). The drugs were administered intraperitoneal route 0.45mg and 0.25 mg.

Pharmacokinetic study protocol

After collecting the 0 h blood sample (blank), the drugs in the study were administered intraperitoneal route. No food or liquid other than water was permitted until 4 h following the administration of the drugs. Blood samples (2 ml) were collected from reterorbital route at 0,1,2,3,4,5,6,7,8,9,10,11,and12 h after administration. The blood samples were collected into K2EDTA tubes and were centrifuged at 10000 rpm for 10 min and the plasma separated was collected into dry tubes. All the samples were stored under refrigerated conditions prior to assay on the same day.

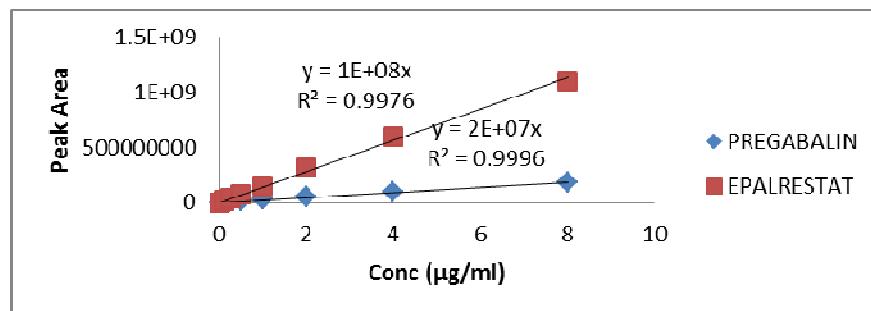


Figure-1 calibration curve of epalrestat and pregabalin

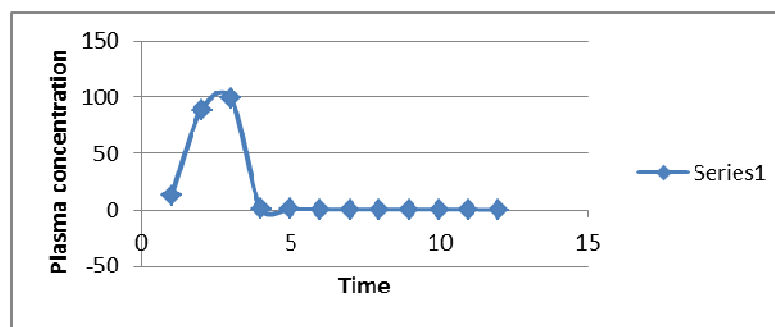


Figure-2 plasma concentration vs time profile

CONCLUSION

A novel bioanalytical RP-HPLC method has been developed and validated for the simultaneous estimation of Epalrestat and Pregabalin in bulk and dosage forms. The method was validated as per ICH guidelines. The method proved the selectivity, precision, accuracy and mobile phase used to provide simple and economic application. Consequently, the stability indicates the power of the method can be assessed. The developed method is simple, sensitive, and rapid linear, precise, and rugged specific and robust.

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FORMULATION AND EVALUATION OF PARACETAMOL TABLETS USING BORASSUS, YAM & BASIL AS NATURAL BINDING AGENTS & BASIL SEED COAT AS A SUPER DISINTEGRANT

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ABSTRACT

Fast dissolving tablets are those which disintegrates rapidly within the mouth and taken without consumption of water. Paracetamol is a slightly water soluble drug belongs to BCS Class IV, used in various pain managements & in management of fever. The objective of this study was to improve the rapid onset of action of the drug. The fast dissolving tablets of Paracetamol were prepared using wet granulation technique by employing Basil, Borassus & Yam as natural binders and Basil seed coat as super- disintegrant. The prepared tablets were evaluated for pre-compression parameters and post-compression parameters. From the results obtained, it was found that tablet formulation (F13) with Yam (2.5%) & Basil (25mg) has shown 100% of drug release within 10minutes and disintegration time was found to be 03 sec. Also, the hardness, friability, weight variation, and drug content of prepared tablets were found to be in the acceptable limits. The *in-vitro* release kinetics reveals that formulation (F13) follows zero order kinetics.

KEYWORDS: *Natural binders, Super Disintegrant, Fast dissolving tablets, Disintegration.*

INTRODUCTION

Innovative drug delivery system known as Fast/mouth dissolving/disintegrating tablets (**FDTs/MDTs**) are novel types of tablets that dissolve in saliva within few seconds without water. MDTs are to improve patient's compliance, rapid onset of action, increased bioavailability and good stability. ^[1-3] USFDA defined ODTs as "A solid dosage form containing medicinal substances or active ingredients which disintegrates rapidly within a few seconds when placed up on tongue".^[4,5] The bioavailability of some drugs may be increased due to absorption of drugs in oral cavity and also due to pre-gastric absorption of saliva containing dispersed drugs that pass down in to the stomach. Moreover the amount of drug that is subjected to first pass metabolism is reduced as compared to standard tablets ^[6, 7]. Paracetamol ^[8,9] is a widely prescribed antipyretic and analgesic drug for all age groups. Binders are the agents employed to impart cohesiveness to the granules. Natural binders like starch, gums, mucilage, dries fruits possesses binding capacity as well as some other properties like Disintegrant, filler & natural polymers are safe and economical than polymers like PVP. Basil *Ocimum basilicum* is used for stomach spasms, loss of appetite, intestinal gas, kidney conditions etc. Yam is a rich source of energy and also Vitamin B Complex, Vitamin C, antioxidants, minerals, fiber and complex carbohydrates. The Borassus flabellifer leaves are used for thatching, mats, baskets, fans, hats, umbrellas, and as writing material. The objective of the present study is to formulate and evaluate fast dissolving tablets (FDTs) of paracetamol employing three binders namely Basil, Borassus & Yam and Basil seed coat as a superdisintegrant. In each case three concentrations of binders (2.5%, 5%, and 7.5%) were used and the tablets were prepared by wet granulation method and were evaluated.

MATERIALS & METHODS

Materials: Paracetamol was obtained from SK Health Care Formulations Pvt Ltd, Hyderabad. Magnesium stearate, Talc, MCC were obtained from Loba chemical Pvt.Ltd, Maharastra. Natural binders are from local market.

Formulation (Table 1) and preparation of Paracetamol tablets: Fast dissolving tablets of Paracetamol were prepared by wet granulation method employing various super disintegrants.

Table 1: Composition of Paracetamol tablets

S.NO	Ingredients in mg	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
1	Paracetamol	120	120	120	120	120	120	120	120	120	120	120	120	120
2	Basil (%)	2.5	5	7.5	-	-	-	-	-	-	-	-	-	-
3	Borassus (%)	-	-	-	2.5	5	7.5	-	-	-	-	-	-	-
4	Yam (%)	-	-	-	-	-	-	2.5	5	7.5	-	-	-	2.5
5	Starch (%)	-	-	-	-	-	-	-	-	-	2.5	5	7.5	-
6	Super disintegrant	-	-	-	-	-	-	-	-	-	-	-	-	25
7	Mg .Sterate	2	2	2	2	2	2	2	2	2	2	2	2	2
8	Talc	2	2	2	2	2	2	2	2	2	2	2	2	2
9	MCC	126	126	26	126	126	126	126	126	126	126	126	126	101

Evaluation of Blend

Angle of repose ⁽¹¹⁾: The angle of repose of powder blend was determined by the funnel method. The diameter of the powder cone was measured and angle of repose was calculated using the formula: $\tan \theta = h/r$

Bulk Density

a) Loose Bulk Density (LBD): Apparent bulk density in gm. /mL was calculated by the following formula:
Loose bulk density (LBD) = Weight of powder / Bulk volume

b) Tapped bulk density (TBD): Calculate the tapped bulk density in gm. /mL by the following formula:
Tapped bulk density (TBD) = Weight of powder / Tapped volume

Compressibility Index: The compressibility index is calculated using measured values for bulk density (Db.) and tapped density (Dt) as follows: *Compressibility Index = (Dt-Db/Dt×100)*

Hausner's Ratio: Hausner's ratio is a number that is correlated to the flow ability of a powder or granular material. *Hausner's Ratio = TBD/ LBD*

EVALUATION OF TABLETS: The formulated tablets were evaluated for the following physicochemical characteristics:

General appearance: Shape, color, texture, and odour of the formulated tablets were assessed.

Thickness: The tablet thickness is determined by diameter of die. The thickness of the tablets was measured by Vernier calipers. It is expressed in mm.

Hardness: Hardness of tablet was determined by using the Monsanto hardness tester.

Weight Variation: 20 tablets were selected and weighed collectively and individually. From the collectively weight, average weight was calculated. Each tablet weight was then compared with average weight to ascertain whether it was within the permissible limits or not.

Friability Test: 20 previously weighed tablets placed in the friability apparatus. Which was given 100 revolutions at 25rpm and the tablets were reweighed. The percentage friability was calculated by using the following formula,

$$\text{Percentage friability} = [\text{initial weight} - \text{final weight} / \text{initial weight} \times 100]$$

$$\% \text{friability} = [(W_o - W_f) / W_o] \times 100$$

W_o = initial weight of tablets,

W_f = Final weight of tablets.

Disintegration Time: The USP device to test the disintegration was six glass tubes that are "3 long, open at the top and held against 10" screen at the bottom end of the basket rack assembly. One tablet is placed in each tube and the basket rack is positioned in 1 liter beaker of distilled water at $37 \pm 2^\circ \text{C}$.

Drug content: The tablets were powdered, and 100mg equivalent weight of Paracetamol accurately weighed and transferred into 100ml volumetric flask. To this 5ml of alcohol was added and shaken for 10min. Thereafter, the volume was made up to 100ml with 5.8 PH buffer. Subsequently, the solution in volumetric flask was filtered and 1ml of filtrate was diluted and analyzed at 275nm using UV visible spectrophotometer.

RESULTS & DISCUSSION

Preformulation studies

1. Solubility: Paracetamol freely soluble in acetone, alcohol, and methanol & sparingly soluble in water.

2. Compatibility- FT-IR STUDIES: An FTIR spectroscopy study was carried out separately to check the compatibility between the drug and the excipients used for the preparation of tablets.

Evaluation studies

Micromeritic properties

Pre-compression parameters: Compressibility index of the prepared blends fall in the range of 6.1 to 15.5 indicates that the blends have excellent compressibility. Hausner's ratio of the prepared blends fall in the range 1.08 to 1.74 indicated that the blends have the required flow property and strength for compression.

Post compression parameters (Table 2)

Table 2: Post compression parameters

Formulation Code	Thickness(mm) (mean $\pm$ S.D)	Hardness(kg/cm ²) (mean $\pm$ S.D)	%Friability (mean $\pm$ S.D)	Weight variation(mg) (mean $\pm$ S.D)
F1	4.27 $\pm$ 0.18	3.39 $\pm$ 0.234	0.8 $\pm$ 0.03	249 $\pm$ 2.88
F2	4.18 $\pm$ 0.12	4.3 $\pm$ 0.1	0.9 $\pm$ 0.01	251 $\pm$ 4.50
F3	4.11 $\pm$ 0.117	4.1 $\pm$ 0.16	0.70.15	250 $\pm$ 2.08
F4	4.29 $\pm$ 0.375	4.3 $\pm$ 0.1	0.8 $\pm$ 60.05	246 $\pm$ 2.0
F5	4.19 $\pm$ 0.282	4.1 $\pm$ 0.11	0.82 $\pm$ 0.03	249 $\pm$ 2.64
F6	4.06 $\pm$ 0.06	4.2 $\pm$ 0.108	0.72 $\pm$ 0.04	250 $\pm$ 1.547
F7	4.49 $\pm$ 0.105	4.2 $\pm$ 0.25	0.75 $\pm$ 0.06	249 $\pm$ 2.08
F8	4.17 $\pm$ 0.125	4.4 $\pm$ 0.28	0.9 $\pm$ 0.03	250 $\pm$ 2.88
F9	4.42 $\pm$ 0.125	4.2 $\pm$ 0.1	0.6 $\pm$ 0.03	248 $\pm$ 2.04
F10	3.73 $\pm$ 0.267	4.07 $\pm$ 0.04	0.95 $\pm$ 0.01	247 $\pm$ 1.64
F11	4.06 $\pm$ 0.06	4.3 $\pm$ 0.1	0.8 $\pm$ 60.05	248 $\pm$ 1.25
F12	4.09 $\pm$ 0.105	4.4 $\pm$ 0.28	0.9 $\pm$ 0.03	250 $\pm$ 0.96
F13	3.67 $\pm$ 0.125	4.2 $\pm$ 0.1	0.6 $\pm$ 0.03	248 $\pm$ 0.98

All values represents mean $\pm$ standard deviation [n=3]

Thickness: The dimensions determined for formulated tablets were tabulated in Table 2., tablets mean thickness were almost uniform in all formulations and were found to be in the range of 3.67 to 4.49.

Hardness test: Hardness of three tablets of each batch checked by Monsanto hardness tester and the data were shown in Table 2. The results showed that the hardness of the tablets was in the range of 3.39 to 4.4 .

Friability test: Friability of ten tablets of each batch was checked by Roche Friability tester and the data was shown in Table 2. The results showed that the friability of the tablets was in the range of 0.6 to 0.95. As per official requirements of IP, the formulation tablets shown good mechanical resistance.

Weight variation test: Tablets of each batch were subjected to weight variation test, difference in weight and percent deviation was calculated for each tablet and was shown in Table 2. The average weight of the

tablet is approximately 250mg: so the permissible limit is 7.5%. The results of the test showed that, the tablet weight was within Pharmacopoeial limit.

Disintegration time (Table 3): Tablets of each batch were evaluated for Invitro disintegration time and the data was shown in the Table 3. The results have shown that the disintegration time of prepared tablets were in the range of 3 to 174 sec. The internal structure of tablets, water penetration into tablets and swelling of disintegration substance are suggested to be the mechanism of disintegration. On comparing all the formulations for disintegration time, the rapid disintegration was seen in the formulation containing yam as a binder and basil as a super disintegrant. This is due to high capillary nature of the disintegrant in the tablets. Rapid penetration of buffer medium and rapid disintegration of the tablet was observed.

Table 3: Evaluation of compressed tablets

Formulation	Disintegration time in sec	%Drug content	Formulation	Disintegration time in sec	%Drug content
F1	43	97.83±1.258	F8	43	98.45±1.32
F2	90	98.33±1.527	F9	65	98.91±0.047
F3	157	101±1	F10	11	99.18±1.123
F4	38	99.18±0.797	F11	56	101.48±1.17
F5	56	99.2±1.044	F12	174	99.93±1.03
F6	144	100±2.03	F13	3	99.49±0.457
F7	28	99.18±0.487	F8	43	98.45±1.32

*All values represents mean± standard deviation [n=3]

Drug content

Drug content uniformity study was carried out on the tablets of every batch and the data was shown in the Table 3, the content uniformity of all the formulations was found to be in the range of 97.83 to 101.48 which showed that there was uniform distribution of the drug throughout the batch. The IP standard says that Paracetamol tablets must contain not less than 97.0% and not more than 110.0% of the stated amount of Paracetamol. Thus all the FDT formulations of Paracetamol comply with IP limit for assay.

Drug Release Kinetics

Kinetic model study: The dissolution data of Paracetamol formulations were analysed as per zero order and first order equation. The drug release of the optimized formulation was found to be Zero order kinetics. (Fig 1,2,3)

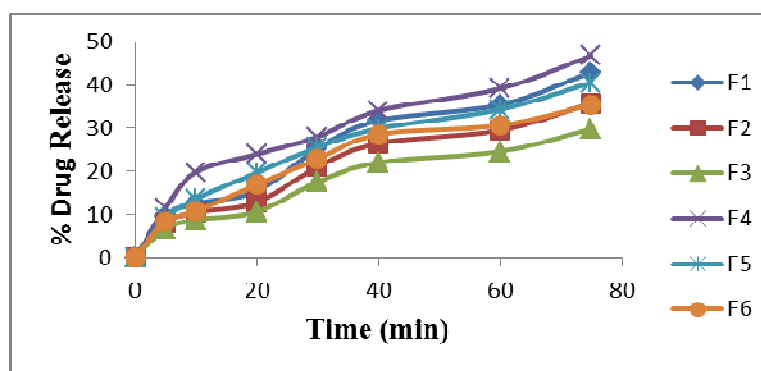


Fig 1: In-vitro drug release of formulations F1 to F6.

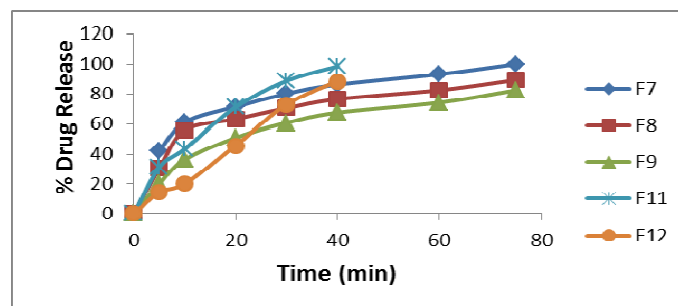


Fig 2: In-vitro drug release of formulations F7 to F12

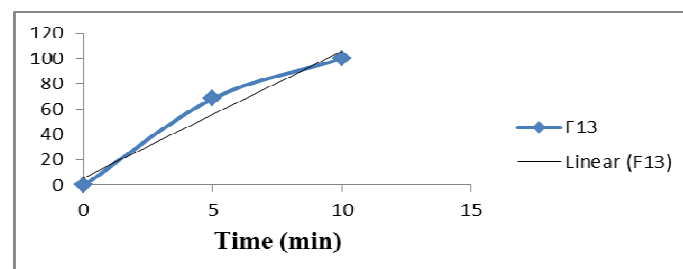


Fig 3: In-vitro drug release of optimized formulation F13

Stability studies: During the stability studies the formulations does not show any significant change in physical and chemical parameters of the tablets and the data were shown in the table 4. The drug content did not alter more than 1-2%. There was no significant change in drug content, and disintegration time of PCM tablets even after 3 months, hence the formulations were found to be stable.

Table 4: Stability parameters of F13 formulation stored at room temperature

Parameters	Optimised F13	After 1 month	After 2 months
Drug content (%)	99.49	99.46	99.45
Disintegration time (sec)	3	3	4

CONCLUSION

Among 13 Formulations, F13 formulation containing Yam (2.5%) & Basil (25 mg) has shown better drug release (100%) within 10 minutes & these ingredients can be used as natural excipients in the formulation of fast dissolving tablets & it is cost effective & beneficial to health.

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SCREENING OF ANTIHYPERLIPIDEMIC ACTION OF MACROTYLOMA UNIFLORUM (LAM.) YOUNG STEM EXTRACT

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ABSTRACT

Antihyperlipidemic action is to prevent or counteract the accumulation of lipids and lipoproteins in the blood, an antihyperlipidemic drug. These antihyperlipidemic drugs also known as Lipid lowering drugs. The main biological functions of lipids include energy storage, signalling and acting as Structural Components of cell membranes. Due to high levels of fats, cholesterol in blood may lead to heart diseases. In order to prevent here, we use the Macrotyloma uniflorum which is used as antihyperlipidemic drug. Macrotyloma uniflorum is also known as Horse gram. The whole part of the plant (*Macrotyloma uniflorum*) is used in cure of heart diseases, and also used for the persons who are suffering from jaundice or Water retention and also used as a part of weight loss diet. It also plays a major role in human nutrition and has a rich source of proteins, minerals, and vitamins. The present research proving scientific action of *Macrotyloma uniflorum* on reduction of hyperlipidemia levels in blood. All the lipid profile parameters were determined. Total Cholesterol (TC), triglycerides (TG), High density lipoproteins (HDL), very low density lipoproteins (VLDL), low density lipoproteins (LDL) were analysed from serum.

KEYWORDS: Antihyperlipidemic, *Macrotyloma uniflorum*, High fat diet, Total Cholesterol, LDL-Cholesterol, Phytochemical, Pharmacological activities.

INTRODUCTION

Macrotyloma uniflorum (horse gram, kulthi bean, hurali, Madras gram ¹ is one of the lesser known beans also called as Poor Man's Pulse. *Macrotyloma uniflorum* is derived from Greek Word Makros = large; tylos = Knob, and loma = Margin in reference to knobby sutures on the pods.² *Macrotyloma uniflorum* are the legumes of tropics and subtropics. Hyperlipidemia in blood is caused due to having high level of low density lipoprotein Cholesterol (LDL-C) is a major risk factor for atherosclerotic cardiovascular disease (ASCVD). Because the LDL-C contributes to fatty build-ups and narrowing of arteries (atherosclerosis), it's often called the "Bad Cholesterol" (LDL-C). Infact high LDL-C at any age it may increase fatty deposits in arteries and the risk of blockages and may lead to cardiovascular diseases. The 2018 Guideline on the management of Blood Cholesterol presents a new guideline which is an update to the 2013 guideline on diagnosing, treating and monitoring high cholesterol. The levels of lower rates of heart disease and stroke are in this level i.e the total cholesterol level should be about 150mg/dL, with LDL-C at or below 100mg/dL, and adults with LDL-C. The World Health Organization estimates that 80% of the population depends on traditional medicine for their primary health especially in developing countries.³ *Macrotyloma uniflorum* has been used as an excellent remedial values due to presence of non-nutritive bioactive substances (such as phytic acid, phenolic acid, fiber, enzymatic/proteinase inhibitors) which have significant and metabolic and physiological effects.⁴ The *Macrotyloma uniflorum* is rarely found in Africa, Australia, Bhutan, India, Indonesia, Myanmar, Nepal, Pakistan, Philippine and Sri-Lanka⁵.

MATERIALS AND METHOD

PLANT PROFILE OF MACROTYLOMA UNIFLORUM

Synonym: *Dolichos biflorus* Auct. And *Dolichos uniflorus* Lam.

Biological Source:

Macrotyloma uniflorum (Lam.) Verdc, is an annual or perennial herb, climbing, prostrate or rarely erect obtained from exploited legume crop.⁶

Taxonomical Classification⁷:

Kingdom : Plantae

Class	: Magnoliopsida – Dicotyledons
Subclass	: Rosidae
Order	: Fabales
Family	: Fabaceae
Subfamily	: Faboideae
Tribe	: Phaseoleae
Subtribe	: Phaseolinae
Genus	: Macrotyloma (Wight & Arn.) Verdc – macrotyloma
Species	: Macrotyloma uniflorum (Lam.) Verdc.

Materials and Methods

- Wistar albino rats of either sex (150-200gm)
- Test extract of 100mg/kg b.w.p.o
- High cholesterol diet pellets
- 5% Aqueous gum acacia
- Animal Models: Wistar albino rats
- Standard drug: Atorvastatin- ATOCOR TM 80 Supplied by Dr. Reddy Labs.

Preparation of Plant Extracts

Young Stems of *Macrotyloma uniflorum* were extracted by soxhlation process extracts were made solvent free using rota evaporator further tested for Phyto-chemical compounds.

Experimental Protocol

Healthy Wistar albino rats weighing between 150-200gm were acclimatized to the laboratory at temperature (25±1) °C, relative humidity (50±15)%, 12 hrs light-dark cycles, kept in standard polypropylene cages and given standard diet and water ad-libitum. The animals were divided into control, toxic, standard and test groups of 100mg/kg, b.w.p.o, suspended in 5% gum acacia solution, daily once. Each comprising of 6 animals in all sets of experiments. Animals in the normal control group, received normal saline orally. Except control group test other groups were fed with rich cholesterol diet pellets supplied by M/s Rayans biotechnologies Pvt. Ltd., Hyderabad. Standard group received Atorvastatin 10mg/kg b.w.p.o suspended in 5% gum acacia solution. The treatment was given for 20 days. In between mean body weight of the animals was checked time to time. Feeding the animals with cholesterol supplied diet induces hyperlipidemia, especially hypercholesterolemia and hypertriglyceridemia. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in experimental animal. On 21st day the blood samples were withdrawn from the arterial damage. All the lipid profile parameters were determined. Total Cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), very low density lipoproteins (VLDL), low density lipoproteins (LDL) were analysed from serum.

STATISTICAL ANALYSIS

All the data expressed as mean± S.E.M and analyzed statistically using ANOVA followed by Dennett's test and compare with respective control group. A value was of $p < 0.05$ was considered significantly and $p > 0.05$ is ns=non significant.

RESULTS AND DISCUSSION

Macrotyloma uniflorum improved the serum lipid profile in rats by decreasing serum TC, TG, LDL, VLDL and increasing serum HDL⁸.

Phytochemical Results

Test	Results
1. Alkaloids	+
2. Amino Acids	+
3. Carbohydrates	+
4. Lignin	-

5. Flavanoids	+
6. Glycosides	+
7. Antraquinone glycosides	+
8. Cardiac glycosides	+
9. Saponin glycosides	+
10. Tannins	+
11. Steroid and Triterpenoids	a. + For Libermann-Burchard b. – For Salkowski

Pharmacological Results

Effect of Test extracts on body weight of hyperlipidemic rat Models.

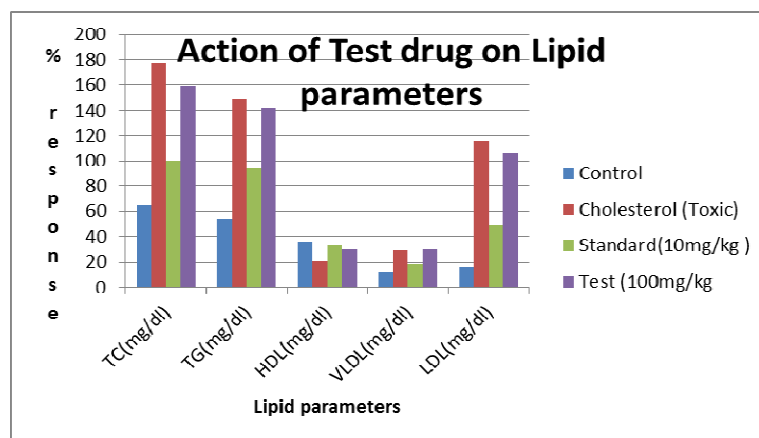
Days	Mean body weight (gm) change in body weight			
	Normal (Water)	Cholesterol	STD(10mg/kg)	TEST(100 mg/ml)
0 th day	140	142	143	141
5 th day	143	149	145	149
10 th day	148	167	152	156
15 th day	151	175	160	169
20 th day	155	199	170	182

Effects of Test extracts on Cholesterol parameters cholesterol induced hyperlipidemic rat model.

GROUPS	TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)	VLDL(mg/dl)	LDL(mg/dl)
Control	64.89±2.280	53.90±1.666	36.15±1.125	11.76±0.3387	16.00±2.656
Cholesterol (Toxic)	177.20±2.69 8**	149.13±2.165**	20.7±1.221*	29.23±0.4326*	116.26±3.507*
Standard (10 mg/kg)	100.22±0.96 57**	93.95±1.205	32.51±0.70 98 ^{ns}	18.78±0.02407*	48.89±0.7986*
Sample (100 mg/kg)	159.11±2.323*	142.14±2.516	30.07±0.611*	30.11±0.516*	106.33±1.45*

Values are mean± SEM, n=6, ***p*<0.01, when compared with control Group **p*<0.01, when compared with Toxic Group

Graphical representation of Sample extract on cholesterol diet induced hyperlipidemic model in wistar albino rats.



CONCLUSION

Finally, the reported Phytochemical and Pharmacological studies supported that *Macrotyloma Uniflorum* have the property of decreasing the hyperlipidemia levels in blood. Now- a- days various researches have been conducted to prove the efficacy of *Macrotyloma uniflorum* in various health problem like cardiovascular diseases, Diabetic, Diuretics, Kidney stones, asthma, bronchitis, inflamed joints, fever, sinus wounds and localized abdominal tumours. This collection will be helpful to generate interest towards the

plant may be useful in developing new medicinal formulations which are more effective and have more therapeutic values.

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QUANTIFICATION OF PERMETHRIN & DIAFENTHIURON PESTICIDES BY ATTENUATED TOTAL REFLECTANCE - FOURIER TRANSFORM INFRARED SPECTROSCOPY

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1.ABSTRACT

A Fourier transform infrared (FTIR) method was developed for simultaneous estimation of Permethrin and Diafenthiuron in agricultural crops. The method involves the direct determination of the drug through peak measurement in the 1538cm^{-1} for Permethrin and Diafenthiuron in 1045cm^{-1} using a base line correction between 1020cm^{-1} to 1650cm^{-1} . The method provides a limit of detection for Permethrin is $4.8\mu\text{g/ml}$ and for Diafenthiuron is $2.3\mu\text{g/ml}$ for 1 gm of sample. The result obtained by Fourier Transform Infrared Spectroscopy shows the developed procedure and offers a good alternative for simultaneous determination of Permethrin and Diafenthiuron in agricultural crops.

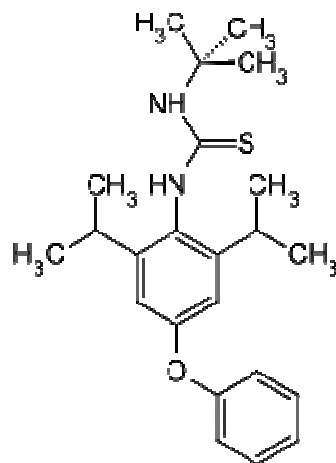
KEYWORDS: *Permethrin diafenthiuron, Fouriertransform infrared spectroscopy*

2.INTRODUCTION

Fourier transform infrared (FTIR) spectrometry was developed in order to overcome the limitations encountered with dispersive instruments. The FTIR can measure the sample without its further preparation. The signal can be measured very quickly, usually on the order of one second or so. Thus, the time element per sample is reduced to a matter of a few seconds rather than several minutes. Permethrin and diafenthiuron are the mostly used pesticides regarding soyabean, cotton, maize crop¹ out of these pesticides permethrin is available in liquid form where as the diafenthiuron is available in the form of wettable powder². **Permethrin**, sold under the brand name **Nix** among others, is a medication and insecticide³⁻⁴. As a medication, it is used to treat scabies and lice⁵. It is applied to the skin as a cream or lotion. As an insecticide, it can be sprayed on clothing or mosquito nets to kill the insects that touch them. Side effects include rash and irritation at the area of use⁵. Use during pregnancy appears to be safe. It is approved for use on and around people over the age of two months. Permethrin is in the pyrethroid family of medications. It works by disrupting the function of the neurons of lice and scabies mites. Diafenthiuron is a chemical compound from the group of thioureas. It was introduced in 1990 by Ciba-Geigy as an insecticide and acaricide.⁶ Diafenthiuron is a colorless solid that is insoluble in water.⁷ According to the European Chemicals Agency, this substance is toxic by inhalation, is very toxic to aquatic life, is highly toxic to aquatic life with long lasting effects and may cause damage to organs through prolonged or repeated exposure.⁸ Diafenthiuron itself is a propesticide which is converted to the active carbodiimide form in the plant.



Permethrin



Diafenthiuron

Various experiments are conducted in estimation of permethrin namely Gas chromatography⁹, Mass spectrometry⁹, High Performance Liquid Chromatography¹⁰, Enzyme Linked Immune Sorbent Assay¹¹ and diafenthiuron by liquid-liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high performance liquid chromatography with ultraviolet detection¹². Quantification of pesticides in plants plays a key role in agriculture. As most of the pesticides are used during the the culturing of crops some amount of pesticide residues will be remained in the final crop out put .As no possible experiments are performed on permethrin and diafenthiuron those two pesticides are selected in the present research.

3.MATERIALS AND METHODS

A BRUKER FTIR SPECTROMETER, designed with a modified deuterated triglycine sulphate detector and a global IR source was employed for FTIR spectra acquisition, using a cell with Zn, Se windows and a pathlength of 0.11mm by averaging 16 scans per spectrum with a nominal resolution of 4cm⁻¹. Permethrin was formulated by AGASTYA AGRO LIMITED and diafenthiuron was formulated by ADAMA INDIA PRIVATE LIMITED were purchased from local store.

3.1 Preparation of sample (permethrin)

6 test tubes were taken and 1ml, 2ml, 3ml, 4ml, 5ml & 6ml of permethrin is placed in each of the six test tubes and volume is made upto 10 ml with methanol and the absorbance values are observed for each dilution. Wave number and calibration curve and the R² values are recorded.

3.2 Preparation of sample (diafenthiuron)

6 test tubes were taken and 1ml, 2ml, 3ml, 4ml, 5ml & 6ml of diafenthiuron is placed in each of the six test tubes and volume is made upto 10 ml with methanol and the absorbance values are observed for each dilution the wave number and calibration curve and the R² values are recorded.

3.3 Preparation of samples

The plants of maize and cotton which are cultured under suitable environmental conditions at location of narakoduru village, chebrolumandal, guntur district, Andhra Pradesh were collected the leaves are separated from the plant and 500 mg of leaves were weighed and placed in a centrifuge tube and 5 ml of methanol was added and allowed to centrifuge at 350 RPM for 15 minutes of time and supernatant liquid was seperated and the absorbance was measured using FTIR .

3.4 Methodology

Tangent lines are drawn on each spectrum and their absorbances are calculated accordingly by using the formula and then the calibration curve is constructed for each drug and then the peak value of pure drug is taken on y axis that is absorbance and equivalent line towards the calibration curve was drawn and the unknown concentration of the pesticide present in the plant was measured according to ICH guidelines

4.RESULTS

FIGURE 1
INFRARED SPECTRA OF 1 ml PERMETHRIN IN METHANOL

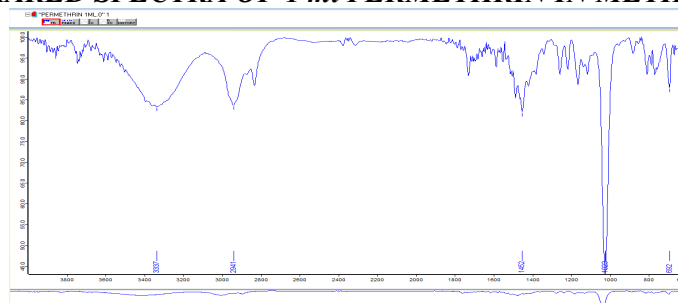


FIGURE 2
INFRARED SPECTRA OF 2 ml PERMETHRIN IN METHANOL

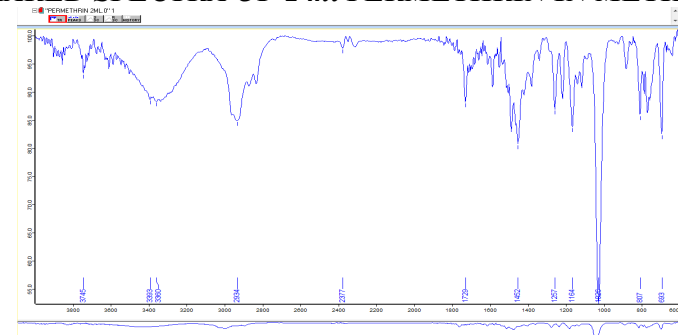


FIGURE 3
IR SPECTRA OF 3 ml PERMETHRIN IN METHANOL

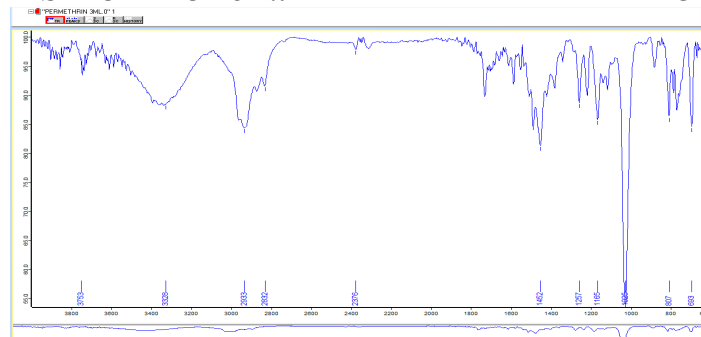


FIGURE 4
IR SPECTRA OF 4 ml PERMETHRIN IN METHANOL

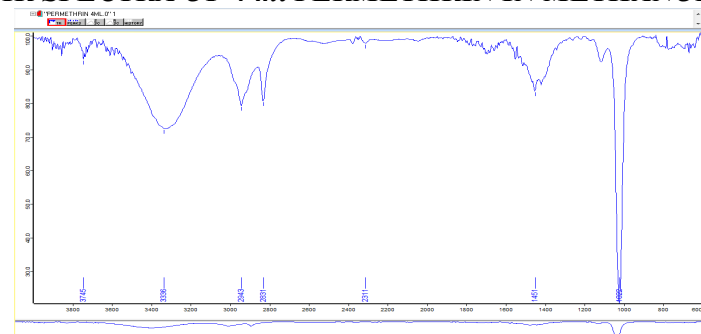


FIGURE 5
IR SPECTRA OF 5 ml PERMETHRIN IN METHANOL

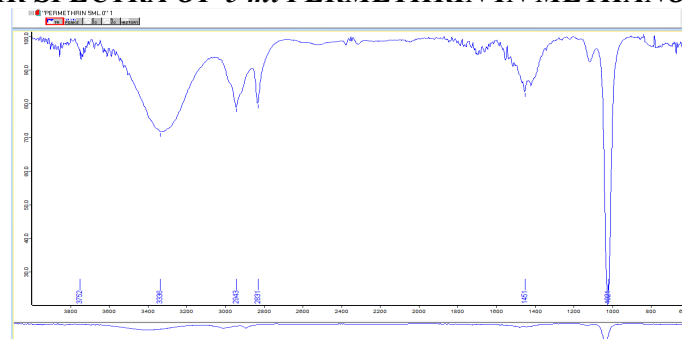


FIGURE 6
IR SPECTRA OF 6 ml PERMETHRIN IN METHANOL

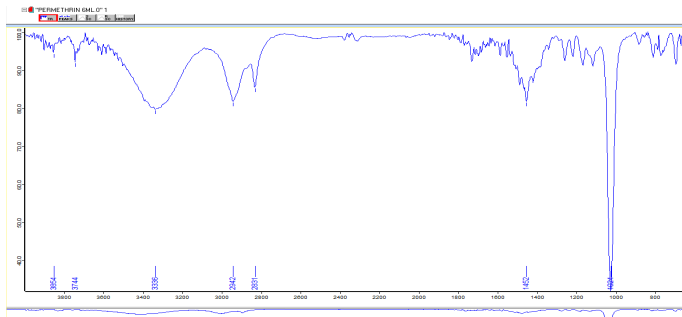


FIGURE 7
OVERLAY SPECTRA OF DIAFENTHIURON SOLUTION CONTAINING 1-6 ml IN METHANOL

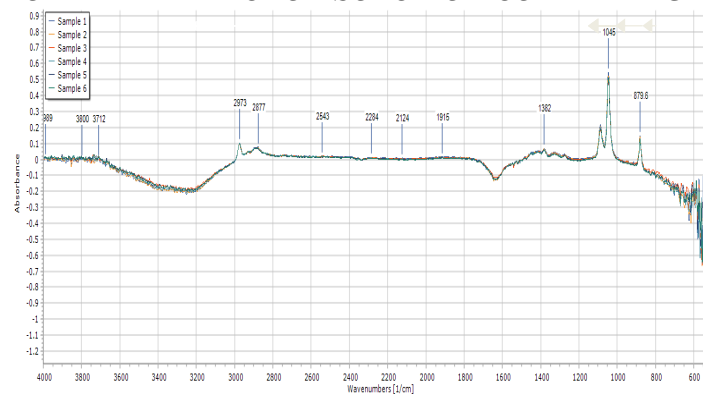


FIGURE 8
INFRARED SPECTRA OF METHANOL EXTRACT OF COTTON LEAVES

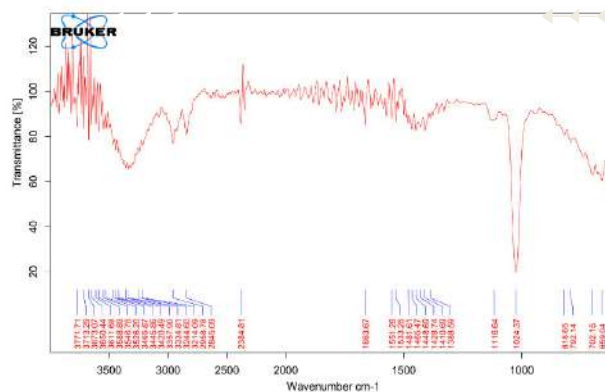


FIGURE 9
INFRARED SPECTRA OF METHANOL EXTRACT OF MAIZE LEAVES

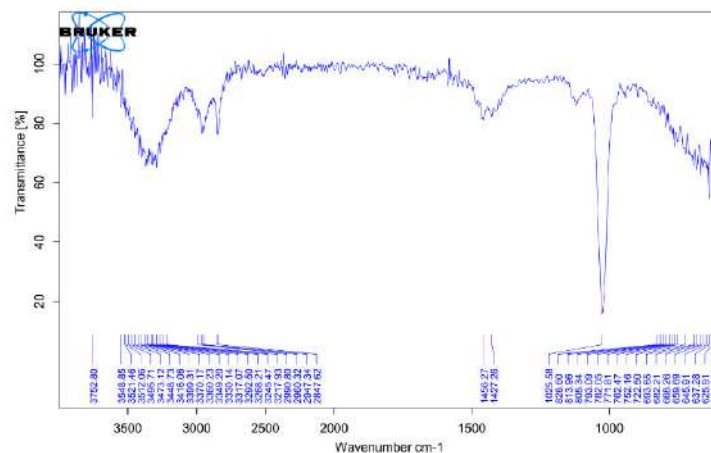


FIGURE 10
CALIBRATION CURVE OF DIAFENTHIURON

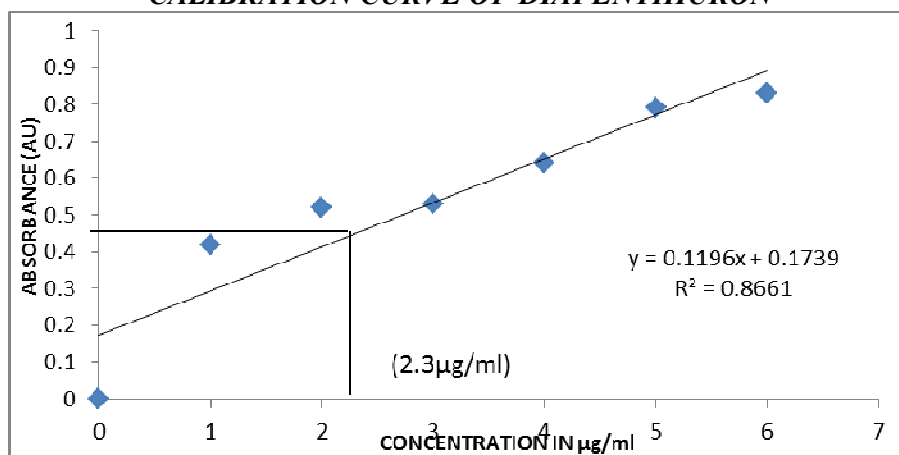
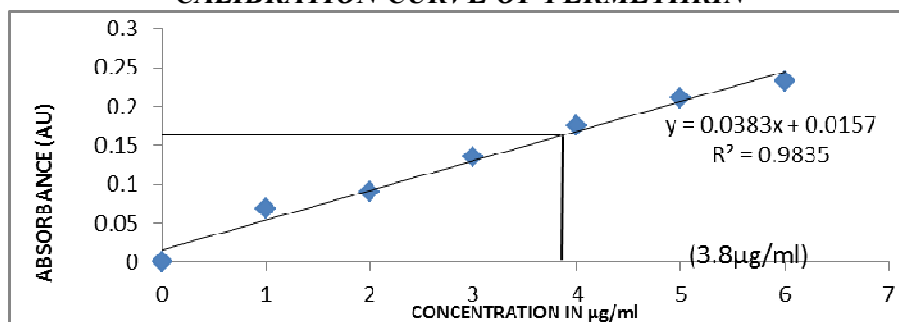


FIGURE 9
CALIBRATION CURVE OF PERMETHRIN



From the above obtained spectra the absorbance values were calculated for each concentration of pesticide in samples of cotton and maize, the unknown concentration of drug present in sample was found to contain 2.3μg/ml of diafenthiuron in soyabean plant and the R^2 value was found to be 0.866. And 3.8 μg/ml of permethrin in maize and R^2 value was found to be 0.983.

5.DISCUSSION

Quantification of pesticides can be considered as a validated and fast procedure in analytical field of pharmaceuticals mainly that deals with pesticides. As the modern practices of agriculture have more drawbacks regarding the pesticide residues this method is helpful in analysis of food.

6.CONCLUSION

So, it can be concluded that the FTIR procedure developed is simple, fast and accurate alternative for the quality control analysis of pesticide formulations containing permethrin and diafenthiuron, and provides enhanced methodology as compared with previous study of permethrin and diafenthiuron determination.

7.ACKNOWLEDGEMENTS

Authors acknowledge the support of Nirmala College of pharmacy who helped in carrying out this study.

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A CLINICAL CASE REPORT ON SUPERIOR MESENTRIC ARTERY SYNDROME

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ABSTRACT

Superior mesenteric artery syndrome (SMAS) is a rare cause of small bowel obstruction (SBO) but potentially life-threatening condition. SMAS is a gastro-vascular disorder (digestive condition) that occurs when the 3rd portion of the duodenum (the first part of the small intestine) is compressed between two arteries (the aorta and the superior mesenteric artery). This compression of two arteries causes partial or complete blockage of the duodenum. Usually acute occurrence is observed in SMA syndrome, such as a duodenal obstruction, or more insidiously, such as our patients who presented with long-standing vague abdominal pain, early satiety, anorexia and recurrent episodes of abdominal pain associated with vomiting. We present the case of 30 years old male patient who presented with right-sided abdominal pain from 3 months, insidious in onset, gradually progressive associated with nausea, early satiety, and difficulty in passing stools. He had pain associated with intake of food and no radiation in pain. A CECT Abdomen revealed that narrowed 3rd part of duodenum compressed between SMA and abdomen aorta, decreased aortomesenteric distance. A CT scan can also be done. This case can be implicated to the patients without history of weight loss and the superiority of duodenojejunostomy compared to gastrojejunostomy

KEYWORDS: *superior mesenteric artery, small bowel obstruction, gastro-vascular disorder.*

INTRODUCTION

Superior mesenteric artery syndrome (Wick's syndrome) is due to decreased in aortomesenteric angle. The surgical management of SMA Syndrome in the modern era is reserved for medically refractive cases¹. The first therapy for this is usually Nasojejunal feeding². When is recommended, a laparoscopic duodenojejunal anastomosis is now the stand of care. It is atypical of proximal intestinal obstruction, most frequently occurring young patients who have had an important weight loss. High insertion of the ligament of Treitz as well as surgery for spinal deformities is another potential cause for the occurrence of SMA syndrome³. The severity of the symptoms mostly depends on the degree of the compression as reflected by the aortomesenteric angle. SMA Syndrome can be diagnosed by CT scan and Contrast-enhanced computed tomography (CECT). Conservative therapy mainly consists of weight gain achieved orally or parenterally, with the aim of reconstituting the mesenteric fat pad and increasing the aortomesenteric angle⁴. In this paper, we describe a case of SMA Syndrome in a young male with characteristic symptoms of right side abdominal pain from 3 months, insidious in onset, gradually progressive associated with nausea, early satiety, difficulty in passing stools. He had pain associated with intake of food and no radiation in pain. We present this case to heighten the awareness of this syndrome and the need for early management to prevent diagnosis delay and serious complications⁵.

CASE REPORT

A 30-year-old male patient was brought to the hospital with right-sided abdominal pain from 3 months, insidious in onset, gradually progressive associated with nausea, early satiety, difficulty in passing stools. He had pain associated with intake of food and no radiation in pain. They don't have significant past history. He is addiction to occasional alcohol intake. His appetite can be reduced and bowel sounds can be stimulant. On Examination revealed that he was a thin patient with normal vitals and abdominal examination had soft. Based upon clinical features serum Creatinine (0.70mg/dl), lymphocytes (15.0%) had decreased and neutrophils (29.0%) had increased. A Contrast-enhanced computed tomography (CECT) Abdomen revealed that narrowed 3rd part of duodenum compressed between SMA and abdomen aorta, decreased aortomesenteric

distance. A CT Scan revealed that reduced aorta mesenteric distance noted measuring 5.8 mm. Reduced aorta mesenteric angle. Features suggest SMA Syndrome. Main portal vein, right and left branches and splenic vein are dilated. No evidence of thrombosis/collateral/splenomegaly.

DISCUSSION

SMA Syndrome is one of the rare syndromes. In literature, the incidence of Superior mesenteric artery Syndrome was reported 0.12-0.3%⁶. The most common cause is significant weight loss which leads to loss of retroperitoneal fat. The aim of the treatment is to reverse or correct the symptoms that have arisen because of narrowed 3rd part of duodenum compressed between SMA and abdomen aorta. However, there are no proper guidelines for the treatment of SMA Syndrome⁷. The longest treatment as lasted for is 169 days. If it is diagnosed early, measures can be taken to reverse it by removing the causes responsible. For weight loss, nutritional supplements should be given. If it is a late diagnosis, duodenojejunostomy is performed which is successful in 90% of the cases⁸. In our case, the laboratory details are right side abdominal pain from 3 months, insidious in onset, gradually progressive associated with nausea, early satiety, difficulty in passing stools. Laboratory parameters done for this patient serum creatinine (0.70mg/dl), lymphocytes (15.0%) had decreased and neutrophils (29.0%) had increased. Diagnostic criteria for SMA Syndrome was CECT Abdomen. Based on this standard diagnostic criteria this patient was diagnosed as SMA Syndrome which reveals that narrowed 3rd part of duodenum compressed between SMA and abdomen aorta, decreased aortomesenteric distance⁹.

CONCLUSION

The diagnosis of SMA syndrome is often missed and rare entity. Diagnostic criteria for SMA Syndrome was CECT Abdomen. Based on this, standard diagnostic criteria this patient was diagnosed as SMA Syndrome which reveals that narrowed 3rd part of duodenum compressed between SMA and abdomen aorta, decreased aortomesenteric distance. There remains less controversy about the diagnosis of SMA syndrome as symptoms of the disorder do not necessarily correlate well with the abnormal anatomic findings. Furthermore, the symptoms which are seen in SMA Syndrome may not resolve completely following treatment. We feel that SMA syndrome is underdiagnosed. High clinical suspicion is of utmost importance, especially in patients with weight loss and symptoms of gastric distension. Surgical stress should be considered as a trigger factor of SMA syndrome. Interdisciplinary teamwork provides the most therapeutic results and beneficial diagnosis in this often underestimated disease. Lastly, we would like to point out the difficulty in achieving an accurate diagnosis of SMA syndrome in an atypical clinical setting similar to what happened in our case.

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ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF HORDEUM VULGARE

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ABSTRACT

Hordeumvulgare has various medicinal uses belonging to the poaceae family and it is also a member of the grass family. Barley is used as the source of fermentable material for beer. The objective of the present work was to assess the anti inflammatory effect of hordeumvulgare (EEHV) by sub dermal injection of carrageenan-induced hind paw edema model. Wistar rats (190–210 g) were randomly divided into five groups six of each and each rat was treated with oral administration for 6 days. The groups are taken as follows Group I Received 10% of DMSO solution; Group II Received diclofenac 10 mg/kg bd.wt.; Groups III Received EEHV (50 mg/kg bd.wt.); Group IV Received EEHV (150 mg/kg bd.wt.); Group V Received EEHV (300 mg/kg bd.wt.). The final results show that the possible mechanism of action of MEHP is involved with inactivation of COX in carrageenan-induced paw edema.

KEYWORDS: *Hordeumvulgare, Anti-inflammatory effect, sub dermal injection, wistar rats*

INTRODUCTION

Inflammation (from latin; inflammatio) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants¹. It is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair¹. Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation². Inflammation is a protective immunovascular response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair³. Acute inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus⁴. Inflammatory pathways impact the pathogenesis of a number of chronic diseases, and involve common inflammatory mediators and regulatory pathways. Inflammatory stimuli activate intracellular signaling pathways that then activate production of inflammatory mediators. Primary inflammatory stimuli, including microbial products and cytokines such as interleukin-1 β (il-1 β), interleukin-6 (il-6), and tumor necrosis factor- α (tnf- α), mediate inflammation through interaction with the tlr, il-1 receptor (il-1r), il-6 receptor (il-6r), and the tnf receptor (tnfr)⁵. In this the study deals with anti-inflammatory activity of methanolic extract of hordeumvulgare where the barley is used priorly. Barley is among the most ancient cereal crops grown in the world today. Archeological evidence suggests the existence of barley in egypt along the river nile around 17,000 years ago⁶.

Phytochemical analysis

Barley contains an assortment of phytochemicals in varying concentrations usually determined by genotypic or environmental factors or the interaction of both factors. Phytochemicals in barley may exist in free, conjugated or bound forms and are categorized into several major classes including phenolic acids, flavanoids, lignans, tocopherols, phytosterols and folates⁷.

MATERIALS AND METHODS

EVALUATION OF ANTI INFLAMMATORY ACTIVITY

Carrageenan - Induced Rat Paw Edema Model To evaluate the anti inflammatory effect of EEHV by sub dermal injection of carrageenan-induced hind paw edema model. Wistar rats (CPCSEA reg no: 010/iaec/NCPA/B.Pharmacy/2018-2019) (190–210 g) were randomly divided into five groups six of each and each rats were treated with oral administration for 6 days as follows:

Group I Received 10% of DMSO solution

Group II Received diclofenac (10 mg/kg bd.wt).

Groups III Received EEHV (50 mg/kg bd.wt).

Group IV Received EEHV (150 mg/kg bd.wt).

Group V Received EEHV (300 mg/kg bd.wt).

METHOD

100 μ L of 1% carrageenan suspension in normal saline was injected into the plantar side of right-hind paw. Paw volume was measured with plethysmometer at 5th hr after carrageenan injection.

RESULTS AND DISCUSSIONS

Effect of ethanolic extract of *Hordeum vulgare* (MEHP) on Carrageenan induced hind paw edema.

Table 1. Carrageenan (1% 0.1ml sub plantar Injection)+Received 10% of DMSO solution.

S.No	Rat	Paw edema volume (mm.)	
		Before treatment	5th hrs. of After injection
1	R 1	2.2	3.6
2	R2	2	3.2
3	R3	2	3.6
4	R4	2.1	3.6
5	R5	2.2	3.9
6	R6	2.1	3.5
	Mean	2.1	3.6
	SD	0.09	0.23
	SEM	0.04	0.1

Carrageenan (1%, 0.1 ml sub Plantar Injection) + Received 10% of DMSO solution.

Table 2. Carrageenan (1% 0.1ml sub Plantar Injection)+Diclofenac 10mg/kg bd.wt.paw Volume(mm).

S.No	Rat	Paw edema volume (mm.)	
		Before treatment	5th hrs. of After injection
1	R 1	2.2	2.2
2	R2	2	2.1
3	R3	2	2.5
4	R4	2.1	2.9
5	R5	2.2	3.1
6	R6	2.1	2.3
	Mean	2.2	2.5
	SD	0.03	0.40
	SEM	0.02	0.2

Carrageenan (1%, 0.1 ml sub Plantar Injection) + Diclofenac 10 mg/kg bd.wt. Paw Volume (mm).

Table 3. Carrageenan (1% 0.1ml sub Plantar Injection)+EEHV (50 mg/kg), Volume(mm).

S.No	Rat	Paw edema volume (mm.)	
		Before	5th hrs. of

		treatment	After injection
1	R 1	2.2	3.2
2	R2	2	3.1
3	R3	2	3.4
4	R4	2.1	3.2
5	R5	2.2	3.2
6	R6	2.1	3.5
	Mean	2.1	3.3
	SD	0.09	0.15
	SEM	0.04	0.1

Carrageenan (1%, 0.1 ml sub Plantar Injection) + EEHV (50 mg/kg), Paw Volume (mm).

Table 4. Carrageenan (1% 0.1ml sub Plantar Injection)+EEHV (150mg/kg), Paw pawvolume(mm).

S.No	Rat	Paw edema volume (mm.)	
		Before treatment	5th hrs. of After injection
1	R 1	2.2	3.1
2	R2	2	3.3
3	R3	2	3.1
4	R4	2.1	2.9
5	R5	2.2	2.8
6	R6	2.1	3.1
	Mean	2.1	3.1
	SD	0.09	0.18
	SEM	0.04	0.1

Carrageenan (1%, 0.1 ml sub Plantar Injection) + EEHV (150 mg/kg), Paw Volume (mm).

Table 5. Carrageenan (1% 0.1ml sub plantar Injection)+EEHV (300mg/kg),paw volume

S.No	Treatment Group	Paw edema volume (mm.) [Mean $\pm$ SEM]	
		Before treatment (0 hr)	5 th hrs. of After injection
1	Group I	2.1 $\pm$ 0.04	3.6 $\pm$ 0.1***a
2	Group II	2.2 $\pm$ 0.02	2.4 $\pm$ 0.2
3	Group III	2.2 $\pm$ 0.03	3.3 $\pm$ 0.1
4	Group IV	2.1 $\pm$ 0.02	3.1 $\pm$ 0.1
5	Group V	2.1 $\pm$ 0.04	2.8 $\pm$ 0.1**b

Carrageenan (1%, 0.1 ml sub Plantar Injection) + EEHV (300 mg/kg), Paw Volume (mm).

Data were expressed as Mean $\pm$ SEM. Significant at ^a***P< 0.001, ^b**P< 0.01 compare with compared before treatment group rats.

Table 6. Effect of EEHV on Carrageenan Paw Edema in Wistar Albino Rats. (mm).

S.No	Rat	Paw edema volume (mm.)	
		Before treatment	5th hrs. of After injection

1	R 1	2.2	2.9
2	R2	2	2.7
3	R3	2	3.1
4	R4	2.1	2.8
5	R5	2.2	2.9
6	R6	2.1	2.6
	Mean	2.1	2.8
	SD	0.09	0.18
	SEM	0.04	0.1

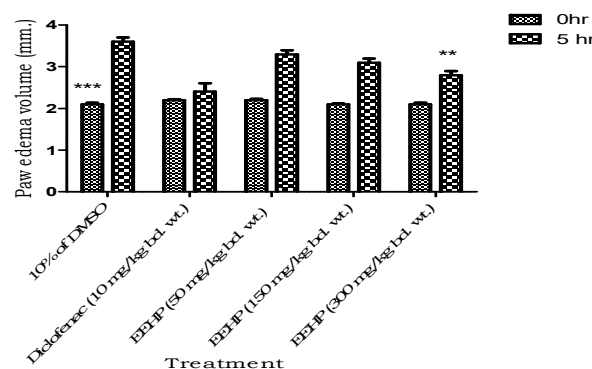


Figure1-Effect of EEHV on Carrageenan induced hind hind paw edema in Wistar albino rats

DISCUSSION

Inflammation is a complicated biological and bio-chemical processes consisting of vascular tissues and nonspecific reactions activated by natural immune responses against irritants, infection, injury and injured cells. The microcirculation is the central playground where the course of inflammatory events was assessed and examined. Inflammation contains a lengthy sequence of molecular reactions and cellular actions, which are intended to renovate a tissue from simple skin cut or to cure numerous burn wounds. An inflammatory process in cellular and tissue levels consist of a chain of events with dilation of arterioles and venules, increased blood vessel permeability, and blood flow with infiltration of leukocytes into the tissues (Antonisamy et al., 2017; Schmid-Schönbein GW. 2006). Medicinal plants showed essential roles as foundations of effective anti-inflammatory agents. According to the World Health Organization (WHO), nearby three-quarters of the world's inhabitants depend on traditional medicines for their healthiness.

CONCLUSION

In our work, EEHV at doses of 50, 150 and 300 mg/kg of EEHV treatment inhibited edema formation in the phase of carrageenan induction with a similar effect to COX antagonist (nonsteroidal anti-inflammatory) used as reference drug). The above data states that the mechanism of action of MEHP is involved with inactivation of COX in carrageenan-induced paw edema.

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CONFLICT OF INTEREST

There is no conflict of interest.

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A REVIEW ON XENO TRANSPLANTATION

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ABSTRACT

Organ transplantation is the one of the most phenomenal success stories in 20th century. Solid organ transplantation is the one of the most successful phenomenon. With the increased population in the world many people are suffering from organ failure. The availability of organs and donors are less but demand is more. In order to avoid this problem xenotransplantation is one of the best phenomenons in organ transplantation that is the organs are transplanted from human beings. As the xeno transplantation is useful to human being but have some defects like hyperacute xenograft rejection, cellular xenograft rejection, acute vascular xenograft rejection, chronic xenograft rejection. To avoid rejection of xenotransplanted organs immunosuppressive agents and depletion of antigalactose antibodies by immunoadsorption techniques are used.

KEYWORDS: *Autograft, Allograft, Dominant transplant, Tissues.* **ABREVIATIONS:** *ABO-incompatible (ABOi), ABO-compatible (ABOc)*

INTRODUCTION

Organ transplantation may be a procedure within which Associate in nursing organ is far away from one body and placed within the body of a recipient, to exchange a broken or missing organ. The donor and recipient are also at identical location, or organs are also transported from a donor website to a different location. Organs / tissues that are transplanted within the same person's body are called autografts. Transplants that square measure performed between 2 subjects of identical species square measure referred to as allografts. Allografts will either be from a living or dead body supply. Organ donors may be living, brain dead, or circulatory death.¹ Tissue may be recovered from donors who died of circulatory death,² as well as of brain dead – up to twenty four hours past the stop of heartbeat. Unlike organs, most tissues (with the exception of corneas) may be preserved and keep for up to 5 years, which means they'll be "banked".

TYPES OF TRANSPLANT

1. Autograft
2. Allograft and allotransplantation
3. Xenograft and xenotransplantation
4. Domino transplants
5. ABO-incompatible transplants
6. Transplantation in obese individuals

DEFINITION OF DONAR

A person or animal donating blood, an organ, bone marrow or other biological tissue for transplantation is considered as donor.

TWO TYPES OF DONAR ARE PRESENT

1. Living donor
2. Deceased donor

1.LIVING DONOR

A person or animal donating blood, an organ, bone marrow or other biological tissue for transplantation is considered as donor.

Organ donation is that the act of donating Associate in nursing organ by an individual in order that it is transplanted by surgery within the body of the recipient.

2.DECEASED DONOR

Deceased donors (formerly cadaveric) square measure people that has been declared as brain-dead and whose organs square measure unbroken viable by ventilators or different mechanical mechanisms till they're excised for transplantation. Apart from brain-stem dead donors, WHO have fashioned the bulk of deceased donors for the last twenty years, there's increasing use of donation-after-circulatory-death-donors (formerly non-heart-beating donors) to extend the potential pool of donors as demand for transplants continues to grow. Prior to the recognition of brain death in the 1980s, all deceased organ donors died of circulatory death. These organs have inferior outcomes to organs from a brain-dead donor.

XENOTRANSPLANTATION

Xenotransplantation or heterologous transplant is that the transplantation of living cells, tissues or organs from one species to a different.³ It is in contrast with allotransplantation (from other individual of same species), syngeneic transplantation or isograft transplantation (grafts transplanted between two genetically identical individuals of the same species) and autotransplantation (from one a part of the body to a different within the same person). Xenotransplantation of human tumour cells placed in mice may be a analysis technique often utilized in pre-clinical medicine analysis. Human organ transplant offers a possible treatment for end-stage organ failure, a major ill health in components of the industrialised world. It also raises many novel medical, legal and ethical issues.⁴ A continuing concern is that many animals such as pigs have a shorter lifespan than humans meaning that their tissues age at a quicker rate. The first serious attempts at xenotransplantation (then called heterotransplantation) appeared in the scientific literature in 1905, when slices of rabbit kidney were transplanted into a child with chronic kidney disease.⁷

Table - 01. LIST OF XENOTRANSPLANT PERFORMED

S.NO	XENOTRANSPLANT ORGAN	XENOTRANSPLANT ANIMAL	SCIENTIST PERFORMED	SUCCESS OR FAILURE	REFERENCE
1.	Kidney	Rabbit	Princetone et al 1905	failed	8
2.	Kidney	Pig	Jaboulay et al 1906	failed	9
3.	Kidney	Goat	Jaboulay et al 1906	failed	10
4.	Kidney	Non-human primate into man	Unger et al 1910	failed	11
5.	Kidney	Lamb	Neuhof et al 1923	Failed	12
6.	Kidney	Chimpanzee	Tulane university in New Orleans et al 1964	failed	13
7.	Heart	Chimpanzee	Hardy and others et al 1964	failed	14
8.	Kidney	Chimpanzee	Starzl et al 1964	failed	15
9.	Blood	Animal to human	Denis et al 17th century,	failed	16
10.	Blood vessel	Hog	Alexis Carrel et al 1907		17
11.	Testis	Chimpanzee	Serge et al Voronoff 1920	success	18
12.	Kidney	Same species animals	Voronoff et al 1933	success	19
13.	Glandular tissues	Goat	John et al Brinkley	success	20
14.	Kidney	Chimpanzee	Kieith et al Remtma 1960	success	21
15.	Heart	Chimpanzee	James Hardy et al 1963	failed	22
16.	Liver, kidney	Chimpanzee	Thomas et al Starzl 1960	success	23
17.	Heart	Baboon	Leonard et al Bailey 1983	success	24
18.	Pancreatic islet	Pig	Carl Groth et al 1993	Success but no use	25

CONCLUSION

Organ transplantation is one of the major problems in present society. In a multipopulated country like India the demand for organs is more but the sources are inadequate. It led to evolution of xenotransplantation. Success rates may be few but still advanced researches are going on in this area. Major issues like appearance of new disease symptoms, immune reactions and less survival rates are generally observed. Nothing is impossible in this magic world. Still more research and production of transgenic species for xenotransplantation may overcome this massive problem. More and more scientific researches with success rate may be a good alternative to organ demand.

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DESIGN, SYNTHESIS CHARACTERISATION AND LOCAL ANAESTHETIC ACTIVITY OF NOVEL SUBSTITUTED BENZANILIDE DERIVATIVES

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ABSTRACT

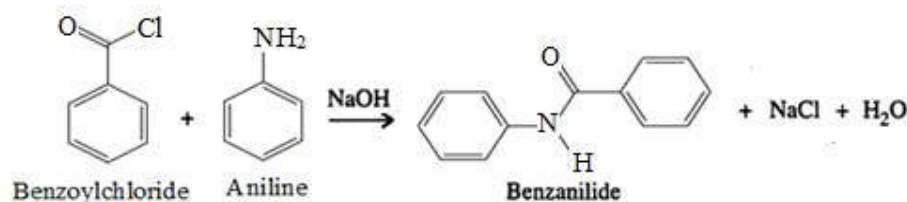
Present aim of the study is to design novel benzanilide derivatives with potent local anaesthetic activity. Novel benzanilides derivatives were synthesized by the condensation reaction of aniline with para substituted benzoyl chlorides. The prepared derivatives are subjected to the M.P, I.R, NMR, MASS spectroscopy. The test compounds were showed characteristic IR peaks amide $1640-1650\text{ cm}^{-1}$, aromatic $\text{C}=\text{C}$ peak at 2230 cm^{-1} . ^1H NMR values for aromatic, $-\text{NH}$ and other substitutions were in the proper positions. All are showed characteristic molecular ion peaks in Mass spectroscopy. The test compounds were subjected to the local anaesthetic activity on frog. Among all the compounds test compound 6,8 were showed optimum activity, test compound 5,6 were showed good activity. All these compounds were having electron donating groups and making the ring active. All the four compounds can be taken as a lead compounds for further investigation.

KEYWORDS: *Benzanilide derivatives, Synthesis, Characterisation, Local anaesthetic activity*

INTRODUCTION

Chemistry is the scientific discipline involved with compounds composed of atoms, i.e. elements and molecules, i.e. combinations of atoms their composition, structure, properties, behavior and the changes that occur during the reaction with other compounds. Characterisation of the binding behavior plays a vital role in rational designs of drugs as well as to elucidate fundamental biochemical processes by blocking depolarization thereby reversibly loss of sensory impulses occurs without causing any structural damage to the nerve cell upon topical administration².

SCHEME



MATERIALS

Aniline, 10% NaOH, Ethanol, Benzyl Chloride, P-Chloro Benzoyl Chloride, P- Bromo Benzoyl Chloride and all the chemicals used were from Sigma Aldrich.

METHODOLOGY

CHEMICAL METHODS

1. METHOD OF PREPARATION OF BENZANILIDE

Weighed accurately 2.6 gm of aniline and add 25 ml of 10% NaOH and were taken in a clean and dry erlenmayer flask (conical flask). To that added 4.3 gm of benzoyl chloride in small proportion with continuous stirring or vigorous shaking for 1 min after every addition. Cork the flask and stirred

vigorously for 10 mins to complete the reaction..M.P: 140⁰c IR Data: Amide stretching (1754 cm⁻¹), Aromatic stretching (2257 cm⁻¹), N-H stretching (2643cm-1). H¹ NMR: 6.12(Ar, 5 H, Multiplet), 7.93 (Ar ,5 H, Multiplet), 2.67 (NH, Singlet), MASS. M+1 Peak 198.12.

2. METHOD OF P-CHLORO BENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and add 25 ml of 10% NaOH and were taken in a clean and dry erlenmayer flask (conical flask).To that added 4.3 gm of chloro benzoyl chloride in small proportion with continous stirring or vigorous shaking for 1 min after every addition.Cork the flask and stirred vigorously for 10 mins to complete the reaction..M.P: 140⁰c, IR Data: Amide stretching (1752 cm⁻¹), Aromatic stretching (2253 cm⁻¹), N-H is stretching (2643 cm-1). H¹ NMR: 6.42 (Ar, 5 H, Multiplet), 7.87 (Ar, 5 H, Multiplet), 2.67 (NH, Singlet), C-Cl Stretching (753 Cm⁻¹) MASS. M+1 Peak 208 56

3. METHOD OF PREPARATION OF P-BROMOBENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and 25 ml of 10% NaOH were taken in a clean and dry erlenmayer flask (conical flask). To that added 4.3 gm of P-Bromo benzoyl chloride in small proportion with continous stirring or vigorous shaking for 1 min after every addition. Corked the flask and stirred vigorously for 10 mins till the reaction to be completed. The product was filtered and washed several times with ice cold water and recrystallised the product from boiling alcohol solution. M.P: 160⁰c, IR Data: Amide stretching (1746 cm⁻¹), Aromatic stretching (2262 cm⁻¹), N-H stretching (2678 cm-1),C-Br stretching (1078 Cm⁻¹) . H¹ NMR: 6.23(Ar, 5 H, Multiplet), 7.85 (Ar ,5 H, Multiplet), 1.85 (NH, Singlet), MASS. M+1 Peak 278.9

4. METHOD OF PREPARATION OF P-NITRO BENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and 25 ml of 10% NaOH were taken in a clean and dry erlenmayer flask (conical flask).To that added 4.3 gm of P-Nitro benzoyl chloride in small proportion with continous stirring or vigorous shaking for 1 min after every addition.Corked the flask and stir vigorously for 10 mins till the reaction to be completed. IR Data: Amide stretching (1736 cm⁻¹), Aromatic stretching (2256 cm⁻¹), C-NO₂stretching (748 Cm⁻¹), N-H stretching (2635 cm-1). H¹ NMR: 6.46(Ar, 5 H, Multiplet), 7.98 (Ar, 5 H, Multiplet), 2.45 (NH, Singlet), MASS. M+1 Peak 243.8

5. METHOD OF PREPARATION OF P-METHYL BENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and 25 ml of 10% NaOH were taken in a clean and dry erlenmayer flask (conical flask).To that added 4.3 gm of P-Methyl benzoyl chloride in small proportion with continous stirring or vigorous shaking for 1 min after every addition.Corked the flask and stir vigorously for 10 mins till the reaction to be completed.M.P :1740⁰ C : IR Data: Amide stretching (1734 cm⁻¹), Aromatic stretching (2278 cm⁻¹), C-CH₃ stretching (798 Cm⁻¹), N-H stretching (2657 cm-1). H¹ NMR: 6.489(Ar, 5 H, Multiplet), 7.23 (Ar, 5 H, Multiplet), 2.18 (NH, Singlet), 3.67 (triplet, 3H), MASS. M+1 Peak 213.8

6. METHOD OF PREPARATION OF P-ETHYL BENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and 25 ml of 10% NaOH were taken in a clean and dry erlenmayer flask (conical flask).To that added 4.3 gm of P-Ethyl benzoyl chloride in small proportion with continuous stirring or vigorous shaking for 1 min after every addition.Corked the flask and stir vigorously for 10 mins till the reaction to be completed.. M.P: 184⁰ C, IR Data: Amide stretching (1756 cm⁻¹), Aromatic stretching (2457 cm⁻¹), C-C₂H₅stretching (798 Cm⁻¹), N-H stretching (2634 cm-1). H¹ NMR: 6.489(Ar ,5 H, Multiplet), 7.23 (Ar ,5H, Multiplet), 2.18 (NH, Singlet), C- C₂H₅(Multiplets,5H) MASS. M+1 Peak 213.8

7. METHOD OF PREPARATION OF P-METHOXY BENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and 25 ml of 10% NaOH were taken in a clean and dry erlenmayer flask (conical flask). To that added 4.3 gm of P-Methoxy benzoyl chloride in small proportion with continuous stirring or vigorous shaking for 1 min after every addition.Corked the flask and stir vigorously for 10 mins till the reaction to be completed. M.P:145⁰ C, IR Data: Amide stretching (1756 cm⁻¹), Aromatic stretching (2457 cm⁻¹), C-C₂H₅stretching (798 Cm⁻¹), N-H stretching (2643 cm-1). H¹ NMR: 6.489(Ar, 5 H, Multiplet), 7.23 (Ar, 5H, Multiplet), 2.18 (NH, Singlet), C- C₂H₅ (Multiplets, 5H) MASS. M+1 Peak 213.8

8. METHOD OF PREPARATION OF P-ETHOXY BENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and 25 ml of 10% NaOH were taken in a clean and dry erlenmayer flask (conical flask). To that added 4.3 gm of P-Ethoxy benzoyl chloride in small proportion with continuous stirring or vigorous shaking for 1 min after every addition. The product was crystallised. M.P: IR Data: Amide stretching (1756 cm^{-1}), Aromatic stretching (2457 cm^{-1}), N-H stretching (2655 cm^{-1}). ^1H NMR: 6.134(Ar, 5H, Multiplet), 7.89 (Ar, 5H, Multiplet), 2.67(NH, Singlet), C- C_2H_5 (3.67, Multiplets, 5H) MASS. M+1 Peak 213.8

9. METHOD OF PREPARATION OF P-ACETYLAMINO BENZANILIDE DERIVATIVE

Weighed accurately 2.6 gm of aniline and 25 ml of 10% NaOH were taken in a clean and dry erlenmayer flask (conical flask). To that added 4.3 gm of P-Acetylamino benzoyl chloride in small proportion with continuous stirring or vigorous shaking for 1 min after every addition. The product was crystallised. M.P : 170°C , IR Data: Amide stretching (1675 cm^{-1}), Aromatic stretching (2356 cm^{-1}), N-H stretching (2650 cm^{-1}). ^1H NMR: 6.768(Ar, 5 H, Multiplet), 7.98 (Ar, 5 H, Multiplet), 2.35 (NH, Singlet), - C_2H_5 (3.98, Multiplets, 5H) MASS. M+1 Peak 212.6.

LOCAL ANAESTHETIC ACTIVITY ON FROG

Frog was dissected by using pithing needle remove the upper part of the spinal cord and expose the spinal nerve in the cavity open and cut the abdomen and removed all the abdominal organs (fig 2). Place the frog in the frog board by hanging two of its hind legs free from the board. Consider the leg as the control and right as the test and perform the experiment Immersed the leg in the beaker containing 0.1N HCL. Record the brisk reflex withdrawal of the leg. Washed the immersed (right) leg with flowing water or by dipping the leg in the beaker containing saline. Repeated the same with the right hind leg. Wash the immersed leg. Placed about 10 ml of 1% w/v lignocaine HCL in the abdominal pouch. The sciatic nerve flex is now exposed to the local anaesthetic action of the drugs. Allow the drug to act for 5 mins. Immersed both left and right leg succession in the beaker containing acid solution as before. Recorded the delay in the reflux withdrawal of legs. Repeated the procedure with the sample solution of 10 ml of 1% test in the beaker. Note the reflux in the leg after immersed the legs in the acid solution as before. Compared the results obtained from the test sample with that of the standard.

RESULTS AND DISCUSSIONS

Novel benzanilide were prepared by the simple condensation reaction of aniline and para substituted benzoyl chlorides. The obtained derivatives were recrystallised by using aqueous ethanol. The melting point was noted by using open capillary tube method and they are subjected to characterization by using IR, NMR and MASS. In IR spectra, we found characteristic amide function group peak at $1740\text{--}1750\text{ cm}^{-1}$ and para substituted such as -Cl, -Br, - NO_2 , - CH_3 , - C_2H_5 , - OCH_3 , - OC_2H_5 and - NHCOCH_3 , correspondingly and the fifth derivative (T) NMR spectra is observed characteristic aromatic protons in the region of 7.2-8.4 and 3 protons of aliphatic methyl group in the region of 1.6-1.8, -NH group proton at 4.2. Characteristic mass spectra of compound (T_5) (M+1) is 212.2. All the derivatives are shown corresponding mass peaks. The derivatives are subjected to local anaesthetics activity by using standard drug lignocaine (table no 2). The compounds were having good local anaesthetic activity. In that (T_8 and T_9) are having potent local anaesthetic with that of standard drug (fig 3).

CONCLUSION

Novel benzanilides were synthesized by using condensation reaction. They were purified by recrystallisation process by using ethanol. All showed sharp melting points. They were showed respective IR peaks Amide Group ($1640\text{--}1650\text{ cm}^{-1}$), aromatic $\text{C}=\text{C}$ ($2230\text{--}2240\text{ cm}^{-1}$) and peaks related to the para substitutions. NMR data obtained were matched with corresponding structures. They were showed respective molecular ion peaks. Novel derivatives are subjected to local anaesthetic activity the compounds 7,8,9 were showed good activity because of presence of electron donating groups remaining halogen substituted are moderate active and nitro derivative is less active. The test compounds 7,8,9 can be taken as a lead compounds for further investigations.

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A CASE REPORT ON NON STEROIDAL ANTI INFLAMMATORY DRUG INDUCED NEPHROPATHY

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ABSTRACT

Non – steroidal anti – inflammatory drugs (NSAIDS) are the most commonly used analgesics globally. NSAID'S exert anti-inflammatory, analgesic & antipyretic effects by decreasing prostaglandins (PG) synthesis through inhibition of enzyme cyclooxygenase (COX). They majorly target Gastro intestinal tract & Kidney and results in many complications such as gastritis, nephritis ending up even in chronic renal failure (CRF) and end stage renal disease (ESRD). Renal effects of NSAID's include decreased GFR, abnormal renal excretion. It was known from the studies that the incidence of usage of NSAID's increasing from December 2015 to 2017, out of 1830 patients on analgesics, 49 are induced with nephropathy. This case report illustrates about the patient who is on NSAID's since 3 months for the treatment of chronic inflammatory polyarthritis had developed chronic kidney disease. This article summarizes how NSAID's may affect the kidney functioning under clinical conditions like polyarthritis.

KEY WORDS: *Non steroidal Anti Inflammatory Drugs, Polyarthritis, Analgesic, Nephropathy, Chronic Kidney Disease.*

1. INTRODUCTION

Non steroidal anti-inflammatory drugs (NSAIDs) are very commonly prescribed analgesics worldwide. Most of the people widely use these drugs in the treatment of pain, fever with an estimation usage of greater than 30million people/day.¹ NSAIDs majorly target Gastro intestinal tract & Kidney and results in many complications such as gastritis, nephritis ending up even in chronic renal failure (CRF) and end stage renal disease (ESRD).² NSAID'S exert anti-inflammatory, analgesic & antipyretic effects by decreasing prostaglandins (PG) synthesis through inhibition of enzyme cyclooxygenase. It exists in two isomers i.e., COX-I & COX-II .Both enzymes promote PG's synthesis that promote inflammation, pain & fever. Cox-I produce PG's that activate platelets and protect the stomach and intestinal linings ,while Cox-II produce PG's and results in inflammation. Non selective NSAID'S inhibit both COX-I & COX-II leads to suppression of PG's levels. PG (PGE2, PGI2) has major role in maintaining the glomerular filtration rate (GFR), hence in case of usage of NSAIDs inhibitionof these PG synthesis results in decreased renal blood flow & decreased GFR which will affect the kidney functioning .² Renal hemodynamic & GFR is mainly affected and controlled by COX-I & while COX-II maintain the salt and water excretion .³ In case of NSAID'S usage people inhibit both COX-I&II results in the alterations in the renal hemodynamic and GFR rates. Therefore the chronic use of NSAID'S have an increased risk of developing acute and chronic renal failure. NSAID'S induced nephropathy is a disease resulting from the frequent use of NSAID'S medications leading to significant impairment of renal function .² Approximately 2.5 million Americans experience NSAID's induced renal effects². A data analysis conducted on about 3,600 patients who are using NSAID's it was reported that 27.88% was suffering with renal complications(nephropathy, acute kidney injury, chronic kidney failure , nephritis)⁴. NSAID's produce many side effects which include stomach bleeding , heart bur ,stomach ulcers, (mainly associated with intake of steroids like prednisone) dizziness, ringing in the ear, liver problems, leg swelling , high blood pressure.⁵ Patient in our case study using NSAID'S since months having co morbidities like chronic inflammatory polyarthritis resulted in chronic kidney failure (nephropathy).

2.CASE REPORT

A 47 years male patient was admitted in the special ward at manipal hospital with chief complaints of decreased appetite, decreased intake of food, low grade fever, red color urine, (hematuria) dark color stools (Melina) since one month, vomiting associated with blood (hematemesis). Patient had past history of shortness of breath and pitting type of edema in feet since 3 days (side effect of NSAID's) and known case of chronic inflammatory polyarthritis and been using ibuprofen and had no history of diabetes, hypertension, epilepsy. Past medication include tab wysolone 5mg and NSAID's on consulting the local primary health centre. No significant family history. Personal history; he is a vegetarian, bowel and bladder found to be normal, sleep was adequate. He is a known smoker and alcoholic since 20 years. Vitals (Pulse; 98 beats/minute; blood pressure 130/80 mm of Hg; respiratory rate 21 beats/minute; temperature 98.5°F). Patient was found to be pallor positive. Systemic examination i.e., cardiovascular system, respiratory system, central nervous system and per abdomen was found to be normal. Hence the provisional diagnosis was done as chronic inflammatory polyarthritis and on NSAID's continuously resulted in nephropathy and mast cell activation syndrome synovitis positive, bilateral ankle edema. Ultrasonography of abdomen was examined and the report was found to be bilateral I/II renal parenchymal change, left renal calculus, bilateral pleural effusion, small umbilical hernia of size 12mm viral markers were negative. Clinical pathology of urine was examined and found to be as urine turbid and albumin +3.2-D ECHO was found to be normal. Biochemistry profile was found to be increased creatinine, uric acid, blood urea nitrogen. Prothrombin time-14.2 sec.

Table 1: Laboratory Investigations

Test	31/10/19	1/11/19	2/11/19	3/11/19	4/11/19	5/11/19	6/11/19
Serum creatinine	6.30mg/dl	6.50mg/dl	7.10mg/dl	6.3mg/dl	6.0mg/dl	5.5mg/dl	4.0mg/dl
Serum sodium	134mmol/l	136mmol/l	136mmol/l	136mmol/l	136mmol/l	136mmol/l	136mmol/l
Serum potassium	3.4mmol/l	3.8mmol/l	3.8mmol/l	3.8mmol/l	3.8mmol/l	3.8mmol/l	3.8mmol/l
Serum calcium	7.6mg/dl	-	-	-	-	-	-
Serum phosphate	-	8.9mg/dl	-	-	-	-	-
Serum albumin	2.2mg/dl	2.2mg/dl	-	-	-	-	-
Serum uric acid	11.6mg/dl	11.6mg/dl	11.0mg/dl	-	-	-	-
Blood urea nitrogen	53mg/dl	-	50mg/dl	-	-	-	-
RBC	2.80million cells/ Cub.mm	2.80million Cells/cub.m m	-	-	-	-	-
Hb	7.6g/dl	7.6g/dl	-	-	-	-	-
Heamatocrit	23.1%	23.1%	-	-	-	-	-
RDW	18.3%	18.3%	-	-	-	-	-
Platelets	2,80,000/cub. mm	2,80,000/cu b.Mm	-	-	-	-	-

Heamodialysis was done on 2/11/2019, 3/11/2019, 4/11/2019 and the creatinine levels were slowly decreased. The patient was symptomatic for the above conditions with proton pump inhibitor (injection pantop-40mg), antiemetic (injection zofer -4mg), antibiotics (injection monocef-1gm), antipyretic (tablet dolo-650mg), to decrease uric acid levels prescribed with tablet febuget-40mg, tablet perinorm-10mg, TID. The prescription was changed in place of ibuprofen tab. hydrochloroquine-200mg & low dose of tablet prednisone-5mg to treat chronic polyarthritis is prescribed.



Figure1: pitting type of edema

3.DISCUSSION

Based on subjective evidence like pitting type of edema (figure1) Melina, hematuria, hematemesis (figure2), and history of usage of ibuprofen & objective evidence like increased creatinine, BUN, uric acid levels, USG report the patient is finally diagnosed as NSAID's induced nephropathy and known history of chronic polyarthritis due to continuous NSAID (ibuprofen) intake.

3.1. MECHANISM OF NSAID's

The mechanism of NSAID's action is inhibition of cyclooxygenase enzyme (COX) both peripherally and centrally, thus interfere with the conversion of arachidonic acid into PGE₂, PGI₂ and thromboxane. Prostaglandins have a vasodilatation property which is very essential for preglomerular resistance, glomerular filtration rate maintenance and preserving renal blood flow⁶. NSAID's can inhibit either COX-I or COX-II. Based on these enzymes the NSAID's are classified as non selective NSAID's (aspirin, ketorolac, naproxen), these non selectively inhibit both COX-I & COX-II, next is preferential COX-II inhibitors (meloxicam, nimesulide, etodolac) and highly selective COX-II inhibitors (celecoxib). Most of the side effects of NSAID's are associated with COX-1 inhibition because it is associated with kidney functioning, the action of COX-II associated with water and electrolyte balance.⁶



Figure 2. Hematemesis

3.2. PATHOPHYSIOLOGY OF NSAID's-RELATED NEPHROPATHY

The kidneys are important organs for the excretory function of body. In order to adequately perform their filtration function, these organs have regulatory mechanisms such as prostaglandin synthesis, which maintain glomerular filtration rate (GFR) and renal homeostasis.⁷ In case of NSAID's it inhibits the production of arachidonic acid, either selectively or non-selectively, which affects prostaglandin synthesis (PGE₂, PGI₂, PGD₂ act on afferent arteriole and increase renal perfusion, with distribution of the cortex flow to nephrons in the renal medullary region) causes vasoconstriction, limit performance of rennin-angiotensin-aldosterone system which can lead to acute kidney failure followed by chronic condition.⁸ In addition to vasodilatation action of prostaglandins they also play a role in maintenance of sodium, chloride and water balance in body, by inhibition of prostaglandins (PGE₂) allow sodium and chloride transport in ascending loop of Henle and in collecting duct by inhibition of EP1 receptors leads to diuresis or abnormal urine production.

Longer usage of NSAID's showing long term effects on the kidney functioning. Some research data interpreted that daily use of NSAID's for more than one year increases the risk of developing chronic

kidney disease.⁹ There may be progression of the disease status if the patient doesn't discontinue the usage of NSAID's. Recent studies conducted on patients taking analgesics and people affected with drug induced nephropathy mentioned in table 2.

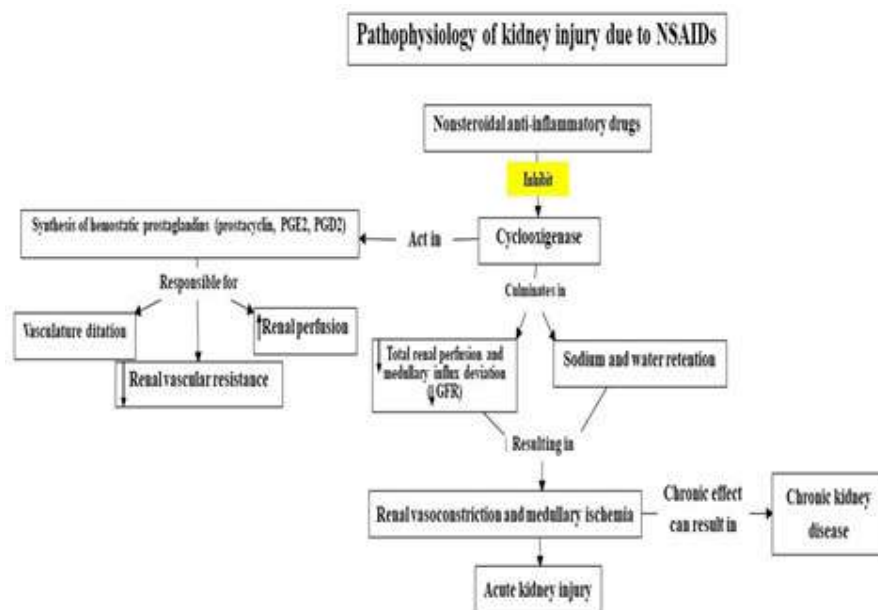


Figure3: The main effects of NSAID's on the function of kidney.

Table 2. Statistical analysis of patients on NSAID's and drug induced nephropathy.¹³

S.NO	Month/year	Patients on NSAID's	Drug induced nephropathy
1.	DEC 2015	21	1
2.	JAN 2016	152	7
3.	FEB 2016	112	2
4.	MAR 2016	180	9
5.	APRIL 2016	109	3
6.	MAY 2016	94	5
7.	JUNE 2016	81	2
8.	JULY 2016	170	1
9.	AUGUST 2016	1021	1
10.	SEPT 2016	1274	1
11.	OCT 2016	551	2
12.	NOV 2016	1107	3
13.	DEC 2016	1120	1
14.	JAN 2017	1050	4
15.	FEB 2017	910	6

CONCLUSION

Nowadays NSAID's represent the most widely used drugs in the clinical practice. Usage of NSAID's had increased from past few years. The acute and chronic kidney effects associated with the use of NSAID's have been extensively discussed in this case. NSAID's are associated with many side effects out of which nephropathy is the major side effect during recent times. Hence NSAIDS should be used with caution and periodical monitoring of renal function tests need to be done in long term usage.

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CLINICAL PROFILE AND OUTCOMES OF ORGAN DYSFUNCTION AMONG SEPSIS PATIENTS: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Sepsis is outlined as life-threatening organ dysfunction caused by a dysregulated host response to infection. It's become the foremost necessary explanation for Multiple Organ dysfunction Syndrome (MODS). The present study was an attempt to analyze the clinical characteristics of the sepsis patients, their symptoms, and the origin of infection, co-morbidities and outcomes of sepsis with organ dysfunction. It was a prospective observational study conducted for about 6 months. Data was collected from the ICU and analysed using SPSS software version 20.0. In the present study a total of 51 cases were enrolled. Mean age of patients was 59.74 years. Male and females were 37 and 14 respectively. Most common symptom was found as dyspnoea and fever. Cellulitis was found as the most common cause of infection with incidence rate of 32% followed by pneumonia 24%. All the cases some kind of organ dysfunction present among which respiratory, CVS and renal dysfunction were predominant. Diabetes mellitus was the most common comorbid condition in these cases. Sepsis is a vulnerable disease can affect any age group but it is found to be more mortal in elders with less significance value and more comorbidity. The overall mortality of sepsis was found to be 29%.

KEYWORDS: *Sepsis, Organ dysfunction, Mortality rate, SOFA score.*

1. INTRODUCTION

Sepsis is outlined as life-threatening organ dysfunction caused by a dysregulated host response to infection.¹ It's become the foremost necessary explanation for Multiple Organ dysfunction Syndrome (MODS).² Though sepsis is vulnerable regardless of the cohort effect, elders (>65yrs of age) were found additionally liable to MODS, bigger mortality rates and fewer seemingly to be survived.³ There's a scarcity of information from India that describes the epidemiology of sepsis and its clinical characteristics concerning organ dysfunction. It is difficult to ascertain the global epidemiology of sepsis yet it is estimated to affect greater than 30 million people worldwide every year and causing 6 million deaths among. Anyone having an infection can be lead to sepsis conditions but some populations such as the elderly, pregnant women, neonates, diabetics, people with HIV/AIDS, liver cirrhosis, kidney disease, and autoimmune diseased patients are at higher risk⁶. Pathophysiology of sepsis is complex with varied host response to the invading pathogen.² It contributes to control the infection as well as the tissue damage that leads to organ dysfunction.⁴ Severe sepsis can lead to a drastic decrease in blood flow to the major organs inducing septic shock, organ dysfunction, and certain death⁵. The present study was an attempt to analyze the clinical characteristics of the sepsis patients, their symptoms, and the origin of infection, co-morbidities and outcomes of sepsis with organ dysfunction.

2. METHODOLOGY :

It is a prospective observational study carried out in the intensive care units of a tertiary care hospital in Vijayawada in the state of Andhra Pradesh from April 2019 to November 2019. Ethical approval was obtained from the Institutional Ethics Committee (IEC). In this study, a total of 51 patients diagnosed with sepsis satisfying the inclusion and exclusion criteria.

2.1 Inclusion Criteria (According to International Sepsis Definitions Conference criteria.⁷)

- Male or Female (age >18 years) with clinical and laboratory evidence of sepsis.

As per ACCP/SCCM guidelines¹⁹, septic shock was characterized as sepsis with hypotension (i.e., MAP <70mmHg) for at least one hour even with adequate fluid resuscitation.

2.2 Exclusion Criteria

- Children & Pregnant and lactating women

Data collected were tabulated and statistically analysed by using SPSS Version 20.0 for windows.

3. RESULTS & DISCUSSIONS

In the current study, a total of 29 patients fall at the age group of 50-70 years followed by 11 cases aged >70 years and the least (3%) are aged less than 30 years. Around 74% of the patients are male and 26% are females affected with sepsis. In contrast to our study, Bhattacharya PK et al and Gaieski DF et al had found that majority of patients are in age group of >65 years^{8,9} and Paary et al and Todi et al had found that male patients (64.2%) and female patients (57.71%) in their studies which is similar to our study.^{10,11} (TABLE 1)

Table 1: Age and Sex Distribution

Age (in years)	Male (%)	Female(%)	No. of Patients (n=51)
<30	2(5%)	1 (7)	3
30-50	7 (19)	1 (7)	8
50-70	21(56)	8 (57)	29
>70	7 (19)	4 (29)	11
Grand Total	37 (74%)	14 (26%)	51

In this study, a total of 68% of patients were to have sepsis among which 82.3% have survived and 17.6% were expired. Around 24 % of cases have severe sepsis among which 41.6% patients have survived and 58.3% people died. Septic shock patients were around 12% among that majority of patients expired due to cardiac arrest.(TABLE 2)

Table 2: Distribution of Sepsis

DIAGNOSIS	SURVIVAL STATUS		
	Expired	Survived (%)	Grand Total (%)
Sepsis	6 (17.6)	28 (82.3)	34(68)
Septic shock	7 (58.3)	5 (41.6)	12(24)
Severe sepsis	2 (33.3)	4 (66.6)	6(12)
Grand Total	15	36	51

Mortality rates of each patient were assessed using Sequential Organ Failure Assessment (SOFA) Score. Maximum mortality rate was seen in male patients with mortality rate of 74% and female have less incidence and as well as less mortality rate 28% in our study.(TABLE 3)

Table 3: Percentage Mortality in Males and Females

% MORTALITY	No. of Patients		
	Female	Male	Grand Total
<10%	0	3	3
SURVIVED	0	3	3
15-20%	7	11	18
SURVIVED	7	11	18
40-50%	2	9	11
EXPIRED	1	1	2
SURVIVED	1	8	9
50-60%	3	6	9
EXPIRED	2	2	4
SURVIVED	1	4	5
>80%	0	2	2

EXPIRED	0	2	2
>90%	2	6	8
EXPIRED	2	5	7
SURVIVED	0	1	1
Grand Total	14	37	51

Fever was the most common symptom present in all cases of the sepsis patients followed by dyspnoea, decreased urination and altered sensorium in 86%, 71%, 43% and 37% respectively.(TABLE 4).

Table 4: Distribution of Symptoms Among Cases of Sepsis

Symptoms	No. of Patients	%
Fever	44	86%
Dyspnoea	36	71%
Pain	9	18%
Swelling	13	25%
Vomiting	10	20%
Diarrhoea	7	14%
Altered sensorium	19	37%
Decreased Urination	22	43%
Jaundice	15	29%
Chills	10	20%

In the present study, the major cause of sepsis was found to be cellulitis with the incidence of 32% followed by pneumonia 24%, UTI (10) and 22% of unspecified cause of infection. Mortality was highest in cellulitis with 14% followed by pneumonia remaining have lesser mortality rates ranging from 2-4%. TTS Paary et al, had observed that source of infection in the respiratory tract (37.2%) whereas Simon et al, Karlson et al, Engel et al, showed different sources of infections.^{10,12-15} In this study, cellulitis occupies the highest mortality rate whereas Leligdowicz A et al, reported ischemic bowel disease as the common cause of mortality among severe sepsis cases.^{16,17}(TABLE 5)

Table 5: Mortality Based on Source of Infection

SOURCE OF INFECTION	MORTALITY		
Source of Infection	Expired (%)	Survived (%)	Grand Total (%)
Bed Sore	1(2)	0	1(2)
Cellulitis	7(14)	9(18)	16(32)
Gastroenteritis	0	1(2)	1(2)
Peri Anal Abscess	0	2(4)	2(4)
Pneumonia	2(4)	10(20)	12(24)
Splenic Abscess	1(2)	0	1(2)
Trauma	1(2)	1(2)	2(4)
Unspecified	2(4)	9(18)	11(22)
UTI	1(2)	4(8)	5(10)
Grand Total	15(29)	36(71)	51(100)

In the current study, diabetes mellitus was the most common comorbidity, present in 33 patients(65%) followed by hypertension in 23 cases(45%), 13(25%) patients was found to have cardiac diseases, 15(29%) patients were renally impaired. Bhattacharya PK et al and Martin GS et al also found Diabetes mellitus as the most common comorbid condition in their studies.^{8,18} (TABLE 6)

Table 6: Comorbidities with Sepsis

CO-MORBIDITES	NO.OF PATIENTS (%)
Diabetes	33(65)
Hypertension	23(45)
Cardiac Diseases	13(25)

Cerebrovascular Disease	4(8)
Kidney Disease	15(29)
Liver Disease	5(10)
Thyroid Disorders	4(8)
Pulmonary Disease	7(14)
Anaemia	3(6)
Cancer	3(6)
Psychological	2(4)
Others	4(8)
None	1(2)

There were around 28% of patients suffering with two comorbidities, 26% patients have three comorbidities, 24% of patients have single comorbidity 16% patients have more than 3 comorbidities where 8% patients do not have any comorbidities.

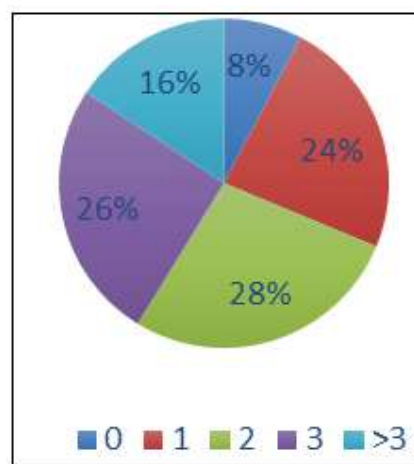


Fig 1: Multiple comorbidities

Here, organ dysfunction in male was highest than in females. Haematological system changes were found in 43(84%) of cases followed by respiratory system with incidence of 37(73%) patients, cardiac dysfunction in 28(55%) patients. Renal dysfunction in 25(49%) patients. Mortality was higher with increase in number of organ dysfunction. This is similar to the result observed by Bhattacharya PK et al.¹⁶ (TABLE 7).

Table 7: Outcomes of Organ Dysfunction with Percentage Mortality

Organ Dysfunction	Survived	Expired	Total (%)
CVS	19	9	28(55)
Respiratory	28	9	37(73)
Renal	15	10	25(49)
Hepatic	5	5	10(20)
Haematological	29	14	43(84)

In our study, mortality was lowest in single organ dysfunction due to low incidence and highest with 28(39%) when more than two organ dysfunction was present. There was a statistical significantly increase in mortality with a progressive rise in number of organ dysfunctions.(TABLE 8)

Table 8: Multiple Organ Dysfunction Due to Sepsis

Organ Dysfunction	Total	Survived	Expired	%Mortality
Single Organ Dysfunction	4	3	1	25%
Two Organ Dysfunction	19	16	3	15%
More than two Organ Dysfunction	28	17	11	39%

Grand Total	51	36	15	79%

From this study, it is observed that there is no significant relation between age and mortality in sepsis cases. Mortality has a significant correlation with No. of comorbidities, as the comorbidities increases there is an increase in mortality rate and multi organ dysfunction. Sepsis mortality can also be related with no. of organs dysfunction due to it. There was a rise in mortality in patients with advanced age associated with organ dysfunction.^{26,9}

Table 9: Correlation With Mortality

PARAMETER	MEAN(SD)	P Value
Age	59.74 (14.41)	0.078
No. of Comorbidities	2.196 (1.233)	0.007
Multi Organ Dysfunction	2.823 (0.973)	0.005
LOS	4.98 (2.64)	0.381

4. CONCLUSION

Pathophysiology of sepsis is complex and multifaceted. In severe conditions sepsis can lead to septic shock, multi organ failure and death. In the current study, number of females was less compared to males yet mortality rate was higher in females. Cellulitis was the most common cause of infection. Respiratory system was the most common organ dysfunction. Mortality has no significant correlation with age but it is related with number of comorbidities and number of organ dysfunction. It is clear that the presence of organ dysfunction has an impact on severity and outcomes of sepsis and can modulate prognosis and decrease survival.

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A REPORT ON DRUG RELATED PROBLEMS IN CHRONIC KIDNEY DISEASE PATIENTS ADMITTED IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Drug related problems were found to have higher incidence among chronic kidney patients due to polypharmacy. A Drug-Related Problem is an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes. The main objective of our present study was to identify number and type of drug related problems and to demonstrate the importance of clinical pharmacist intervention in ensuring rational drug therapy and improving therapeutic outcome in chronic kidney disease patients. A Prospective observational study was carried out in chronic kidney disease patients of 350 bedded private multispecialty hospitals. The type, Number and incidence of DRP's were identified and documented according to PCNE classification v.6.2. A total of 152 patient case sheets were evaluated during study period of 6 months, out of which 224 DRPs were identified in 108 patients. The most common drug related problem was therapeutic failure due to drug interactions, overdose (11.53%), and mild incidence of adverse drug reactions. The current study demonstrates that majority of drug related problems are preventable. So continuous identification, monitoring and intervening improves therapeutic outcome and patient quality of life.

KEYWORDS: *Drug related problems, Intervention, Chronic kidney disease, Polypharmacy*

INTRODUCTION

Kidney is a bean shaped organ weighs about 150 to 300gms in male and 120 to 125 mg in female. The kidney usually located superiorly on top of adrenal gland, the tail of the pancreas lies medial to the left renal hilum, and the colon is present anterior to both kidneys and posteriorly with diaphragm. The main function of kidney is filtration and excretion of waste products such as ammonia and urea. Also, regulation of blood volume and blood pressure by renin Angiotensin Aldosterone system. Hormones such as erythropoietin stimulates red blood cells production is a regulatory function of kidney.¹ Kidney diseases are one of the global health problems requiring early detection and treatment to prevent its progression. According to the national kidney foundation reports about 10% of population in worldwide is affected by chronic kidney diseases. In the age of 64, one in every five men and one in every four women are suffering from chronic kidney diseases.² Over 2million had received treatment for kidney failure by kidney transplantation, dialysis but they are still alive. According to global burden of disease study 2010, chronic kidney diseases places at 27th rank.³ chronic kidney disease is a progressive loss of kidney function over a period of time. Each kidney consists of millions of tiny filters called nephron. If these nephrons are damaged, blood supply is reduced which may lead to kidney failure. Early diagnosis there is no problem but untreated it may lead to life threatening conditions. The decreased kidney functions lead to many complications like metabolic abnormalities, endocrine complications and increased risk of cardiovascular diseases.⁴ Diagnosis of CKD is by blood tests, measuring glomerular filtration rate, ultra sound method is a underlying caused diagnosed method and urine test.⁵ In the year of 2000, National kidney foundation had appointed an advisory board for development of clinical practice guidelines to classify stages in chronic kidney diseases.⁶ Drug related problem is an event (or) circumstances involving drug treatment that interferes with patient achieving an optimum outcome of medical care. DRP'S starts from prescribing phase to drug dispensing phase. DRP'S may also occur due to improper collection of family history and patient follow up medication. DRP'S may occur in paediatric, geriatric population and co morbid conditions.⁷ Detection and prevention of DRP'S can save lives along with enhancing patients and quality of life and optimizing health care costs. In January

1999, Pharmaceutical Care Network Europe was classified a scheme for drug related problems. This classification set consists of scheme, reporting forms and cases for training and validation. PCNE is used for prevalence and incidence of DRP'S. PCNE contains 16 domains such as 3 primary domains for problems, 8 primary domains for causes, 5 primary domains for interventions. In 2000, a scale was added to solve the problems, has 4 primary domains and 7 sub domains.⁸ The present study aims to report the incidence of DRPs in Chronic Kidney Disease (CKD) patients and also assess the severity of the reported adverse drug reactions.

2. METHODOLOGY:

2.1. Study design and duration of the study

It was a Prospective Observational study conducted at Manipal Super speciality Hospitals, Vijayawada, and Andhra Pradesh for a period of six months from October 2017 to March 2018. Patients of either sex admitted to the hospital during the study period were included in the study.

2.2. Inclusion Criteria

Patients of age >18 years of either sex, with two or more drugs prescribed and with length of stay >2 days were included in the study.

2.3. Exclusion criteria

Patients below 18 years age, with only one or two drugs prescribed, Length of stay < 2 days and Pregnant women were excluded from the study.

2.4. Source of Data: All the necessary and relevant data were collected during clinical rounds in consultation with the treating physician.

S.NO	Source	Data Collected
1.	Patients history Record	Relevant history with diagnosis, laboratory reports of investigations ordered and past history regarding drugs are collected
2.	Treatment Chart	List of all the medications prescribed, along with dose, frequency, route and duration of treatment
3.	In patient follow-up records	Disease course, development of new signs and symptoms and change in laboratory repeat investigations if any

2.5. Study procedure

2.5.1. Data collection

Regular ward rounds were carried out in the hospital during the study period and all the necessary information from the case sheets of patients was collected using a data collection form. A total of 152 cases had been collected during the study period.

2.5.2. Data Analysis

The collected data from the prescriptions was thoroughly analysed and screened for possible drug related problems using the Lexicomp (Up-to-date software), Medscape, and Drugs.com. Principal researcher (pharmacist) identified the occurrence of DRPs through the patient interview, discussion with nurses and assessment of patients' medication charts and medical records. The identified DRPs were validated and classified using Pharmaceutical Care Network Europe/PCNE classification scheme for drug-related problems 6.2. According to PCNE DRP classification scheme, DRPs were categorised into four primary domains for DRP problems and eight primary domains for DRP causes. The rate of DRPs was defined as the rate of DRPs per 76 medication orders and the number of DRPs per each patient. Further, DRPs were divided into actual and potential DRPs. The suspected ADRs were analyzed to establish causal relationship with suspected drug using various standard scales like Naranjo's probability scale to categorize them as certain/definite, probable, possible, unassessable/unclassifiable, unlikely, conditional/ unclassified, conditional etc., Hartwig et al. severity scale, preventability scale viz. Modified Schumock and Thornton scale which categorized as definitely preventable, probably preventable and not preventable.

RESULTS & DISCUSSION

Table 1. Incidence of DRPs Gender wise distribution

Table 2. of DRPs different	S.No	Gender	Number of Patients	Number of Patients With DRPs	Incidence (%)	Incidence among age groups:
	1	Male	96	74	77.08	
	2	Female	56	34	60.71	
S.No	Age Group		No Of Patient's	No Of Patient's With DRPs	Incidence	
1	Adults		84	68	80.9	
2	Geriatrics		68	40	58.8	

Table 3. Distribution of patients based on number of co morbidities

No of Co-Morbidities	No of Patients	Percentage of Patients(%)
None	15	9.86
1	36	23.68
2	66	43.42
3	12	16.44
4	05	6.57

Table 4 Type of Drug Related Problem

S.No	Type of Problem	Frequency(Percentage%)
1	P1- Treatment effectiveness	70(31.25)
2	P2- ADR'S	4(1.78)
3	P3- Treatment Costs	20(8.92)
4	P4- Others	18(8.03)

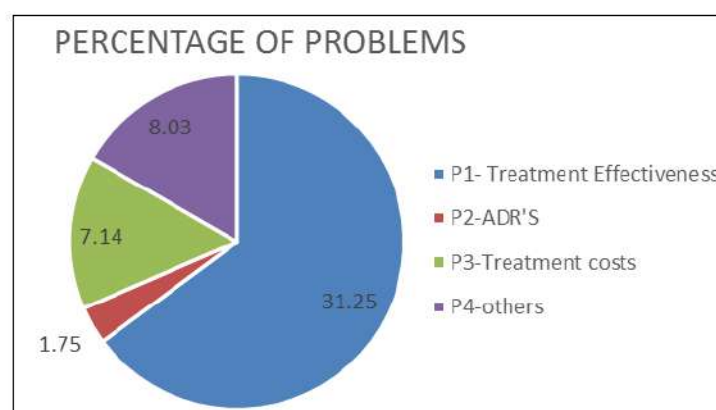


Table 5 Causes of DRPs

S.No	Causes Of DRP'S	Number And Percentage (%)
1	Drug selection	64(28.57)
2	Dosage form	0(0)
3	Dose selection	34(15.17)
4	Treatment duration	4(3.57)
5	Treatment compliance	0

6	Others	10(4.46)

Table.6 Causality Assessment of Reported Adverse Drug Reactions (ADR's)

	Definite/ Certain	Probable	Possible	Unassesable/ Unclassified	Unlikely	Conditional
Naranjo's probability scale	0	0	4	N/A	0	N/A

Table.7 Severity Assessment of Reported Adverse Drug Reactions (ADRs)

Hartwig Scale	Severity	No of ADR'S	% of ADR'S
Level 1		0	0
Level 2		0	0
Level 3		2	50
Level 4		2	50
Level 5		0	0
Level 6		0	0
Level 7		0	0

Table.8 Preventability Assessment of Reported Adverse Drug Reactions (ADRs)

Modified Schumock and Thornton Scale	No of ADR'S	%ADR'S
Definitely preventable	4	100
Possibly preventable	0	0
Not preventable	0	0

Drug-related problems are the most common health care problems which are responsible for the increased morbidity and even mortality on some patients and increased health care costs.⁹ A total of 152 patients with chronic kidney disease (CKD) were enrolled in the study out of which 108 patients were found with 224 DRPs. Fasipe Olumuyiwa et al., in their study in Nigeria reported that the treatment of chronic kidney disease (CKD) patients often requires the use of multiple drugs due to high number of comorbidities associated with the kidney disease like Diabetes Mellitus, Hypertension etc. Use of more number of drugs predispose to severe DRPs like drug-drug interactions which may increase the risk of morbidity, mortality, length of hospital stay and health-care costs.¹⁰ In this study, the incidence of DRPs was highest among male patients (77.08%) among the age group adults which was similar to the result of other research studies where adults above the age of 50 years were the most affected people with Renal disease.^{11,12} In the present study patients with at least two co morbidities were highest (43.42%) and with no co morbidities were (9.86%). Hence it was found that chronic kidney disease is associated with other co morbidities. The reported DRPs were classified using PCNE guidelines version 6.2 and given codes (P1, P2, P3, P4) accordingly. Treatment failure (31.25%) was found to be the most common type of DRP in CKD patients followed by treatment cause, and ADR's accounting the least (1.78%). Drug selection (28.57%) and dose selection (15.17%) were observed to be the most common causes of DRPs in CKD patients. This study result matches with the study conducted by Dlearhassanossman, et al., in Research gate, June 2015 which reported that under dose (29%) and treatment failure (27.7%) were the most common DRPs.¹³ Different drug dosage recommendations had been approved by United States Food and Drug Administration (FDA). Despite their availability it was revealed in a study conducted by Rachel W. Flurie, et al., in ASN Kidney News Journal that as many as 19 to 69 percent of drugs prescribed for elderly CKD patients exceed the recommended dose. Thus, it is very important that physicians need to monitor the renal function of the elderly CKD patient, identify the need for dose modification since there would be alterations in the pharmacokinetic parameters in elderly when compared to the others and then prescribe the correct dosage regimen based on the approved resources.¹⁴ Treatment effectiveness and adverse reaction domains contributed to the majority of DRPs in CKD adults in a study conducted by Hesty u. Ramadaniati, et al.¹⁵ where as in a study conducted by B. Kovacova, et al., in Pharmazie it was reported that the most frequent DRPs were clinically significant drug-drug interactions (n = 121; 23.5%), incomplete or missing drug history (n = 73; 14.2 %) and inappropriate

time of administration (n = 67; 13.0 %).¹⁴Causality, severity and preventability assessment of reported ADR's was done by using naranjo's probability scale, Hartwig severity and Schumock and Thornton scale respectively which confirmed that all the observed ADR's were possible, level 3 and 4 severity and definitely preventable. Studies showed differences in the incidence of type of DRPs among CKD patients but it was evident from most of the studies that the risk of DRPs was higher among renal patients due to their associated co morbidities. The present study also reports the incidence of DRPs in CKD patients is higher.

4. CONCLUSION

Chronic kidney disease was the most common problem encountering the adults aged above 50 years of age. The incidence of drug related problems is higher in these patients since CKD is associated with the use of multiple drugs due to many associated co morbidities and alterations in pharmacokinetic parameters. Hence proper dosage regimens should be followed to minimise the treatment effectiveness failure domains. Other potential effects like adverse drug reactions were found to be 100 percent possible and preventable and are of level 2 and level 3 severities which can be controlled by proper monitoring of the patients during the therapy. So continuous identification, monitoring and intervening improves therapeutic outcome and patient quality of life.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

Authors declare no conflict of interest

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CASE REPORT ON THERAPY RELATED MYELOYDYSPLASTIC SYNDROME-EB2

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ABSTRACT

Myelodysplastic syndrome is a heterogeneous group of haematological disorders. It is mainly characterized by cytopenias. It carries a high risk of progression to acute myeloid leukemia. MDS commonly presents with symptoms related to anemia, and approximately two-thirds will develop thrombocytopenia, a rare, but potentially lethal complication that increases complexity in treatment and morbidity, and may be due to unique genetic mutations leading to refractory thrombocytopenia, ultimately leading to an overall reduction in survival. here we present a 59 years old male patient admitted with the complaints of fever, exertional breathlessness, left eye peri orbital swelling and burning, right thigh swelling and burning. On basis of peripheral smear, HRCT (High Resolution Computed Tomography) & Bone marrow examination he was finally diagnosed as MDS-EB2.

KEYWORD: Myelodysplastic syndrome, haematological disorders, cytopenias, HRCT, MDS-EB2

INTRODUCTION

Myelodysplastic syndrome is a heterogeneous complex group of clonal stem cell disorder that mainly describes the ineffective and dysplastic hematopoiesis resulting in apoptosis in the bone marrow and peripheral one or more cytopenia and varying of predisposition to develop acute myeloid leukemia^{2,3} MDS mainly arise without primarily instigating event or may be related to previous treatment with either radiation or chemotherapy. In incidence in the general population has been reported as five new MDS diagnoses per 100,000 people. It affects men frequently than it does women and incidence rate increased with age. At present, the majority of MDS patients are aged >60 years.⁵⁻⁶ Epigenetic mutation is reported as founder mutation in case of MDS. Beyond age, other risk factors include male gender, obesity, smoking and prior receipt of radiation or chemotherapy, but most of cases remain idiopathic. cytogenetically, MDS patients fall into three groups they are Normal karyotype, Balanced chromosome abnormality causing generation of the fusion oncogenes (Bcr-Acl, PML-RAL alpha, -5, del (5q), -7, +8, 11q-, 13q-, etc), complex karyotype. To diagnose the patient who developed MDS Immunophenotype, cytogenetic analysis and peripheral blood smear, Bone marrow aspiration, are used for confirmation of MDS^{7,8}. There are several types of MDS, including refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, refractory cytopenia with multilineage dysplasia, and myelodysplastic syndrome associated with an isolated del (5q) chromosome abnormality (5q-syndrome).⁹

Case summary

History of present illness: A 59 years old male patient admitted with a history of left eye periorbital swelling and burning, right thigh swelling and pain.

Chief complaints- fever for 3 days, exertion breathlessness.

Past medical history: The patient has history of DM, TB (treated with ATT for 9 months, 1 month before diagnosis of MDS-EB2), Bilateral pneumonia (2 months after diagnosis of MDS-EB2).

Past medication history: He received 3 cycles of chemotherapy and on the 4th cycle he received INJ.AZACYTIDINE-100mg

Social history: He is a non-smoker and alcoholic, with no illicit substance and toxic exposure.

Physical examinations:

General: Well dressed and well-nourished. In no acute distress.

Vital signs: Temperature: 100F°; BP:120/80mmhg,PR:92/min,RR:20/min and his height,weight found to be as 160 cm & 60 kg.

Head Ears Eyes Nose Throat: No pallor, icterus. Oropharynx clear.

Neck: Supple, no jugular venous distention, no significant lymphadenopathy.

Respiratory: Clear to auscultation.

Cardiovascular: Regular rate and rhythm, no murmurs.

Abdomen: Soft, no tenderness and good bowel sounds in all 4 quadrants. No organomegaly.

Extremities: No edema, cyanosis, clubbing.

Diagnostic procedures and lab investigations: On admission, physical examination was normal except for temperature-slightly increased up to 100°F. complete blood count revealed that Hb:7.4gm/dl (last month it is 4.9gm/dl) platelet count:2, 000cells/cu.mm, packed cell volume(PCV):23.3%.serum enzymes are found to be as follows:Bilirubin (direct):0.55mg/dl, serum albumin:2.7gm/dl, serum globulin:4.3gm/dl,serum creatinine:0.6mg/dl,serum sodium:132mmol/lit.lab details found to be as-prothrombin time(PTT):18.8sec,fibrinogen:505.4mg/dl,HRCT of chest done,in that the findings are like-fibrotic lesions in apical segments of both upper lobes,ground glass lesions are seen in both lower limbs and in right upper lobe (right>left),multiple enlarged pre tracheal,pre-vascular,carnial lymph nodes noted largest measuring 18×12mm,bone marrow examination reveals trilineage dysplastic with excess blasts and bone marrow smear examination reveals 8%blast; 23%myelocyte; 14%metamyelocyte,megakaryocytes are reduced and granulopoiesis is increased, during the stay he received 5 units of packed red cell,3 units of platelets (SDP), 8units of (RDP) transfusion, MRI study of orbits shown that subcutaneous edema in left zygomatic region, mild perceptual edema in left orbit, minimal mucosal thickening in bilateral maxillary and ethmoid sinuses and screening of brain shows that the fluid in bilateral mastoid air cells,flair hyperintense foci in bilateral centrum semiovale, corona radiata with no diffusion restriction small vessel ischemic changes. For the thigh (right) ultrasound is done, it shows the evidence of bulky biceps femoris & semi membranous semi tendinosis muscular on focal region)

DISCUSSION

Myelodysplastic syndromes are well-established conditions in adult patients. Most authors agree that such syndromes are related to the transformation of a multipotent stem cell or of a pluripotent progenitor, which acquires a proliferative advantage, while maintaining the daughter cell's ability to differentiate, giving rise to monoclonal population of mature cells. Bone marrow and circulating cells can exhibit various functional or morphological abnormalities⁹. Diagnosing MDS is difficult because of the similarities with other hematologic disorders including acute myeloid leukemia (AML), hypoplastic anemia and hereditary sideroblastic anemia. However, research efforts have been made to uncover the disease mechanisms that lead to MDS.¹⁰ MDS is complicating, deadly set of bone marrow disorder for which there are no easy cures. Most patients in the initial stages of MDS experience anemia. Anemic patients typically have pale skin and experience fatigue along with shortness of breath.¹¹ For proper treatment, proper diagnosis is needed, for that classification is done by WHO in that several major changes have been made in the categorization of myelodysplastic syndromes (MDS) in the fourth edition of the WHO classification. These changes include the incorporation of refractory cytopenia with uniline age dysplasia (RCUD) and refractory cytopenia of childhood (RCC) as separate entities. There are 7 different types are present in this category. These diseases not already mentioned include refractory anemia with ringed sideroblasts, refractory cytopenia with multilineage dysplasia, refractory anemia with excess blasts (RAEB-1 and 2), MDS with isolated del (5q), and MDS unclassifiable.^[11] The category of RAEB-1 also has been redefined to include cases with 2–4% blasts in the PB to emphasize the prognostic significance of increased blasts in the PB. A list of cytogenetic abnormalities that are considered presumptive evidence of MDS in the presence of a refractory cytopenia but without morphologic evidence of dysplasia also has been introduced in the current WHO classification. The common symptoms of Myelodysplastic syndrome include fever, shortness of breath, frequent infections all these symptoms are seen in this patient, so the symptoms are related to myelodysplastic syndrome.¹¹ The common diagnostic tests that are done for diagnosing the myelodysplastic syndrome was Bone marrow examination, complete blood count, mean corpuscular volume.¹³⁻¹⁴ for this patient these all tests were done and all are showing a positive reaction to myelodysplastic syndrome. Bone marrow smear examination reveals trilineage dysplastic with excess blasts and reveals 8% blast; 23% myelocyte; 14% metamyelocyte, megakaryocytes are reduced and granulopoiesis is increased. Complete blood count revealed that Hb: 7.4gm/dl (last month it is 4.9gm/dl) platelet count: 2,000cells/cu.mm, packed cell volume(PCV): 23.3%.serum enzymes are found to be as follows:Bilirubin(direct): 0.55mg/dl,serum

albumin: 2.7gm/dl, serum globulin: 4.3gm/dl, serum creatinine: 0.6mg/dl, serum sodium: 132mmol/lit.lab details found to be as-prothrombin time(PTT): 18.8sec, fibrinogen; 505.4mg/dl.¹⁵ The standard treatment for myelodysplastic syndrome is "Azacytidine", Supportive Therapy & Transfusions. For This Patient Drug Inj. Azacytidine is given, as patient is suffering from the shortness of breath, he was given with Neb. dulolol, budesonide, INJ. Deriphyllin and for this patient transfusion of 5 units PRC, 3 units SDP, 8 units RDP and regular monitoring of vitals and other lab investigations are done.¹²

CONCLUSION

The rational therapy of myelodysplastic syndrome with refractory anemia (MDS-RA) in hypertensive patients is a serious issue and needs an unusual intention of health professionals. In addition, it is required to check elderly patients for hypertension and close monitoring of blood pressure is recommended. Moreover, they are major obstacles for the therapy to cure this disease such as the high cost of anti-MDS medications and non-affordability. Most of the patients do not continue the therapy due to major side effects and their high cost. Therefore, drugs with low dose and high potency, high progress against the disease, affordable, and with fewer side effects will help in treating MDS patients in a better way. The goal of treatment for patients with truly lower-risk MDS is to improve symptomatic cytopenias and reduce the transfusion burden. For non-del (5q) MDS patients, treatment considerations include immunosuppressive therapy for those younger than 60 years, azacytidine, and lenalidomide for patients with adequate platelet and neutrophil counts. Clinical trials remain the standard of care at any point in managing lower-risk MDS.

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RECENT ADVANCES IN DENGUE TREATMENT AND MANAGEMENT

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ABSTRACT

Dengue has been an emerging threat to billions of people worldwide. Since the past 20 years this incidence has increased by four-fold and this trend appears to be continuing. This review article is based on dengue clinical features, epidemiology, pathogenesis, vaccine development, and prevention placed in a context of observations made over the previous half century. The dengue viruses are comprised of four serotypes DENV1 through DENV4, which are transmitted by urban cycle mosquitoes causing diseases whose severity, is influenced by the interacting factors such as age, virus, immunity of the host, and human genetic variability. DENV causes a variety of diseases in wide ranges from acute febrile illness associated dengue to life threatening dengue hemorrhagic fever or Dengue shock syndrome. Dengue fever is self limited though it causes illness, fever, headache, retro orbital pain, myalgia, arthralgia, and skin rashes. Whereas dengue hemorrhagic fever or dengue shock syndrome has similar characteristics as dengue fever followed by increased vascular permeability, thrombocytopenia, and abnormal flow of blood which may lead to vascular collapse and death. Based on recent evidences of in-vivo and in-vitro studies, the tissue damaging DENV non-structural protein 1[NS1] has emerged as the most prominent patho-physiological features of severe dengue. Hence, drugs directed at the viral targets can be used safely to reduce disease severity and fatalities are still needed to reduce the risk of dengue.

KEY WORDS: Dengue, flavivirus, dengue, drug discovery, antivirals, dengue prophylaxis, dengue therapeutics.

INTRODUCTION

Dengue fever is a mosquito-borne disease caused by the Dengue virus.¹ Symptoms usually begin in three to fourteen days. Once infection sets in it results in high fever, headache, vomiting, muscle cramps and joint pains.^{1,2} Recovery sometimes takes two to seven days. Dengue is carried by many species of female mosquitoes of the genus *Aedes*, and mainly *A. aegypti*.^{2,1} The virus has five species,⁴⁵ and infection with one kind generally provides long immunity. An immunogen for dengue has been approved and is commercially available in the market.³ Treatment of acute dengue fever requires administration of fluids either orally or intravenously for gentle or moderate disease conditions. For more severe cases, hospital admission is required.² Paracetamol (acetaminophen) is suggested rather than associated inflammatory non-steroidal anti-inflammatory drugs NSAIDs for fever reduction and pain relief in breakbone fever since there is an increased risk of harm from NSAID use.²⁶ Dengue has spread to more than one hundred ten countries.¹⁰ In 2013 it caused six million symptomatic infections worldwide.¹¹ In cities and cities; the virus is primarily transmitted by *A. aegypti*. In rural settings the virus is transmitted to humans by *A. aegypti* and totally different species of genus *Aedes* sort of *A. albopictus*. In all settings the infected lower primates or *humans greatly increase* the quantity of *current* breakbone fever viruses, during a method known as amplification.¹⁶

CLASSIFICATION

The World Health Organization's a pair of 2009 classification divides dengue fever into 2 groups: uncomplicated and severe.^{17,18} This replaces the 1997 WHO classification, that required to be simplified because it had been found to be too restrictive, although the older classification remains wide used including by the globe Health Organization's Regional workplace for South-East Asia as of 2011. Severe breakbone fever is outlined as that associated with severe injury, severe organ dysfunction, or severe plasma escape whereas all different cases area unit uncomplicated. The 1997 classification divided breakbone fever into undifferentiated fever, dengue, and breakbone fever viral infection^{20,19} breakbone fever viral infection was

divided more into grades I–IV. Grade I is that the presence solely of simple bruising or a positive patch take a look at in somebody with fever, grade II is that the presence of spontaneous harm into the skin, grade III is that the clinical proof of shock, and grade IV is shock so severe that pulse can't be detected.¹⁹ Grades III and IV area unit spoken as "dengue shock syndrome".^{18,19}

SIGNS AND SYMPTOMS

Typically, folks infected with dandy fever virus are symptomless (80%) or have solely gentle symptoms like uncomplicated fever.^{17, 21-22} Others have additional severe unwellness (5%), and during a little proportion it's dangerous.^{17,21} The period (time between exposure and onset of symptoms) ranges from three to fourteen days, however most frequently it's four to seven days. Children usually expertise symptoms the same as those of the respiratory disease and inflammatory disease (vomiting and diarrhea) and have a bigger risk of severe complications²³ though initial symptoms are typically gentle however body carries high fever.²²

CLINICAL COURSE

The characteristic symptoms of dengue fever are sudden-onset fever, headache (typically situated behind the eyes), muscle and joint pains, and a rash. The name for dengue fever, "breakbone fever", comes from the associated muscle and joint pains.²⁴ The course of infection is split into 3 phases: febrile, critical, and recovery.⁵ The febrile part involves high fever, over forty °C (104 °F), and is related to generalized pain and a headache; this typically lasts 2 to seven days.^{[24][25]} Nausea and vomit might also occur.²⁵ Rash is observed in 50–80% of these with symptoms²⁴ in the primary or second day of symptoms as flushed skin, or later within the course of unwellness (days 4–7), as a measles-like rash. A rash represented as "islands of white during a ocean of red" has conjointly been determined. Some petechial (small red spots that don't disappear once the skin is ironed, that are caused by broken capillaries) will seem at now, as might some gentle haemorrhage from the mucose membranes of the mouth and nose.²⁷ The fever itself is classically biphasic or saddleback in nature, breaking so returning for one or 2 days. In some folks, the unwellness issue to a essential part as fever resolves. During this period, there's escape of plasma from the blood vessels, usually lasting one to 2 days. This might end in fluid accumulation within the chest and bodily cavity likewise as depletion of fluid from the circulation and attenuated blood offer to very important organs.²⁵ There might also be organ dysfunction and severe haemorrhage, usually from the alimentary tract.²⁵ Shock (dengue shock syndrome) and hemorrhage (dengue haemorrhagic fever) occur in but five out of all cases of dengue; but, those that have been infected with alternative serotypes of dengue fever virus ("secondary infection") are at increased risk.

CAUSE

VIROLOGY

Dengue fever virus (DENV) is mainly transmitted by arthropods (mosquitos or ticks), and are arboviruses (arthropod-borne viruses).²⁷ The dengue fever virus order (genetic material) contains concerning eleven, ester bases, that code for the 3 differing types of supermolecule molecules (C, prM and E) that type the virus particle and 7 alternative non-structural supermolecule molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are found in infected host cells solely and are needed for replication of the virus.^[15] There are five strains⁴ of the virus, referred to as serotypes, of that the primary four are remarked as DENV-1, DENV-2, DENV-3 and DENV-4. The fifth kind was declared in 2013.⁴ The distinctions between the serotypes are supported their antigenicity.

MECHANISM

When a mosquito carrying dengue fever virus bites someone, the virus enters the skin along with the mosquito's secretion. It binds to and enters white blood cells, and reproduces within the cells whereas they move throughout the body. The white blood cells respond by manufacturing variety of signature proteins, like cytokines and interferons that are to blame for several of the symptoms, like the fever, the flu-like symptoms, and also the severe pains. In severe infection, the virus production within the body is greatly hyperbolic, and a lot of other organs (such because the liver and also the bone marrow) will be affected. Fluid from the blood leaks through the wall of tiny blood vessels into body cavities thanks to capillary porosity. As a result, less blood circulates within the blood vessels, and also the pressure becomes thus low that it cannot provide adequate blood to very important organs. Due to infection of the stromal cells platelet count decreases.³¹

VIRAL REPLICATION

Once within the skin, dengue fever virus binds to Langerhans cells.³¹ The virus enters the cells through binding between microorganism proteins and membrane proteins on the Langerhans cell, specifically the C-type lectins referred to as DC-SIGN, mannose receptor and CLEC5A.^[32] Meanwhile, the virus order is translated in membrane-bound vesicles on the cell's endoplasmic reticulum, wherever the cell's supermolecule synthesis equipment produces new microorganism proteins that replicate the microorganism polymer and start to make microorganism particles. Immature virus particles are transported to the vesicle, the part of the cell wherever a number of the proteins receive necessary sugar chains (glycoproteins). The currently mature new viruses are discharged by exocytosis. They're then ready to enter different white blood cells, like monocytes and macrophages.³² The initial reaction of infected cells is to provide antiviral drug, a protein that raises variety of defences against virus infection through the innate system by augmenting the assembly of an outsized cluster of proteins mediated by the JAK-STAT pathway.

SEVERE DISEASE

It is not entirely clear why secondary infection with a unique strain of dengue fever virus places folks in danger of dandy fever haemorrhagic fever and dandy fever shock syndrome. The foremost wide accepted hypothesis is that of antibody-dependent improvement (ADE). The precise mechanism behind potable is unclear. it should be caused by poor binding of non-neutralizing antibodies and delivery into the incorrect compartment of white blood cells that have eaten the virus for destruction.^{32,15} Severe illness is marked by the issues of capillary porosity (an allowance of fluid and supermolecule commonly contained among blood to pass) and disordered curdling.³⁴ These changes seem related to a disordered state of the epithelial tissue glycocalyx, that acts as a molecular filter of blood elements.²³

DIAGNOSIS

The diagnosing of dengue fever is usually created clinically, on the premise of rumoured symptoms and physical examination; this is applicable particularly in endemic areas.^[17] but, early unwellness will be tough to differentiate from different infective agent infections.^[20] A probable diagnosing is predicated on the findings of fever and 2 of the following: nausea and ejection, rash, generalized pains, low white blood corpuscle count, positive bandage take a look at, or any serious warning call in somebody World Health Organization lives in a pestilence space. Warning signs usually occur before the onset of severe dengue fever. The bandage take a look at, that is especially helpful in settings wherever no laboratory investigations square measure without delay obtainable, involves the appliance of a vital sign cuff at between the beat and blood pressure for 5 minutes, followed by the enumeration of any petechial haemorrhages;^{35,25} The earliest laboratory investigations could be a low white blood corpuscle count, which can then be followed by low platelets and acidosis.²⁰ A moderately elevated level of aminopherase (AST and ALT) from the liver is usually related to low platelets and white blood cells.^[23] In severe unwellness, plasma outpouring leads to hem concentration (as indicated by a rising haematocrit) and hypoalbuminemia.^[10] serous membrane effusions or pathology will be detected by physical examination once massive,¹⁰ however the demonstration of fluid on ultrasound could assist within the early identification of dengue fever shock syndrome.^[0,17] The use of ultrasound is proscribed by lack of accessibility in several settings.¹⁷ dengue fever shock syndrome is gift if pulse pressure drops to $\leq$ twenty millimetre of mercury at the side of peripheral vascular collapse.²³ Peripheral vascular collapse is decided in kids via delayed capillary refill, speedy vital sign, or cold extremities.²⁵

LABORATORY TEST

The diagnosing of dengue could also be confirmed by microbiological laboratory testing.^{35,38} This will be done by virus isolation in cell cultures, super molecule detection by PCR, infective agent substance detection (such as for NS1) or specific antibodies (serology).^{15,39} Virus isolation and super molecule detection square measure additional correct than substance detection, however these tests don't seem to be wide obtainable because of their larger price.³⁹ Detection of NS1 throughout the symptom section of a primary infection could also be larger than ninetieth sensitive but is simply 60–80% in resulting infections. All tests could also be negative within the early stages of the unwellness these laboratory tests square measure solely of diagnostic worth throughout the acute section of the unwellness with the exception of medical science. Tests for dengue fever virus-specific antibodies, sorts Ig and immune globulin will be helpful in confirming a diagnosing within the later stages of the infection. Each Ig and immune globulin square measure created when 5–7 days. The best levels (titres) of immune globulin square measure detected

following a primary infection, however immune globulin is additionally created in reinfection. Immune globulin becomes undetectable 30–90 days when a primary infection, however earlier following re-infections. IgG, against this, remains detectable for over sixty years and, within the absence of symptoms, could be a helpful indicator of past infection when a primary infection, Ig reaches peak levels within the blood when 14–21 days. In resulting re-infections, levels peak earlier and therefore the titres square measure typically higher. each Ig and immune globulin offer protecting immunity to the infecting serotype of the virus.^{15,24,40]} In testing for Ig and immune globulin antibodies there could also be cross-reactivity with different flaviviruses which can end in a false positive when recent infections or vaccinations with yellow jack virus or Japanese encephalitis.²³

PREVENTION

Prevention depends on management of and protection from the bites of the virus that transmits it⁴¹ the World Health Organization recommends associate degree Integrated Vector management program consisting of 5 elements:²⁹ The primary methodology of dominant *A. aegypti* is by eliminating its habitats. This is finished by obtaining eliminate open sources of water, or if this can be inconceivable, by adding pesticides or biological management agents to those areas. Generalized spraying with insecticide or pyrethroid pesticides, whereas generally done, isn't thought to be effective. Reducing open collections of water through environmental modification is that the most well-liked methodology of management, given the issues of negative health effects from pesticides and larger provision difficulties with management agents

VACCINE

In 2016 a part effective immunogen for breakbone fever became commercially obtainable within the Philippines and Republic of Indonesia.⁴³ It is conjointly been approved to be used by United Mexican States, Brazil, Salvador, Costa Rica, Singapore, Paraguay, abundant of Europe, and also the United States^{43,44,45} The immunogen is merely to be utilized in people that have antecedently had a dandy fever infection as otherwise there was proof it should worsen ensuing infections.^{[43][44][45]} In Republic of Indonesia it prices regarding US\$207 for the suggested 3 doses. The immunogen is made by Sanofi and goes by the brand Dengvaxia.⁴⁶ It's supported by a weakened combination of the yellow jack virus and every of the four dengue fever serotypes.⁴⁹

MANAGEMENT

There aren't any specific antiviral medicine for dengue; but, maintaining correct fluid balance is vital.²³ Treatment depends on the symptoms.⁵⁴ In those with severe dengue fever care ought to be provided in a vicinity wherever there's access to associate medical care unit.⁵⁴ Blood vessel administration, if needed, is usually solely required for one or 2 days.⁵⁴ In youngsters with shock because of dengue fever a speedy dose of 20 mL/kg is cheap. Invasive medical procedures like nasogastric cannulisation, contractile organ injections and blood vessel punctures square measure avoided, visible of the haemorrhage risk. Paracetamol (acetaminophen) is employed for fever and discomfort whereas NSAIDs like Advil and anodyne square measure avoided as they could worsen the danger of haemorrhage.⁵⁴ Transfusion is initiated early in patients presenting with unstable important signs within the face of a decreasing haematocrit, instead of looking ahead to the Hb concentration to decrease to some planned "transfusion trigger" level.¹ Packed red blood cells or blood square measure suggested, whereas platelets and recent frozen plasma square are not.

CONCLUSION

Dengue is one amongst the world's most rapidly spreading diseases, and as incidence continues to rise in endemic areas, and transmission in new regions of the globe becomes established. There are recent advances in our understanding of the medical specialty, risk factors for severe disease, and pathologic process, and the identification of therapeutic targets, which can cause novel treatments. Improved animal and human infection models ought to cause higher understanding of sickness evolution and assist drug development. Additionally, the advance within the study of human organism antibodies has unfolded a brand new avenue for immunogenic development, that ought to focus on causing the potent neutralizing anti-EDE antibodies against all four sero-types and avoid anti-prM antibodies that have low neutralizing activity and high potential to boost virus infection through fruit drink. Future irregular controlled trials of novel medicine and fluids, as well as in adults, are going to be needed to guide evidence-based follow all told patient teams.

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A NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF DOLUTEGRAVIR AND LAMIVUDINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Lamivudine and Dolutegravir are the two drug antiretroviral approved combination widely used in the treatment of retrovirus infections. A new simple, precise, selective, accurate and rapid reverse phase chromatographic method was developed and validated for bulk dosage form. The separation was achieved with Inertsil ODS with retention time 2.16 and 6.36 minutes respectively. The recovery percentage for Lamivudine and Dolutegravir were found to be in the range of 98.01 -101.5% and 98.35 - 102.14%. Calibration curve constructed between peak area vs concentration resulted good linearity in range of 14.98µg/mL -91.25µg/mL for Lamivudine and 2.54µg/mL -15.35µg/mL for Dolutegravir. The effect of acid hydrolysis, base hydrolysis, oxidation, thermal, photochemical, UV i.e. degradation studies in various conditions were also studied which resulted in undisturbed main peak. The results are analyzed statistically and are found to be acceptable according to ICH guidelines. Hence this method can be successfully employed for routine analysis of active analytes in bulk and pharmaceutical dosage form.

KEYWORDS: *Lamivudine, Dolutegravir, Methanol, Acetonitrile, Buffer, RP-HPLC.*

INTRODUCTION

Lamivudine¹ (LAM) is a nucleoside reverse transcriptase inhibitor (NRTI) active against human immunodeficiency virus type I (HIV-I) and Hepatitis B. Dolutegravir² (DOL) is a newly developed human immune deficiency virus (HIV-1) integrase inhibitor. It is given in a combination with novel drugs Lamivudine, Dolutegravir and many other drugs. The drugs prescribed individually, as well as multicomponent dosage forms available in the market. The chemical structures of Lamivudine and Dolutegravir are shown in

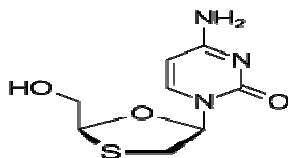


Figure 1
Chemical Structure of Lamivudine

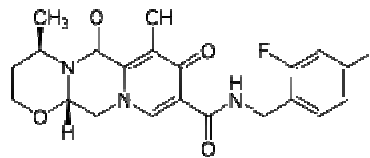


Figure 2
Chemical Structure of Dolutegravir

Lamivudine and Dolutegravir (Dovato), manufactured by GSK Healthcare, is a combination antiretroviral agent approved by the FDA with fixed-dose combination of 50mg Dolutegravir and 300mg Lamivudine³. Various UV, HPLC and LC/MS/MS assay methods were reported in the literature. These methods include HPTLC method⁴, HPLC method⁵⁻⁹ and LC/MS/MS¹⁰⁻¹¹. An attempt has been made to develop and validate a new method for stability-indicating simultaneous determination of Lamivudine and Dolutegravir in bulk drug and pharmaceutical dosage form.

MATERIALS AND METHOD

Chemicals and solvents

Lamivudine and Dolutegravir were obtained as gift samples from Mylan Laboratories Limited, Hyderabad, India. The commercial Pharmaceutical tablets of Dovato containing 50 mg of Dolutegravir, and 300 mg of Lamivudine respectively (manufactured by GSK healthcare) were procured from pharmacy.

Instrumentation

Separations were done by using HPLC-Waters (model-2695) which possesses an inbuilt auto sampler, a column and detector (2996 PDA). Data was collected through Empower – 2- software. Column used was Inertsil ODS with dimensions 250×4.6mm, 5 µm. to enhance dissolution of the compound meltronics sonicator was used. pH was adjusted using buffer and weighing was done using sartorius balance (model AE-160)

Optimized Chromatographic conditions

Instrument	:	Waters 2695, High performance Liquid Chromatography
Flow rate	:	1ml/min
Column	:	Inertsil ODS-3V, 250 x 4.6 mm, 5µ.
Detector wave length	:	257 nm
Column temperature	:	30°C
Injection volume	:	10 µL
Run time	:	10 min
Mode of separation	:	Isocratic mode

Buffer was prepared by taking 1.36gm of potassium dihydrogen orthophosphate in 900ml with HPLC grade water, and pH adjusted to 3.00.

Mobile phase is Phosphate buffer pH 3.0, Acetonitrile and methanol at ratio 50:20:30%v/v/v.

Standard solution and sample solutions were prepared at concentration of 60 µg/mL Lamivudine and 10 µg/mL Dolutegravir

VALIDATION

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision, Specificity, Forced degradation studies, Linearity, Accuracy, Limit of detection and Limit of quantification¹².

System Suitability

The system suitability was determined by making six replicate injections from freshly prepared standard solutions. The results summarized in Table-1.

System suitability parameters	Result		Acceptance criteria
	LAM	DOL	
Retention Time	2.169	6.367	For information
% RSD for area count of six replicate injection of standard	0.7	0.8	NMT 2.0
Tailing factor	1.34	1.12	NMT 2.0
Theoretical plates	7643	55924	NLT 2000
Resolution	N/A	22.51	NLT 2.0

Table-1: System suitability results

Linearity

The linearity for lamivudine and dolutegravir was performed by preparing standards at different concentrations within the range of 14.98µg/mL-91.25µg/mL and 2.54µg/mL-15.35µg/mL. The values obtained and their corresponding calibration curve was given in table-2 and fig-3.

Table-2: Linearity study results

S.No.	Analyte Name	Linearity Range (µg/mL)	Equation of Calibration curve	Correlation Coefficient

1	Lamivudine	14.98 – 91.25	$y = 11,326.640x + 5,694.835$	0.999
2	Dolutegravir	2.54 – 15.35	$y = 49,345.031x - 1,016.567$	0.999

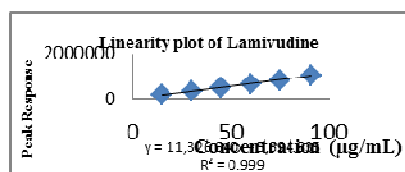


Figure 3: Linearity plot of Lamivudine

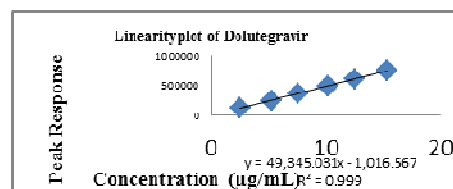


Figure 4 : Linearity plot of Dolutegravir

Precision

Intra-day and inter-day analysis was considered for conforming the precision. Percentage relative standard deviation was calculated within acceptable criteria of not more than 2.0. The results obtained were displayed in table-3, 3A.

INJECTIONS	Peak Response	
	Lamivudine	Dolutegravir
1	562424	409726
2	565574	412864
3	575969	414115
4	561345	426527
5	578738	413765
6	586696	411675
AVG	571791	414779
S.D	10231.19	5971.93
%RSD	1.78	1.44

Table-3 precision

Sample Preparations	%Assay	
	Lamivudine	Dolutegravir
Sample-1	99.7	99.6
Sample-2	100.1	100.1
Sample-3	99.6	99.7
Sample-4	100.8	99.2
Sample-5	99.5	100.4
Sample-6	100.3	100.0
Average	100.0	99.8
S.D	0.50	0.42
%RSD	0.5	0.4

Table-3A Method precision

System Accuracy

Accuracy was determined by method of standard additions to measure the recovery of drugs. The results were given in table-4.

Table-4: Accuracy study results

% of Target concentration	Lamivudine (%Recovery)	Dolutegravir (% Recovery)
50	100.16	101.48
100	99.03	99.21
150	100.21	100.48

Mean % Recovery (n=3)	99.8	100.4
% RSD	0.67	1.13

Ruggedness

Sample analysis was performed by different analyst using different instruments on different days to conform ruggedness. No remarkable changes were observed which defines that the method developed was rugged in nature. The results were mentioned in table-5.

Table-5: Intermediate precision Results

Sample Preparations	%Assay	
	Lamivudine	Dolutegravir
Sample-1	98.9	99.2
Sample-2	100.1	100.0
Sample-3	99.4	99.6
Sample-4	100.2	98.9
Sample-5	99.7	100.1
Sample-6	100.4	99.9
AVG	99.8	99.6
S.D	0.56	0.48
%RSD	0.6	0.5

Robustness

Three parameters namely changes in flow rate (± 0.1 ml/min), mobile phase composition ($\pm 5\%$) and column temperature ($\pm 5^\circ\text{C}$) were optimized in chromatography to check our robustness. Reproducibility in values was observed. The results were mentioned in table -6A and 6B.

Table-6A: Robustness results for Lamivudine

S.No	Parameter	As such method	Used	%RSD for peak area(n=6)	Plate count	Tailing Factor
1	Flow Rate (± 0.1 ml/min)	1.0 ml/min	0.9 ml/min	0.2	9457	1.35
			1.0 ml/min	0.7	7651	1.32
			1.1 ml/min	1.0	9145	1.30
2	Column Temperature ($\pm 5^\circ\text{C}$)	30°C	25°C	0.3	8640	1.25
			30°C	0.7	7657	1.31
			35 °C	1.5	9128	1.33
3	Mobile phase composition	Buffer:ACN:Methanol :55:20:30 %v/v	55:25:25	0.5	8648	1.30
			55:20:30	0.7	7653	1.31
			55:15:35	0.9	8475	1.33

Table-6B: Robustness results for Dolutegravir

S.No.	Parameter	As such method	Used	%RSD for peak area(n=6)	Plate count	Tailing Factor
1	Flow Rate (± 0.1 ml/min)	1.0 ml/min	0.9 ml/min	0.8	50185	1.12
			1.0 ml/min	0.9	55945	1.14
			1.1 ml/min	1.2	53434	1.06
2	Column Temperature	30°C	25°C	0.5	52290	1.11
			30°C	0.9	55945	1.16

	($\pm 5^\circ\text{C}$)		35 $^\circ\text{C}$	1.5	53457	1.07
3	Mobile phase composition	Buffer:ACN:Methanol :55:20:30 %v/v	55:25:25	0.7	52235	1.08
			55:20:30	0.9	55985	1.13
			55:15:35	1.2	51769	1.04

Limit of Detection and Limit of Quantification (LOD AND LOQ)

LOD and LOQ were calculated using the waters empower software for signal to noise ratio method. Limit of Detection and Limit of Quantification were found to be 0.40 $\mu\text{g/ml}$ and 0.88 $\mu\text{g/ml}$ respectively for Lamivudine and 0.04 $\mu\text{g/ml}$ and 0.11 $\mu\text{g/ml}$ respectively for Dolutegravir and the signal to noise ratio values for LOD and LOQ were found to be 3.3 and 10.2 for Lamivudine and 3.1 and 9.7 for Dolutegravir and observed all the obtained results were found to be within the acceptance criteria as per ICH guidelines. The results were shown in Table-7.

Table-7: Limit of Detection and Limit of Quantification

S.No.	Analyte Name	Signal to Noise ratio values	
		LOD	LOQ
1	Lamivudine	3.3	10.2
2	Dolutegravir	3.1	9.7

Estimation of Lamivudine and Dolutegravir Tablet Dosage Forms

10 tablets were weighed and crushed them to a fine powder and a quantity of tablet powder equivalent to 1 tablet was transferred into a 100 mL volumetric flask, add 3/4th volume of diluent and sonicate for 30 minutes, further the volume made up with diluent and filter through 0.45 μm nylon filter. Pipette out 0.5mL into a 25 mL volumetric flask with diluent to get the working sample solution. The assay results were shown in Table 8.

Table-8: Assay results of Lamivudine and Dolutegravir formulation

Drug	Label claim (mg)	Amount found $\pm$ SD	Assay (%)	% RSD
Lamivudine	300	298.7 $\pm$ 1.18	99.56	0.39
Dolutegravir	50	49.8 $\pm$ 0.14	99.60	0.28

Specificity

Specificity was optimized by injecting blank solution without any drug followed by drug with volume 10 μl into column to check separation of molecules namely Lamivudine and Dolutegravir from impurities. It was identified that there was no change in retention time which proves the specificity of compound. The chromatograms were copied in figure 5,6,7.

Figure 5 Typical Chromatogram of Placebo

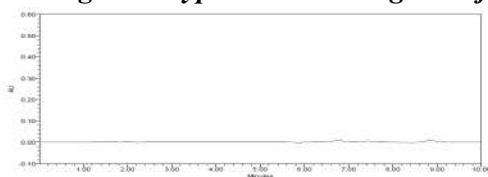
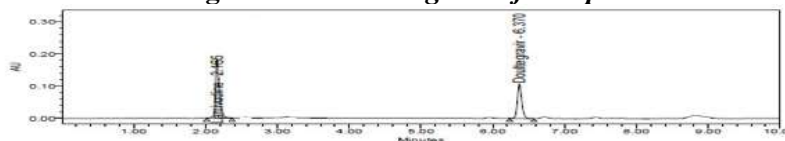


Figure 6 Chromatogram of Blank



Figure 7 Chromatogram of Sample



Forced Degradation Studies

Forced degradation studies were performed to demonstrate the optimized method. Sample was treated with 1ml of 2N HCl (Acid stability), 1ml of 2N NaOH (Alkaline stability), 1ml of 20% H₂O₂ (Oxidative degradation), exposure of sample drug solution at 105 $^\circ\text{C}$ for 6 hrs (dry heat degradation), photo stability

degradation (exposure of drug at 200 watt hours/m²) and neutral degradation (refluxing with water at 60°C for 6 hours. Peaks were recorded for degraded products where no interference with analyte peak was observed. The peak purity values were found to be within acceptable limits. The results were shown in Table-9 (9A, 9B).

Table-9 Forced Degradation Studies:
Table-9A Forced Degradation study results for Lamivudine

S.No	Injection	%Assay	%Degradation	Purity Angle	Purity Threshold	Purity Flag
1	Controlled sample	100.1	--	0.310	1.375	No
2	Acid Degradation	95.1	5.0	0.325	0.463	No
3	Base Degradation	96.9	3.2	0.575	1.081	No
4	Peroxide Degradation	94.8	5.3	1.612	2.175	No
5	Thermal Degradation	98.3	1.8	0.268	0.294	No
6	UV Degradation	99.7	0.4	0.318	0.335	No
7	Water Degradation	98.4	1.7	0.264	0.298	No

Table-9B Forced Degradation study results for Dolutegravir

S. No	Injection	%Assay	%Degradation	Purity Angle	Purity Threshold	Purity Flag
1	Controlled Sample	99.9	---	0.122	0.378	No
2	Acid Degradation	95.1	4.8	0.397	0.475	No
3	Base Degradation	96.3	3.6	0.761	1.199	No
4	Peroxide Degradation	91.4	8.5	1.618	2.154	No
5	Thermal Degradation	97.6	2.3	0.397	0.498	No
6	UV Degradation	98.1	1.8	0.454	0.492	No
7	Water Degradation	98.6	1.3	0.292	0.458	No

RESULTS AND DISCUSSION

A new chromatographic method was developed for quantification of Lamivudine and Dolutegravir from bulk and pharmaceutical dosage forms. Analytical method was developed using phosphate buffer pH 3.00: Acetonitrile: methanol in the ratio of 50:20:30 %v/v at a flow rate 1.0 ml/min. The analysis is done at 257 nm with volume 10 µL and separation is done using Inertsil ODS-3V (250 x 4.6 mm, 5µ) column. The retention time of Lamivudine and Dolutegravir were found to be 2.169 and 6.367 respectively. The proposed column was selected which gave a sharp and symmetrical peak with 1.34 tailing factor and theoretical plates of 7643 for Lamivudine, and 1.12 tailing factor and theoretical plates of 55924 for Dolutegravir. The resolution between Lamivudine and Dolutegravir is 22.51 and found to be within the acceptable limit (not less than 2.0). The calibration curve was linear over the concentration range of 14.98µg/mL-91.25µg/mL for Lamivudine and 2.54µg/mL -15.35µg/mL for Dolutegravir. Stability of the product was assured by peaks obtained during forced degradation peaks. RSD values for accuracy and precision were less than 2.0%. The results obtained due to all analytical methods gives a clear definition that the method developed was stability indicating.

CONCLUSION

The new method developed for estimating Lamivudine and Dolutegravir in Bulk and pharmaceutical dosage form is accurate, simple and selective that is stability indicating. Attempts were not made to estimate the multidrug mixture. The results obtained for system suitability, linearity, accuracy, robustness and specificity, limit of detection and limit of quantitation supported the specificity of product. Resultant peaks obtained during forced degradation studies remains undisturbed with main peak. This leads to a conclusion that the method developed was adopted conveniently for the routine quality control analysis in bulk and combined formulations.

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CONFLICT OF INTEREST

Nil

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A QUALITATIVE STUDY EXPLORING PATIENT PERCEPTION ON THE ROLE OF PHARMACIST BASED PATIENT COUNSELLING IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Patient counseling refers to the process of providing information, advice and assistance to help patient use their medication appropriately. A prospective observational study was carried out in patients admitted to Dr. Pinnamneni Siddhartha Institute of Medical Sciences and Research Foundation for a period of 6 months. Questionnaire was divided into 3 parts and was analyzed using Likert's scale. Statistical analysis was done using graph pad prism. Results obtained from the study shows that 324(32.45) of patients agreed and 296(29.6%) of patients strongly agreed to the patient counseling. 464(46.4%) patients felt that pharmacists provide medication counseling, 490(49.0%) of patients perceived that pharmacists had given enough time. A large number of participants 489(48.9%) considered pharmacist as an expert in drug related matters. Majority of participants 806(80.6%) in the study felt that pharmacist provided them with thorough medication counseling which is quite evident that participants are very clear about the primary responsibilities of pharmacist and deliver the same in the optimal by encouraging them to ask questions. As the counseling was done in regional language Telugu 990(99%) of participants were satisfied with the language used by the pharmacist during counseling session. The results of the study shows that a pharmacist based patient counseling services are positively associated with pharmacist knowledge related to drugs, time provided by the pharmacist to the patients, medication history interview and language used by the pharmacist.

KEYWORDS: *Pharmacist, patient counseling, public perception, patient adherence*

INTRODUCTION

Over the past four decades the pharmacist profession has made considerable efforts to shift its focus from medication supply to direct patient care¹. Patient counseling refers to the process of providing information, assessing the patient, understanding of drug therapy, proper use and adverse effects of medications and motivating the patient to take an active role in health management²⁻³. Pharmacies are recognized as the most accessible health care setting due to high number of patients using their services⁴. Hence, pharmacists are well positioned to help in improving medication adherence, educating patient about disease, reducing medication errors, management of patients medical condition and improving patient outcomes and satisfaction with care⁵. The pharmacist's features for better patient counseling includes establishing trust among the patients, with effective verbal and non-verbal communication skills, asking open-ended questions, providing privacy and confidentiality and motivating the patients⁶⁻⁷. A constructive pharmacist-patient relationship is essential for effective health care practice and optimal well being of the patient⁸. The profession of pharmacy, pharmaceutical care and patient counseling services has become well popularized all over the world⁹. In India patient counseling services are not well established and most of the patients unaware about the pharmacist's participation and patient counseling¹⁰⁻¹¹. Hence this study was initiated to know public perceptions towards counseling and to know whether they are satisfied with their services. We made an effort to spread the importance of patient counseling among public and to set-up patient counseling services in every hospital.

MATERIALS AND METHODS

We have conducted a prospective observational study over a period of 6 months from Nov 2018-April 2019 at Dr. PSIMS& RF which is a 1000 bedded tertiary care teaching hospital Chinnoutpally, GannavaramMandal, Krishna district, Andhra Pradesh. A total of 1000 subjects who met the inclusion criteria were recruited in the study. Patients of age 18-75 years and Patients attending out-patient department in tertiary care teaching hospital were included in the study, for accuracy of results patients of age <18 years and > 75 years were excluded as they are unable to provide us with suitable answers. Initially study protocol was approved by IEC of Dr. PSIMS & RF. Data was collected using a predesigned data collection form which consists of three parts. First part includes socio-demographic details of the participant like age, gender, educational level. Second part consists of perception related questions and third part consists of satisfaction related questions. As per the 5 point Likert scale response to the questions was made ranging from strongly agree to strongly disagree. The questionnaire was designed in regional language Telugu inorder to facilitate better understanding to the patient. The data collection form was given to the participants followed by verbal explanation. On an average, the participants were given 10-15 minutes to complete the self-administered questionnaire. The responses of perception and satisfaction related questions were assessed by estimating the percentages of participants answering the individual responses of strongly agree to strongly disagree.

RESULTS

Table 1 depicts majority of the respondents 295(29.5%) are in the range of 42-53 years, 252(25.2%) are in range of 54-65 years, 244(24.4%) are in the range of 30-41 years, 111(11.1%) are above 65 years and 98(9.8%) are in the range of 18-29 years. Among the subjects 608 (60.8%) were females and 392(39.2%) were males. Majority of patients 467(46.7%) were uneducated, 215(21.5%) passed pre high school, 238(23.8%) passed the high school and 80(8%) were graduated. Table 2 explains perception related questions: Question 2 illustrates a majority of participants were agreed that they feel comfortable about seeking advice from pharmacist 584(58.4%). In perception related questions most of the participants were strongly agreed that pharmacist are expert in matters related to drugs 324 (32.4%), thorough medication counseling 464(46.4%), giving enough time in discussing medication related problems 490(49%) and patients believing that pharmacist checks prescription accurately 482(48.2%). Table 3 depicts satisfaction related questions: In satisfaction related questions 507(50.7%) subjects are agreed that they are satisfied with the questions asked by the pharmacist before counseling. 510(51%) participants strongly agreed with the language used by the pharmacist in discussing drug related matters. Majority of the participants were satisfied with the knowledge that pharmacist demonstrate in drug related issues 403(40.3%) and time spent by the pharmacist 439(43.8%).

TABLE 1

AGE	FREQUENCY/PERCENT
18-29	98 (9.8%)
30-41	244 (24.4%)
42-53	295 (29.5%)
54-65	252 (25.2%)
65 above	111 (11.1%)
GENDER	
Female	608 (60.8%)
Male	392 (39.2%)
EDUCATION	
Graduated	80 (8.0%)
High school	238 (23.8%)
Pre high school	215 (21.5%)
Un educated	467 (46.7%)

TABLE 2

PERCEPTION RELATED QUESTIONS	Strongly disagree	Disagree	Neutral	Agree	Strongly agree

1. Do you consider the pharmacists as an expert in matters related to drugs ?	6 (0.6%)	5 (0.5%)	309 (30.9%)	324 (32.4%)	296 (29.6%)
2. When I am in the pharmacy I feel totally at ease about asking the pharmacist for advice	1 (0.1%)	34 (3.4%)	113 (11.3%)	584 (58.4%)	268 (26.8%)
3. When I am at the counseling session pharmacist provides me with thorough medication counseling and encourages me to ask questions	13 (1.3%)	43 (4.3%)	138 (13.8%)	342 (34.2%)	464 (46.4%)
4. When I am at the counseling session the pharmacist gives me enough time to discuss my problem and listens to me carefully	10 (1.0%)	36 (3.6%)	172 (17.2%)	490 (49.0%)	292 (29.2%)
5. I trust the pharmacist for the information on the use of medicines	0	31 (3.1%)	252 (25.2%)	403 (40.3%)	314 (31.4%)
6. Does pharmacist advice patients on general health issues other than about drugs	6 (0.6%)	33 (3.3%)	138 (13.8%)	489 (48.9%)	334 (33.4%)
7. Does pharmacist check my prescription for accuracy in terms of drug name, dos	3 (0.3%)	22 (2.2%)	78 (7.80%)	415 (41.5%)	482 (48.2%)

TABLE 3

SATISFACTION RELATED QUESTIONS	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1. I am satisfied with the type and amount of information discussed by the pharmacist on drug related matters.	6 (0.6%)	55 (5.0)	200 (20.0%)	421 (42.1%)	318 (31.8%)
2. I am satisfied with the questions asked by the pharmacist before counseling like any history of previous drug allergy and disease details.	5 (0.5%)	48 (4.8%)	149 (14.9%)	507 (50.7%)	291 (29.1%)
3. I am satisfied with the level of knowledge that pharmacist demonstrate in drug related issues.	3 (0.3%)	15 (1.5%)	194 (19.4%)	403 (40.3%)	385 (38.5%)
4. I am satisfied with the language used by the pharmacist in discussing drug related matters.	1 (0.1%)	1 (0.1%)	8 (0.8%)	480 (48%)	510 (51.0%)
5. I am satisfied by the amount of time spent by the Pharmacist with each patient.	4 (0.4%)	27 (2.7%)	148 (14.8%)	438 (43.8%)	383 (38.3%)

DISCUSSION

Determining patient's views on services provided by the pharmacists, effective patient counseling and offering standards of care, all this enables the pharmacist's role to be judged for overall quality and satisfaction. As this study was conducted in the rural area majority of the participants are uneducated 467(46.7%). The study shows that high number of participants 620(62%) agreed that pharmacists are specialists in matters related to drugs, participants perception 739(73.9%) satisfied with the level of knowledge that pharmacist demonstrated related to drugs and the remaining negative response is influenced by the fact that the patients are not correctly responded to the pharmacist regarding drugs. Majority of participants 806(80.6%) in the study felt that pharmacist provided them with thorough medication counseling which is quite evident that participants are very clear about the primary responsibilities of pharmacist and deliver the same in the optimal by encouraging them to ask questions and 798(79.8%) participants were satisfied with the questions asked by the pharmacist like any history of previous problems.

The greater number of participants 821 (82.1%) were satisfied with the time provided by the pharmacists to discuss their problems this indicates that enough time is given to each patient to discuss their doubts regarding the drug, disease and life style modification. A vast majority of participants 823(82.3 %) agreed that pharmacists provided them with advice on general health issues other than drugs and 717(71.7%) of participants agreed that they trust pharmacists for the information on the use of the medicines and the negative response indicated that the participants esteems physician as the first person to consult regarding medication-related issues, primarily because they had more trust in the physicians ability to answer their questions and counsel them. As the counseling was done in regional language Telugu 990(99%) of participants were satisfied with the language used by the pharmacist during counseling session.

CONCLUSION

From our results we can conclude that pharmacist based patient counseling services is positively associated with pharmacist knowledge related to drugs, time provided by the pharmacist to the patients, medication history interview and language used by the pharmacist which helps in improving the quality of life of patient. These findings concluded that the patient counseling services provided by the pharmacist were satisfied by large number of patients in improving their health, we were of the opinion that the provision of patient counseling services are important to the advancement of pharmacy profession and in providing patients with a high level of care.

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STUDY OF ANTIMICROBIAL EFFECT OF POLYPHENOLS EXTRACTED FROM SKINS OF ROYAL GALA AND GRANNY SMITH

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ABSTRACT

Apples contain many useful human health related contents. Polyphenols exhibit antimicrobial effects against human pathogens. In this study the polyphenolic contents are extracted from the skin of Royal Gala and Granny Smith, which are the two varieties of apples. The phenolic compounds were extracted from fresh fruit peels of Royal Gala and Granny Smith with the solvents: A-(water), B-(ethanol: water). The antimicrobial activities of the extracts were tested against *Escherichia coli* and *Staphylococcus aureus* bacteria by the agar well diffusion method. The extracts from both apples by solvent A showed higher antimicrobial activity as compared to extracts by solvent B. Among the two varieties of apples the Granny Smith extracts showed a superior antimicrobial effect as compared to Royal Gala extracts against the selected microorganisms *E. coli* and *S. aureus*. The zone of inhibition by the apple peel extracts is more in case of *S. aureus* compared with *E. coli*; this reveals that the organism *S. aureus* is more sensitive to polyphenolic compounds than *Escherichia coli*. With this study it was concluded that the water extract of Granny Smith peels showed superior antimicrobial activity due to higher amounts of polyphenolic compounds as compared to Royal Gala and the best solvent to extract polyphenolic compounds from apple peels is water.

KEYWORDS: *Antimicrobial, Royal Gala and Granny Smith, polyphenol*

1. INTRODUCTION

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.¹⁷ Phenolic compounds are well known for their health benefits related to antioxidant activity. In addition, this kind of compounds can be extracted from natural sources, such as olives, grapes, apples, fruits, vegetables, rice, spices, herbs, tea and algae. In fact, other important characteristics associated to phenolic compounds are the antimicrobial activity, because phenolics have the capacity of retarding the microbial invasion in some products and avoiding the putrefaction of others, mainly fruits and vegetables. These properties allow phenolic compounds to be suitable for numerous food preservation applications. Active packaging is an innovative strategy where phenolic compounds can play an important role for improving the global assessment and extend the shelf life of commercial products.¹⁵ Polyphenols are classified according to their structure as phenolic acids derivatives, flavonoids, and tannins. Apples contain many types of phenolic derivatives and flavonoids (flavan-3-ols, flavonols, procyanidins, chalcones, and anthocyanins).^{3,8} Apple skin contains the flavonoids, quercetin glycosides and cyanidin glycosides.⁷ Epidemiological studies associate phenolic consumption with lower mortality, especially caused by coronary diseases. They present multiple biological properties, which are of growing interest for consumers due to the high antioxidant, anti-inflammatory, anti-allergic, anti-thrombosis and antimicrobial activities.^{1,2,4,9,10,11} Furthermore they act as antideposit of triglyceride, anticholesterolemic, and antiviral agents, among others phenolic compounds may affect microbial growth and metabolism.^{6,7,12,13} The objectives of present study are to examine the antimicrobial activity of phenolic compounds from skin of two apple varieties, Royal Gala and Granny Smith, against pathogenic bacteria and to find out the variety of apple with superior activity, and to find out the preferable solvent for extraction of polyphenols from apple peels.

Royal Gala (yellowish-red skin)- Synonyms-Royal Gala, Local Name-Red apple, *malus pumila*, Biological source-Dried ripe fruit of *malus pumila*, *malus sieversii*, Family-Rosaceae. Description -royal Gala apples are covered in a thin yellow to orange skin, highlighted with pink to red stripes that vary in hue dependent upon the apples maturity. Their dense flesh is creamy yellow and crisp, offering a mildly sweet flavor and flora aroma. Gala's that are allowed to reach the peak of their sweetness on the tree will have a deeper red hue and a slightly sweeter flavor.¹⁸



Figure:1 Royal Gala (yellowish-red skin) and granny smith

Granny Smith (greenish-white skin)- Morphology: Synonyms-Granny smith, Local Name-Green apple, *malus pumila*, Biological Source-Dried ripe fruit of *malus pumila*, *malus sylvestris*, Family-Rosaceae. Granny Smith apples have a bright green skin that is often speckled with faint white lenticels (spots). Medium to large in size and round in shape, they are a firm and juicy apple with thick skin. Their flesh is bright white and crisp in texture sweet flavor.¹⁸

2. MATERIALS

Two Apple varieties one is Red apple- Royal Gala and another one is green apple-Granny Smith, agar, peptone, sodium chloride, Beef extract/Yeast extract, distilled water, Ethanol, Micro-organisms–Gram positive and Gram negative (*E. coli*, *S. Aureus*).

3. METHODOLOGY

3.1. Collection of peels- healthy Apples of *Royal Gala* and *Granny Smith* were collected and washed with distilled water. The fresh peels of *Gala* and *Granny Smith* were collected and authenticated by Botanist. Spread the peels on the sterile clean green net in a well-ventilated room. Peels completely dry within 4 days, the dried peels are grinded in mortar by pestle to make powder, then this peel powder dried at 50°C for 30 minutes to reduce moisture content and protected from light and humidity.¹⁴



Figure 2 : Peels and powder of Royal Gala and Granny Smith.

3.2. Polyphenol extraction- The peel powder of 6 g of each apple variety was chopped up finely. Extractions were carried out twice for 8 hrs at 60°C with 40 ml of the following solvents: A, water; B, ethanol: water (1:1) and Extracts were then concentrated in a evaporator by avoiding direct contact with light and oxygen.^{5,9}

3.3. Extract clarification- Clarification was carried out with 1 g of activated carbon in 20 ml of each extract. Extracts were shaken and then membrane-filtered.⁹

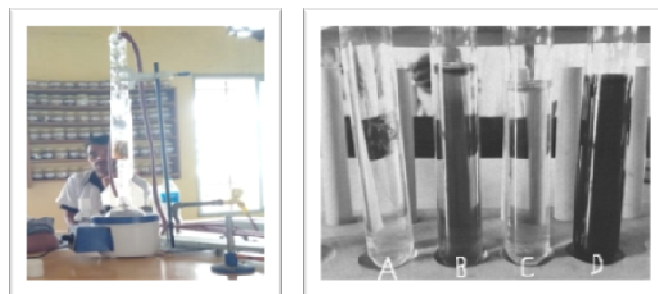


Figure 3: Extraction assembly & Extracts of Royal Gala & Granny Smith.

A: Granny Smith water extract, B: Royal Gala water extract, C: Granny Smith ethanol: water extract, D: Royal Gala ethanol: water extract.

3.4. Antimicrobial activity

Agar plate media was prepared by suspending 28 g of nutrient agar powder in 1 liter of distilled water and heated this mixture while stirring to fully dissolve all components. Autoclaved the dissolved mixture at 121⁰ C for 15 minutes. Once the nutrient agar has been autoclaved, allowed it to cool but not solidify. Then inoculated the selected microorganism in to nutrient agar medium and poured into plates and set aside on the sterile surface until the agar has solidified.¹⁷ In this cup-plate method/ agar well diffusion method, when the agar has solidified, holes about 9 mm in diameter are cut in the medium with a sterile cork borer. The antimicrobial agents (apple peel extracts) directly placed in the holes. The the plates are incubated at 30 to 35°C for 2 to 3 days. The zone of inhibition is observed after incubation period, the diameter of the zone of inhibition was measured in millimeters.^{14,17} the incubated plates shown figure 4.

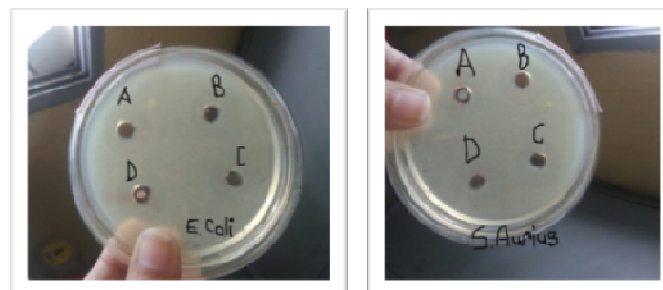


Figure: 4 . Zone of inhibition of E-coli and S -aureus (Diameter in mm)

A: water extract (Granny Smith), B: ethanol: water Granny Smith).C: water extract(Royal Gala), D: ethanol: water(Royal Gala).

4. RESULT AND DISCUSSION

ZONE OF INHIBITION

Table1. Effect of the apple skin extracts from Royal Gala and Granny Smith varieties against bacterial pathogen

Extracts		E coli	S. Aureus
Granny Smith	A	7.0	9.0
	B	2.0	3.0
Royal Gala	A	3.0	4.0
	B	1.0	2.0

A: water, B: ethanol: water. Diameter of inhibition zone in mm

In Table1. The values of zone of inhibition by aqueous and alcoholic extracts of two apple varieties are given. it was observed that the major inhibitory effect for both varieties due to the poly phenolic compounds present in apple peels. It is known fact that the zone of inhibition is directly proportionate to the

antimicrobial activity. So These results shows that when compare with water extract and water: ethanol extract, water extracts showing more antimicrobial effect than water: ethanol extracts. Water extract of Granny Smith is more effective than that of Royal Gala. The zone of inhibition is more in *S. aureus* inoculated plates compare with *E-coli* inoculated plates, so this indicates the *S-aureus* is more susceptible microorganism to phenolic compounds than *E-coli*. The Granny Smith and Royal Gala are more effective in water extract but Granny Smith having more activity than the Royal Gala in both extracts.

5. CONCLUSION

Based on our study results it is concluded that the best solvent for extraction of polyphenols from apples is water, the Granny Smith peels extracts are more effective antibacterial agents than those from Royal Gala, because there is a direct relationship between phenolic content and antibacterial effect. And it also reported that the *S. aureus* is more susceptible than *E- coli* to polyphenols. The antibacterial effect of apple poly phenols demonstrated in this study can be added to the already known beneficial properties of apple to the human health.

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IMPORTANCE OF VARIOUS DRUG DELIVERY APPROACHES FOR EFFECTIVE MANAGEMENT OF RHEUMATOID ARTHRITIS WITH BETTER PATIENT COMPLIANCE

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic progressive autoimmune disease, affecting the joints with varying severity among patients. RA can occur in all races and ethnic groups. Around 40/100,000 of the general population is suffering from this disease worldwide. Till now, there is no permanent cure has been developed for RA, but there are various therapeutic approaches are there to relieve pain and inflammation and improve or maintain joint function. Rapid diagnosis and initiation of treatment before irreversible joint damage is required to maintain productive and normal active life. In this review risk factors, diagnosis, pathophysiology of RA is briefed out and various conventional drugs used to treat and manage RA are listed with mechanism of action. The importance of various novel drug delivery approaches in treatment of RA is discussed and is listed with respect to better patient compliance and effective management of RA with lesser side effects.

KEY WORDS: Rheumatoid arthritis, Therapeutic approaches, Novel drug delivery approaches, Patient compliance

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease that causes pain, swelling, stiffness, and loss of function in various joints. The respective joint's lining becomes inflamed, leading to tissue damage, as well as chronic pain, unsteadiness, and deformity. RA can also affect extra-articular sites, including the eyes, mouth, lungs, and heart.¹ Arthritis can make it very difficult for an individual to remain physically active, contributing to an increased risk of obesity, high cholesterol or vulnerability to heart disease. Individuals with arthritis are also at increased risk of depression.³ With early intervention and appropriate treatment, symptoms can be ameliorated for a certain duration.² RA can occur in all races and ethnic groups. Around 40/100,000 of the general population is suffering from this disease worldwide. The prevalence of RA in developed countries is 0.5% to 1% of the population (0.6% in the U.S.). RA onset generally occurs in middle age and is more common in older adults, but it can also develop in children and young adults. The lifetime risk of developing an inflammatory autoimmune rheumatic disease is 1 in 12 (8.3%) for women and 1 in 20 (5%) for men.⁵ Specifically, the lifetime risk of developing adult-onset RA is 1 in 28 (3.6%) for women and 1 in 59 (1.7%) for men but trends in RA incidence, prevalence, and mortality vary based on the studied population.^{1,2}

Causes: The exact cause of RA is still unknown, but genetic factors, environmental factors, and hormones may be involved in its autoimmune development and progression.¹

Risk factors¹: Some risk factors which increase the risk of RA are

Older age - highest incidence in people aged ≥ 60 years, Gender - higher incidence in women, Genetics - especially human leukocyte antigen [HLA] class II genotypes, such as HLA-DRB1, Smoking, early life exposures (if mother smoked, child has greater risk of RA), Obesity - higher risk with increasing body weight, Patients who are seropositive for anticitrullinated protein antibodies (ACPAs) or rheumatoid factors (RFs) also have an increased risk of RA.

Signs and Symptoms of Arthritis³

- Inflammation and redness of a joint, Joints will be sensitive to touch

- Deformation of limbs and extreme pain and warmth
 - Stiffness of limbs during morning session and Recurrent back pain
 - Ankylosed joints (immobile joints due to the union of bones because cartilage has been destroyed)
- figure 1 shows classical example of joint deformities associated with rheumatoid arthritis.

Figure 1. A classic example of joint deformities associated with rheumatoid arthritis.⁴



Boutonniere deformity is visible in the 5th digit of the right hand, Swan neck deformity in the 5th digit of the left hand, and hallux valgus can be seen in the foot.

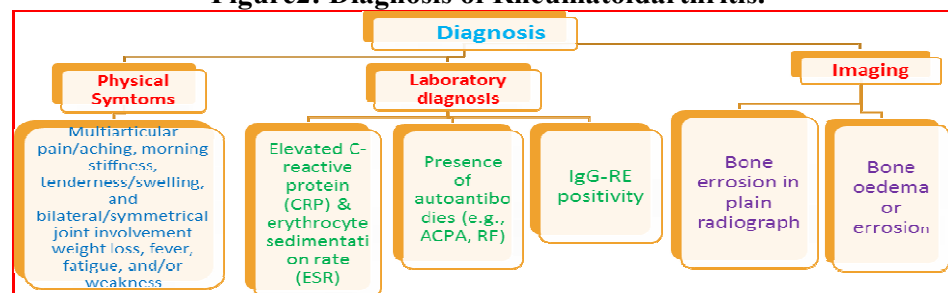
Pathophysiology

Joint swelling in RA is usually synovial-membrane inflammation, with cytokine and chemokine involvement. The most relevant components in the inflamed space include tumour necrosis factor (TNF), interleukin-6 (IL-6), and granulocyte-macrophage colony-stimulating factor. Cytokines and chemokines induce or aggravate the inflammatory response by activating endothelial cells and promoting immune-system cell accumulation within the synovial compartment. Activated fibroblasts, B cells, T cells, monocytes, and macrophages can eventually trigger osteoclast generation via receptor activator of nuclear factor kappa-B ligand (RANKL), which is expressed on B cells, T cells, and fibroblasts. The RANK receptor is present on macrophages, dendritic cells, and preosteoclasts. In addition, the cartilage matrix within joints is eventually degraded by metalloproteinases and other enzymes.¹ A pre-RA phase lasts for month to years and it may be characterized by the presence of circulating autoantibodies, increasing concentration and range of inflammatory cytokines and chemokines, and altered metabolism. Clinical disease onset comprises synovitis and systemic comorbidities affecting the vasculature, metabolism, and bone.

Diagnosis¹

RA is diagnosed based on physical symptoms, laboratory tests, imaging of joints.(fig.2)

Figure2: Diagnosis of Rheumatoidarthritis.



Treatment and management of Rheumatoid arthritis

Till now, there is no permanent cure has been developed for RA, but there are various therapeutic approaches are to relieve pain, inflammation and improve or maintain joint function. Rapid diagnosis and initiation of treatment before irreversible joint damage is required to maintain productive and normal active life.² Treatment regimens consist of combinations of therapeutics, exercise, educating patients about the disease, and rest. Treatments are generally customized to a patient's needs and depend on their overall health. This includes factors such as disease progression, the joints involved, age, overall health, occupation, compliance, and education about the disease.⁴

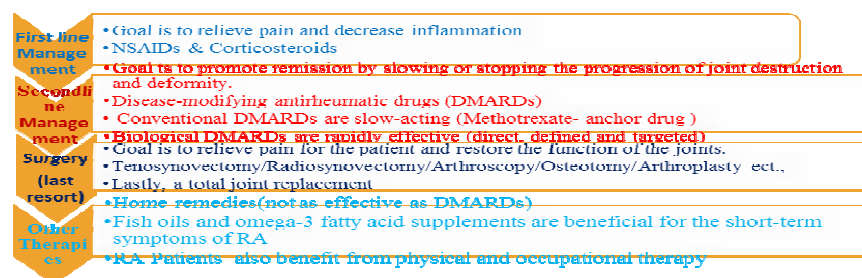
The Current therapeutics for RA treatment include (mentioned in table.1)

Table 1. Conventional therapeutic agents for Rheumatoid Arthritis^{1,2,4}

Drug classification	Example drugs	Mechanism of action	Side effects
NSAIDs	Aspirin, naproxen, ibuprofen, and etodolac, Indomethacin, Diclofenac, ect	Inhibiting cyclo-oxygenase to prevent the synthesis of prostaglandins, prostacyclin, and thromboxanes.	Nausea,abdominal pain, ulcers, and gastrointestinal (GI) bleeding
	Celecoxib	Selective Cox-2 inhibitor.	Less risk of GI side effects compares with other NSAIDs
Glucocorticoids/Corticosteroids	Prednisolone, Dexamethasone	Preventing the release of phospholipids and decreasing the actions of eosinophils, thereby decreasing inflammation	Bone-thinning,weightgain, diabetes, Hypertension and immunosuppression
Conventional DMRDs	Methotrexate (MTX) ⁵	low-dose MTX increase adenosine level and reduce the pro-inflammatory while increase the anti-inflammatory cytokine levels.	Liver problems, cirrhosis, and bone marrow deterioration.
	Hydroxy chloroquine	Decreases the secretion of monocyte-derived proinflammatory Cytokines	Problemsin the GI tract, skin, and central nervous system
	Leflunomide	Inhibits the synthesis of ribonucleotideuridine monophosphate pyrimidine. It retards the progression of RA	Hypertension, GI upset, liverdamage,leukopenia, interstitial lung disease, neuropathy,rash, and bone marrow damage
	Sulphasalazine	May reduce secretions of interleukin (IL)-8and monocyte chemoattractant protein (MCP)	GI and central nervous system symptomsas well as rash
	Aurothioglucose, auranofin,gold sodium thiomalate	Accumulation of gold by macrophages inhibits both phagocytosis and of the activities of lysosomal enzymes ⁶	Damage tothe bone marrow and kidneys
Biological DMRDs	Etanercept,infliximab ,adalimumab, golimumab,and certolizumabpegol	Tumor necrosis factor (TNF) inhibitors	Increased risk of infections, neurologic diseases like multipleSclerosisand lymphoma(expensive, contraindicated in patients with congestive heart failureof demyelinating diseases)
	Rituximab	Anti B-cell	
	Anakinra (Kineret)	AntiIL-1(chemical messenger of inflammation)	
	Tocilizumab (Actemra),Sarilumab	Anti IL-6,	
TARGETED SYNTHETIC DMRDs	Baricitinib, tofacitinib(Xeljanz)	Blocks Januskinases within cells(enzymes of inflammation)	Increased risk of infections

Since decade ago, different conventional therapeutics such as analgesics, steroids & NSAIDs were used in RA as first line drugs which relieve pain and decrease inflammation. But with due to the course of disease progression they became ineffective, so to slowing or stopping the progression of joint destruction and deformity, Disease-modifying antirheumatic drugs (DMARDs) are used as second line management of rheumatoid arthritis. After all nonsurgical approaches have failed, at that point (intractable joint pain or functional decline due to joint destruction), the disease is considered "end-stage". Then Surgery is a last resort for the treatment of RA. A patient who needs surgery should be evaluated based on their customized needs because many different types of surgical treatments are there. The management of Rheumatoid arthritis is briefed out in fig.3 as it is in the review by Jacqueline Bullock et al.⁴

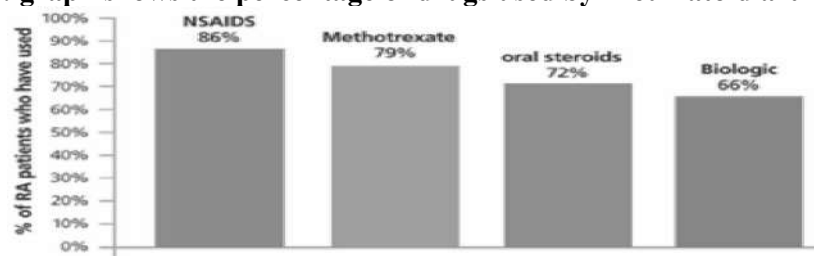
Figure 3: The management of Rheumatoid arthritis



IMPORTANCE OF DRUG DELIVERY SYSTEMS IN MANAGEMENT OF RA:

The figure 6 showing 86% patient use NSAIDs, 79% patient use disease modifying anti-rheumatic drug (methotrexate), 72% patient use oral steroids and 66% patient use biologic DMARDs.³

Figure 6 . : graph shows the percentage of drugs used by rheumatoid arthritis patient



Citation: Health Union, LLC. RA in America, 2013. Available at: <http://rheumatoidarthritis.net/RA-in-america-2013/>

Even though most of the RA patient using NSAIDs and overall cost burden of treatment of NSAIDs is less compared with other drugs, they can only effective in first line therapy moreover long-term use of NSAIDs and steroidal drugs may lead to severe effects. The conventional DMRDS, Methotrexate (MTX) is used by 79 % patients and MTX is the initial second-line drug, and is considered as Gold standard drug for RA treatment, Even with the newer biological agents, MTX continues to serve as a reference point and there is still a role for MTX in the treatment of RA patients but it causes toxicities like stomatitis, gingivitis, glossitis, ulceration, and bleeding of the mucous membrane when given orally and hematological effects like leucopenia, thrombocytopenia, anaemia, haemorrhage from various sites in single-dose intravenous administrations, and also some hepatic toxicities by administering as conventional dosage forms.^{7,8} Biological DMRDS are considered to be a more "direct, defined and targeted" method of treatment, but with these cause serious side effects, such as increased risk of infections, neurologic diseases like multiple sclerosis and lymphoma,⁴ and these are costlier drugs compare with other. In addition, because of short half-life and insufficient concentration of therapeutic agents in site of action, high and frequent dosing is required which may be resulted in severe side effects as well as high cost. To overcome these limitations, development of new agents or therapeutic strategies has been proposed. But it is better to use already existing drug molecules rather than new drug invention. Considering this point new therapeutic strategies, like novel controlled, sustained, extended release dosage forms and delivery systems are developed.

Sustained release (SR) or controlled release dosage form is better than conventional dosage form because SR dosage form are designed to achieve prolong therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose while conventional dosage form multiple dose administration is necessary. Various advantages of novel sustained release drug delivery systems are⁹

- Improved patient convenience and compliance due to less frequent drug administration.
- Reduction in fluctuation in steady-state levels and therefore better control of disease condition.
- Reduced intensity of local or systemic side effects.
- Increased safety margin of high potency drugs due to better control of plasma levels.
- Increase effectiveness of the drug by localization at the site of action.
- Maximum utilization of drug enabling reduction in total amount of dose administration

By considering these advantages various drug delivery approaches were developed for the effective management of Rheumatoid arthritis with better patient compliance.

Amandeep Kaur et al.,¹⁰, Rupali Shukla et al.,³, and Mohanty *et al.*,² in their reviews discussed and reviewed the various novel therapeutic strategies of various drugs used in Rheumatoid Arthritis like controlled and delayed release tablets, capsules, sustain release pellets, Suppositories, topical formulations, and novel drug delivery systems like microcapsules, microspheres (beads), microballons, Micro Sponge, nanoparticles, nanoemulsions, nanocomposites, nanosuspensions, dendrimers, micelles, liposomes, neosomes, pharmacosomes, transdermal patches, emulgels etc. And so on as these are the promising tools having successful applications in overcoming the limitations associated with conventional drug systems for effective treatment of Rheumatoid Arthritis. These are listed in table 2.

Table:2 Novel drug delivery approaches and their importance in RA management

DRUGS	DELIVERY APPROACH	IMPORTANCE
NSAIDS (Nonsteroidal anti-inflammatory drugs)		
Diclofenac	Liposomes	Enhance skin delivery
	Pellets	Increase bioavailability
	Microcapsules	Better efficacy
	Microspheres	Good targeting efficiency
	Nanocomposites	Prolonged action
	Soft gels	Acceptable less side-effect
	Suppositories	Avoid gastric irritation
	Pharmacosomes	High drug loading
	Microsponges loaded capsules ¹¹	Controlled release, reduce the dosing frequency and gastric irritation
Aceclofenac	Topical Gel containing microsponges ¹²	Prolonged release for proficient arthritis therapy
Ibuprofen	Nanosuspensions	Decrease side-effects
	Microemulsions	Good stability
	Microspheres	Effective therapy
	Lipospheres	good anti inflammatory and analgesic properties
	Pulsatile Capsules	sustained release increase efficacy and safety
Indomethacin	Capsules	Better safety
	Pellets	Faster release of drug
	Liposomes	Minimum side effects
	Microballoons	Good floating ability
	Microspheres	Prolonged delivery
	Nanoemulsions	Transdermal delivery
	Suppositories	More therapeutic efficacy
	Dendrimers	Improve bioavailability
	Microsponges ¹³	Controlled release
Acelofenac	Microspheres	prolonged drug release.
	Matrix Tablets	sustained release, uniform therapeutic effect.
Naproxen	Tablets, Nanoparticles	Better safety Implantable sustain release
Ketoprofen	Tablets	Improve bioavailability
	Microspheres	Increase in-vitro release
	Microcapsules	High drug loading

	Microsponges	Usage in dermatology
	Nanoemulsions	Modify drug release
	Transdermal patch	More skin permeation
	Suppository	Good efficacy profile
	Floating microparticles	Good flow property
Mefenamic acid	Matrix Pellets	Controlled release, lowering the risk of side effects and improving patient convenience
	Microparticles	Avoid adverse effects, and improved patient compliance
Celecoxib-	Liposomes	more effective to reduce the pain and cartilage protection
	Patches	Reduced frequency of administration, and thus may improve the patient compliance.
Lornoxicam	Press Coated Tablets	Sustained release lornoxicam show more effective for treating RA
	Compression coated tablets	Sustained release more effective
	Microsponge tablet	Prolonged release
Tenoxicam	Patches	Efficacious, safe, stable and non-irritant to skin in RA.
Meloxicam ¹⁴	Microsponges (transdermal)	Enhanced release and the dissolution, minimize gastrointestinal side effects and improve patient compliance
Piroxicam ¹⁵	Microsponges Tablet	Enhanced solubility and dissolution rate for better absorption
Steroidal drugs		
Prednisolone	Liposomes	Better targeting
	Microspheres	Fast release of drug
	Nanoparticles	Prolonged release
Dexamethasone	Micromethason liposomes	Reduces the side effects for prolonged period therapy
	Novel liposomes	Improved hematological profile of RA
	Multilamellar liposomes	Reduces knee joint inflammation
Betamethasone ¹⁶	Microsponge Loaded Topical Gel	Minimize the frequent dosing, improve effect and patient compliance
DMARDS (Disease modifying Anti-Rheumatic Drugs)		
Methotrexate	Microspheres	Better targeting
	Sustain release tablets	Good efficacy
	Niosomes	Reduce its renal, hepatic, gastrointestinal toxicity and to sustain the effect of drug release.
	Tripolyphosphate Nanoparticles	Enhance the efficacy
	Sodium alginate Nanoparticles	Controlled drug release for a prolonged period
	Ethyl cellulose Nanoparticle	controlled drug delivery strategies used to enhance the efficacy of the drug.
	Microspheres	Sustained release improved bioavailability and avoid the inconvenience and expense of continuous infusion of long duration
Sulfasalazine	Delayed release tablets	Improved efficacy
Azathioprine	Sustain release tablets	Better safety
Tacrolimus	Solid dispersion	Potent suppressive activity
Leflunomide	Sustain release Tablets	More safety profiles
	Microcapsules	Better sustain action
	Microspheres	Rapid action of drug
Auranofin	Sustain release Tablets	More efficacy
	Injectables	Produce high drug concentration at target site

CONCLUSION

In spite of various advancements in research of RA, neither single drug nor combination therapy has permanently cure the disease or has fruitful results. RA can control by currently available therapeutics, but these therapies are accompanying with significant side effects. Therefore, development of a new therapeutic strategy for RA treatment is essential. The various drug delivery strategies as mentioned in this review, due to their controlled release, selective accumulation and reduced systemic toxicity outweighs the conventional RA therapies and promises to improve patient compliance. Moreover, it is beneficial to develop new drug delivery strategies of already existing drug molecules rather than inventing new drugs. Therefore, every new approach in the targeted therapy of rheumatoid arthritis could contribute to the effectiveness in treating the chronic disease. So, it is advisable to the researchers to develop cost effective, controlled release, targeted new delivery systems of existing drug molecules for effective management of RA with better patient compliance.

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DEVISE AND EVALUATION OF CURCUMIN LOADED PERIODONTAL FILMS USING HPMC E15, HPMC E5 AND PVP K30

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ABSTRACT

In present study periodontal films of curcumin were devised and evaluated for faster on set of action using hydrophilic polymers, HPMC E5, HPMC E 15 and PVP K90. Periodontal films were prepared by employing solvent casting method. Propylene glycol was selected as permeation enhancer and plasticizer. Formulations were prepared with the varying concentrations polymers ranging from F1-F12, and all the formulations were evaluated for various physical parameters such as physical appearance, weight variation, thickness, folding endurance, tensile strength, drug content, moisture uptake, moisture content and all the results were found to be were found to be with in the required limits. In vitro drug release studies were conducted by modified method using SS disc with caynoacrylate adhesive. Among all the 12 formulations F6 which contains HPMC E5 350mg shown 94.2% cumulative drug release within 30 min as compared to those those of other HPMC E5, HPMC E15 and poly vinyl pyrrolidine-K90. Formulations of HPMC E 15 showed more retarded drug release pattern.

KEY WORDS: *Periodontal films, Local drug delivery, Curcumin, HPMC E15, HPMC E5, PVP K90, HPMC E 15.*

INTRODUCTION

Periodontal disease is an infection that involves the inflammatory process and the immune response. The presence of periodontal pathogens such as *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* are responsible for periodontal destruction. Periodontitis refers to acute and chronic disorder of the soft tissues surrounding the teeth which eventually leads to loss of supporting bone.¹ There is an unmet medical need for new products to confront the high percentage of people with periodontal diseases. Retention of the drug dose at the point of application would facilitate long-term release and more effective treatments.² Apart from scaling and root planing, systemic antibiotic therapy is employed in treating periodontitis.³ Systemic antimicrobials such as adjuncts to mechanical therapy have had a positive effect on clinical as well as microbiological parameters.⁴ But the impact of this approach is reduced by the fact that the antibiotic is normally difficult to maintain in therapeutic concentrations at the site over the course of the treatment period. Due to these negative effects, the use of local drug delivery devices containing antibiotics may be explored. These devices can maintain extremely high local concentrations of drug for one month. Several implantable devices like fibers, films⁵ and gels were studied. Various biodegradable polymers such as poly (glycolidecold-lactide), polyester poly (caprolactone), glycerol mono-oleate, crosslinked atelo-collagen, hydroxypropylcellulose, chitosan^{6,7} and alginates were employed as drug carriers. In our earlier studies cross linked sodium alginate^{8,9,10} and chitosan were successfully employed as rate retarding polymer for periodontal films for controlled release of drugs. Curcumin is a hydrophobic polyphenolic compound derived from the rhizomes of *Curcuma longa*, shows wide spectrum of antibacterial, anti-inflammatory, and antioxidant properties against a number of periodontal pathogens and hence selected for site-specific delivery in the treatment of periodontitis.¹¹ Clinical applications of Curcumin as oral gel, prevention of plaque and gingivitis, mouthwash, subgingival irrigant, treating periodontal diseases are reported.^{12,13,14,15} As dietary spices, curcumin has been consumed for centuries up to 100 mg/day.¹⁶ Curcumin loaded films of gelatin,¹⁷ ethyl cellulose, HPMC K4M, and Eudragit are formulated and Clinical studies on curcumin-PLGA nanoparticles.¹⁸ mucoadhesive films¹⁹ and oral curcumin gel were

evaluated.²⁰ Literature survey showed no research on curcumin loaded periodontal films using HPMC E15, HPMC E5 AND PVP K30 polymers. In the present study periodontal films of curcumin were prepared using solvent casting technique and evaluated using these polymers.

MATERIALS AND METHODS

Curcumin procured commercially from Yarrow Chem Ltd., Mumbai, India. HPMC E15, HPMC E5, PVP K30, Propylene Glycol, Citric Acid, Aspartame were procured from Loba Chemicals Pvt., Ltd. Mumbai, India.

Fabrication of periodontal films

HPMC E5 and HPMC E15 were weighed in required ratios and they were then dissolved in water (Cold water) as solvent. Curcumin (100 mg) and propylene glycol was added as plasticizer to the above dispersion under continuous stirring. The uniform dispersion was poured in the petri plate. The rate of evaporation of solvent was controlled by inverting cut funnel over the thin films. After 24h, the dried thin film were taken out and stored in desiccator. The formulations of ingredients used are summarized in the formulation Table 1.

Table 1: Formulations of curcumin periodontal films

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Curcumin	4	4	4	4	4	4	4	4	4	4	4	4
HPMC E 15	300	350	400	450	-	-	-	-	-	-	-	-
HPMC E 5	-	-	-	-	300	350	400	450	-	-	-	-
PVP K90	-	-	-	-	-	-	-	-	300	350	400	450
Propylene glycol	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Citric Acid	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Aspartame	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Water	15	15	15	15	15	15	15	15	15	15	15	15

Characterization of periodontal films

Compatibility studies were conducted using Fourier transform infrared (FTIR) spectroscopy, of the polymer along with the drug. Physicochemical properties such as organoleptic properties, thickness of the film, disintegration time, dissolution time, folding endurance, pH, percentage of moisture uptake, tensile strength of the film, swelling property and transparency, were determined.

Drug excipients interaction studies were conducted using FTIR Samples of drug alone, polymer alone and polymer along with the drug were dried to constant weight and triturated with an equal quantity of KBr. Each sample was then compressed to obtain discs for IR analysis. The spectra of these discs were recorded on a Perkin Elmer RXI, IR spectrophotometer (USA) in the spectral region of 500-4000 cm^{-1} . IR spectral analysis was carried out using FT-IR by the KBr disc method. The sample and KBr were triturated and compressed to get the discs. Organoleptic properties were evaluated using special controlled human taste panels for psychophysical evaluation of the films prepared. Thickness of the dried films was measured using micrometer (model 2050-08, Mitutoyo, Japan). Three measurements were taken at randomly selected points on each specimen and the mean thickness was taken. Disintegration time is the time at which the film begins to break down when brought into contact with water. It can be determined by keeping the films of desired sizes (7×5 mm) in a petri dish containing water and noting the time it takes to break down. A 2 ml of distilled water was placed in a petri dish and one film was added on the surface of the water and the time measured until the periodontal film was totally dissolved. Weight uniformity of films were checked by taking 7×5 mm size films, weighed on electronic balance and the average weight was calculated. This was repeated for three sets of each film and the standard deviation was calculated. In order to carry out the endurance study, the strip of film is repeatedly folded at the same place until it breaks. The number of times the film is folded at the same place prior to breaking gives the folding endurance. Folding Endurance was evaluated by repeatedly folding a small strip of film of 2×2 cm size at the same place till it broke. The film surface pH measurement was carried out by keeping the film in contact with distilled water, and after 1 hour, the pH of the solution or dispersion is measured. The pH value was determined by dissolving one periodontal film in 2 ml distilled water and measuring the pH of the obtained solution. The pH was

measured by using pH paper. Differences were expected because various polymers were used as well as the addition of API. The assay was performed to ensure the drug loading onto each film. This test was performed by dissolving a 6 cm² 6.8 phosphate buffer with stirring. The resultant solution was filtered using a whatman filter paper, and the filtrate was diluted to 100 ml with the same buffer in a volumetric flask. Then 1 ml of the filtrate was further diluted to 10 ml with buffer. This solution was analyzed using a spectrophotometer at 421 nm. Content The content uniformity test was used to ensure that every film contains the intended amount of drug substance with little variation among films area of film in 50 ml of a patch. Three pieces, each 6×2 cm, were cut from the whole patch, and assayed for drug content. Same procedure was repeated for all the nine batches.**Error! Bookmark not defined.**The in vitro drug release study of films was carried out using a USP 23 type 2 rotating paddle dissolution test apparatus (Disso 2000, LabIndia, Mumbai, India). 250 ml of phosphate buffer (pH 6.8) was used, and maintained at 37±5°C while the basket was set at 50 rpm. A film sample of 4 cm² was fixed onto the specially designed SS disk with the help of cyano acrylate adhesive. The disk was put at the bottom of the dissolution vessel so that the patch remained on the upper side of the disk. Five milliliters of samples were taken at a interval of 60 sec., and the same amount was replaced with fresh buffer. Moisture uptake test was done by keeping previously weighed films in desiccators at a particular temperature and relative humidity. After three days, the film is taken out and reweighed to determine the percentage of moisture uptake. Percentage of moisture uptake can be calculated as follows.²¹

$$\text{Percentage of moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100.$$

Moisture content was determined by previously weighed films are stored in a desiccator for 24 hours. The final weight is noted when there is no further change in the weight of individual film. Percentage of moisture content can be calculated as follows,

$$\text{Percentage of moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{initial weight}} \times 100$$

Tensile strength is the maximum stress applied to a point at which the strip specimen breaks The tensile strength of the films was determined by universal strength testing machine. The sensitivity of the machine is 1 g. It consists of two load cell grips. The lower one is fixed and the upper one is movable. The test film of specific size was fixed between these cell grips and force was gradually applied till the film breaks. The tensile strength of the film was taken directly from the dial reading in kilograms.. Tensile strength of the film is determined by using a tensile testing machine like the Instron or Monsanto tester. It is calculated by the applied load at rupture divided by the cross-sectional area of the strip.²² Film swelling studies are conducted using a simulated saliva solution. Each film sample is weighed and placed in a pre weighed stainless steel wire mesh. The mesh containing film sample is submerged into a 15 ml pH 6.8 buffer in a plastic container. An increase in the weight of the film was determined at preset time intervals until a constant weight was observed. Degree of swelling property is calculated by following formula,²³ Swelling Index (SI) = (Wt – Wo) / Wo., Where, Wt is the weight of the film at time “t” and Wo= weight of the film at t = 0.

Accelerated stability studies**Error! Bookmark not defined.**

Optimized formulations were subjected to short-term stability testing. Films wrapped in aluminum foil and kept in a humidity chamber maintained at 40°C ± 2°C/75% ± 5% RH for 3 months as per the International Conference on Harmonization (ICH) guidelines. Changes in the physical appearance, surface pH, folding endurance, and drug content of the stored films were investigated during the period and after 3 months.

RESULTS AND DISCUSSION

Periodontal films were successfully prepared by employing solvent casting method. Propylene glycol was selected as permeation enhancer and plasticizer. Formulations were prepared with the varying concentrations polymers from F1-F12, and all the formulations were evaluated for various physicochemical parameters. The results of physicochemical properties of curcumin periodontal films are presented in Tables 2a and 2b. Thickness of films was found in the range of 0.3447±0.0048 and 0.3576±0.0027 mm; Weight variation was between 21.30±0.09 and 48.30±0.37 mg; Moisture content was found between 3.77±0.12% to 9.67±0.27%; Mositure uptake was from 3.05±0.12% to 19.65±0.26%; Folding endurance was found to be

between 250 ± 2.6 and 288 ± 5.8 times of foldings; Surface pH was from 6.4 to 6.8; Tensile strength was found between 1.26 ± 0.06 and 1.94 ± 0.18 Kg; Swelling index was between 16.2% and 23.3%; Disintegration time from 35 to 95 sec.; Drug content was in between $25.4 \pm 0.6\%$ and $92.5 \pm 3.6\%$.

FTIR studies showed the spectra of curcumin alone and optimized formulation F6. FTIR spectra of the pure curcumin showed characteristic bands at 3856/cm due to C-OH, 1515/cm due to C=O, and 959/cm due to C=C functional groups. FTIR spectra of formulation F6 showed characteristic bands at 3855/cm due to C-OH which 1551.94/cm due to C=O, and 963/cm due to C=C functional groups, indicating the chemical stability of curcumin in the chosen polymeric film of HPMC E5. This also indicates that curcumin is compatible with the polymer employed in the study.

The in vitro drug release studies were conducted by modified method using SS disc with caynoacrylate adhesive. Among all the 12 formulations F6 formulation which contain HPMC E5 350mg and shown 94.2% cumulative drug release within 30 min. And compared to HPMC E5, HPMC E15 and PVP K90, HPMC E 15 showed better drug release profile. All the formulations showed initial burst release and controlled release in later phases, as shown in Figure 2. The percentage drug release from film F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11 and F12 was found to be 91.2, 87.8, 89.9, 79.2, 83.9, 94.2, 42.65, 49.49, 39.84, 57.86, 43.33 and 78.95% of curcumin was released at the end of 300 min respectively. The formulation F6 which contain HPMC E5 350 mg showed 94.2% cumulative drug release within 30 min and compared to those formulations of HPMC E5, HPMC E15 and PVP K90 the formulations of HPMC E5 showed better drug release profile. Table 3 shows drug release kinetics obtained from different plots of formulations F1-F12 showing regression equations and correlation coefficients. The R^2 values of First order kinetics showed higher values as compared to those of zero-order plots and hence the curcumin release from the prepared films followed first order kinetics (Table 3). Higuchi's models were applied to test the release mechanism. The higher values of R^2 showed that the curcumin release mechanism from the formulated films was diffusion controlled.

The folding endurance and drug content of the formulation were found to be decreasing and are reported in Table 4. There were no significant changes observed in other characteristics, which means that the prepared formulations are stable as per ICH guidelines. Out of the twelve formulations, the formulation F6 containing HPMC E5 350 mg showed complete and controlled release with 94.2% cumulative drug release within 30 min and all other physicochemical properties were satisfactory and hence the formulation F6 has been optimized in this investigation.

Table 2a: Physicochemical properties of curcumin periodontal films

Formulation	Mean $\pm$ SD (n=6)			
	Thickness (mm)	Weight variation (mg)	Moisture content (%)	Moisture uptake (%)
F1	0.3569 ± 0.0032	27.46 ± 0.19	3.77 ± 0.12	7.98 ± 0.18
F2	0.3520 ± 0.0044	32.57 ± 0.26	9.2 ± 0.19	16.05 ± 0.22
F3	0.3470 ± 0.0063	29.21 ± 0.21	5.16 ± 0.16	13.09 ± 0.29
F4	0.3496 ± 0.0026	33.65 ± 0.28	5.66 ± 0.21	15.63 ± 0.36
F5	0.3460 ± 0.0048	28.39 ± 0.19	4.87 ± 0.13	11.73 ± 0.21
F6	0.3517 ± 0.0053	35.53 ± 0.31	9.67 ± 0.27	19.65 ± 0.26
F7	0.3568 ± 0.0039	25.36 ± 0.08	6.25 ± 0.11	3.05 ± 0.12
F8	0.3522 ± 0.0062	29.65 ± 0.16	7.86 ± 0.15	9.5 ± 0.28
F9	0.3471 ± 0.0042	36.30 ± 0.24	5.24 ± 0.12	12.4 ± 0.31
F10	0.3484 ± 0.0032	48.30 ± 0.37	6.35 ± 0.14	13.6 ± 0.39
F11	0.3447 ± 0.0048	21.30 ± 0.09	7.89 ± 0.21	15.2 ± 0.29
F12	0.3576 ± 0.0027	22.50 ± 0.11	8.21 ± 0.24	18.3 ± 0.32

Table 2b: Physicochemical properties of curcumin periodontal films

Formulation	Mean $\pm$ SD (n=6)					
	Folding endurance	Surface pH	Tensile strength	Swelling index	Disintegration time (sec)	Drug content

	'n' times (n=6)		(Kg) (n=6)			(n=6)
F1	250±2.6	6.7	1.63±0.15	20.6	39	45.3±1.5
F2	260±4.1	6.5	1.23±0.05	21.2	47	65.5±2.4
F3	265±5.2	6.6	1.45±0.09	22.4	43	57.5±1.8
F4	270±2.9	6.7	1.35±0.07	23.3	56	60.8±1.9
F5	272±2.2	6.8	1.85±0.13	18.3	59	67.5±2.2
F6	278±5.5	6.4	1.94±0.18	18.7	68	92.5±3.6
F7	265±4.9	6.6	1.63±0.12	19.1	54	75.8±2.7
F8	254±6.3	6.5	1.87±0.14	19.5	35	84.6±2.2
F9	265±4.7	6.7	1.54±0.09	16.2	45	65.4±1.7
F10	266±2.8	6.5	1.26±0.06	16.7	85	75.3±2.5
F11	279±3.9	6.8	1.37±0.04	17.0	88	25.4±0.6
F12	288±5.8	6.4	1.42±0.03	17.2	95	36.8±0.9

Figure 2: Dissolution graph of all formulations (F1-F12)

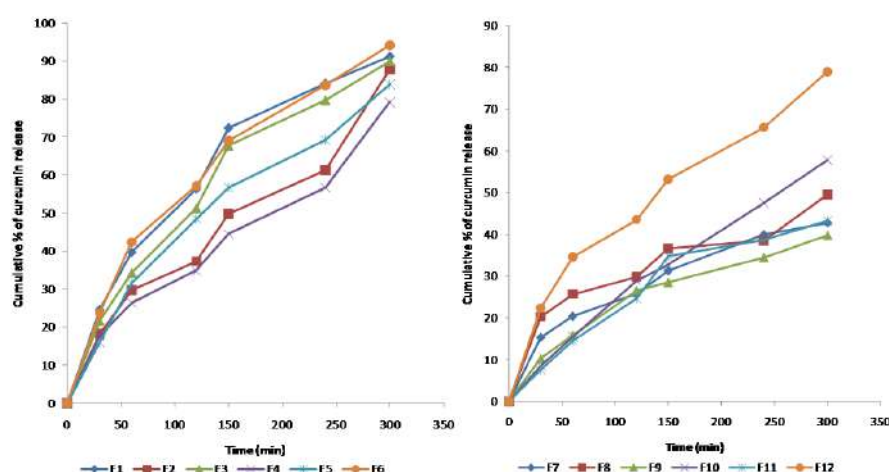


Table 3: Dissolution kinetic values obtained from different plots of formulations F1-F12 showing regression equations and correlation coefficients

Formulation	Zero order	First order	Higuchi model	Korsmeyer-Peppas model
F1	0.904	0.992	0.988	0.929
F2	0.960	0.869	0.941	0.971
F3	0.933	0.984	0.985	0.945
F4	0.965	0.919	0.948	0.985
F5	0.947	0.978	0.984	0.923
F6	0.917	0.970	0.994	0.926
F7	0.898	0.943	0.988	0.982
F8	0.830	0.889	0.941	0.959
F9	0.914	0.949	0.985	0.932
F10	0.989	0.996	0.948	0.961
F11	0.922	0.948	0.984	0.899
F12	0.931	0.976	0.994	0.970

Table 4: Data of stability studies of the optimized formulation F6

Time (days)	Physical appearance	Folding endurance	Surface pH	Drug content (%±SD)
0	No change	278±5.5	6.4	92.5±3.6

10	No change	263±4.6	6.4	92.2±2.4
20	No change	260±5.2	6.2	91.7±3.1
30	Insignificant change in color	256±6.8	6.1	91.4±2.9

CONCLUSION

Local delivery of curcumin in the form of site-specific periodontal films has opened up a new arena for the management of periodontal diseases. Periodontal disease is an infection that involves the inflammatory process and the immune response. Periodontal films were successfully prepared by employing solvent casting method. Propylene glycol was selected as permeation enhancer and plasticizer. Formulations were prepared with the varying concentrations polymers from F1-F12, and all the formulations were evaluated for various physicochemical parameters. FTIR studies showed that curcumin is compatible with the polymer employed in the study. All the formulations showed initial burst release and controlled release in later phases. Accelerated stability studies showed no significant changes observed in other characteristics, which means that the prepared formulations are stable as per ICH guidelines. Out of the twelve formulations, the formulation F6 containing HPMC E5 350 mg showed complete and controlled release with 94.2% cumulative drug release within 30 min and all other physicochemical properties were satisfactory and hence the formulation F6 has been optimized. And compared to HPMC E5, HPMC E15 and poly vinyl pyrrolidone-K90, the formulations of HPMC E 15 showed more retarded drug release pattern.

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ENTERIC COATED CAPSULES FOR EXTENDED RELEASE OF LANSOPRAZOLE MUCOADHESIVE MULTIPLE-UNIT MINI PATCHES

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ABSTRACT

The present study aims to the formulation and evaluation of novel oral mucoadhesive multiple-unit mini patches (MMMP) of lansoprazole (LAN) for extended release. MMMP of LAN in enteric coated capsules were prepared and evaluated using chitosan in combination with xanthan gum, guar gum, tragacanth or acacia in the ratios of 19:1, 9:1, 4:1, 1.5:1 and 0.66:1 employing 2% acetic acid as solvent for chitosan, purified water as solvent for natural gum polymers and glycerin 0.12 mL as plasticizer. The prepared films (0.5 mm dia) were filled into hard gelatin capsules which were enteric coated. The prepared MMMP were characterized for surface texture, thickness, folding endurance, moisture content, moisture uptake, mucoadhesive strength, drug content uniformity, *In vitro* drug release and accelerated stability studies. The SEM photographs showed the rough to smooth pattern of surfaces of patches. The thickness of MMMP found between 43.62 ± 0.27 and 49.53 ± 0.11 μm ; mean weight was between 13.81 ± 0.14 and 14.94 ± 0.15 mg; percentage of swelling was between 215 ± 5.39 and 473 ± 6.72 ; moisture content was between 1.04 ± 0.06 and 2.67 ± 0.24 ; mucoadhesion was found between 5.5 ± 0.2 and 12.3 ± 0.6 h and the drug content was found between $95.08 \pm 3.42\%$ and $99.16 \pm 4.73\%$ for all formulations. The FTIR and DSC spectra indicated no drug-polymer interactions. The *in vitro* dissolution studies showed extended release of LAN from MMMP in pH 6.8 phosphate buffer where as no significant drug release found in acidic environment showing that MMMP in enteric coated capsules could be employed to delivery LAN to intestine directly. In all the cases the drug dissolution was reciprocal to the polymer concentration in the formulations. Among all natural polymers employed in this specification the decreasing order of drug release from the polymers was xanthan gum > tragacanth > guar gum > acacia. The prepared novel mucoadhesive multiple-unit mini patches (MMMP) in enteric coated capsules for controlled release of lansoprazole using natural polymers and could be successfully employed for intestinal delivery while minimizing the drug degradation and providing extended release of the drug.

KEY WORDS: *Enteric coated capsules, extended release, lansoprazole, mucoadhesive multiple-unit mini patches, MMMP*

INTRODUCTION

Intestinal patches mostly millimeter sized provide a unique platform for oral delivery of drugs which possess poor oral bioavailability, necessitating their administration by injections. They are inspired by transdermal patches, which in spite of similar conceptual design; operate in very different physiological environments¹. Several gastrointestinal patch systems provide bioadhesion, drug protection from acidic environment and unidirectional release^{2,3,4}. In addition, these devices create a high concentration gradient for drug transport, which facilitate uptake of loaded proteins through the intestinal membrane⁵. Consequently, intestinal patch-based devices are being developed for oral delivery of several drugs such as insulin,⁶ exenatide, calcitonin, interferon- α , erythropoietin and human granulocyte colony-stimulating factor for the treatment of diabetes, osteoporosis, hepatitis or chemotherapy. **Error! Bookmark not defined.** A patent described pH dependent mucoadhesive patches for unidirectional release of drugs⁷. Commonly used mucoadhesive polymers are chitosan and its thiolated derivatives, pectin, polyacrylic acids, carbopol, alginates, polyvinyl alcohol and cellulose derivatives such as sodium carboxymethyl cellulose (SCMC), hydroxypropylmethyl cellulose and

hydroxypropyl cellulose. Adhesion to the intestinal mucosa occurs primarily through charge-based interaction between cationic polymers and negative sialic acid residues on mucin or through hydrogen bonding and polymer chain entanglement^{8,9}. Recent trend towards the use of plant based and natural products demands the replacement of synthetic additives with natural gums.¹⁰ Natural polymers are biocompatible and safe it is assigned the GRAS (generally recognized as safe) label – as well as being relatively cheap to produce, being non-toxic, biocompatible, and biodegradable and play important role in the stomach-specific drug delivery, intestinal delivery, and colon-specific drug delivery. Lansoprazole (LAN) is an anti-ulcer agent acts as proton pump inhibitor with a chemical name; 2-({3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-ylmethane}sulfinyl)-1H-1,3-benzodiazole¹¹. As lansoprazole is acid sensitive, it degrades in stomach environment and hence is it not possible to formulate a gastroretentive dosage forms. Lansoprazole enteric coated pellets were formulated using Eudragit L/HPMCAS Blend by Fang *et al.*¹². There is no research found in literature on the mucoadhesive multi-unit mini patches (MMMP) in enteric coated capsules for controlled release of small molecules for delivery to small intestine using natural polymers. Therefore this study was aimed to formulate MMMP in enteric coated capsules for controlled release of lansoprazole and to evaluate the characteristics thereof.

MATERIALS AND METHODS

Lansoprazole from Dr. Reddy's Laboratories, Hyderabad, India; chitosan and tragacanth from Central Drug House Pvt. Ltd., Delhi, India; xanthan gum from Edict pharmaceuticals, Chennai, India; were gratis samples. Guar gum was purchased from S.D. Fine-Chemicals Ltd., Boisar, Mumbai, India. Acacia, glycerin, HCl, acetic acid and other reagents were purchased from S.D Fine Chemicals Pvt. Ltd., Mumbai, India.

Preparation of MMMP in enteric coated capsules for controlled release of LAN

Solvent casting technique was employed for the preparation of MMMP. The patches were prepared by dissolving the specified quantity of chitosan in 10 mL of 2% acetic acid solution along with the selected natural polymer gum (Guar gum, Xanthan gum, Tragacanth or Acacia) in 10 mL of water. Mix both the solutions then disperse 212 mg of LAN drug in homogenous polymer solution. Glycerin 0.12 mL was used as a plasticizer in the preparation of polymer patches. Twenty mL of the solution was poured in Teflon mould (6 cm internal diameter) evenly and allowed for drying in hot air oven for 6 h at a temperature of 50°C. After drying MMMP was cut into pieces of 2 × 2 cm or punched as 5 mm diameter mini patch (MMMP), packed in aluminium foils and stored in desiccators until further studies¹³. All formulations contained 212 mg of LAN, 380, 360, 320 and 240 mg of chitosan in each polymer blend; xanthan, guar, tragacanth and acacia were 20, 40, 80 and 160 mg in formulations F1 to F4 for each of the gums. The ratios between chitosan and other polymers (xanthan gum, guar gum, tragacanth or acacia) were 19:1, 9:1, 4:1, 1.5:1 and 0.66:1.

The MMMP filled capsules are enteric coated by the method reported by Fang *et al.***Error! Bookmark not defined.**Five mini patches of 0.5 mm diameter size equivalent to 30 mg of lansoprazole are filled into hard gelatin capsules. They were coated by HPMCAS/Eudragit L dispersion with blend ratio of 1:3. The aqueous polymer dispersion were separately plasticized overnight with 30% w/w TEC plasticizer and adjusted to 10% (w/w) polymer content with purified water. The blends were obtained by adding the HPMCAS dispersion into the Eudragit L dispersion under the condition of electromagnetic stirring. Coating level was made to 30% weight gain of total weight of filled in hard gelatin capsules.**Error! Bookmark not defined.** All the mucoadhesive multi-unit mini patches (MMMP) were evaluated for the following parameters.

Characterization of MMMP of LAN

The MMMP were visually inspected for colour, clarity, flexibility and smoothness. The free MMMP prepared were evaluated for physical appearance by visual observation. Thickness of the MMMP was measured by a 'Mitutoyo (Japan) Dial Gauge'. The mean of the five observations were calculated.¹⁴The thickness was measured at six locations on the strips using a micrometer (Mitutoyo, Japan). Individual weights of five mini discs containing 30 mg of lansoprazole were measured on top loading electronic balance (Shimadzu, Japan) and the mean weight of mini-patches (0.5 cm dia) was measured. The folding endurance was measured manually for the prepared MMMP. A strip of polymer patch (2×2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the MMMP could be folded at the same place without breaking gave the exact value of folding endurance.¹⁵After determination of

original MMMP weight and diameter; the samples were allowed to swell on the surface of agar plate kept in an incubator maintained at $37 \pm 0.2^\circ\text{C}$. Increase in the weight of the MMMP was determined at different time intervals.¹⁶ Moisture content was determined by weighing MMMP and keeping in a desiccators containing calcium chloride at room temperature for 24 h. The MMMP were weighed again after a specified interval until they showed a constant weight and the percent moisture content was calculated.¹⁷ Moisture uptake was determined by keeping the MMMP in desiccators at room temperature for 24 h. These were then taken out and exposed to 84% relative humidity using saturated solution of potassium chloride in a desiccators until a constant weight is achieved % moisture uptake is calculated. To analyze the drug content five patches of MMMP equivalent to 30 mg of LAN was soaked in 100 ml each of pH 1.2 dissolution medium (0.1N HCl) and pH 6.8 phosphate buffer for 24 h. Then it was sonicated for 5 min and filtered through 0.45 μm Whatman filter paper. The filtrate was suitably diluted with 0.1N HCl or pH 6.8 phosphate buffer and the absorbance of resultant solution was measured spectrophotometrically at 285 nm using a double beam UV spectrophotometer (Jasco V530, Japan). The drug content was determined employing the calibration curves of the drug.¹⁸

Instrumental analysis of MMMP

FTIR spectra were recorded with FTIR spectrophotometer (Bruker Alpha, UK). Samples were prepared by KBR disc method (2 mg sample in 200 mg KBR) and examined in transmission mode. LAN and prepared MMMP were evaluated. The scanning range was $400\text{--}4000\text{ cm}^{-1}$, and the resolution was set to 1 cm^{-1} . The recorded spectrum was further subjected to analysis by Opus FT-IR software V 6.5 software, Bruker UK.¹⁹ The thermal behaviors of MMMP were estimated in terms of their melting endotherms using Shimadzu DSC 60, Kyoto, Japan with 3 mg cut from MMMP samples in standard aluminium pans. The samples were heated from 30°C to 300°C at a constant rate of $5^\circ\text{C}/\text{min}$ under nitrogen.²⁰ Scanning electron microscopy (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The instrument employed was Hitachi S 3700N, Japan in the present study. Samples are gold plated and placed on stub and the surface characteristics were observed.

Ex-vivo mucoadhesion property of MMMP formulations was determined as follows. A 5 cm long piece of freshly cut pig intestine was obtained from a local abattoir within 1 h of sacrificing of animal, and was cleaned by washed with pH 6.8 phosphate buffers. Accurately weighed numbers of MMMP was placed on the mucosal surface, which was attached to a polyethylene plate that was fixed at an angle of 40° relative to the horizontal plane. The buffer was warmed at $37 \pm 1^\circ\text{C}$ and passed at a rate of 5 mL/min over the tissue. The time required for detaching all the MMMP from mucosal surface of the pig intestine was recorded by visual inspection.²¹

Filling of MMMP in into hard gelatin capsules

The MMMP filled capsules are enteric coated by the method reported by Fang et al. The MMMP are filled into hard gelatin capsules were enteric coated by HPMCAS/Eudragit L dispersion with blend ratio of 1:3. The aqueous polymer dispersion were separately plasticized overnight with 30% TEC plasticizer (w/w, based on the total polymer mass) and adjusted to 10% (w/w) polymer content with purified water. The blends were obtained by adding the HPMCAS dispersion into the Eudragit L dispersion under the condition of electromagnetic stirring. Coating level was made to 30% weight gain of total weight of filled in gelatin capsule.

In vitro drug dissolution study

Dissolution studies were carried out by using USP XXIII dissolution test apparatus (basket type, Disso 2000, Lab India). Five patches of MMMP totalling 30 mg of LAN was filled in a hard gelatin capsule and enteric coated as discussed above. The dissolution medium containing 900 mL of the dissolution medium (pH 1.2) for first 2 h followed by 900 mL of pH 6.8 phosphate buffer were employed in the study. Rotation speed was 100 rpm and temperature was maintained at $37 \pm 0.5^\circ\text{C}$. Five milliliters of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples at specified intervals of 0.5 h each were analyzed at 285 nm, by UV absorption spectroscopy. **Error! Bookmark not defined.** and the cumulative percentage release was calculated over the sampling times.²² The rate and the mechanism of release of LAN from the prepared MMMP were analyzed by fitting the dissolution data into, zero-order equation, $Q = Q_0 - k_0 t$ (1), where Q is the amount of drug released at time t , and k_0 is the release rate. First order equation, $\ln Q = \ln Q_0 - k_1 t$ (2), where k_1 is the release rate constant and Higuchi's equation, $Q =$

$k_2 t^{1/2}$ (3) where Q is the amount of the drug released at time t and k_2 is the diffusion rate constant. The dissolution data was further analyzed to define the mechanism of release by applying the dissolution data following the empirical equation, $M_t/M_\infty = Kt^n$ (4), where M_t/M_∞ is the fraction of drug released at time t. K is a constant and n characterizes the mechanism of drug release from the formulations during dissolution process.²³

Stability studies of optimized MMMP

The optimized MMMP of chitosan containing various proportions of xanthan gum, guar gum, tragacanth or acacia were separated in to two groups. Each group of formulations were placed separately in stability chamber which is maintained at $40 \pm 5^\circ\text{C}/75\%$ RH for three months and every month the formulations from each group were subjected to dissolution studies and % drug release was calculated.^{24,25,26} The drug content and drug dissolution profile of the exposed samples were determined. Significance of difference for floating lag time, assay values and dissolution profiles of the optimized formulation before and after accelerated stability testing was calculated based on Student's t-test. The table value of 2.57 at 95% confidence limits is used for determining the level of significance. The similarity factor (f_2) given by SUPAC guidelines for a modified release dosage form was used as a basis to compare dissolution profiles before and after stability studies in the present study.^{27, 28}

RESULTS AND DISCUSSION

An interesting advancement of mucoadhesive approach is mucoadhesive devices that delivery drugs into small intestine. Mucoadhesive multiple-unit mini patches (MMMP) were successfully prepared using chitosan in various combinations with guar gum, xanthan gum, tragacanth or acacia.

Characterization of MMMP

The results of various properties of prepared formulations were summarized in Table 2. Texture of patches appeared rough surface for those prepared using xanthan gum and acacia; where as those prepared using guar gum and tragacanth showed nearly smooth surface. The SEM photographs further showed the same pattern of surfaces at 100x (Figure 1 A-D). The MMMP could be filled into capsules which were further treated with formaldehyde to attain enteric coating (Figure 1 E and F). The thickness was found between 43.62 ± 0.27 and 49.53 ± 0.11 μm for all formulations. The folding endurance was found between 179 ± 6 and 346 ± 5 number of times of folding. The flatness for all the patches was 100%. The swelling was between 215 ± 5.39 and $473 \pm 6.72\%$; moisture content was between 1.04 ± 0.06 and 2.67 ± 0.24 ; mucoadhesion was found between 5.5 ± 0.2 and 12.3 ± 0.6 h29 and the drug content was between $95.08 \pm 3.42\%$ and $99.16 \pm 4.73\%$ for all formulations (Table 1).

Fourier Transform Infrared (FT-IR) Spectroscopy

The results of FTIR are showed in Figure 2-I. The FTIR spectrum of pure LAN drug showed the characteristic absorption peaks of LAN appeared at 3215.96, 2982.36 & 2928.56, 1578.50, 1281.32, 1114.58 denoting stretching vibration of -NH-, -CH₂, aromatic ring, C-O and ether bond, respectively. Similarly, the FTIR spectrum of formulation LXF5 showed the characteristic absorption peaks of LAN appeared at 3218.57, 2982.44 & 2928.86, 1578.79, 1281.37, 1114.52 denoting stretching vibration of -NH-, -CH₂, aromatic ring, C-O and ether bond, respectively. Further, the FTIR spectrum of formulation LGF5 showed the characteristic absorption peaks of LAN appeared at 3219.73, 2982.94 & 2928.92, 1579.37, 1281.34, 1114.22 denoting stretching vibration of -NH-, -CH₂, aromatic ring, C-O and ether bond, respectively. Again, the FTIR spectrum of formulation LTF5 showed the characteristic absorption peaks of LAN appeared at 3221.34, 2982.96 & 2929.32, 1578.73, 1281.22, 1114.55 denoting stretching vibration of -NH-, -CH₂, aromatic ring, C-O and ether bond, respectively. Finally, the FTIR spectrum of formulation LAF5 showed characteristic absorption peaks of LAN appeared at 3220.36, 2982.67 & 2929.69, 1579.33, 1281.02, 1114.69 denoting stretching vibration of -NH-, -CH₂, aromatic ring, C-O and ether bond, respectively.

Differential scanning calorimetry (DSC) studies

The DSC thermograms showed an endothermic peak for pure lansoprazole at 181.36°C which corresponds to melting process. Thermogram of formulation LXF5 showed two endothermic peaks at 181.49°C and 267.56°C . Similarly for formulation LGF5 two endothermic peaks were observed at 83.28°C and 181.24°C .

Formulation LTF5 showed two endothermic peaks at 150.29°C and 181.29°C. The thermogram of formulation F9 indicated endothermic peaks at 181.49°C and 274.14°C (Figure 2-II). The distinct endothermic peaks in all formulations indicate that drug and polymers co-exist as physical mixture and there is no interaction between them.

***In vitro* dissolution studies**

upto 12 h. Among formulations LXF5 showed extended drug release upto 98.85±7.04% for 14 h; LGF5 94.07±7.25% upto 12.5 h; LTF5 showed 99.04±7.34% upto 12.5 h; and LAF5 showed 94.9±4.39 upto 12 h as compared to other formulations. In all the cases the drug release was reciprocal to the polymer concentration in the formulations (Figure 3). The *in vitro* release time of LAN from all formulations was delayed significantly with increase in polymer concentration. On the other hand the increase of gums content resulted in a decrease in the amount of LAN released. The apparent slow-release of LAN from all these formulations during the release study seems to be dependent on the drug:polymer ratio. The *f*-ratio value and *p*-values obtained for xanthan, guar, tragacanth and acacia gums were 2.70775, 0.040516; 2.91575, 0.029336; 2.63078, 2.63078; and 2.8205, 0.030824 respectively. The result was significant at *p*<0.05. The mechanism of drug release for the above formulations was determined by calculating the correlation coefficient (*R*² value) for the kinetic models, as summarized in Tables 2. All the formulations followed zero order drug release kinetics. The 'n' values of Korsmeyer–Peppas model for the best formulations were in the range of 0.45–0.85. Therefore, the most probable mechanism of release was found to be non-Fickian diffusion or anomalous diffusion for the formulations tested. The time required for dissolution of 50% (*T*₅₀) and 90% (*T*₉₀) were determined. It was seen that the duration of release gradually increased with an increase in natural polymers proportion, as against chitosan. Formulations FXF5, FGF5, FTF5 and FAF5 showed higher rate of drug release compared to that of other formulations of respective polymers. Among all natural polymers employed in this specification the decreasing order of drug release from the polymers is as Xanthan gum > Tragacanth > Guar gum > Acacia.

Stability studies

Accelerated stability studies showed no significant change in appearance of the patches studied. Student t-test was conducted on drug content and the *p*-values obtained were 0.3216, 0.4138, 0.3141 and 0.4221 for formulations LXF5, LGF5, LTF5 and LAF5 respectively which were lesser than the table value of 2.57 at 95% confidence limits and hence the result is *not* significant at *p*<0.05. There was no significant difference observed in the drug content uniformity before and after the stability studies. Student t-test was conducted on drug dissolution before and after stability testing for optimized formulations. The *p*-values obtained were 0.436992, 0.419959, 0.429062 and 0.435343 for formulations LXF5, LGF5, LTF5 and LAF5 respectively which were lesser than the table value of 2.57 at 95% confidence limits and hence the result is *not* significant at *p*<0.05. There was no significant difference observed in the drug dissolution before and after the stability studies. Similarity factor (*f*₂) for optimized formulations LXF5, LGF5, LTF5 and LAF5 compared before and after stability testing was found to be 85, 80, 86 and 85 respectively which were between 50 and 100.30,31 Therefore the dissolution profiles of optimized formulations before and after stability testing are considered to be similar.

Table 1: Characterization of MMMP

Formulation Code	Thickness (µm)	Weight of mini patch (mg)	Folding endurance (#)	% Moisture Content	% Moisture Uptake	Muco-adhesive Strength (h)	Drug Content (%)
FXF1	46.34±0.12	13.83±0.17	180±3	1.12±0.07	2.33±0.9	10.23±1.12	95.12±0.07
FXF2	47.28±0.14	13.89±0.20	183±3	1.25±0.05	2.45±0.11	11.47±1.15	96.74±0.04
FXF3	48.13±0.09	14.10±0.23	196±4	1.34±0.09	2.47±0.16	11.56±1.47	97.28±0.09
FXF4	48.82±0.16	14.16±0.26	201±4	1.43±0.04	2.48±0.19	11.59±1.58	98.46±0.06
FXF5	49.34±0.12	14.94±0.15	346±2	1.52±0.07	2.67±0.16	12.3±0.6	99.04±0.01

FGF1	45.12±0.14	13.86±0.15	194±3	1.14±0.06	1.45±0.7	6.02±1.15	96.41±0.08
FGF2	46.34±0.11	13.89±0.22	219±5	1.27±0.04	1.52±0.09	7.48±1.12	97.69±0.05
FGF3	47.28±0.04	13.90±0.23	272±6	1.36±0.08	2.63±0.11	7.56±1.31	98.36±0.04
FGF4	48.18±0.13	14.16±0.21	297±9	1.45±0.06	2.68±0.08	7.94±1.28	98.53±0.07
FGF5	47.86±0.14	14.86±0.21	300±4	1.47±0.01	2.65±0.11	8.5±0.4	98.59±0.09
LTF1	43.28±0.07	13.90±0.17	186±5	1.10±0.03	2.27±0.04	9.2±1.08	97.53±0.07
LTF2	46.57±0.09	13.99±0.19	213±4	1.18±0.04	2.36±0.09	9.4±1.11	97.97±0.11
LTF3	47.52±0.06	14.23±0.14	267±3	1.24±0.08	2.49±0.11	9.6±1.21	98.14±0.06
LTF4	48.13±0.11	14.26±0.18	276±6	1.26±0.05	2.61±0.10	10.2±1.18	98.65±0.05
LTF5	48.32±0.09	14.28±0.16	289±2	1.28±0.03	2.67±0.16	11.6±0.5	99.08±0.09
LAF1	43.47±0.09	13.89±0.17	178±6	1.04±0.06	1.21±0.08	4.12±1.58	97.18±0.08
LAF2	46.13±0.06	13.98±0.19	209±5	1.12±0.03	1.34±0.13	4.19±0.6	97.88±0.05
LAF3	47.68±0.08	14.28±0.22	261±4	1.23±0.07	1.46±0.11	5.02±1.15	98.26±0.03
LAF4	48.66±0.17	14.93±0.23	279±3	1.35±0.05	1.57±0.09	5.48±1.12	98.46±0.04
LAF5	48.82±0.16	14.11±0.19	304±5	1.43±0.04	1.84±0.13	5.75±0.3	98.64±0.06

Table 2: In vitro drug release kinetic data of LAN MMMP formulated

Formulation	T ₅₀ (h)	T ₉₀ (h)	Zero order		First order		Higuchi	Korsmeyer Peppas	
			R ²	K ₀ (mg.h ⁻¹)	R ²	K ₁ (h ⁻¹)	R ²	R ²	N
LXF1	3	5	0.9699	15.048	0.5162	0.573	0.8222	0.9584	1.3754
LXF2	3.5	6.5	0.9831	12.409	0.6552	0.383	0.8548	0.9647	1.3953
LXF3	4.5	8	0.9931	10.208	0.7571	0.308	0.8981	0.9745	1.3958
LXF4	5	9	0.9904	9.407	0.7619	0.291	0.8977	0.9857	1.5416
LXF5	5.5	10	0.9899	8.178	0.8428	0.259	0.8851	0.9745	1.5843
LGF1	3.5	6.5	0.9781	13.040	0.6676	0.384	0.8421	0.9745	1.3924
LGF2	4	7.5	0.9866	11.073	0.6801	0.330	0.8674	0.9830	1.3736
LGF3	5	9	0.9942	9.050	0.6729	0.293	0.9048	0.9783	1.3427
LGF4	5.5	10	0.9918	8.457	0.7615	0.243	0.9048	0.9703	1.4546
LGF5	6.5	11	0.9906	7.464	0.8932	0.170	0.9016	0.9777	1.4830
LTF1	3.5	5.5	0.9790	13.749	0.6193	0.446	0.8440	0.9718	1.2541
LTF2	4	7	0.9864	11.523	0.6152	0.365	0.8671	0.9632	1.3105
LTF3	4	8	0.9894	10.554	0.6647	0.376	0.8880	0.9729	1.3670
LTF4	5	9	0.9890	9.641	0.6822	0.284	0.8785	0.9706	1.5112
LTF5	6	10.5	0.9924	7.872	0.9207	0.229	0.8871	0.9753	1.5869
LAF1	4	7	0.9942	11.819	0.6997	0.375	0.9152	0.9922	1.1234
LAF2	4	7.5	0.9959	10.793	0.6983	0.337	0.9006	0.9923	1.1535
LAF3	4.5	8	0.9975	9.929	0.7045	0.324	0.9129	0.9949	1.2309
LAF4	5	9.5	0.9975	8.767	0.7405	0.265	0.9163	0.9948	1.2399
LAF5	5.5	9.5	0.9967	8.466	0.8644	0.204	0.9130	0.9851	1.3596

Figure 1: SEM photographs of MMMP prepared from Chitosan in combination with (A) Xanthan gum, (B) Guar gum, (C) Tragacanth (D) Acacia showing magnifications of 100x, (E) Photograph of MMMPdiscs (F) MMMP in enteric coated capsule

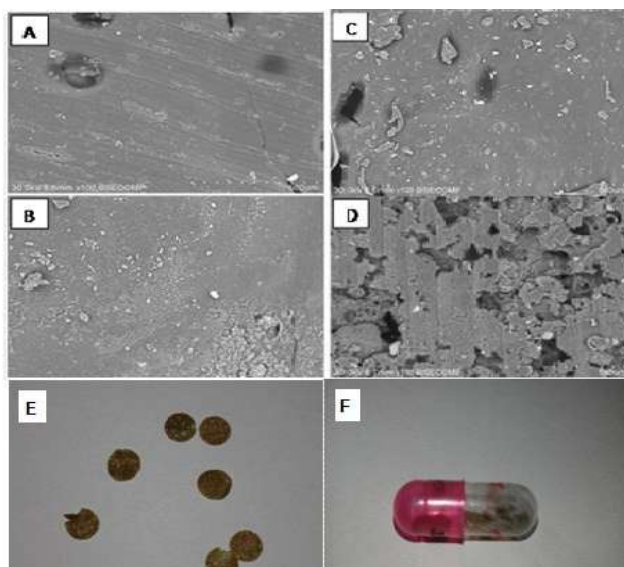


Figure 2: (I) FTIR spectra of (A) LAN, (B) xanthan gum, (C) chitosan, (D) LXF5, (E) guar gum, (F) LGF5, (G) tragacanth, (H) LTF5, (I) acacia and (J) LAF5; and (II) DSC thermograms of (A) LAN, (B) LXF5, (C) LGF5, (D) LTF5 and (E) LAF5

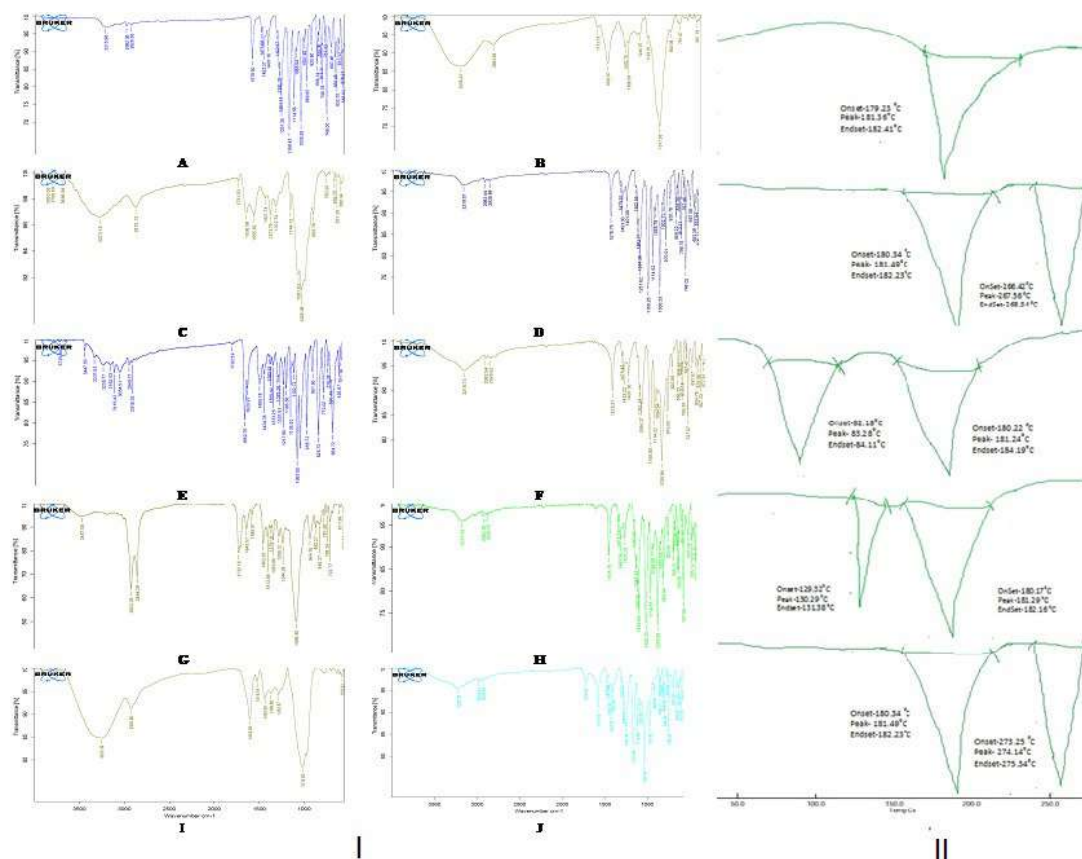
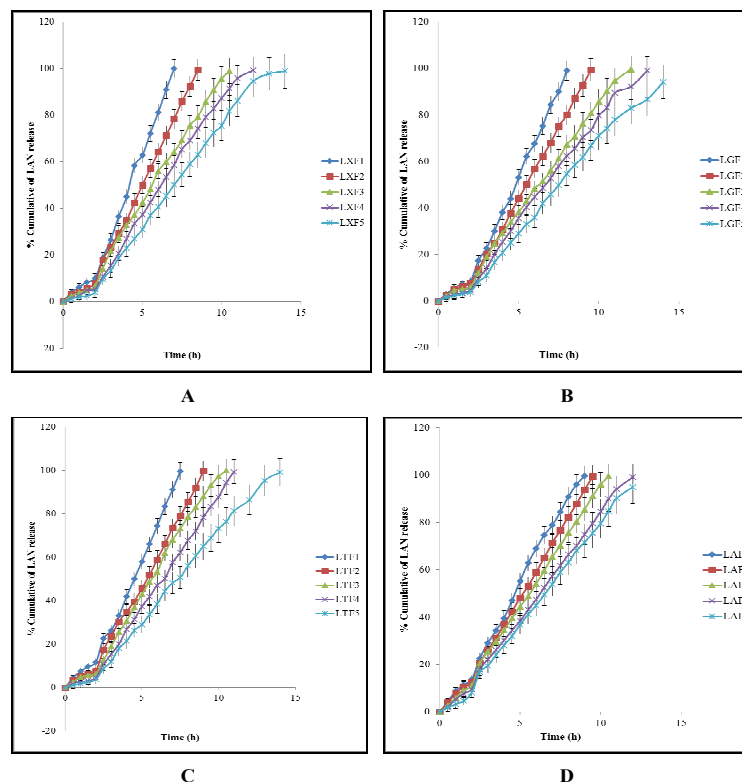


Figure 5: Comparative in vitro drug release profile of LAN MMMP formulated using chitosan with (A) xanthum gum, (B) guar gum, (C) tragacanth and (D) acacia



CONCLUSION

This study discusses the preparation of MMMP of LAN using natural polymer chitosan with xanthan gum, guar gum, tragacanth or acacia. The type of polymer affected the drug release rate and the mechanism. Polymer swelling was crucial in determining the drug release rate flotation. The order of drug release with respect to selected polymers was found as: xanthan gum > tragacanth > guar gum > acacia. The optimized formulations, LXF5, LGF5, LTF5 and LAF5 offered best controlled release along. Good stability was observed for three months during accelerated stability studies. Since the formulation showed sufficient release for prolonged period, the dose could be reduced and the possible incomplete absorption of the drug could be avoided.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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DRUG DELIVERY SYSTEMS OF INSULIN

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ABSTRACT

Diabetes is one of the most common diseases expanding all through the world. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease. All patients with T1DM majorly and in minor amount with long standing T2DM require insulin treatment. Drug delivery systems of insulin have been increasing day by day with advancement in research. Despite the fact that subcutaneous insulin conveyance is the standard course of insulin organization, it is related with infusion torment, needle fear, lipodystrophy, resistance and fringe hyperinsulinemia. In this manner, the need exists for conveying insulin in a negligibly intrusive or noninvasive and in most physiological manner. Inhaled insulin was the first approved noninvasive and alternative way to deliver insulin, but it has been not success in the market. Technologies are being updated to make the noninvasive delivery of insulin possible. Some of the routes of insulin administration that are under investigation are oral, buccal, nasal, peritoneal and transdermal. This article has concentrated on various potential courses of insulin organization with its focal points and impediment and conceivable extension for the new medication improvement. It focuses on the past, present and future of various insulin delivery techniques.

KEYWORDS: *Diabetes mellitus, insulin conveyance, oral insulin, innovation, insulin conveyance.*

INTRODUCTION

Diabetes is one of the most common diseases expanding all through the world. The International Diabetes Federation assessed 366 million individuals had diabetes in 2011 and is required ascent to 552 million by 2030.¹ Though type 2 diabetes mellitus (T2DM) represents 85-95% of population, the pervasiveness of type1 diabetes mellitus(T1DM) has expanded by 2-3% in specific pieces of Europe and USA.^{1,2} Revelation of insulin is one of the best therapeutic disclosures. All patients with T1DM majorly and in minor amount with long standing T2DM require insulin treatment to accomplish great glycemic control.^{3,4} In past insulin was taken from cow and porcine pancreas and were treated from immunological responses, lipodystrophy and erratic insulin retention from subcutaneous tissue.⁴ Drastic improvement in insulin delivery systems resulted in quick and long acting insulin analogs in the last five decades.⁴ The milestone Diabetes Control and Complication Trial (DCCT), exhibited the significance of concentrated insulin treatment (IIT) in T1DM for aversion of smaller scale and full scale vascular complications.^{3,4} It about expanded hazard for hypoglycemia, which is a significant snag in accomplishing glycemic targets.^{5,6} Accentuation has advanced to accomplishing tight glycemic control with negligible hypoglycemia by concentrating on conveying insulin that emulates endogenous insulin emission by the pancreas.⁴ Insulin when taken orally inactivated by gastric corrosive. Intradermal route does not favor proper release. Intravenous and intramuscular routes are not possible because of its large molecular weight. Subcutaneous route is generally favored due to the simplicity of self-organization with minor side effects like impediments at infusion site, lipodystrophy, resistance by the patient, etc.⁴ The more up to date techniques for insulin conveyance mean to convey insulin with negligible obtrusiveness in an exact and exact way and to decrease persistent weight.

INSULIN DELIVERY METHODS-FROM PAST TO PRESENT

Insulin can be managed subcutaneously through different strategies, for example, vial and syringe, insulin pen and Ceaseless Subcutaneous Insulin Implantation (CSII) [Figure 1]. The points of interest and inconveniences of each subcutaneous insulin conveyance framework are investigated here and abridged in Table 1.

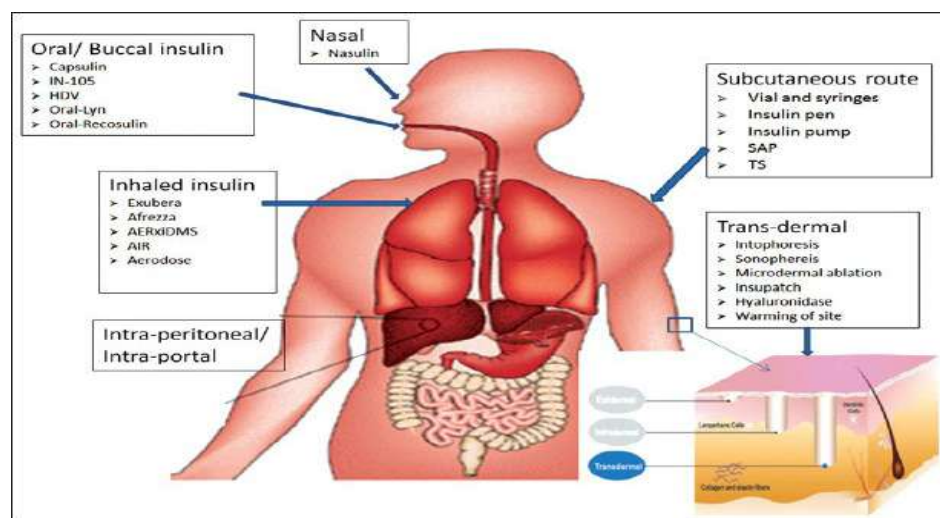


Figure-1: Different courses for insulin organization

Table 1: Advantages and disadvantages of dosage forms of insulin.

METHODS	ADVANTAGES	DISADVANTAGES
Viral and syringe ⁶	Expensive versus pen and CSII	Pain versus pens psychosocial issues inconvenience to carrying it. Accurate versus pens Patient friendly
Pen device ⁷	Convenient pain versus syringes More accurate, precise versus syringes Easier to use	More expensive versus syringes Cant't mix two insulins
CSII (Accu-Chek Spirit, One Touch Ping MiniMed Paradigm, DANA Diabecare IIS, OmniPod, Nipro Amigo, T-slim) ⁸	Continuous delivery of insulin Better glycemic control. Patient compliance and acceptance	Cost Risk of DKA if pump fails injection site infection
SAP (any of the above pumps+Dexcom G4, Minimed, FreeStyle Navigator) ⁹	Same as CSII+better glycemic control. Hypoglycemia	Same as CSII CGM accuracy .
TS (Paradigm Revel 2.0 insulin pump and Enlite glucose sensor) ¹⁰	All the advantage of SAP+reduce hypoglycemia by 30% versus CSII	SameasCSII AND SAP Hypoglycemia algorithms not predictive
Intra-pentoneal (MIP 2007) ¹¹	Direct insulin delivery to the portal vein More physiological	Invasive Cost Infection, portal vein thrombosis risk
Inhaled (Exubera, Technosphere, AERx, insulin Diabetes Management System) ¹²	Noninvasive patient compliance Rapid onset of action (10-15 min) Better PPBG control	Bioavailability Inhalational devices issues Lung function
Oral (Capsulin, ORMD-0801, IN-105) ¹³	Portal insulin concentration Noninvasive Patient friendly	Transient cough GI degradation of insulin Bioavailability
Buccal (Oral-Lyn, Recosulin) ¹⁴	Same as oral+bypass GI degradation	Bioavailability
Nasal (Nasulin) ¹⁵	Same as oral and buccal insulin+no interference with pulmonary functions	Bioavailability (15-25%) Local irritation

Transdermal (Microneedles, iontophoresis, electrophoresis, sonophoresis microdermalabrasion) ¹⁶	needle free	Nasal irritation Skin irritation, blister, pain and redness

OTHER NONCONVENTIONAL ROUTES

Visual course¹⁷

Until date, no human preliminary has been accounted for with this course and a creature study neglected to accomplish huge plasma insulin concentration.

Rectal course¹⁸

Rectal gels and suppositories indicated reasonable outcomes. Be that as it may, this course isn't industrially practical.

Intra-tracheal¹⁹

Organization of insulin was accounted for in 1924 however isn't down to earth so not taken up for further advancement.

CONCLUSION

There is a long history of research concentrating on different course of organization for insulin that is negligibly or noninvasive, powerful, protected, helpful and financially savvy for patients. Each course and conveyance strategy has its own potential points of interest and weaknesses. Be that as it may be effective, elective courses of organization could alter the treatment of diabetes mellitus and help improve patients' personal satisfaction. Still more advancements in nanoparticle drug delivery may be beneficial in efficacy.

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PREVALENCE AND IMPACT OF PATIENT COUNSELLING IN MANAGEMENT OF DIABETES MELLITUS

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ABSTRACT

In India diabetes is fast gaining status of a possible epidemic. According to ICMR National Diabetic Report in 2011 62 million people are with type 2 diabetes and 77 million people are with pre diabetes. It is expected that these projected figures will increase to 101 million by the year 2030. The main aim and objective of the study are to assess the Prevalence and Impact of patient counselling in the management of Diabetes Mellitus. It is a Prospective Observational study was conducted rural area of Andhra Pradesh around 6 months of duration. Based on age-wise distribution we found that 50-60 age group people are more prone to diabetes when compared to other age group people. Based on lifestyle changes before counselling 70% of people are not following lifestyle changes and after counselling 61.9% are not following lifestyle changes. Before counselling 3.8% people are with social history and after counselling 26% are with social history. Improvement in medication adherence was observed with increase in % values of moderate and high adherence from 3.7%, 16.9% to 57.6%, and 25.3% respectively. This study concludes that improving the quality of health education in a rural area had a large effect on raising the awareness and practices among the population in general. In the present study, there was a significant improvement in the adherence to medication in diabetes taking part in the awareness in educational session which is focused on the importance of medication and its adherence.

KEYWORDS: *Counselling, Adherence, Diabetes mellitus, Lifestyle, Education.*

1. INTRODUCTION

Diabetes mellitus (DM) may be a serious and quickly growing public ill health that affects millions of individuals. It usually co-exists with other medical conditions, and its prevalence is increasing year by year reaching epidemic proportions.¹ In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively.² In 2009, 387 million people have DM³ and are expected to reach 483 million and 592 million by the year 2030⁴ and 2035³ respectively, in which type 2 DM is accounting 90% of cases.⁵ DM is responsible for more than 77% of morbidities and 88% of deaths in developing countries.^{3,6} is fast gaining the status of a potential epidemic in India⁷. In India, as per the Indian council of medical research – India diabetes national study report; in 2011, 62.4 million people with type 2 diabetes and 77 million people with pre-diabetes. It is expected that these projected figures will increase to 101 million by the year 2030⁸. There is a disproportionate allocation of health resources between urban and rural areas, and also poverty in rural areas may be multi-faceted. Physical activity, an unhealthy diet increases the popularity of soft drinks and artificially sweetened fruit juices, and smoking is becoming a part of the culture in Saudi Arabia. All of these factors have a direct impact on the risk of obesity, coronary artery disease (CAD), hypertension and type 2 diabetes mellitus.⁹ Lifestyle changes are the largest challenge to the health status of the population in the rural area. Counselling to patients about diabetes, both in the form verbal and written of tailored patient education, or participation in health education seminars on general topics, have a profound effect on the patient's knowledge and understanding of the risk involved with carelessness about their health. Counselling is the most effective way to disseminate information and encourage people to adopt healthy lifestyles. Adoption of a healthy lifestyle not only helps within the prevention of diseases, however additionally in reducing the chance of complications ensuing from these diseases. Medication non-adherence is of accelerating concern for health care providers despite the well-known benefits of recent treatment regimens, with the prevalence reported in one study to be in more than 50 % of diabetic patients.¹⁰ The

WHO has identified non-adherence as a multi-factorial problem caused by the interplay of factors from any of the following 5 areas: 1) the patient, 2) the condition, 3) the type of therapy prescribed, 4) socioeconomic factors, and 5) health system-related factors. Adherence to prescribed medications is one of the key dimensions of health care quality, that is defined as the proportion of prescribed doses of medication taken by a patient over a specified period of your time.¹¹ Adherence to or compliance with medications has important economic and therapeutic consequences¹², as a result of non-adherence patients are at larger risk of developing complications affecting their health status and overall quality of life. Adherence to their anti-diabetic medications is a critical and important factor to prevent serious undesirable complications and to reduce the health care resource utilizations.¹³ There was a significant improvement in the adherence to medication in diabetics taking part in an educational session focusing on the importance of anti-diabetes medication and adherence to it.¹⁴

2. Methodology

2.1 Study Design

2.1.1 Study Site:

A Prospective Observational study was conducted in a rural area of Mangalagiri, Andhra Pradesh.

2.1.2 Study Period:

The study was conducted for 6 months August -January (2018-19)

2.2 Inclusion Criteria:

The patients who are suffering from diabetes mellitus and diabetes with co-morbidities above 18 years

2.3 Exclusion Criteria:

- The patients who are below 18 years
- Pregnant women and lactating mother
- The people who are not willing to participate

2.4 Study Method:

- The study was conducted in a rural area of Mangalagiri, Andhra Pradesh.
- Subjects who satisfy study criteria taken into the study and informed consent form was taken from each patient and collected information through a data collection form and it contains the data regarding demographic details, diagnosis, lifestyle parameters (dietary practice, exercise, social history), current medications prescribed were collected and details of subjects were secured.
- Patient counselling have done to every patient in each review.
- Morisky Medication Adherence Scale (MMAS-8)¹⁸ was filled by each subject before and after counselling. Score was calculated by (MMAS-8).

3. RESULTS & DISCUSSION

We surveyed the prevalence of diabetes mellitus in a rural area of Andhra Pradesh. Out of 1872 people, we found 189 people with diabetes mellitus. The prevalence was found to be 10.9%

3.1 Age & Gender Wise Distribution

Based on gender-wise distribution females are prone to diabetes when compared to males. People with females are 107 and People with males are 82. During six months of our study, we collected the prevalence and patterns of diabetes mellitus living in a rural area (Atmakur village) and it was found to be 10.9%. A population-based study included 1872 adults aged (33.8% of 50-60 years age group people and 54% women) were more prevalent when compared to other age groups and male gender in our study as same as Rui Wang et. al.,¹⁵

3.2 Diabetes with Co morbidities

In this study our findings suggest diabetes with hypertension more prone when compared to other co morbidities. diabetes with hypertension are with 64 people, diabetes with thyroid 21 people, diabetes with hypertension and thyroid 14 people, diabetes with others 8 people.

3.3 Lifestyle Parameters

In figure 2: Based on lifestyle changes before counselling 70% of people are not following lifestyle changes and after counselling 61.9% are not following lifestyle change.

3.4 Social History

In the total number of 189 people, Out of that people with social history before counselling was 73 people and after counselling it was reduced to 49 people. Based on the results before counselling 0.04% people are with smoking, 12% people are alcoholic and 22.2% people are with smoking + alcoholic, after counselling 0.03% people with smoking, 0.095% people are alcoholic and 0.13% people are with smoking + alcoholic based on our study we found improvement. The P-value was 0.139 significant

3.5 Exercise& food habits

Mainly we found that intake of carbohydrate (rice) food is more in this area. So we counselled the patients about dietary changes. In 189 people before counselling rice intake of per day was Rice 3 Times 53 people, Rice 2 Times 104 people, Rice 1 Time 32 people, Fresh Fruits 98 people, Vegetarians 65 people, and Non-Vegetarians 124 people. After counseling Rice 3 Times 33 people, Rice 2 Times 107 people, Rice 1 Time 49 people, Fresh Fruits 129 people. Our finding suggests that the patient counselling was successful in achieving some small but significant changes in the lifestyles among diabetes patients in this rural area. The dietary patterns improved, with some strange results: there was a decrease in consumption of alcohol and smoking p-value was found to be 0.139 significant, exercise p-value was found to be 0.808 non-significant and change in food habits p-value was found to be 0.013 highly significant as same as Sharaf et. Al.,¹⁶

3.6 Morisky medication adherence

In table 1: The medication adherence score was calculated by considering yes as +1 and no as 0. In this study medication adherence was assessed based on MMAS-8 score. It is able and validated scale which is designed to facilitated identification of barriers to behaviours associated with adherence to medication. The finding of the study suggests that medication adherence was low (78.8%) before counselling as same as Kishorkhotkar, Sameer Chaudhari et. Al. and it was reduced to 19% after counselling.¹⁷ Improvement in medication adherence was observed with increase in % values of moderate and high adherence from 3.7%, 16.9% to 57.6%, and 25.3% respectively.

QUESTIONS

1. Do you sometimes forget to take your pills?
 2. People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your medicine?
 3. Have you ever cut back or stopped taking your medicine without telling your doctor because you felt worse when you took it?
 4. When you travel or leave home, do you sometimes forget to bring along your medicine?
 5. Did you take all your medicine yesterday?
 6. When you feel like your symptoms are under control, do you sometimes stop taking your medicine?
 7. Taking medicine every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?
 8. How often do you have difficulty remembering to take all your medicine?
- A. Never/rarely
 - B. Once in a while
 - C. Sometimes
 - D. Usually
 - E. All the time

S.NO	BEFORE		AFTER	
	YES	NO	YES	NO
Question 1	63	126	29	160
Question 2	50	139	38	151
Question 3	24	165	24	165
Question 4	73	116	36	153
Question 5	189	0	180	9
Question 6	0	189	0	189
Question 7	0	189	0	189
Question 8	0	189	0	189

Table 1: medication adherence

Paired T - test

By observing paired t-test data, there is a significant improvement in medication adherence after counselling by a mean difference of 4.6 ± 0.47 and of $p\text{-value} < 0.0001$ which is extremely significant when compared to before counselling.

CONCLUSION

This study concludes that improving the quality of health education in a rural area had a large effect on raising the awareness and practices among the population in general, but particularly among patients with diabetes mellitus. It follows that specially designed patient education programs conducted in a rural area will have more effect. Adherence to medication among diabetics in India continues to be below. In the present study there was a significant improvement in the adherence to medication in diabetes taking part in the awareness in educational session which is focused on the importance of medication and its adherence. The results of such a pilot program would be of utmost value in supporting the implantation of bigger trials or even a national educational program in India which up to today is still lacking.

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Conflict of interest

Authors are not having any conflict of interest.

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PREDICTORS OF MORTALITY ASSESSMENT IN SEPSIS PATIENTS IN INTENSIVE CARE UNIT AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Infectious diseases are the most leading cause of morbidity and mortality among worldwide. The second leading cause of death is sepsis; septic shock is a life-threatening condition that increases the rate of hospitalization and mortality. Severe sepsis can be categorized by presence of one or more organ dysfunction i.e., metabolic acidosis, acute encephalopathy, oliguria, hypoxemia, coagulation or persistent hypotension. To find out the mortality rate of the patients with sepsis or severe sepsis can be done by using various scoring systems like GCS (Glasgow coma score), APACHE II and III, SOFA score. This can improve the patient care and also used for predicting the mortality of the sepsis or severe sepsis patients admitted in the ICU. It was a prospective observational study conducted for about 6 months. The study includes the patients with sepsis, severe sepsis and septic shock. The study excludes patients not with sepsis, pregnancy and lactating females. Data was collected from the ICU and analyzed by using SPSS software 20.0 for windows. Our study was aimed to estimate the mortality among adult patients admitted in the medical ICU with severe sepsis, sepsis. The total sample of 55 patients admitted to medical ICU and the mortality rate among them is 39%, and survival rate in them is 61%. The major co-morbidities observed in this study are a history of fever, diabetes mellitus, and respiratory co-morbidity. Patients who are positive to sepsis or septic shock were having higher mean CRP (170.22+129.01) when compared with survival group (131.88+112.05). Hence, higher level of CRP indicated the high mortality rate. Present study showed 39% of mortality with increased CRP, serum lactate levels and body temperature are found to be clear predictors of mortality in sepsis, severe sepsis and septic shock by using SOFA score.

KEYWORDS: *Sepsis, mortality risk assessment, SOFA score, Predictors of mortality.*

1. INTRODUCTION

Infectious diseases are the most leading cause of morbidity and mortality among worldwide¹ the second leading cause of death is sepsis, sepsis or septic shock is a life-threatening condition that increases the rate of hospitalization and mortality^{2,4}. It adversely affects the quality of life of those individuals who effected with sepsis³. In 2016 new definitions were developed for sepsis or septic shock the major difference between old sepsis (sepsis-2) and new sepsis (sepsis-3) alters by epidemiology and systemic inflammatory response syndrome of (SIRS) criteria⁵. The severe organ dysfunction is usually noted based on the presence or absence of refractory hypotension, respiratory failure, abdominal infections, urinary tract infections and major trauma conditions⁶. Sepsis can majorly be identified by the presence of bacteremia; it means isolation of one or more micro-organisms with SIRS positivity. Severe sepsis can be categorized by the presence of one or more organ dysfunction i.e., metabolic acidosis, acute encephalopathy, oliguria, hypoxemia, coagulation or persistent hypotension. To find out the mortality rate of the patients with sepsis or severe sepsis can be done by using various scoring systems like GCS (Glasgow coma score), APACHE II and III, SOFA score⁸. By using the scoring systems that can save them time, improve the patient care and also used for predicting the mortality of the sepsis patients admitted in the ICU, each scoring system has different parameters to evaluate the mortality rate of the sepsis patients admitted in ICU⁹. Increased levels of lactate in critically ill patients with sepsis this can help more easily for identifying the mortality by the bedside of the patient¹⁵. Sepsis represents a deranged systemic inflammatory response to infection that can progress to multi-organ dysfunction and also leads to shock, this inflammatory response can be triggered by microbial antigens, intrinsic factors released that are released into blood circulation mainly by trauma or infectious conditions or other health-related diseases¹⁰. Most of the epidemiological data were obtained from western

countries¹¹, in 1995 750,000 people were hospitalized due to sepsis or severe sepsis was increased rapidly. Diagnosing sepsis early is crucial so that appropriate treatment can be started promptly and patients given the best possible chances of survival¹². In India overall mortality rate of all sepsis patients is 14% and severe sepsis was alone higher that is approximately 50%. Sepsis majorly caused by bacteria like methicillin-resistant staphylococcus aureus bacteria, vancomycin-resistant Enterococcus species of bacteria, Neisseria, Candida and other gram-negative bacteria like E-coli, influenza in neonates¹³. The average cost of spending on sepsis for the length of stay in the hospital, laboratory tests, culture tests, medications cost, and other costs are in high amount and it also depends on the patient severity on an average hospital stay from 1 to 5 days is Rs.28,275 and for 10 to 20 days is Rs. 50,699. This may be variant from hospital to hospital¹⁴

2. METHODOLOGY

2.1 Study design, study site, and duration of the study

It was a prospective observational study done at medical intensive care units in a tertiary care hospital. The study was done for about 6 months that is from June 2019- November 2019.

2.2. Inclusion Criteria and Exclusion Criteria

- The inclusion criteria of the study are including both the genders, patients admitted in ICU with SIRS, sepsis, severe sepsis and septic shock.
- The study excludes children, patients with no sepsis and pregnant and lactating women.

2.3 Study material and procedure

In this study, we collected patient demographic details, comorbidities, presence of sepsis before admission or after admission, details for any trauma conditions these initial parameters were collected from the case reports. Presence of sepsis can be identified by systemic inflammatory response syndrome (SIRS) with suspected or presence of microbial culture in blood, pus, epithelial and urine were identified and SIRS include any two of the following conditions may indicate the presence of sepsis in the individual patient along with above parameters; those are 1. Hyper and Hypothermia (body temperature greater than or less than 98.6°F), 2. Respiratory rate (greater than 20 /min), 3. Heart rate (greater than 90 beats/min), 4. Hyperventilation (PaCO₂ less than 32 mmHg), 5. WBC count (greater than 12000/mm³ or less than 4000/mm³), 5. Hyperglycemia (>140 mg/dl), 6. Serum lactate levels (<18 mg/dl), 7. Creatinine (>2.0 mg/dl). All patients were subjected to CRP levels (C-reactive protein), complete blood count, hepatic and renal function tests, atrial blood gases, serum electrolytes, prothrombin time and blood cultures and culture specimens from the primary infection site. Mortality of sepsis was scored within 24 hours and identifies the 7th-day mortality of patients with sepsis, septic shock, and severe sepsis, by using the SOFA scoring system.

2.4 Statistical analysis and Ethical Approval

(SPSS) 20.0 for windows was used to analyse the statistical data. The study was approved by the Institutional Ethics committee (IEC) of Nirmala College of Pharmacy, Atmakur, Guntur district, Andhra Pradesh.

3. RESULTS AND DISCUSSION

3.1 Assessment of mortality rate

It is interesting to note that most of the biomarkers we identified have been tested clinically and not experimentally. Clinical and standard laboratory tests are not very helpful because most critically ill patients develop some degree of inflammatory response, whether or not they have sepsis. Even microbiological assessment is unreliable because many culture samples do not yield microorganisms in these patients (16). Patients who died with sepsis tended to be older adults with multiple co-morbidities and recent hospitalizations, and underlying causes of death were mostly associated with severe chronic comorbidities (17). Other important predictors of mortality were identified in our study, namely lactate level and mean urine output (19).

3.2. Comparison of risk factors and mortality

Our study was aimed to estimate the mortality among adult patients admitted in the medical ICU with severe sepsis, sepsis. The total sample of 55 patients admitted to the medical ICU and the mortality rate among them is 39%, and survival rate in them is 61%. Among fifty-four patients the age less than 60 was 20 members

among them mortality rate was 45% and patients with greater than 60 were 34 members among them mortality rate was 56%. The major co-morbidities observed in this study are a history of fever, diabetes mellitus, and respiratory co-morbidity. Out of these 54 patients, the culture reports were positive and mortality among them is 78.5% and isolated at least one micro-organism from blood, urine, sputum, and other specimens. The most common bacteria isolated are E.Coli, KlebsiellaPneumoniae, AcinetobacterBaumannii, Streptococcus Species, Candida Albicans, Proteus Mirabilis. The mean age of those patients who succumbed to sepsis (67.22 ± 10.8) was lower than the survival group (58.75 ± 14.37). The mean of temperature in the mortality group (99.45 ± 1.71) and it was higher than survival group (99.16 ± 0.97) and serum lactate levels were also compared this shows higher in mortality group (41.31 ± 28.87) when compared with survival group (25.69 ± 19.59). Patients who are positive to sepsis, severe sepsis or septic shock were having higher mean CRP (170.22 ± 129.01) when compared with survival group (131.88 ± 112.05). Hence, a higher level of CRP indicated a high mortality rate. Other factors like sodium, potassium, bilirubin, SGOT, SGPT were normalized in ratio between mortality and survival group. The mean SOFA score among who are with sepsis, severe sepsis or septic shock were higher in mortality group (14.95 ± 2.214) when compared with survival group (9.15 ± 2.93)

RISK FACTORS	CLASSIFICATION (n)	MORTALITY n (%)
Age	<60 (20)	9(45%)
	>60 (34)	28(56%)
Gender	Males (39)	21 (53.84%)
	Females (15)	11(73.33%)
Diabetes mellitus	Present (32)	28 (87.5%)
	Absent (22)	15 (68.18%)
Respiratory comorbidites	Present (14)	12 (85.71%)
	Absent (40)	29 (72.5%)
CLD and CKD	Present (24)	11 (45.83%)
	Absent (30)	7 (23.33%)
History of fever	Present (34)	27 (79.41%)
	Absent (20)	8 (40%)
Culture reports	Positive (14)	11 (78.5%)
	Negative (40)	15(37.5%)

Table-1: Comparison of risk factors and mortality
CKD: chronic kidney disease, CLD: chronic liver disease

Risk factors	Survival (33) Mean±SD	Mortality (21) Mean±SD	P-value (<0.05)
Age	58.75 (14.37)	67.22 (10.80)	0.44
Temperature	99.16 (0.97)	99.45 (1.71)	0.16
Bp(map)	93.84 (23.69)	85.86 (18.671)	0.16
RR	23.63 (7.82)	28.18 (6.70)	0.02*
HR	106.81(30.22)	120.68(14.448)	0.03*
Pao2	114.70 (54.28)	115.13 (82.71)	0.98
Wbc	253.3 (272.4)	218.50 (248.87)	0.62
Neutrophils	72.54 (17.25)	73.77 (19.39)	0.81
Platelets	235267 (145641)	317227.2 (262476)	0.16
Hb	10.47 (2.24)	9.52 (1.11)	0.04*
PCV	34.33 (5.74)	36.68 (8.88)	0.28
CRP	131.88 (112.05)	170.22 (129.01)	0.26
Ser.lactate	25.69 (19.59)	41.31 (28.87)	0.03*
Creatinine	2.60 (2.04)	3.26 (1.46)	0.16
Sodium	132.27 (7.28)	132 (8.18)	0.90
Potassium	4.53 (0.83)	4.25 (0.68)	0.18
Bilirubin	1.79 (1.40)	3.42 (3.15)	0.03*
SGOT	55.12 (45.01)	156.5 (209.5)	0.03*
SGPT	40.57 (31.71)	106.22 (182.58)	0.10

INR	2.14 (1.89)	5.16 (13.44)	0.30
SOFA score	9.15 (2.93)	14.95 (2.214)	0.0001***

MAP- mean arterial pressure, CRP- C-reactive protein, SOFA- sequential organ failure assessment

Table-2: the relationship between risk factors and mortality

CONCLUSION

The present study showed 39% of mortality with increased CRP, serum lactate levels and body temperature are found to be clear predictors of mortality in sepsis, severe sepsis, and septic shock by using SOFA score. Asepsis mortality prediction formula based on SOFA scores and CRP levels and serum lactate levels has greater predictive power of scoring the mortality in sepsis patients admitted in ICU.

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A COMPARATIVE STUDY ON THE EFFECTIVENESS OF ANALGESICS USED IN THE TREATMENT OF KNEE OSTEOARTHRITIS AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Osteoarthritis (OA) is a chronic disease that involves the deterioration of cartilage in joints resulting in friction between the bones causing joint stiffness, pain, and restricted movement. It's the foremost common type of arthritis which accounts for approximately 75% of the disease. Worldwide, it ranks as one of the top 5 diseases that cause disability. This is a prospective, observational, comparative study of the effectiveness of analgesics like NSAIDs, Acetaminophen and Opioids in knee osteoarthritis patients, designed according to the American College of Rheumatology (ACR) guidelines which is done for a period of 4 months in a total of 52 patients meeting their criteria. The study included weekly follow-up for 2 months and the data was calculated using statistical analyses. The study results involved seven treatment groups of drugs used in knee osteoarthritis, given alongside with other class of drugs. The results were obtained by equal efficacy shown by all the drugs but, greater level of significance was seen by Acetaminophen + Tramadol (P=0.0) and Aceclofenac + Pregabalin (P=0.0) followed by other dual therapy and triple therapy agents. These next level significance agents included were selective COX-II inhibitor etc. Hence, we concluded that dual and triple therapies are more effective than others and can provide a quicker pain relief in knee osteoarthritis and these opioids and coxibs use in combination with other drugs will be more effective.

KEYWORDS: *Osteoarthritis (OA), NSAIDs, Opioids, Acetaminophen, WOMAC.*

1. INTRODUCTION

Osteoarthritis (OA) is a long-term chronic disease characterized by the deterioration of cartilage in joints which results in bones rubbing together and creating stiffness, pain, and impaired movement. The disease most commonly affects the joints in the knees, hands, feet, and spine and is relatively common in shoulder and hip joints.¹ It's the foremost common type of arthritis which accounts for approximately 75% of the disease. Worldwide, it ranks as one of the top 5 diseases that cause disability. Mostly, adults are more prone, of which >65 years' population are more affected and is generally confirmed using X-ray.² The current American College of Rheumatology (ACR) guidelines for the management of osteoarthritis recommends acetaminophen, as the first-line oral pharmacotherapy drug of choice for treating OA pain. Some of the professional societies suggest that NSAIDs and tramadol i.e., a low-potent opioid are used as primary pharmacological therapy in knee OA. However, some of the clinical trials proved that NSAIDs showed additional efficacy than acetaminophen in some population groups with OA.^{3,4,5} The significant care generally includes analgesics, NSAIDs, corticosteroid injections and other supportive measures such as counselling including education, application of ice or heat, joint rest, weight loss, use of devices, physiotherapy, arthroscopy and total joint (knee/hip) replacement⁶. This comparative study is done using WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) Score which is widely used to measure the physical difficulty and symptoms in the patients with knee/hip osteoarthritis.^{7,8,9} It consists of 24 parameters of which includes 5 for pain, 2 for stiffness and 17 for functioning as its sub-scores with scores of 0-20, 0-8, 0-68 respectively with a total score of 96.¹⁰ It is used to evaluate the significant change in knee/hip OA patients and is now available in around 90 languages.^{11,12} The study of Yashika garg et al. showed the comparison of effectiveness and safety of newer NSAIDs (etodolac & lornoxicam) to diclofenac using patient's and physician's assessment, VAS and WOMAC composite scores.¹³ Johanne Martel-Pelletier et al. study compared the efficacy and safety of NSAIDs and Analgesics.¹⁴ S.R Smith et al. study compared oral NSAIDs and Opioids for their effectiveness in knee OA.¹⁵ So, the current study was designed to compare the effectiveness of NSAIDs, Acetaminophen and Opioids in the treatment of knee osteoarthritis.

2. MATERIALS AND METHODS

2.1. Study Design and Site: This is a prospective, observational, comparative study of the effectiveness of analgesics like NSAIDs, Acetaminophen and Opioids in osteoarthritis patients, designed according to the American College of Rheumatology (ACR) guidelines. The study was carried out in the Department of Orthopedics at Manipal Super Speciality Hospital, Vijayawada for a period of 4 months (August 2019 to November 2019) after the approval from Institutional Review Board (IRB).

2.2. Sample Size: 52 patients were selected and enrolled in the study with specified inclusion criteria.

2.2.1. Inclusion Criteria:

- The patients >30 years including both the genders, who visited both in-patient and out-patient wards of Orthopedic department.
- Patients diagnosed with OA irrespective of their co morbidities.
- Only the patients who are willing to participate in the study and have given written informed consent were included.

2.2.2. Exclusion Criteria:

- Patients who are not willing to participate were excluded.
- Pregnant and Lactating women were also excluded.

2.3. Informed Consent Form: This consent form was provided for the all the patients who are willing to participate in this study in 2 languages (English and Telugu) with which patients were comfortable and written confirmation was taking including their name, address, phone number, e-mail ID (if present), date of consent taken, along with the patient's signature (patient's attender signature was taken in case of any difficulty). Data was collected from both inpatient and outpatient case sheets.

2.4. Data Analysis & Follow-Up: The data of all the patients was then segregated into seven treatment groups according to mono, dual and triple therapies. Weekly follow-up was done for 2 months i.e., 8 weeks. All 52 patients were contacted through telephone and were asked to answer for all the 24 parameters of WOMAC score i.e., 5 for pain (sub-score for 20), 2 for stiffness (sub-score for 8) and 17 for function/difficulty (sub-score for 68) with an overall total score of 96 in the ranges allotted i.e., none, slight, moderate, severe and extreme, which is denoted as post-follow-up WOMAC score. All the patients were involved in the follow-up but; follow-up for 1 patient was missed at the sixth week due to sudden cardiac arrest of the patient. The level of significance, P-value was calculated by two-way ANOVA analysis, Pearson's test using SPSS 20.1 version.

3. RESULTS AND DISCUSSION

A total of 52 patients were enrolled in our study which includes the subjects aged >40 years, of which 61-70 aged group population, were noted to be high. Our study showed that female subjects are seen to be dominated when compared to males. The study of Sharon L. Hame et.al states the reason for this as women have anatomical changes regarding their knee cartilage volume i.e., their total tibial and patella cartilage volume is lesser than males and also some hormonal changes which includes majorly post-menopausal women where estrogen levels are seen to be lowered at this time.¹⁶ In our study, for all the patients BMI was calculated to assess the number of obese patients. They are categorized into underweight, healthy, overweight and obese according to WHO-BMI calculator.¹⁷ Here, obese people are seen to be more when compared to others and overweight patients are next higher later to obese. The study of Huaqing Zheng et.al specified that an increase in 5kg/m^2 showed a 32% significant increased cause for osteoarthritis and an increase of 200Pm of serum leptin, a protein released from adipocytes lead to 11% significant increase of development of OA.¹⁸ Moqi Yan et al. study reasoned this association as the serum leptin levels will be high in women compared to men and it involves in the inflammation process causing cartilage wear and degradation resulting in OA.¹⁹ In this study, the risk factors for all the patients were assessed, which includes smoking, obesity, joint injuries, occupation [farmers, home-makers and others (employees & self-employees)], genetics, bone deformities, certain metabolic diseases like diabetes mellitus. In these, joint injuries noted were accidents to knee and bone deformities include polio. Among these, home-makers ranked first with OA. The study of Jessica Immonen et al. discussed that the home-makers showed degeneration of their medial tibial plateau's articular cartilage due to above average joint use and potential

shearing because of child care, home cleaning and spending more time on pursuing recreational activities put more strain on joint.²⁰(Table 1).

Table 1. Distribution of patients based on Age, Body Mass Index(BMI), Risk Factors.

Table 1a.

PARAMETER	FREQUENCY	%
Age		
40-50	5	9.61
51-60	18	34.61
61-70	20	38.46
>70	9	17.31
Gender		
Male	22	42.31
Female	30	57.7
BMI		
Underweight	1	1.92
Healthy	7	13.5
Overweight	21	40.38
Obese	23	44.23

Table 1b.

Riskfactors			
Smoking		4	7.69
Obesity		21	40.38
Joint injuries		5	9.61
Occupation	Farmers	14	26.92
	Home makers	29	55.77
Others		9	17.31
Genetics		0	0
Bone deformities		1	1.92
Certain metabolic diseases (DM)		16	30.77

In our subjects, treatment was given as both single and dual agents. Their treatment groups were divided into 7 i.e., mono, di and tri-therapies. Mono included Aceclofenac, 200mg(ACE) which is given as single agent and also Acetaminophen + Thiocolchicoside, (ACE+THIO), given alongside with other combination drug. Di-therapy included 5 combinations i.e., Acetaminophen+Tramadol, 325+37.5mg(ACT+TRM), Aceclofenac+Pregabalin, 200mg+75mg (ACE+PREG), Aceclofenac + Thiocolchicoside, 100mg+4mg (ACE+THIO), Aceclofenac+Acetaminophen, 100mg+325mg (ACE+ACT) & Etoricoxib + Thiocolchicoside, 60mg+4mg (ETO+THIO). Tri-therapy includes Thiocolchicoside + Aceclofenac + Acetaminophen, 4mg+100mg+325mg (THIO+ACE+ACT). Over all these therapies, Acetaminophen+Tramadol was given to majority of the subjects i.e., 34 patients (Table 2).

DRUG THERAPY	FREQUENCY	%
Mono-therapy		
Acetaminophen	1	1.92
Aceclofenac	1	1.92
Thiocolchicoside	1	1.92
Dual-therapy		
Acetaminophen + Tramadol	34	65.38
Aceclofenac + Pregabalin	7	13.46
Aceclofenac + Thiocolchicoside	4	7.5
Aceclofenac + Acetaminophen	3	5.77
Etoricoxib + Thiocolchicoside	1	1.92
Triple-therapy		
Thiocolchicoside + Aceclofenac + Acetaminophen	2	3.85

Table 2. Distribution of patients based on the prescribed number of analgesics.

They are also prescribed with antibiotics and hypnotics that includes Linezolid, Cefuroxime, Amoxicillin + Clavulanic acid and Zolpidem respectively. Among antibiotics, Linezolid was prescribed in more patients and Zolpidem was the only non-benzodiazepine hypnotic prescribed in 43 out of 52 patients (Table 3).

ANTIBIOTICS	FREQUENCY	%
Linezolid	18	38.29
Amoxicillin + Clavulanic acid	17	36.17
Cefuroxime	12	25.53
BENZODIAZEPINES	FREQUENCY	%
Zolpidem	43	82.7

Table 3. Distribution of prescribed category of drugs other than analgesics in OA patients.

The effectiveness of all the drugs was assessed using WOMAC 3.0 score by taking pre-followup WOMAC score before the use of drugs at the time accepting consent i.e., one(1 total questionnaire) score for each patient and post-followup WOMAC scores were collected by conducting weekly follow-up for 2 months through telephone in one group with Aceclofenac consisting of 1 patient, second group with Acetaminophen + Tramadol in 34 patients, third group with Aceclofenac + Pregabalin in 7 patients, fourth group with Aceclofenac + Thiocolchicoside in 3 patients, fifth group with Etoricoxib + Thiocolchicoside in 1 patient and sixth group with Thiocolchicoside + Aceclofenac + Acetaminophen in 2 patients(Table 4).

Table 4. WOMAC Scores in all different treatment groups.

DRUG THERAPY	WOMAC								P-value
	Pain score		Stiffness score		Function score		Total score		
	Pre-followu	Post-followu	Pre-followu	Post-followu	Pre-followu	Post-followu	Pre-followu	Post-followu	
	p WOM AC score	p WOM AC score	p WOM AC score	p WOM AC score	p WOM AC score	p WOM AC score	p WOM AC score	p WOM AC score	
ACE	11	12.5	8	5	49	44.25	68	61.75	0.03
ACT+TRM	13.26	4.58	4.29	4.09	43.97	42.58	56.94	59.81	0.0
ACE+PREG	14.57	12.57	4.71	3.58	47.71	41.64	67	57.76	0.00
ACE+THIO	15.75	13.37	3.5	4.21	44	42.75	63.25	60.87	0.001
ACE+ACT	14.66	13.12	2.33	4.54	43.33	43.33	60.33	61.37	0.02
ETO+THIO	17	12.75	4	5	47	42.5	68	60.25	0.002
THIO+ACE+ACT	15	13.37	2	5.06	54.5	45.87	71.5	64.75	0.0
Note: All scores are mentioned in mean.									

The correlation coefficient was calculated and all the drugs in our study showed significant improvement in patients related to their pain, stiffness and function. Among all the drug therapies, dual therapy followed by triple-therapy showed more significant results than mono-therapy. Greater level of significance was seen with dual therapy drugs, ACT+TRM and ACE+PREG with a P-value of (0.0) and (0.00) respectively. Alongside, tri-therapy drugs i.e., THIO+ACE+ATM with a P-value of (0.0) also showed the similar level of significance with that of the dual therapy drugs. Other dual therapy drugs such as ETO+THIO & ACE+THIO showed the next level of significance with a P-value of (0.002) & (0.001) respectively. Compared to all the drugs, ACE has showed the lower level of significance with a P-value of (0.03) (Table 5).

Table 5. Reported level of significance in all treatment groups.

DRUG THERAPY	REPORTED LEVEL OF SIGNIFICANCE	EXPECTED LEVEL OF SIGNIFICANCE
ACE	0.03	0.01
ACT+TRM	0.0**	0.01
ACE+PREG	0.00**	0.01
ACE+THIO	0.001**	0.01
ACE+ACT	0.02	0.01
ETO+THIO	0.002**	0.01
THIO+ACE+ACT	0.0**	0.01
Note:- Two-way ANOVA analysis using Pearson's test.(SPSS20.1 version)		

4.CONCLUSION

This study concluded that all the drugs that are given irrespective of their combinations had shown their efficacy by a significant decrease in scores in all the patients individually and improved patients functional status. So, all the drugs can be used for pain in OA. As, the 4 dual therapies and a triple therapy were effective than the mono-therapy, Tramadol, Pregabalin and Thiocolchicoside can be used along with other drugs in combination as dual therapies for more efficacy and quick relief from osteoarthritic pain. Hence, it is better to use NSAIDs, Acetaminophen and Opioids in combination than using as a single agent in the treatment of Knee Osteoarthritic pain.

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A STUDY ON ANXIOLYTIC ACTIVITY ON SOLANUM NIGRUM LINN. LEAF EXTRACTION IN EXPERIMENTAL MODEL

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ABSTRACT

Anxiety is a part of daily life. While mild levels of anxiety can be positive, moderate to severe level can cause intense distress. When anxiety interferes with a person's ability to function, it warrants treatment. Generalized anxiety disorders (GAD) is a chronic disabling condition characterized by at least 6 months of frequent worries and three of the following symptoms: fatigue, restlessness, poor concentration, irritability, muscle tension, and unsatisfying sleep. The primary treatment for anxiety is pharmacotherapy. Medication prescribed for anxiety has shifted from exclusive benzodiazepine therapy to a combination of benzodiazepine therapy to combination of benzodiazepine and antidepressant drugs. The principal disadvantages of benzodiazepines are their long-term use with associated physical dependence, tolerance, and withdrawal symptoms. For *in vivo* studies, most investigators use a battery of anticonvulsive tests, anti-aggressive tests and evaluation of conditioned behaviour. The GABA receptor is involved in anxiety and that its direct activation would have an anxiolytic effect. Based in these findings various *in vitro* tests have been developed for the treatment of treatment of anxiety disorder.

KEYWORDS: *anxiety disorder, benzodiazepines-non benzodiazepines, psychiatric studies.*

1. INTRODUCTION

Anxiety is an emotion that predates the evolution of man. Its ubiquity humans, and its presence in a range of anxiety disorders, make it an important clinical focus. Developments in epidemiology and psychobiology have significantly advanced our understanding of the anxiety disorders in recent years¹. Advances in pharmacotherapy of these disorders have brought realistic hope for relief of symptoms and improvement in functioning to patients. This complex characterization of anxiety in man already indicates the difficulties to find appropriate pharmacological models. Therefore, several tests have to be performed to find a spectrum of activities which can be considered to be predictive for therapeutic efficacy in patients. Anxiety enables the individual to recognize danger and to deal with an unknown or vague internal or external threat. Fear is a similar alerting signal, but differs from anxiety in that it is regarded as response to a known, definite, non-conflictual threat. Clinicians assessing anxiety distinguish between 'normal' and 'pathological' anxiety. An epidemiological study of pediatric OCD revealed that 84% of youth diagnosed with OCD had comorbid disorders, including major depression (62%), social phobia (38%), alcohol dependence (24%), and dysthymia (22%)². The most common comorbid diagnoses include frequently experience other psychiatric conditions, including attention deficit/hyperactivity disorder and the disruptive disorders⁴.

2. METHODOLOGY

Materials

Plant materials: the plants *solanum nigrum* belongs to the family Solanaceae. The description, history, cultivation and constituents of which have been already described. The plant is largely found in the Tamil nadu. The leaves of the plant are collected from the local area of RR district in winter, Diazepam I.P-

Elevated plus maze model

Elevated plus maze (EPM) one of the commonly used animal model (exteroceptive aversion stimulus model) for testing anti-anxiety drugs was employed to assess anxiolytic activity of sample under study. EPM is based on the apparent natural aversion of rodents to open and high spaces animals have tendency to spend more time in the open arms⁵.

Procedure

Albino mice of either sex weighing between 18-25gms were dividing into six experimental groups of six animals each.

- Group 1- control (2% gum acacia).
- Group 2- standard drug (Diazepam 2mg/kg i.p.)
- Group 3- ethanolic extracts dose EESN1 (200 mg/kg p.o).
- Group 4- ethanolic extract dose EESN2 (400 mg/kg p.o).
- Group 5- aqueous extract dose AESN1 (200 mg/kg p.o).
- Group 6 - aqueous extract dose AESN2 (400 mg/kg p.o).

Standard drug diazepam was administered 45 min prior to testing and extracts were administered p.o. 45 min prior to testing. Anxiolytic activity was measured using the elevated plus maze test⁶. The maze consisted of two open (28cm x 5cm) and two closed (28cm x 5cm x 14cm) arms, extending from the central platform (5cm x 5cm) and elevated up to the height of 40 cm above the floor. The entire maze was made of clear Plexiglas. Mice were individually placed on the center of the maze facing an open arm, and the number of entries and the time spend in closed and open arm were recorded during a 5 min observation period. Arm entries were defined as entry of all four paws into the arm. The % of open arm entries (100 x open/total entries) was calculated for each animal.

The light / dark transition test

The light/dark transition test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodent in response to mild stressors, that is, novel environment and light⁷. A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined results of these tendencies in novel situations. Thus, in the light/dark, test drug induced increases in behavior in white part of a two compartments box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity⁸.

Procedure

Albino mice of either sex weighing between 18-25 gm. were dividing into four groups of six animals each. Standard drug (Diazepam) was administered 30 min prior to testing and extracts were administered p.o 45 min prior to testing.

- Group 1- control (2% gum acacia).
- Group 2- standard drug (diazepam 2mg/kg i.p.)
- Group 3- aqueous and ethanolic extract dose EESN1 (400 mg/kg p.o).
- Group 4- aqueous and ethanolic extract dose EESN2 (400 mg/kg p.o).
- Group 5- aqueous and ethanolic extract dose AESN1 (200 mg/kg p.o)
- Group 6- aqueous and ethanolic extract dose AESN2 (400 mg/kg p.o).

The apparatus for light/ dark transition test consist of two compartments: One light area (27 L x 27 W x 27 H cm), illuminated by 100 W desk lamp was painted white, and the other dark area (18 L x 27 W x 27 H cm) was painted black. The two compartments were separated by a partition with a tunnel (7.5 x 7.5 cm) to allow passage from one compartment to the other. The experiments were performed between 09:00 and 14:00. Animals were placed in the center of the light area with its back to the opening. The following parameters were recorded during 5 min: Latency time for the first crossing to the dark compartments, the number of transitions between the light and dark compartment (tunnel crossing), and the total time spent in the light compartment. The apparatus was cleaned thoroughly between trials.

3. RESULTS

Acute toxicity study

Acute toxicity studies for ethanolic, chloroform, aqueous extracts of *Solanumnigrum* (*Solanaceae*) were conducted as per OECD guidelines 420 using albino Swiss mice. Each animal was administered aqueous and ethanolic and ethanolic extracts by oral route. The animals were observed for any changes continuously

for the first 2 hrs and up to 24hrs for mortality, there were no mortality and noticeable behavioral changes in all the groups tested. The extracts were found to be safe up to 2000 mg/kg body weight.

Elevated plus maze test

Ethanol extracts of SN on EPM

The behavioral effects of aqueous extract E ESN1, EESN2, and diazepam, on the behavior of the behavior of mice in the elevated plus maze test were summarized in table. Diazepam has increased the % of time spent and of arm entries in open arms significantly ($P \leq 0.05$, Figure.ure.7) with no entry in to open arm as compare to control. The students with that of EESN2 show significant results as control group. EESN2 and AESN2 has significantly increased the % time spent and arm entry in open arms ($P \leq 0.001$, figure.ure.7 and 9) where as in closed arm it has decreased ($P \leq 0.001$, figure.7 and 9) as compared to control group.

The light/dark transition test:\

Aqueous extract of AESN on L/D test:\

Results of the light/dark test of shows in table 5 and figure 13 to 15. Diazepam treatment group showed significantly increased time in light area, latency in to enter dark compartment and number of tunnel crossing. The SN1 has not significant in above paradigms as compare to control group, whereas SN2 showed significantly increased the time in light area ($P \leq 0.01$, figure 1), latency in to enter dark compartment ($P \leq 0.05$, figure 1) and number of tunnel crossing.

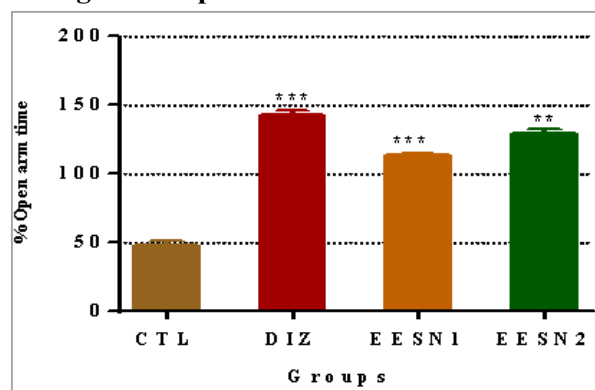
Table 1- Effects of ethanolic extracts of SN from elevated plus maze test in mice.

Treatment	open arm time (sec)	Closed arm Time(sec)	Open arm entry
Control	52.6±3.04	216±3.94	4.03±3.02
Diazepam (2 mg/kg)	145.3±0.52***	52±1.571***	8.8±3.04***
EESN1 (200 mg/ kg)	113.6±3.853*	76±1.22*	6.9±3.76***
EESN 2 (400 mg/kg)	134±4.587***	54±3.040***	7.0±3.16***

All values are mean $\pm$ SEM, (n = 6), one-way ANOVA, followed by Dunnet's test * $P \leq 0.05$, *** $P \leq 0.001$, when compared to vehicle treated group.

Effects of aqueous extracts of SN leaves from elevated plus maze test after acute treatment with 200 mg/kg and 400 mg/kg in mice

Figure- 1 Open arm time in 5-min EPM.



4. DISCUSSION

The study was to evaluate the anxiolytic effect of ethanolic and aqueous extracts of *Solanumnigrum* leaves by using behavioural animal models of anxiety. The major finding of present investigation proposes the anxiolytic activity on elevated plus maze test, light/dark transition test in mice⁹. The elevated plus maze is currently one of the most widely used model of animal anxiety and it's validated and use in rats and mice⁷⁶.

The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than evoked by the exposure to an enclosed arm³. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in to the open arm are sensitive to agents thought to act via the GABA_A receptor complex, justifying the use of diazepam as a positive control in this study⁶², even when the compound being screened does not act via benzodiazepine receptors. Diazepam increases the percentage of entries and the time spent in the open arm confirms its anxiolytic effects. The aqueous and ethanolic extract of *Solanumnigrum* leaves had similar effects on these parameters. The aqueous extract dose AESN1 shows increase percentage of time spent in open arm but not entry whereas EESN1 ethanolic extract is insignificant as compare to control. The AESN2 (400 mg/kg) of aqueous and ethanolic extracts had increased the percentage in time spent and entry in to open arm with decreased in closed arm.

5.CONCLUSION

The results obtained from these experimental models clearly confirmed that the anxiolytic activity of aqueous and ethanolic extracts of *Solanumnigrum* leaves. The acute toxicity study conducted for aqueous and ethanolic extracts indicates that they are safe up to 2000mg/kg body weight. The acute treatment with aqueous extract of AESN2 (400mg/kg) clearly demonstrate a dose dependant anxiolytic effect comparable to standard diazepam (2mg/kg; i.p) in all experimental models of anxiety, while EESN2 (400mg/kg) of ethanolic extract had significant anxiolytic activity comparable to standard drug diazepam (2 mg/kg; i.p) in all models. The phytoconstituent like flavonoids (Apigenin) were reported for their anxiolytic effect and these constituents were present in aqueous and ethanolic extracts of *Solanumnigrum* leaves, so this active principle might be responsible for anxiolytic effect.

6. SUMMARY

The study was complete to investigate the anxiolytic property of aqueous and ethanolic extracts of *Solanumnigrum* leaves on elevated plus maze test, light/dark test as behavioural models of anxiety. In this study the 200 mg/kg and 400 mg/kg dose of aqueous and ethanolic extracts has selected after end of fixed dose procedure of acute oral toxicity study and the dose 2000 mg/kg; p.o is safe. The results of both extracts by mentioned above models are summarised that in elevated plus maze test of aqueous and ethanolic extracts of 400 mg/kg has shown significantly increase percentage of time spent and of entry in open arms. In light/dark transition test 400 mg/kg dose of aqueous extract significantly increase the time spent in light chamber, latency to enter dark chamber and tunnel crossings whereas in ethanolic extract 400 mg/kg dose is significant. It is now clear that the aqueous and ethanolic extracts have significant anxiolytic activity which was comparable to diazepam.

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STABILITY INDICATING METHOD FOR SIMULTANEOUS DETERMINATION OF PARACETAMOL AND ACECLOFENAC USING ATR-FTIR ASSISTED BY CHEMOMETRICS

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ABSTRACT

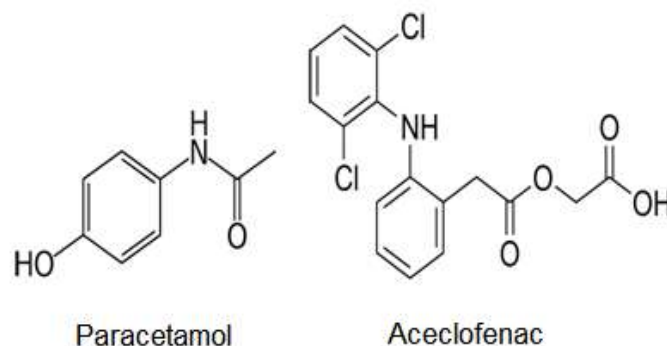
An attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy method was developed for the simultaneous estimation of paracetamol and aceclofenac in Pharmaceutical tablets. The method involves the direct determination in the drug through peak measurement in the 3502 cm^{-1} for paracetamol and 1436 cm^{-1} for aceclofenac using a baseline correction between 3800 and 1000 cm^{-1} . For standardization an external calibration line established from standard solutions of pure paracetamol and aceclofenac were used. The method provides a limit of detection for paracetamol is 9.18 and for aceclofenac is 7.95 for per mg tablet and Limit of Quantification for paracetamol is 27.84 and for aceclofenac is 24.11583 for per mg tablet. The accuracy of the sample was found to be 0.816% for paracetamol and 1.32% for aceclofenac. A relative standard deviation (RSD) for 3 independent measurement of standard and test solution at a concentration level of 1.5 mg/mL for paracetamol and aceclofenac is 0.000577% . Range for the tablet was $10\text{ }\mu\text{g/mL}$ to $250\text{ }\mu\text{g/mL}$ for paracetamol and $10\text{ }\mu\text{g/mL}$ to $300\text{ }\mu\text{g/mL}$ for aceclofenac. The assay of the marketed tablet was found to be 149% and 142% for aceclofenac and paracetamol, the purity of the tablet was 65% . Result obtained by FTIR shows the developed procedure and offers a good alternative for the simultaneous determination of paracetamol and aceclofenac in commercial products.

KEYWORDS: *Chemometrics, simultaneous determination, paracetamol, aceclofenac, ATR-FTIR, purity, validation.*

1. INTRODUCTION

Pharmaceutical analysis comprises of procedures necessary to determine "identity, strength, quality and purity of the drug substances and drug products. Pharmaceutical analysis derives its principles from various branches of sciences like physics, chemistry, microbiology, nuclear science, and electronics.^{1,2} An accurate description of analytical chemistry is "the science of inventing and applying the concepts, principles, and strategies for measuring the characteristics of chemical systems".³ This mainly operate at the extreme edges of analysis, for improving the ability of all chemists to measure on smaller samples, on more complex samples, on shorter time scales, and on species present at lower concentrations.^{2,3} A separation step is usually a necessary part of both a qualitative and quantitative analysis. Pharmaceutical analyst plays a major role in all quality controlling divisions of Industry.^{4,6} A number of existing techniques have been combined to expand the utility of the component methods. Liquid chromatography-mass spectrometry (LC-MS), Gas chromatography-mass spectrometry (GC-MS), inductively coupled plasma - mass spectrometry (ISP-MS) and Gas chromatography-infrared spectroscopy (GC-IR) are examples of successful Hyphenated methods.^{7,8} Fourier transform infrared (FTIR) spectroscopy was developed in order to overcome the limitations encountered with dispersive instruments. The FTIR can measure the sample without its further preparation. The signal can be measured very quickly, usually on the order of one second or so. Thus, the time element per sample is reduced to a matter of a few seconds rather than several minutes.^{9,10} Mostly FTIR employ a beam splitter which takes the incoming infrared beam and divides it into two optical beams. One beam reflects off of a flat mirror which is fixed in place.^{11,12}

The structures of paracetamol¹³ and aceclofenac¹⁴ are:



Standard analytical procedure for the determination of newer drugs or formulation may not be available in pharmacopoeias; therefore, it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid. FTIR spectroscopy was used for determination of various drugs in formulations such as penicillin-cephalexin,¹⁵ cilnidipine¹⁶, ibuprofen-paracetamol¹⁷ and paracetamol¹⁸. However there are no report of ATR-FTIR spectroscopy for simultaneous determination of paracetamol and aceclofenac was found in literature. Therefore the present investigation was aimed to develop and validate a simple ATR-FTIR spectroscopic method for quantitative analysis paracetamol and aceclofenac in bulk and in pharmaceutical preparation and applying chemometrics to it.

2. MATERIALS AND METHODS

A Bruker FT-IR Spectrometer, equipped with a modified deuterated triglycine sulphate detector and a global IR source was employed for FTIR spectra acquisition, using a cell with ZnSe windows and a path length of 0.11 mm by averaging 16 scans per spectrum with a nominal resolution of 4cm⁻¹. Acemiz® Plus (Pinnacle Life Sciences Pvt. Ltd., India aceclofenac-100 mg+paracetamol-500 mg) tablets were purchased from local drug store. Paracetamol and Aceclofenac pure drug was gift sample from Sipra Labs, Vijayawada, AP, India. DMSO was used as the solvent for the dilutions. Infrared spectra of solid samples were recorded over a spectral region from 3800 to 1000 cm⁻¹.

2.1 Method Development

Paracetamol sample solution was prepared by dissolving 50 mg of paracetamol with 50 mL of DMSO to prepare a stock of 1000 µg/mL solution after diluting the sample. From that 2.5 mL was taken and made the volume with DMSO up to 25 mL to make 100 µg/mL solutions. From that again taken 1.5 mL and made the volume with DMSO to obtain 15 µg/mL solutions. Then further dilutions of 25, 35, 45 and 55 µg/mL were prepared. The absorbance and transmittance values for different concentrations were observed and the wave number values and plotted calibration curve were recorded. Aceclofenac sample solution was prepared by dissolving 50 mg of aceclofenac Pure Drug was taken and added 50 mL of DMSO to prepare a stock of 1000 µg/mL solution after diluting the sample. From that 2.5 mL was taken and make the volume with DMSO up to 25 mL to make 100 µg/mL solutions. From that again taken 1 mL and made the volume with DMSO to obtain 15 µg/mL solutions. Then prepared some more dilutions like 25, 35, 45 and 55 µg/mL. Observed the absorbance and transmittance values for different concentrations. Recorded the wave number values and plotted calibration curve. To prepare the solutions of commercial tablet formulations 20 tablets of Acemiz was taken, individually weighed and triturated to form a powder. Weighed about 50 mg equivalent weight of drug and added 50 mL of DMSO to prepare a stock of 1000 µg/mL solution after diluting the sample. From that 2.5 mL was taken and make the volume with DMSO up to 25 mL to make 100 µg/mL solutions. From that again taken 1.5 mL and made the volume upto 10 mL with DMSO to obtain 15 µg/mL solutions. Then prepared dilutions like 25, 35, 45 and 55 µg/mL. Measured the absorbance and transmittance values for different concentrations. Recorded the wave number values and plotted calibration curve.

2.2 Chemometric analysis

Unscrambler® X software version 10.2 (CAMO software AS, Nedre Vollgate 8, N-0158, Oslo, Norway) was used for all the spectra and both groups like calibration and the validations of the present study.

3. RESULTS AND DISCUSSION

Spectra of paracetamol and aceclofenac in pure form and in formulation were successfully obtained using ATR-FTIR spectroscopy. Figures 1 shows the obtained IR overlay spectra of Acemiz brand (combination of paracetamol and aceclofenac tablets) at various concentrations of 15%, 25%, 35%, 45% and 55%. The linearity curves of pure paracetamol and aceclofenac showed correlation coefficients of 0.977 and 0.985 which indicate that they are more linear. The linear regression equation is shown in Figure 2. The formulation showed the correlation coefficients (R^2 values) of 0.988 and 0.982 for paracetamol and aceclofenac respectively.

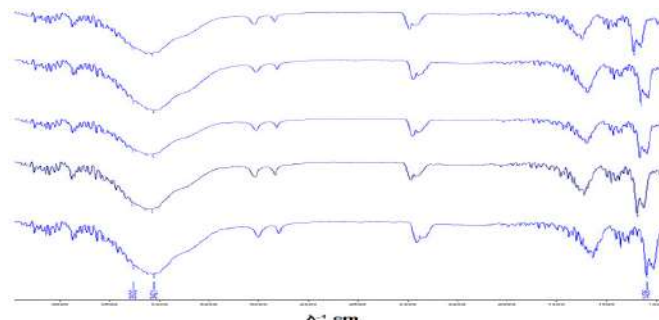


Figure 1.

IR overlay spectra of Acemiz at concentrations of (A) 15%, (B) 25%, (C) 35%, (D) 45% and (E) 55%

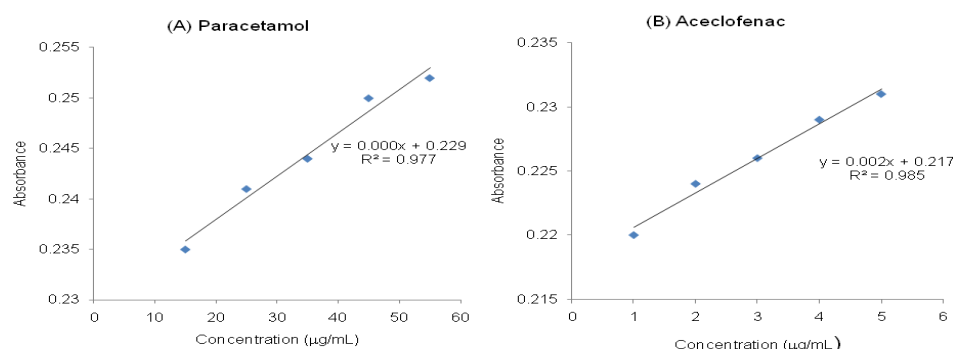


Figure 2. Linearity curves of (A) paracetamol and (B) aceclofenac

Assay of the commercial tablets

The testing of a material to determine its ingredients quality. The assay for paracetamol was found to be 142% and for aceclofenac 84%. The percentage of purity of paracetamol and aceclofenac in Acemiz tablets was found to be 65%. Other validation parameters are summarized in

Table 1.

Table 1: Validation parameters of paracetamol and aceclofenac in pure and tablet formulation

S. No.	Parameters		Results			
			Paracetamol (3502 cm^{-1})	Aceclofenac (1435 cm^{-1})	Acemiz	
					Paracetamol (3502 cm^{-1})	Aceclofenac (1435 cm^{-1})
1.	Linearity	R^2	0.977	0.985	0.988	0.982
2.	Accuracy	%Recovery	0.816 for paracetamol and 1.32 for aceclofenac			
3.	Precision	RSD	0.000577 for both			
4.	Range		10-250 $\mu\text{g/mL}$	10-300 $\mu\text{g/mL}$	10-250 $\mu\text{g/mL}$	10-300 $\mu\text{g/mL}$
5.	Assay	%Assay	-		149	142
		%Purity			65%	
6.	LOD		9.18796	7.900423	6.641881	7.958224

7.	LOQ		27.84230	22.42704	20.12691	22.42704

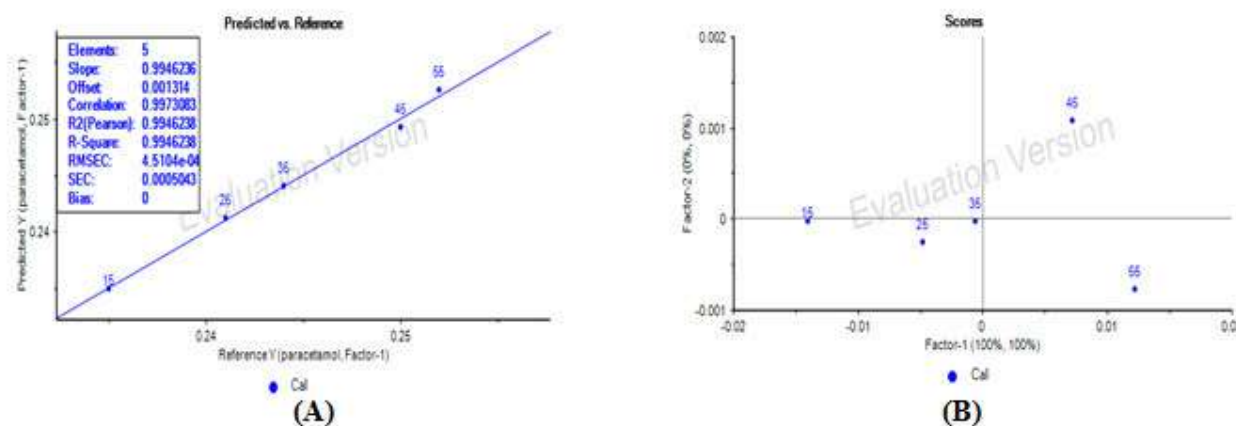


Figure 3

Plots showing (A) PLS difference graph of paracetamol, (B) PLS scores of paracetamol

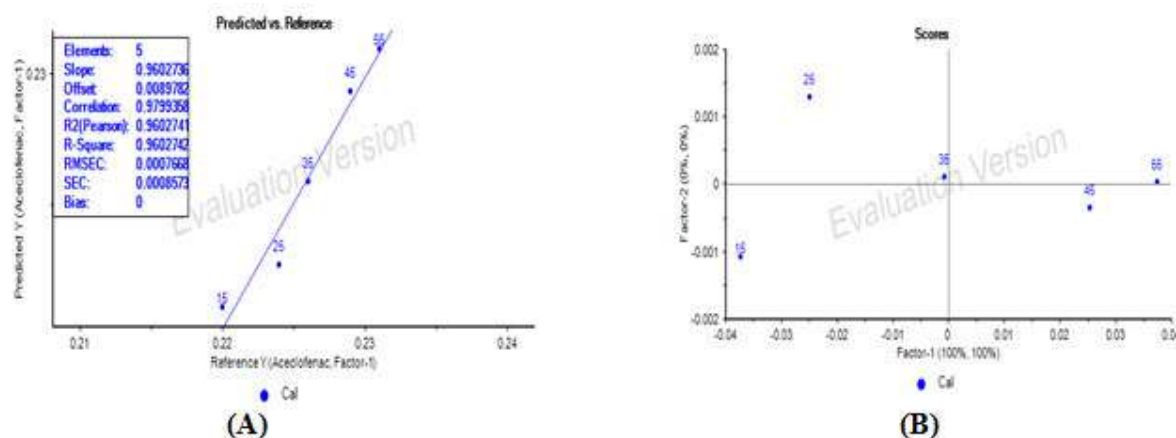


Figure 4

Plots showing (A) PLS difference graph of aceclofenac, (B) PLS scores of aceclofenac

4. CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of Paracetamol and Aceclofenac in tablet dosage form. The method involves the direct determination in the drug through peak measurement in the 3502 cm^{-1} for paracetamol and 1436 cm^{-1} for aceclofenac using a baseline correction between 3800 and 1000 cm^{-1} . For standardization an external calibration line established from standard solutions of pure paracetamol and aceclofenac were used. The method provides a limit of detection for paracetamol is 9.18 and for aceclofenac is 7.95 for per mg tablet and Limit of Quantification for paracetamol is 27.84 and for aceclofenac is 24.11583 for per mg tablet. The accuracy of the sample was found to be 0.816% for paracetamol and 1.32% for aceclofenac. A relative standard deviation (RSD) for 3 independent measurement of standard and test solution at a concentration level of 1.5 mg/mL for paracetamol and aceclofenac is 0.000577%. Range for the tablet was $10\text{ }\mu\text{g/mL}$ to $250\text{ }\mu\text{g/mL}$ for paracetamol and $10\text{ }\mu\text{g/mL}$ to $300\text{ }\mu\text{g/mL}$ for aceclofenac. The assay of the marketed tablet was found to be 149% and 142% for aceclofenac and paracetamol, the purity of the tablet was 65%. Result obtained by FTIR shows the developed procedure and offers a good alternative for the simultaneous determination of paracetamol and aceclofenac in commercial products. The method developed was simple and economical that can be adopted in regular Quality control test in Industries.

5. ACKNOWLEDGEMENTS

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SP-100

A PROSPECTIVE METAPHORICAL STUDY ON SAFETY, EFFICACY AND TOLERABILITY OF TRAMDOL AND DICLOFENAC IN OSTEOARTHRITIS PATIENTS AT TERTIARY CARE HOSPITAL

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ABSTRACT

Osteoarthritis (OA) is one of the most common, chronic musculoskeletal inflammatory disorder which affects knee and hip joint in elderly people. Disease of synovial joint characterized by cartilage loss with accompanying peri-articular bone response. The objective of the study is to assess the safety, efficacy of medication by using different scales and to know about adverse effects of drugs. The study was conducted by collecting demographic detail of patient, medication chart of patient, profile form, patient and attendant interview. And the data collected was analyzed by using WOMAC and VAS scales. Data was collected for a total of 200 patients. And the data was again segregated into age wise, gender wise and prescription pattern of Tramadol v/s Diclofenac. In this study improvement was seen by using VAS and WOMAC scales. But the improvement with tramadol was found to be better in comparison to Diclofenac. The overall incidence of adverse effects in our study was 10% in Tramadol and 20% in Diclofenac group. Tramadol was well tolerated than Diclofenac in terms Nausea and Vomiting, abdominal pain and constipation.

KEYWORDS: *Osteoarthritis, WOMAC, VAS, Diclofenac, Tramadol.*

1. INTRODUCTION

Osteoarthritis (OA) is the most common chronic condition of the joints, affecting approximately 27 million Americans. OA can affect any joint, but it occurs most often in knees, hips, lower back and neck, small joints of the fingers and the bases of the thumb and big toe¹. Although over half of all knee OA patients eventually undergo total knee replacement, nearly all will require at least some amount of pain control.² Elderly (approximately 35% of patients over 65 years old) females, patients with obesity and African Americans are the population with the highest risk of developing OA^{3,4}. The knee is the largest synovial joint in humans, it is composed by osseous structures (distal femur, proximal tibia, and patella), cartilage (meniscus and hyaline cartilage), ligaments and a synovial membrane. The latter is in charge of the production of the synovial fluid, which provides lubrication and nutrients to the avascular cartilage. Unfortunately, given the high use and stress of this joint, it is a frequent site for painful conditions including OA⁵. It was believed that OA was exclusively a degenerative disease of the cartilage, however, latest evidence has proven that OA is a multifactorial entity, involving multiple causative factors like trauma, mechanical forces, inflammation, biochemical reactions, and metabolic derangements⁶. Opioids, which have a dual mode of action on opioid and monoaminergic receptors, comprise another group of analgesic drugs that are efficacious against both nociceptive and neuropathic pain. Among the opioids, tramadol has fewer side effects, such as constipation, respiratory depression, and sedation, compared with the typical strong opioids. Tramadol is now considered to be a first-line analgesic for many musculoskeletal indications⁷. The general recommendation for the management of moderate to severe acute pain is a combination of paracetamol or NSAIDs with opioids, based on increasing pain severity. This combination has an advantage of additive analgesic effects along with a minimized dose of opioids and hence, minimal undesirable side effects. NSAIDs offer an opioid-sparing strategy in which the opioid activity can be potentiated by NSAIDs. This activity is due to an increased conversion of arachidonic acid to 12-lipoxygenase products, which in turn augments the effects of opioids on K⁺ channels⁸. WOMAC Index was developed in 1982 at Western Ontario and McMaster Universities. WOMAC is available in over 65 languages and has been linguistically validated^{9,10}. The WOMAC takes approximately 12 minutes to complete, and can be taken on paper, over the telephone or computer. Both the computerized and the mobile versions of the test have been found to be comparable to the paper form, with no significant difference^{11,12}. The test questions are scored on a scale of 0-4, which correspond to: None (0), Mild (1), Moderate (2), Severe (3), and Extreme (4)¹³. The VAS is widely used to investigate many kinds of subjective experience, including pain¹⁴. When using a VAS to assess pain, subjects are asked to indicate intensity by marking a (usually) 100-mm-long horizontal line that is labeled "no pain" at one end and "worst pain possible" at the other end. This requires the patient to be able to equate the length of the line (as

measured from the left-hand side to the point marked) with the amount of pain they are experiencing. The scales correlate with pain intensity measured by other methods^{15,16}.

2. METHODOLOGY

2.1 COLLECTION OF DATA

Patient who were diagnosed with OA were selected and involved in the study. Patient medication details, demographic details, lab investigations, x-ray of joints affected and adverse effects experienced by the patients were collected from the medication charts and from patient/attendant interview.

2.2 BIFURCATION OF DATA

The collected data was bifurcated based on the medications used for the management of pain in osteoarthritis condition. Mainly into two groups Tramadol group and Diclofenac group.

2.3 EVALUATION OF DATA

The collected data was evaluated by using by WOMAC and VAS scales. The evaluation is done by using the scores obtained from these scales.

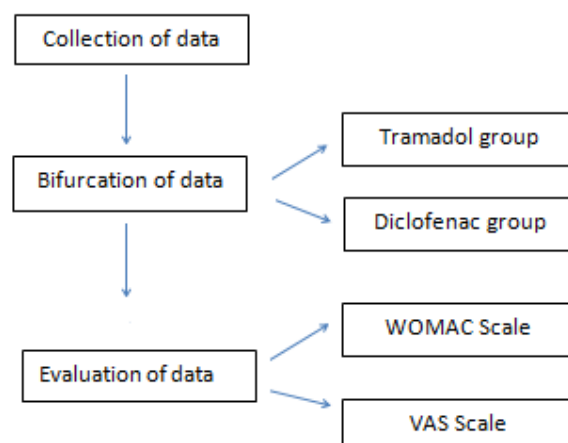


Figure 1: Detailed process about methodology

Sample size

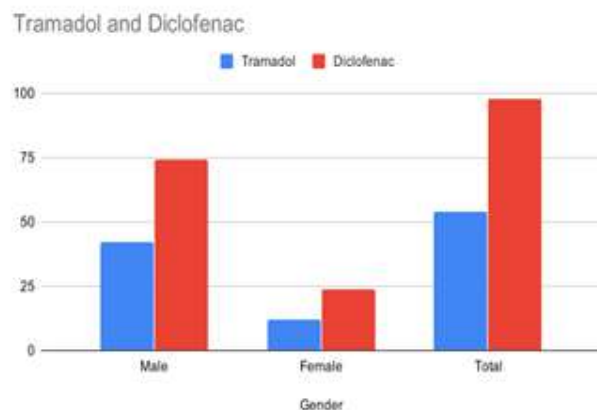
Data was collected for a total of 152 patients.

Age Wise Distribution and Gender Wise Distribution

Out of which 17 patients were between 30-40 years, 42 patients between the age of 40-50 years, 51 patients in between the age of 50-65 years and 42 patients are >65 years of old. The study sample included 54 male and 98 female patients.

Prescription pattern of Tramadol & Diclofenac

Tramadol and Diclofenac are prescribed in total patients of male and female as, 42 tramadol and 12 Diclofenac in male and 74 tramadol and 24 Diclofenac in females.



Prescription pattern of Tramadol & Diclofenac

WOMAC scores in the two treatment group at baseline and after 1st visit and 2nd visit [mean $\pm$ SD]

S.No.	Parameters	Tramadol Group	Diclofenac Group	Unpaired t-test p-value
1	Baseline	94.517 $\pm$ 13.89	95.270 $\pm$ 19.270	<0.0001
2	1 st visit	68.920 $\pm$ 14.257	75.920 $\pm$ 20.883	<0.0001
3	2 nd visit	46.474 $\pm$ 12.173	50.460 $\pm$ 20.169	<0.0001

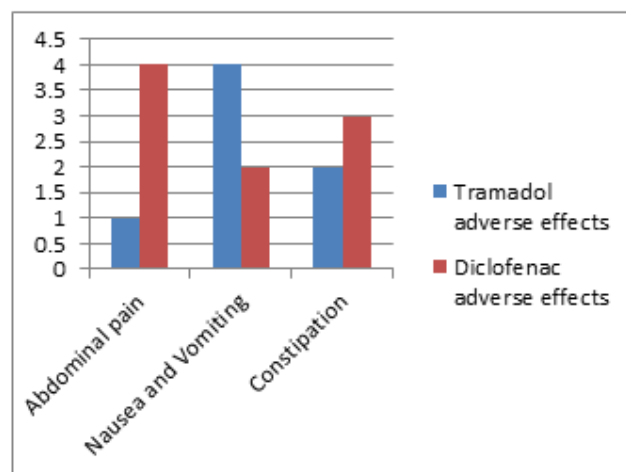
Visual analogue scale (VAS) score for pain in the two treatment group at baseline, after 1st visit and 2nd visit treatment [mean $\pm$ SD]

S.No.	Parameters	Tramadol Group	Diclofenac Group
1	Baseline	5.498 $\pm$ 3.281	5.498 $\pm$ 3.277
2	1 st visit	4.626 $\pm$ 3.466	5.498 $\pm$ 3.476
3	2 nd visit	5.498 $\pm$ 3.488	5.498 $\pm$ 4.832

Adverse Effect

Common adverse events for Tramadol and Diclofenac includes Nausea and Vomiting, Epigastric pain, GI bleeding, Constipation, Abdominal pain, Headache, GI ulcers. Noticed events in the two treatment groups are epi-gastric pain, abdominal pain, Nausea and Vomiting, Constipation .

S. No.	Adverse effects	Tramadol adverse effect patients	Diclofenac adverse effect patients
1.	Abdominal pain	1	4
2.	Nausea and Vomiting	4	2
3.	Constipation	2	3



3. RESULT AND DISCUSSION

The theme of the study is to assess the safety, efficacy and tolerability of Tramadol and Diclofenac in osteoarthritis patients. Tramadol is centrally acting opioid analgesic where Diclofenac is NSAID (Non-Steroidal Anti-Inflammatory Drug). A total of 152 patients were included in this study out of which 54 patients were using Tramadol and 98 patients were using Diclofenac. As most of the patients were using Diclofenac when compared to Tramadol. But according to our study we came to know that Tramadol is more safer drug than Diclofenac. The patients who were receiving Tramadol has experienced less adverse effects in comparison with Diclofenac. The scores taken by using WOMAC and VAS also demonstrated that Tramadol is more effective when compared to diclofenac.

4. CONCLUSION

It is concluded that Tramadol is more safer drug than Diclofenac in treatment of Osteoarthritis patients. Since, it was observed that Tramadol is most effective and showed less adverse effects when compared with Diclofenac.

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ANTI-CANCER ACTIVITY OF FLAX SEED OIL BY USING WHEAT GERM OIL AS PRESERVATIVE

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ABSTRACT

Sunscreen inhibits the transmission of ultra violet (UV) radiations into the skin by reflecting, absorbing or scattering such radiation. In this we have replaced the chemical compounds with all organic ingredients that are easily available in our home. These organic sunscreens have no toxic ingredients such as Oxybenzone, Parabens and fragrances, and environmentally safe. The organic compounds include the following Aloe Vera juice which helps to hydrates and cure the damage caused by sun rays to the skin; Flax seeds are rich in Omega-3 fatty acids which have been linked with protecting skin against free radical damage from sun exposure; Watermelon seed oil is light, easily absorbed, does not clog pores and is rich in Linoleic and Oleic acid. Watermelon seed oil is rich in essential fatty acids which help to restore elasticity of skin; Almond oil helps to protect your skin from UV radiation damage, and helps your skin look smooth, soft and free of fine lines. These fatty acids help your skin retain moisture and can heal chapped and irritated skin; Red Raspberry seed oil blocks UV rays as a natural sunscreen and has high antioxidant booster for fighting free radicals; Coconut oil is composed primarily of nourishing fatty acids and is especially high in lauric acid. It also contains vitamin E and healthy fats, which helps to make your skin smooth; Wheat germ seed oil which is dark brown in color and incredibly rich in Omega-6 fatty acids, protein's and vitamins A,B,C and especially vitamin E, which makes a natural preservative and also prolong the shelf life of the preparation

KEYWORDS: *UV Radiation, Free Radicals, Anti-oxidants, and Nourishment.*

Definition

Sunscreen is a form of lotion, spray, gel, foam, stick or power that absorbs some of the UV radiation from sun and protect against sunburn.

INTRODUCTION

The naturally occurring gaseous layer called ozone is gradually thinning due to release of the chlorofluorocarbons, global warming, nitrogenous compounds, ozone depletion has adverse effects on human health skin cancer, cataracts or blindness, breast cancer, leukemia, suppression of immune response, genetic mutations⁽²⁾. Exposure to UV radiation is the main cause of the most common form of skin cancer and one of the most effective ways to avoid it, of course, is sunscreen⁽²⁾. Sunscreen is also known as sun blockers skin cancer rates are increasing among older generations as they exposed to the radiation for decades earlier in their lives. Sunscreen is the undeniably important tool to fight against skin cancer, formulation of sunscreen may need to be improved to contain safer ingredients⁽²⁾.

MATERIALS METHODS

The active ingredients present in chemical filter sunscreen are oxybenzone, avobenzone, octisalate, octocrylene, homosalate, octinoxate.

The minerals sunscreen mainly contains zinc oxide and/or titanium dioxide.

Natural sunscreen

Natural sunscreen has the ingredient's which are the extractive juices, oils as from the naturally occurring substances like fruit, seeds, bark of the plants.

Natural sunscreen most commonly contains the following ingredients:

- Almond oil

- **Coconut oil**
- **Red raspberry seed oil**
- **Flax seed oil**
- **Aloe**
- **Watermelon**
- **Avocado oil**
- **Wheat germ oil (preservative)**

Method to prepare sunscreens at home

We can prepare sunscreen at home by using the ingredients that are easily available at home although the home made are less efficient and had less fragrances when compared with sunscreen available in the market and homemade sunscreen does not have the good appearances than that of the marketed sunscreens.

I How to make sunscreen at home

- ¼ cup Almond oil
- ¼ cup coconut oil
- 1 tb spoon of red raspberry seed oil
- 2 tb spoon Shea butter (optional)

Optional ingredients

- Essential oils
- Vanilla extract
- Other natural extract (not citrus)

Almond oils

Almond oil is acquired from the almond seeds, which contains proteins, vitamin E and B as an essential minerals, healthy fats and proteins ⁽¹⁾. The sun can be damaging to the skin as its harmful UV rays causes sunburn, wrinkling and other signs of damage associated with aging, as well as skin cancer. You may already protect yourself from the sun with sun block's and sunscreen but almond oil is another tool you can use "Journals of Cosmetic Dermatology" reports a study that status that applying almond oil the skin will not only protect it but can also reserve damage that may have already occurred⁽¹⁾. Along with protecting the skin from seen damage and lings of aging using almond oil as a skin moistening will often and smooth the skin. According to the February 2010 issue of "Complementary Therapies in Clinical Practice", almond oil has emollient properties, which gives it the ability to rejuvenate and soften the skin, there by evening out the skin tone and improving the complexion. The author, Z- Ahmad, from the Department of plastic and reconstructive surgery Salisbury general hospital in the UK, also status that almond oil has been shown to reduce searing that occurs with surgery and should be considered as a possible pos operative treatment⁽⁹⁾.

Skin irritation, such as itching and sweating, can also be reduced with almond. There are many skin conditions, such as psoriasis and eczema that cause inflammation of the skin, as well as redness and an itchy rash ⁽⁸⁾. Topical application of almond oil can temporarily relive the inflammation and the irritation associated with such conditions. For some, however, almond oil can be a skin irritant, so before applying it to the skin, a small patch test should be done to determine if you have an allergy, or sensitivity to almond oil⁽¹⁾.

Avocado oil

Humans have been using vegetable oil, oil and fats to protect their skin. Together with some seed oil, avocado oil has the higher natural SPF amongst vegetable oil. Avocado oil contains a lot of healthy fatty acids such as linoleic acid and oleic acid, and a very large number of natural vitamins. And its high palmitic acid content, makes the avocado oil to absorb easily into the human skin, keeping, protective layer that guards it against direct external influences ⁷.

Watermelon seed oil

Watermelon has high-water content and hydrates the skin and has high content of vitamin C which is an antioxidant that helps to prevent cell damage from free radicals. Watermelon contains citrulline; an amino acid that may increase nitric oxide levels in the body and lowers blood pressure. Watermelon is also

associated with a lower risk of some type of cancer; a study results are mixed and appears to reduce cancer risk by lowering insulin like growth factor, a protein involved in cell division. High IGF levels are linked to cancer⁽⁸⁾.

Flax seeds oil:

Flax seeds oil comes from the seeds of the flax plants. For all-natural skincare, you can apply it both to your skin as well as taking as a supplement⁽⁶⁾. Antioxidants found in flaxseeds oil are highly concentrated; they help the skin to protect against free radicals and UV rays. Its anti-inflammatory properties help to minimize any irritation of the skin or redness, meaning that sunburn or a skin rash may heal quicker. Ones a little of this soothing oil are applied⁽⁶⁾. Flaxseed oil is extremely beneficial for chronic skin conditions such as rosaceous, acne, dermatitis, eczema or psoriasis, the fatty acids act to balance out the skins own oils and reduce any inflammation, helping the skin repair dry and flaky or dull and blemished skin may be the result of the deficiency of omega-3 in the diet. Flax seeds are the rich source of omega-3⁽¹⁰⁾.

Coconut oil

Coconut oil is one of the other most often quoted sunscreen oils in the homemade sun blockers. You may see mentions of coconut oil having a SPF of 7, which may give the impression that it's still possible to use neat coconut oil sunscreen for a short period and be safe in the sun⁽⁷⁾.

This may be true because coconut oil is high in saturated fats, which are great for moisturizing the skin sunburned skin tends to be dry and itching. So, applying coconut oil may help relieve those symptoms by replenishing your skin's moisture⁽⁷⁾.

Red Raspberry seed oils

Red raspberry seed oil is rich in antioxidants, like vitamin E and polyphenols. Vitamin E is a powerful antioxidant that protects cells from free radical damage. Since free radical damage causes wrinkles, loss of elasticity and age stops, vitamin E protects skin from these common signs of aging⁽⁴⁾. Polyphenols are another type of antioxidants. Polyphenols protects the skin from ultraviolet radiations and enhance skin cell regeneration, improving skin tone and elasticity⁽⁴⁾. Scientist concluded that raspberry seed oil provides UVA and UVB protection similar to titanium dioxide with an SPF protection factor against UVA rays of 28-50 and an SPF protection factor against UVA rays of around 8⁽⁴⁾. This natural sun protection is very likely due to the oil's high antioxidant's content the vitamin E and polyphenols which again, neutralize free radicals⁽⁴⁾.

Aloe Vera

Pure aloe Vera acts as a good moisturizer which is extracted from the plants that grows in many arid regions of the world. It was originally grown in regions of Africa and India⁽¹⁾. When applied daily aloe Vera can help to prevent fine lines and wrinkles by keeping the skin hydrating and boosting the skins own natural regenerative abilities⁽³⁾. Some studies indicate that aloe Vera moisturizer may also help to prevent sun damage and heal burns more quickly to prevent wrinkles that occur from sun exposure⁽¹⁾. Some of the best aloe Vera moisturizers contain SPF protection from added defense against the suns harmful U.V. rays⁽³⁾.

Preservatives

Wheat germ oil

Wheat germ is dark brown oil incredibly rich in omega 6 fatty acids, proteins and vitamins A, B, C and especially vitamin E, which makes it a natural preservative for all other carrier oils. We recommend using as a 5- 10% solution to prolong the shelf life of your DIY cosmetic and massage blends⁽⁵⁾.

CONCLUSION

After all sun exposure is necessary for the synthesis of vitamin D and other metabolic process and to increases the blood circulation although the sun exposure on a sunny day afternoons may lead to the conditions like rashes, sunburns, and other sensitivity triggering conditions in the human body hence to avoid all these unwanted conditions; homemade sunscreens are preferred which work under moderate temperature.

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EVALUATION OF BIOEFFECIENCY OF POLY-HERBAL FORMULATION WITH EMU OIL FOR ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

The polyherbal formulation containing Moringa oliefera (leaves), Bellis perennis (flowers / seed), Tamarindus Indica (seeds) is used in the treatment of inflammatory and analgesic based on the long term experience of the ayurvedic physicians. But no scientific study has been carried out so far we have evaluated the Analgesic and Anti-inflammatory activities of poly herbal formulation using different animal models like (rats and mice) such as Hot-plate method, Tail immersion for analgesic activity and anti-inflammatory activity by Histamine induced paw oedema significant analgesic activity of poly herbal preparation was found after 2 hours as compared to the Diclofenac $P < 0.295$ was considered as statistical extremely significant. The preparation also showed sufficient Anti-inflammatory activity chocked against Histamine induced paw oedema. The poly-herbal formulation was tested at dose of 10, 100, 100, 100, and 100, mg/ml per kg body weight and 2 ml per kg body weight. This article contains pharmacognostical evaluation and pharmacological evaluation.

KEY WORDS: *Poly herbal formulation, Anti-inflammatory action, Analgesic action.*

INTRODUCTION

Poly herbal formulation is the use of more than one herb in a medical preparation the concept is found in Ayurvedic and other traditional medicinal systems where multiple herbs in a particular ratio may be used in the treatment of illness. Historically, the Ayurvedic literature "sorangdhar samhitha " dated centuries ago in 1300 A.D has highlighted the concept of polyherbalism in this ancient medicinal system. In this traditional system of Indian medicine, plant formulations and combined extracts of plants are chosen rather than individual ones. It is known that Ayurvedic herbals are prepared in a number of dosage forms, in which mostly all of them are poly herbal formulations. Due to synergism, polyherbalism confers some benefits which are not available in single herbal formulation. An emu is a flightless bird, scientifically named "Dromaius novaehallindiae "the bird native to Australia, butis now found in many countries, because of arising popularity of nutritious emu meat and medicinal emu oil. Emu oil is a bright yellow liquid, made up of mostly fat, which is collected from the deposits below the skin of the bird. It is a widely available commercial product that is having Analgesic and Anti- inflammatory activity. Once the fats are collected and they are passed through full processing in order to reduce bacteria and the most popular benefit of emu oil is its use as an Anti-inflammatory. Industrially refined "emu grade "emu oil is composed of a minimum of 70 % unsaturated fatty acids. The largest component is oleic acid, a monosaturated omega-9-fatty acid. Emu oil also contains roughly 20 % linoleic acid (an omega-6-fatty acid) and 1 to 2 % linolenic acid (omega -3-fatty acid) these chemical constituents are responsible for showing Anti-inflammatory action. The Moringaoliefera which is the most widely cultivated species ingenus moringa, the only genus plant belongs to the family moringaceae. Thechemicalconstituentspresent in seeds helps in showing Analgesic or Anti-inflammatory action are oleicacid (84%) L+or-ascorbicacid- 2,6-dihexadecanoate (9.80), 9-octadecenoic acid (1.88%), methyl ether – hexadecanoic acid (1.31%), 9 octadecenamide (0.78 %) .

MATERIALS AND METHODS

CHEMICALS AND DRUGS OR PLANT MATERIALS

The mature fresh leaves of Moringa oliefera and seeds of Tamarindus Indica, Bellis perennis collected from the local region. The plant materials were identified and authenticated taxonomically at botanical survey of

India and control (vehicle), standard (Diclofenac), poly herbal formulation, *Dromia novae-hollandiae* oil.

PREPARATION OF EXTRACTS

EXTRACTION PROCESS OF MORINGA OLEIFERA LEAF POWDER:⁶

SOXHLETATION

A small amount of hot liquid is made to percolate through a column of drug again and again by evaporation and subsequent condensation using Soxhlet extractor. A Soxhlet extractor consists of a flask holding the menstrum the extractor or a cylindrical percolator provided with an attached siphon called Soxhlet extractor and reflux condenser fitted at the top of the Soxhlet. The material to be extracted usually placed in thimble made of filterpaper and then inserted into extractor. The menstrum is placed in the flask a boiled. The vapour is arising from the flask pass by the side tube into condenser the vapour is condensed and dips into the thimble of extract or aspure menstrum. It percolates through the drug to be extracted dissolving the soluble substance. Menstrum in the main extractor rises above the siphon bend. The extract is drained out flowing through the siphonin to the flask. The alternate filling and emptying and the body of the extract or goes on the continuously until the cycle the distillate obtained from the extract gets delivered upon the drugs. Thus the same quantity of menstrum is recycled every time and complete extraction is achieved with a small volume of the menstrum, extraction process and the preparation obtained was distilled out and extract was prepared by using distillation and then solvent in the allowed to evaporate. Obtained extracts was stored in air tight container until future use.⁸

EXTRACTION PROCESS OF BELLIS PERENNIS SEED EXTRACT

100 grams of seeds are soaked in 1L of distilled water for 12 hours and then add to a blender to separate mucilage from seeds. After blending for 15 minutes he mass passed through the 8 folds of muslin cloth. The mucilage was precipitated by acetone.

EXTRACTION PROCESS OF TAMARINDUS INDICA SEED EXTRACT

200 gms of tamarind seeds were soaked in distilled water for about 24 hrs and then distilled for 5 to 6 hrs to remove the outer dark layer. After removing the dark layer, distilled water was added to inner white portion and boiled for 3 to 4 hrs with continuous stirring to obtain slurry. Resultant solution was cooled and the mare was separated using muslin cloth and the filtrate was concentrated on water bath at 60°C to reduce the volume to 1/3 of its initial volume and cooled. To the filtrate equal volume of ethanol or acetone was poured by continuous stirring to precipitate the mucilage. Precipitated mucilage was separated by filtration, dried at 40°C, passed thorough sieve no.60 and finally stored in air tight container at room temperature.⁸

ANIMALS

Healthy Albino rats weighing 250-300 grams were taken; they were then placed into individual. Restraining cages leaving the tail hanging out freely. The animals are allowed to adapt in the cages. The selected rats are divided into 6 groups the experiments were designed and conduct in accordance with ethical norms approved by committee for the purpose of control and supervision on experiments on animals (CPCSEA) and Institutional animal ethical committee (IAEC).

PREPARATION OF POLY HERBAL FORMULATION

Poly herbal formulation was prepared according to the ED 50 of individual herbs. ED50 of individual plants was found to be Diclofenac (100 mg/ml per kg body weight), Ethandic leaf extract of *Moringa oliefera* (100 mg/ml per kg body weight), seed mucilage of *Bellis perennis* (100 mg/ml per kg body weight) and *Dromia novae-hollandiae* (2 ml per kg body weight).

DRUGS AND DOSAGE

The poly herbal formulations was administered orally at the doses of 10 mg/ml, 100 mg/ml, 100 mg/ml, 2 ml per kg body weight in the form of suspension prepared in double distilled water. Diclofenac and required chemicals are purchased from local market.

ACUTE TOXICITY STUDY

Acute toxicity study was performed in accordance with OFCD guidelines a25 (45) no adverse effect or mortality was detected in Albino rats up to 3 gm/kg. P O of poly herbal formulations during the 24 to 72 hrs observation period for 5 hours for any gross behavioural, neurological, or autonomic toxic effect and lethally

after 24 to 72 hours.¹⁹

DRUG TREATMENT

Swiss Albino mice/Wistar rats were divided into eight groups of 5 animals each. Group I served as Control (1% (w/v) CMC in double distilled water, p.o). Group II was administered standard drug Aspirin (300 mg/kg, p.o). Group III-V served as test groups and treated with poly-herbal formulation (200, 400 and 600 mg/kg, p.o in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC) respectively). Group VI-VIII was treated with test drugs as an individual herb extracts (C.P., 400 mg/kg, p.o; P.P. 300mg/kg, p.o and V.N. 500 mg/kg, p.o). The prepared extract was administered once daily for 3 consecutive days for analgesic activity and once daily for 7 consecutive days for anti-inflammatory activity.

PHARMACOLOGICAL STUDIES

1. Hot plate method or Hot plate reaction time in rat
2. Tail immersion method or Tail immersion reaction time in rat
3. Histamine induced paw edema

1. HOT PLATE METHOD

Experimental models of either sex were randomly selected and divided into 6 groups consisting of 6 rats in each group.

GROUP 1: Rats treated with normal saline orally which serves as control.

GROUP 2: Rats treated with Diclofenac (10 mg/ml per kg body weight) serves as standard. GROUP 3: Rats treated with poly herbal formulation (100 mg/ml per kg body weight).

GROUP 4: Rats treated with ethanolic leaf extract of *Moringa oliefera* (100mg/ml per kg body weight).

GROUP 5: Rats treated with seed mucilage of *Bellis perennis* (100 mg/ml per kg body weight).

GROUP 6: Rats treated with *Dromia novaehollandiae* (2 ml per kg body weight).

The animals were positioned on Eddy's hot plate kept at a temperature of 55 ± 0.5°C. A cut-off period of 15 sec observed to avoid damage to the paw. The reaction time was recorded when animals licked their fore or hind paws or jumped.¹⁴⁻¹⁸

2. TAIL IMMERSION METHOD

Healthy Albino rats weighing 250 to 300 gms were taken then placed into individual restraining cages leaving the tail hanging out freely. The animals are allowed to adapt in the cages. The selected rats were divided into 6 groups.

GROUP 1 : Rats treated with normal saline orally which serves as control. GROUP 2 : Rats treated with Diclofenac (10 mg/ml per kg body weight).

GROUP 3 : Rats treated with poly herbal formulation (100 mg/ml per kg body weight).

GROUP 4 : Rats treated with Ethanolic leaf extract of *Moringa oliefera* (100 mg/ml per kg body weight).

GROUP 5 : Rats treated with seed mucilage of *Bellis perennis* (100 mg/ml per kg body weight).

GROUP 6 : Rats treated with *Dromia novaehollandiae* (2 ml per kg body weight).

The lower 3.5 cm portion of the tail was immersed in a cup of freshly filled water of exactly 55 ± 0.5°C. Prior to analgesic experiment, the animals were screened for the sensitivity test by immersing the tail gently in hot water maintained at 55 ± 0.5°C.

3. HISTAMINE INDUCED PAWEDEMA

Experimental models of either sex were randomly selected and divided into 6 groups consisting of 6 rats in each group.

GROUP 1: Rats treated with normal saline orally which serves as control.

GROUP 2: Rats treated with Diclofenac (10 micro grams per ml per kg body weight) serves as standard.

GROUP 3: Rats treated with poly herbal formulations (200 mg/ml per kg body weight).

GROUP 4: Rats treated with ethanolic leaf extract of *Moringa oliefera* (100 mg/ml per kg body weight).

GROUP 5: Rats treated with seed mucilage of *Bellis perennis* (100 mg/ml per kg body weight).

GROUP 6: Rats treated with Emu oil (100 mg/ml per kg body weight).

Chronic inflammation was produced by injecting 0.1 ml of histamine in to the left hind paw of rat. The paw thickness was measured by using vernier callipers before and after histamine injection. Increase in paw

thickness was calculated by using the formulae $P1 - P0$ where $p1$ is the thickness of the paw at time t and $p0$ is the paw thickness at '0' time. Percentage edema was calculated using the formulae $[(p1-p0) / p1] \times 100$.

RESULT

Statistical Analysis

All results were expressed in mean standard error of mean [SEM] data was analysed by using one way ANOVA and two way repeated measures followed by turkeys multiple comparison and student unpaired t test using IBM SPSS statistical software. $p < 0.05$ was considered as a statistical extremely significant.

Hot plate method

S.NO	TREATMENT	TIME TAKEN IN SECS
1	Control (Vehicle)	8.96 $\pm$ 0.30
2	Standard (Diclofenac) 10mg /ml	21.83 $\pm$ 0.35
3	Polyherbal formulation (50 mg/ml)	19.11 $\pm$ 0.28
4	Moringa olifera leaf extract (100 mg/ml)	18.85 $\pm$ 0.24
5	Bellis perennis seed mucilage (100 mg/ml)	10.71 $\pm$ 0.36
6	Dromia novaehollandiae oil (100 ml /kg)	22.08 $\pm$ 0.28

Tail immersion method

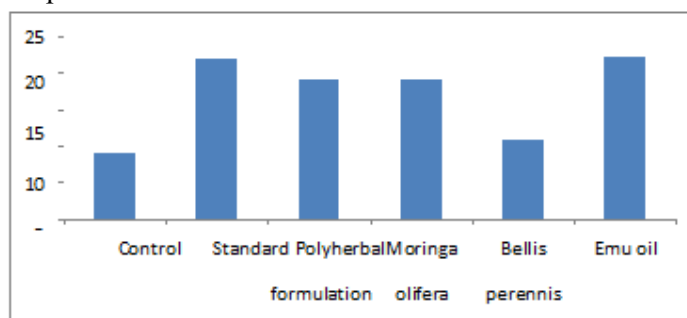
S.NO	TREATMENT	TIME TAKEN IN SECS
1	Control (Vehicle)	2.76 $\pm$ 0.32
2	Standard (Diclofenac) 10 μ g /ml	6.53 $\pm$ 0.37
3	Polyherbal formulation (200 mg/ml)	5.18 $\pm$ 0.48
4	Moringa olifera leaf extract (100 mg/ml)	1.98 $\pm$ 0.20
5	Bellis perennis seed mucilage	2.4 $\pm$ 0.25
6	Emu oil	4.01 $\pm$ 0.27

Anova calculation of above results

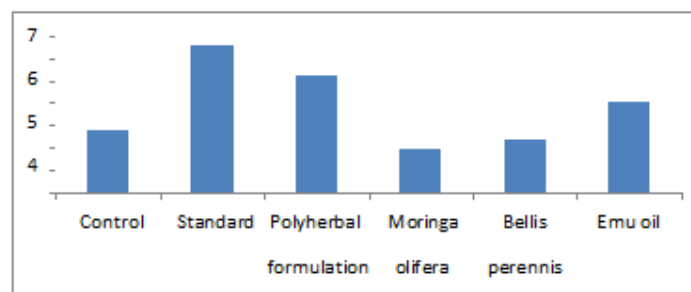
Correlations

		VAR00001	VAR00002
VAR00001	Pearson Correlation	1	.295
	Sig. (2-tailed)		.570
	N	6	6
VAR00002	Pearson Correlation	.295	1
	Sig. (2-tailed)	.570	
	N	6	6

Effect of poly-herbals on hot plate method



Effect of poly herbals on tail immersion method



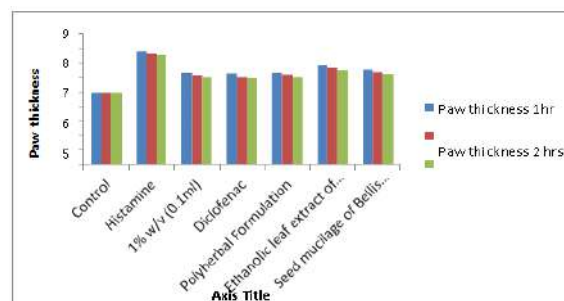
Histamine Induced Paw Edema

Treatment Groups	Dose(mg/kg)	Paw thickness (mm) and % inhibition		
		1 hr	2 hrs	3 hrs
Control	Saline	4.96±0.016	4.96±0.016	4.96±0.016
Histamine 1% w/v (0.1ml)	Saline	7.78±0.041	7.62±0.015	7.53±0.018
Diclofenac	100 mg/kg	6.32±0.020	6.17±0.028	6.04±0.021
Polyherbal Formulation	100 mg/kg	6.28±0.147	6.035±0.021	5.93±0.019
Ethanolic leaf extract of Moringa oliefera	100 mg/kg	6.32±0.018	6.22±0.01	6.02±0.018
Seed mucilage of Bellis perennis	100 mg/kg	6.8±0.018	6.65±0.010	6.51±0.019
Oil of Dromia novae hollandiae	100 mg/kg	6.52±0.029	6.38±0.023	6.23±0.016

ANOVA calculation of above results

Correlations		VAR00002	VAR00003
VAR00002	Pearson Correlation	1	.720**
	Sig. (2-tailed)		.001
	N	18	18
VAR00003	Pearson Correlation	.720**	1
	Sig. (2-tailed)	.001	
	N	18	18

** . Correlation is significant at the 0.01 level (2-tailed).



DISCUSSION

Diclofenacis taken as a standard drug to evaluate Analgesic activity by hot plate method and tail immersion method, we found that. In p retreated animal's the reflux time for paw licking, jumping and withdrawing were very less when compared to the treated group and also differences occur in between control group, standard group and test. (T1, T2, T3 groups). Histamine is injected to control, test, and standard for the development of oedema.

CONCLUSION

From the results obtained in the present study, it may conclude that polyherbal formulations possess significant analgesic and anti-inflammatory activity. The results of the present study shows that the polyherbal formulations possess significant analgesic and anti-inflammatory activities in all the tested experimental animals models indicating inhibition of all phase of inflammation. The polyherbal

formulations possess central analgesic activity which was evaluated using hot plate method and peripheral activity in histamine induced writhing test. Histamine causes analgesia by liberating endogenous substance and many other that excite pain at nerves ending. According to the percentage inhibition on the number of writhes obtained with the various doses of the polyherbal formulations, it was found that the intensity of the analgesic effects was similar to that of aspirin. Aspirin inhibits cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors. To include, polyherbal formulation possesses analgesic and anti-inflammatory effects.

ACKNOWLEDGEMENT

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EVALUATION OF ANTI ULCER ACTIVITY OF BERRIES HYDRO ALCOHOLIC EXTRACT OF *HIPPOPHAE-RHAMNOIDES* IN ASPIRIN INDUCED ULCER IN WISTAR ALBINO RATS

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ABSTRACT

Peptic ulcer is an important and prevailing problem in many developing countries. The cause of ulceration in patients is mainly due to hyper secretion of gastric juice and also hyper secretion of pepsin. Many drugs are available for the treatment of peptic ulcers. But on chronic administration of these drugs causes various adverse effects like dizziness, nausea, headache, diarrhoea, abdominal pain. So nowadays more research work done on medicinal plants for many diseases, and prefer the combination therapy for chronic diseases like Diabetes, cancer for avoiding side effects. Herbal preparations also available in the market, in the literature survey many herbal extracts have been used for the treatment of peptic ulcer. The present study was intended to investigate and evaluate the anti-ulcer activity of ethanolic berry extract of *Hippophae-rhamnoides* using Aspirin induced ulcers in albino rats and to determine its effects between oxidant and anti-oxidant parameters. The extract of *Hippophae-rhamnoides* was tested on Aspirin induced ulcers in albino rats. The anti-ulcer activity of high dose (400mg/kg) and low dose (200mg/kg) of ethanolic extract of *Hippophae-rhamnoides* was assessed by measuring the ranitidine. Pre-treatment of ethanolic extract both high dose and low dose shows significant ($P < 0.001$) decrease in ulcerative area as compared to the control and almost as effective as standard Ranitidine. In conclusion anti-ulcer properties of the extract may be attributed due to its chemical constituents particularly polyphenolic compounds.

KEYWORDS: *Anti-ulcer, Aspirin, ranitidine, Hippophae-rhamnoides*

INTRODUCTION

Peptic ulcer was found to be the most important problem in many developing countries (GW, 2001). It occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors. Many drugs including prostaglandin analogs, proton pump inhibitors, histamine receptor antagonists and cytoprotective agents are available for the treatment of the peptic ulcer. But on chronic administration of these drugs causes various adverse effects ranging from dryness of mouth to achlorhydria, atrophic gastritis, osteodystrophy and encephalopathy (Ariyphisi I, Toshiharu A, Sugimura F, Abe M, Matsuo Y, 1986; KR, 2007). Therefore, herbal medicines seems to be an alternative for chronic drug administration, due to their fewer adverse effects. It was identified that almost 70% of the population were being dependent on traditional and herbal medicines for their primary health care needs (Antwi S, Martey ON, Donkor K, 2009). Since the extensive research is going on in the area of natural medicine, many plants were reported to have anti-ulcer activity. *Hippophae-rhamnoides* commonly known as sea buckthorn belongs to the family Elaeagnaceae. It is native to cold temperate regions of Europe and Asia. It is a spiny deciduous shrub that can grow between 2 and 4 m high. It has a rough, brown, greyish-green crown. The leaves are alternate, narrow and lanceolate, with silvery green upper faces. It is a dioecious, meaning that the male and female flowers grow on the different shrubs. The male inflorescence consists of only one apetalous flower, while the female inflorescence contains only one apetalous flower and also contains one ovary and one ovule. Fertilisation occurs via wind pollination. The oval or lightly roundish fruits grow in compact grapes varying from pale yellow to dark orange. The plants have a very well-developed extensive root system, and the roots live in symbiosis with nitrogen fixing *Frankia* bacteria. Individual fruits weigh between 270 and 480 mg and contain high amounts of vitamin C, vitamin E, carotenoids, flavonoids phenolic compounds, and health beneficial fatty acids, as well as higher amounts of vitamin B12 than other fruits. Since it has got a

traditional significance the aim of the study is to evaluate the anti-ulcer activity of *Hippophaerhamnoides* in wistar rats.

TAXONOMY¹

Hippophae is a small genus of Elaeagnaceae. *Hippophae* classified into three species based on morphological variations: *H. rhamnoides* L., *H. salicifolia* D. Don, and *H. tibetana* Schlecht. *Hippophaerhamnoides* was divided further into nine subspecies: *carpatica*, *caucasica*, *gyantsensis*, *mongolica*, *sinensis*, *turkestanica*, *unnanensis*, *rhamnoides*, and *fluviatilis* Rousi. Liu and He (1978) described a fourth species, *H. neurocarpa* Liu & he found that on the Qinghai-Xizang plateau of China. These classifications were accepted generally by Lian (1988), but taxonomists still disagree on the precise classification of the genus. In ancient times, leaves and young branches from sea buckthorn were supposedly fed as a remedy to horses to support weight gain and appearance of the coat, leading to the name of the genus, *Hippophae* derived from *hippo* [horse] and *phao* [shining].

GEOGRAPHICAL DISTRIBUTION²

Hippophaerhamnoides the common sea buckthorn is the most widespread of the species in the genus with the ranges of its eight subspecies extending from the Atlantic coasts of Europe across to northwestern Mongolia and northwestern China. In Western Europe it is largely confined to sea coasts where salt spray off the sea prevents other larger plants from outcompeting it. In central Asia it is more widely spread in dry semi-desert sites where other plants cannot survive the dry conditions. In central Europe and Asia it also occurs as a sub-alpine shrub above the tree line in mountains, and other sunny areas such as river banks where it has been used to stabilise erosion. They are tolerant of salt in the air and soil, but demand full sunlight for good growth and do not tolerate shady conditions near larger trees. They typically grow in sandy areas.

CULTIVATION

Sea buckthorn is a deciduous, dioecious shrub, usually spinescent, reaching 2 to 4 m in height. It has brown or black rough bark and a thick grayish green crown. Leaves are alternate, narrow and lanceolate with a silver-gray colour on the upper side (Synge, 1974). So this calcicolous species tolerates low temperatures, high pH of 8.0 and salt spray (Bond, 1983). The plant's extensive root system is capable of holding the soil on fragile slopes. Sea buckthorn can plant in marginal soils due to its symbiotic association with nitrogen-fixing actinomycetes (Akkermans et al., 1983; Dobritsa and Novik, 1992). Roots of sea buckthorn also are able to transform insoluble organic and mineral matter in the soil into more soluble states (Lu, 1992). The plant rapidly spreads by rhizomatous roots, and will quickly colonize adjacent areas. The sex of seedlings can't be ascertained until they start to flower (Synge, 1974). Flower buds are formed mostly on 3-year-old wood, differentiated during the previous growing season (Bernath and Foldesi, 1992). The male inflorescence consists of four to six apetalous flowers. Pollen is released in large quantities when the air temperature reaches 6 to 10 °C. The female inflorescence usually consists of one single apetalous flower with one ovary and one ovule. The plant depends entirely on the wind for pollination: neither the male nor the female flowers have nectars and do not attract insects. Cultural management Sea buckthorn normally is transplanted or directly seeded in the spring. Best growth occurs in deep, well drained, sandy loam soil with ample organic matter (Wolf and Wegert 1993).

MATERIALS AND METHODS³⁻⁸

Plant material

Fresh berries powder of *Hippophaerhamnoides* was purchased from Amazon.

Preparation of plant extract

The berries of *Hippophaerhamnoides* powder was packed in Soxhlet at 40°C for 72 hours and extracted using ethanol as a solvent. Extracts were concentrated under vacuum using rotary flash evaporator to get the residues.

Animals

Wistar albino rats of either sex, weighing 150–200 g were taken and maintained under standard laboratory conditions (temperature 25±2 °C, relative humidity 55±10% and 12-h light: 12-h dark cycle). Animals were

allowed to take standard laboratory feed and ad libitum. The experiments were carried out after the prior approval of all experimental protocols by the institutional animal ethics committee.

Approval number: 001/IAEC/NCPA.

Acute toxicity study

Albino wistar rats were used for acute toxicity study. The animals were kept fasting for overnight with water ad libitum, after which the extracts were administered orally at the dose of 200 mg/kg and also 400 mg/kg and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose, i.e., 2000 mg/kg b.w. Two doses were selected for evaluation of anti-ulcer activity, i.e., 200 mg/kg and 400 mg/kg b.w (Handa, S., Anupama, 1990). Aspirin-induced gastric ulcer: (Nair et al., 2010) One day before the induction of ulcers, animals were divided into groups (n=6) randomly and drugs/vehicle was administered as follows.

Treatment Protocol

GROUP	TREATMENT	DOSE (mg/kg)	Route of administration
Group-1(normal control)	Saline	0.9%W/V	Oral
Group-2(negative control)	Aspirin		Oral
Group-3 (standard)	Ranitidine	20	Oral
Group-4 (test)	HRE of low dose	200	Oral
Group-4(only extract)	HRE of high dose	400	Oral

The animals were kept fasted (with free access to water) for a period of 24 h so as to ensure completes gastric emptying. The 24 h fasted animals were again administered drugs/vehicle 1 hr prior to the experiment. Aspirin was administered in a dose of 500 mg/kg body weight orally to I, II & III groups (Parmar NS, 1993)12. Animals were allowed to fast for 5 more hours. Animals were then sacrificed under anaesthesia. Midline abdominal incision was made on stomach and was dissected along the greater curvature. All the gastric content was drained out. The stomach was then cut open along the greater curvature and evenly spread out on a dissection board. The mucosa of animals in each group was examined under microscope. The number of ulcers and their severity (Khandelwal, 2000), were recorded using arbitrary scale as follows. Scoring of ulcer (Kulkarni, 2002) 0 = Normal coloured stomach 0.5 = Red coloration 1 = Spot ulcer 1.5 = Haemorrhagic streaks 2 = Ulcers ≥ 3 but ≤ 5 3 = Ulcers >5 Calculation of ulcer index (Vogel, 2002) $UI = UN + US + UP \times 10^{-1}$ UI = Ulcer Index UN = Average of number of ulcer per animal US = Average of severity score UP = Percentage of animal with ulcer Calculation of % inhibition (Hojage M.G., Hriprassanna R.C. &MathaPati. S., 2010) $100 - \frac{\text{control mean ulcer index} - \text{test ulcer index}}{\text{control mean ulcer index}} \times 100$

The results were expressed as mean $\pm$ SEM. Statistical difference between means were determined by one-way ANOVA followed by Dunnett's post hoc test $P > 0.05$ as the limit of significance (SEM= standard of error of mean). with the drugs/vehicle 1 hr prior to the experiment. Aspirin was administered in a dose of 500 mg/kg body weight orally to I, II & III groups (Parmar NS, 1993)12. Animals were allowed to fast for 5 more hours. Animals were then sacrificed under anaesthesia. Midline abdominal incision was made on stomach and was dissected along the greater curvature. All the gastric content was drained out. The stomach was then cut open along the greater curvature and evenly spread out on a dissection board. The mucosa of animals in each group was examined under microscope. The number of ulcers and their severity (Khandelwal, 2000), were recorded using arbitrary scale as follows. Scoring of ulcer (Kulkarni, 2002) 0 = Normal coloured stomach 0.5 = Red coloration 1 = Spot ulcer 1.5 = Haemorrhagic streaks 2 = Ulcers ≥ 3 but ≤ 5 3 = Ulcers >5 Calculation of ulcer index (Vogel, 2002) $UI = UN + US + UP \times 10^{-1}$ UI = Ulcer Index UN = Average of number of ulcer per animal US = Average of severity score UP = Percentage of animal with ulcer Calculation of % inhibition (Hojage M.G., Hriprassanna R.C. &MathaPati. S., 2010) $100 - \frac{\text{control mean ulcer index} - \text{test ulcer index}}{\text{control mean ulcer index}} \times 100$

Statistical analysis

The results were expressed as mean $\pm$ SEM. Statistical difference between means were determined by one-way ANOVA followed by Dunnett's post hoc test $P > 0.05$ as the limit of significance (SEM= standard of error of mean).

RESULTS

Anti ulcer activity

GROUP	TREATMENT	DOSE (mg/kg)	ULCER INDEX	% PROTECTION
Group-1(normal control)	saline	0.9%W/V	3.66+/_0.41	
Group-2(negative control)	Aspirin			
Group-3 (standard)	Ranitidine	20	1.10+/_0.17	69.94
Group-4 (test)	HRE of low dose	200	2.16+/_0.21	40.98
Group-4(only extract)	HRE of high dose	400	0.41+/_0.09	89.07

Significance is expressed as mean +/_ SEM and * $P < 0.001$

DISCUSSION

Although the etiology of peptic ulcer is unknown in most of the cases, but it is generally accepted that it is caused due to an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. The treatment of peptic ulcer is mainly targeted at reducing the hydrochloric acid secretion, increasing gastric cytoprotection, eradication of *H. pylori* or curing Zollinger Ellison syndrome (Jana U, Bhattacharya D, Bandopadhyay S, Pandit S, Deb-nath PK, 2005). Different therapeutic agents are used to treat peptic ulcer, including plant extracts (Al, 2009) So far, several plants have been screened for antiulcer activity. *Hippophaerhamnoides* extract is one of the herbal agents used in the present study to evaluate the anti-ulcerogenic in aspirin induced ulcers in rats. NSAID's like aspirin causes damage of gastric mucosa by decreasing prostaglandin levels through inhibition of PG synthesis¹⁶. Prostaglandins were found to play a vital role in maintaining mucosal integrity. An Increase in certain endogenous prostaglandins enhances gastric mucosal resistance to ulcerogenic agents (Robert, 1979). The mechanisms involved in prostaglandin action are many, including stimulation of mucus and bicarbonate output (Hogan, D. L.; Ainsworth, M. A. and Ibensberg, 1994), gastric mucosal blood flow (Gaskil, D. L.; Serinek, K. L. and Levine, 1982), decreasing gastric motility, increasing the release of endogenous mediators of gastric injury vaso active amines and leukotrienes and stimulation of cellular growth and repair (Hawkey, C. J. and Rantim, 1985). The preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, proteins; phenolic compounds have been reported for its anti-ulcerogenic activity. the plant extract at dose of 200 mg/kg showed significant gastro protective activity 40.98% compared with standard drug ranitidine 20mg/kg showed 69.94% (Table-I). From this study, it was found that ethanolic extract of *Hippophaerhamnoides* have significant anti-ulcer activity. Further studies need to carry out to characterize and explore the biological activity of the compounds in the extract.

CONCLUSION

From the result we conclude that ethanolic extract of *Hippophae-rhamnoides* have showed significant anti-ulcer activity. Further studies need to carry out to characterize and explore the biological activity of the compounds in the extract.

ACKNOWLEDGMENT

The authors are greatly thankful to the management of Nirmala College of pharmacy for providing all the facilities for the research work.

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A REVIEW ON UTERINE FIBROIDS

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ABSTRACT

Uterine fibroids are also called as uterine leiomyoma. These are major cause of the morbidity in woman of reproductive age. The prevalence in all population in India 17.9% and 30% fibroids is symptomatic. Diagnosed by 2d and 3d ultrasound which is associated by Hysterosonography and hysteroscopy. by the natural treatment we can eliminate all types and sizes of fibroids quickly, naturally and safely and also prevent the heavy periods, pelvic pain and other symptoms of fibroids whereas by surgeries many risk factors are associated such as blood loss, pain, infection, longer recovery and also expensive method.

KEYWORDS: *uterine leiomyoma, Hysterosonography, hysteroscopy, natural treatment.*

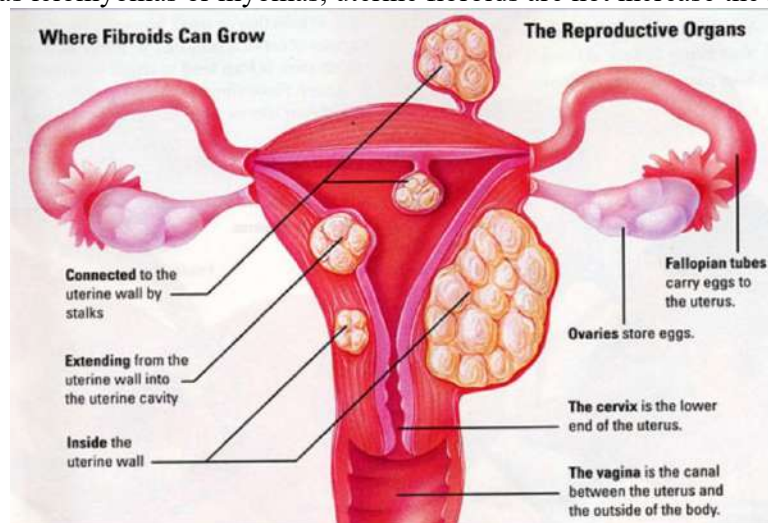
INTRODUCTION

Uterine fibroids are tumours that can grow in a woman's womb (uterus). these growths are typically non-cancerous (benign). A non-cancerous tumour of the uterus can range from the size of the pea to the size of the grape. Fibroids grossly appear as round, well-circumscribed, solid nodules that are white or tan and show whorled appearance on histological section. Small fibroids require no treatment but those causing serious symptoms may need to be removed.

Definition:¹

Uterine fibroids are non-cancerous growth of uterus that often appear during childbearing.

These are also called as leiomyomas or myomas, uterine fibroids do not increase the risk of uterine cancer.

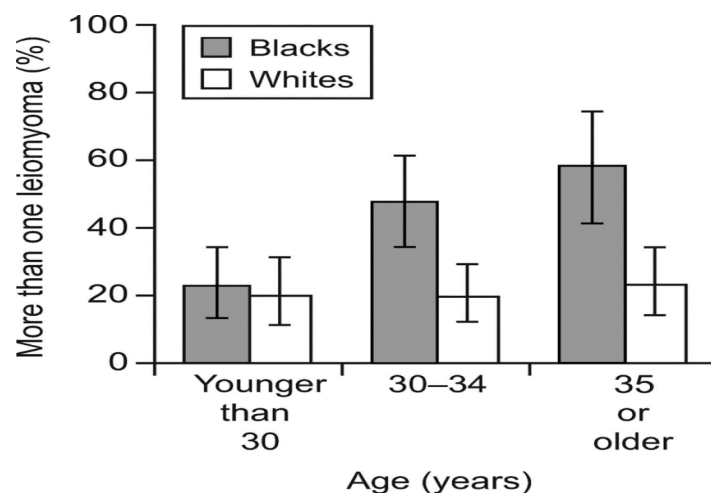
**Epidemiology:**¹

About 20 to 80% of women develop fibroids by the age of 50. In 2013 it was estimated that 171 million women were affected worldwide.

Race: black women are more likely to have fibroids than are women of other racial groups.

Heredity: if your mother or sister had fibroids you are at risk of developing them.

In India the reported prevalence of uterine fibroid is around 17.9%. They are typically found during the middle and later reproductive ages.



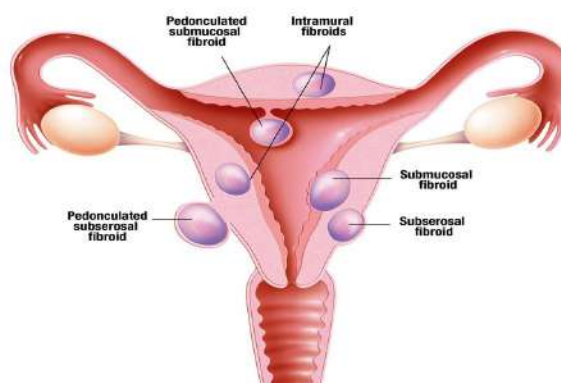
Pregnancy and fibroids

Fibroids usually don't interfere with getting pregnant .however itspossible that fibroids- especially submucosal fibroids-could cause infertility or pregnancy lose fibroids May also raise the risk of certain pregnancy complications such as placental abruption ,foetal growth restriction, preterm delivery

Types

There are 4 types of uterine fibroids

- 1) Submucosal fibroids: Grown in the submucosa, which means just below the thin layer of tissue found in the uterus.these fibroids can protrude in to the uterine cavity. These are the rarest type of fibroids.
- 2) Intramural fibroids: Grown in and are contained with in the uterine wall. These are the most common type of fibroids
- 3) Subserosal fibroids: Grown on the outside of the uterine wall
- 4) Pedunculated fibroids: These are the types of fibroids that grow on stalks or stems.these stems are attached to the uterine wall and can grow either outside the uterus or inside the uterine cavity.



Symptoms:²

the most common symptoms of uterine fibroids include:

- 1)heavy menstrual bleeding
- 2)prolonged menstrual periods
- 3)pelvic pressure or pain
- 4)frequent urination
- 5)difficulty emptying the bladder
- 6)back ache or pain
- 7)constipation
- 8)Anaemia

Causes:³

Genetic changes	Many fibroids contain changes in genes that differ from those in normal uterine muscles.
Hormones	Oestrogen and progesterone, ² hormones that stimulate development of the uterine lining during each menstrual cycle in the preparation for pregnancy appear to promote the growth of fibroids.
Extra cellular tissue	Ecm is the material that makes all cells sticks together like mortar between bricks Ecm increase in fibroids and makes them fibrous. Ecm also stores growth factors and causes biological changes in the cells themselves.
Other growth factors	Substances that help the body maintain tissues such as insulin like growth factor may affect fibroid growth.

Possible causes of uterine fibroids

Drinking alcohol,not eating enough fruits,vitamin D deficiency

Diagnosis

Pelvic examination: can typically identify the larger fibroids.

Sonography - mostly available and least costly to differentiate fibroids.

MRI: can be used to define the depletion of size and location of fibroids within the uterus.Computed tomography (CT): this x-ray procedure shows internal organs and structures in cross section.

Endometrial biopsy: the purpose is to detect the pre-malignant condition of endometrial hyperplasia with cytological endometrial carcinoma.

Hysterosonography: utilizes clean saline to extend the uterine depression,making it easier to get picture of submucosal fibroid and the endometrium.

Risk factors:³ Race-black woman are more likely to have fibroids than woman of other racial groups.

Heredity-if your mother or sister had fibroids you are at increased risk of developing them.

Other factors: Obesity, Drinking alcohol, Eating red eat

Treatment by surgery

Hysterectomy: A hysterectomy is the partial or total removal of the womb. This is considered for treating extremely large fibroids or excessive bleeding a total hysterectomy can prevent the return of fibroids. If a surgeon also removes the ovaries and fallopian tubes, side effects can include reduced libido and early menopause.

Myomectomy: This is the removal of fibroids from the muscular wall of the womb.it can help women who still want to have children. Women with large fibroids or fibroids located in particular parts of the womb, may not benefit from the surgery.

Endometrial ablation: Removing the lining inside of the womb may help if the fibroids are near the inner surface of the womb endometrial ablation may be an effective alternative to a hysterectomy to some women with fibroids.⁴

MRI guided focused ultra sound surgery: MRI guided focused ultrasound surgery: an MRI scan locates the fibroids and high energy ultra sound waves are delivered to shrink them.

Herbal treatment**Therapeutic Activity of the respective phytoconstituents present in the different Plant**

S.No	Plant name	Synonym	Plant part	Phytoconstituent	Effect
1	Allium sativum	Garlic	Bulbs	Allicin	Anti-inflammatory property and anti-tumour property.
2	Zingiberofficin	Ginger	Rhizome	Gingerol	Anti-inflammatory properties

	ale				that reduces pain in the uterus
3	Vitex agnus-castus	Chaste berry, vitex	Fruit	-	Balance the hormones
4	Linseed	Flax seeds		Omega-3-fatty acids, fibres	Balance hormones to shrink the fibroids
5	Castor oil			Ricin oleic acid	To shrink fibroids and to relieve pain
6	Green tea			Epigallocatechin	Inhibits growth of fibroids and anti-inflammatory, anti-proliferative.
8	Indian goose berry	Amla			Used to reduce fibroids
9	Cinnamon	Cinnamon bark	Bark	Biflavone	Used to shrink fibroids
10	Turmeric		Root	Curcumin	Used to reduce uterine fibroids
11	Grapes		Fruit	resveratrol	Used to reduce fibroids and shrink fibroids.

A number of vitamins may also help to ease fibroid symptom: Vitamin b-1, vitamin B-6, vita the doctors may consider the following procedures.

CONCLUSION

It is concluded that following herbal drug are used to treat uterine fibroids Allium sativum, Zingiber officinale. These two having the high efficient to treat Anti-inflammatory property and anti-tumor property.

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SURVEY, SCREENING, ANALYSIS OF HEAVY METALS IN SELECTED MEDICINAL PLANTS BY UV-VISIBLE SPECTROPHOTOMETRY METHOD

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ABSTRACT

A simple, precised, accurate method was developed for the estimation of heavy metals incleome viscose, mimusopselangi, milling toniahortensis, gliriciasepium & murraya paniculatamedicinal plants by uv-visible spectrophotometry method. To determine the inorganic ions or heavy metals in the selected medicinal plants, and perform the evaluation studies by the survey, screening and analysis of heavy metals in selected medicinal plants by uv-visible spectrophotometry method. First survey is done in Acharya nagarjuna university, out of the selected plants the phytochemical screening tests are performed to determine the inorganic ions and heavy metals, then the analysis was performed by uv-visible spectrophotometry method. And to determine the medicinal plants selected are consisting of the essential and non essential ions within the acceptance limits of WHO guidelines.

KEYWORDS: *Cleome viscose, mimusopselangi, millingtoniahortensis, gliriciasepium and murrayapaniculata, UV visible spectrophotometry*

1. INTRODUCTION

The term heavy metals¹ refer to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. Heavy metals are natural components of the Earth's crust. As trace elements, some heavy metals are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Cleome viscose² is an annual herb that grows up to a meter high. It belongs to the family Capparidaceae. The crushed leaves have been investigated as a treatment for stored seeds of cowpea, to prevent weevil infestation. The leaves are used as external application to wounds and ulcers. The seed are anthelmintic and carminative. The juice of the leaves is used as a remedy against discharge of pus from the ear. Mimusops elengi³ Medium-sized evergreen tree found in tropical forests in South Asia. The flowers are sun dried and used to make floral infusions and as an addition to green tea in Thailand. Millingtonia hortensis⁴ Tree jasmine, the sole species in the genus Millingtonia, is a tree native to South Asia & South East Asia. In its synonym, Bignonia suberosa, 'suberosa' derives from 'suberos' which means 'corky' in Latin. Gliricidia sepium⁵ Often simply referred to as Gliricidia is a medium size leguminous tree belonging to the family Fabaceae Tropical, evergreen plant native to South Asia, Southeast Asia and China and Australia bearing small, white, scented flowers. The species is widely grown as an ornamental tree or hedge. Murraya⁶ is closely related to *Citrus*, and bears small orange to red fruit resembling kumquats, though some cultivars do not set fruit. Asian spider flower⁷ (Cleome viscosa) is a usually tall annual herb, up to a meter high, more or less hairy with glandular and glandular hairs. Often found locally abundant as a naturalized weed in cultivated fields, along roadsides, and especially in cane fields. In the name Millingtonia hortensis⁸ The tree grows to height of between 18 and 25 metres and has a spread of 7 to 11 metres. The bark, flowers, fruits, and seeds of Bakula⁹ are used in Ayurvedic medicine in which it is purported to be astringent, cooling, anthelmintic, tonic, and febrifuge. It is mainly used for dental ailments such as bleeding gums, pyorrhea, dental caries, and loose teeth. It reaches maturity between 6 and 8 years of age and lives for up to 40 years. It is a versatile tree which can grow in various soil types and climates with a preference for moist climates Millingtonia¹⁰ is named for Sir Thomas Millington who was an inspiration to Carl Linnaeus the Younger who first described the genus. The specific epithet 'hortensia' derives from 'hortensis' and 'hortus' which in Latin is related to garden.



Figure-1 *Cleome viscosa*



Figure -2 *Mimosa pudica*



Figure-3 *Moringa oleifera*



Figure-4 *Gliricidia sepium*



Figure-5 *Murraya paniculata*

2. MATERIALS AND METHODS

2.1 PLANTS, REAGENTS AND CHEMICALS

Five medicinal plants were provided Acharya Nagarjuna University. Sodium carbonate Hydrochloric acid Potassium hydrogen phthalate Methanol Sulphuric acid Ferric chloride Calcium chloride Magnesium sulphate Sodium hydroxide Iron sulphate Lead acetate arsenic water HPLC from Loba chemie material India limited.

2.2. INSTRUMENTATION

Instrument employed for the study were, Thermo scientific aquamate plus single beam spectrophotometer, Cyber labs sonicator, shital scientific industries hot plate.

3. METHOD DEVELOPMENT

3.1 PREPARATION OF STANDARD SOLUTION:

About 100mg of chemicals were weighed accurately and transferred into 100ml of volumetric flask and prepared solution. Take 10 ml of prepared solution and dilute to 100ml in another volumetric flask..

3.2 PREPARATION OF TEST SAMPLE SOLUTION FOR 5 PLANTS EXTRACTS

Powder sample of the plant leaves is taken in a china dish and is heated to 50° c by placing in a china dish. The ash obtained from the leaves of the sample is dissolved in 100ml of 10% Hydrochloric acid. Then it was filtered to obtain a clear solution from this solution 10 ml of sample is taken and made up to 100ml with 10% HCL.

4. METHOD VALIDATION:

1. Assay:

The ability to estimate and unequivocally the analyte in presence of components, that may be expected to presence of impurities degradation products and other components.

2. Accuracy

Accuracy is nothing but closeness of the test results that are obtained by the true value of the method, It was estimated by the recovery studies at 3 different levels in each level 3 injections are given and the amount of drug present percentage recovery and relative standard deviation were calculated.

3. Precision

Precision is the analytical method of degree of compatibility between individual test results.

The analysis of multiple sampling from the homogeneous samples. The precision of present method

Was estimated in terms of repeatability intra and inter day variations.

4. Linearity and range

Linearity is an analytical method of ability to obtain the results directly proportional to the analyte concentration in the sample within a definite range. The six series of standard solutions are selected to estimate the linearity range. The calibration curve was plotted between peak area and concentration of the

standard solution and then the regression equations are calculated. To calculate the slope, intercept and correlation coefficient by least square method

5 LOD and LOQ

LOD is the lowest amount of the analyte in a sample that can be detected by LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy LOD & LOQ are separated determine based on the calibration curves. The UV VISIBLE spectrophotometric method the LOD and LOQ are calculated as 3.3 s/n and 10 s/n respectively as per W.HO guide lines

Where s/n indicates that the signal to noise ratio

Robustness

Robustness of an analytical procedure is a measure of its ability to remain unaffected by small but measured variations in the parameter of the methods and it provides an indication of its reliability during normal usage.

5. RESULTS AND DISCUSSION

5.1 LINEARITY

The linearity of the method was demonstrated over the concentration range of 2 to 10 µg/ml of the target concentrations. From the working standard solution aliquots of 0.2, 0.4, 0.6, 0.8, 1 ml was transferred in 10 ml volumetric flask and up to the mark with mobile phase to get the concentration of 2, 4, 6, 8, 10 µg/ml, respectively. These solutions were analysed in UV visible spectro photometer as per test procedure, a calibration curve was plotted for concentration vs absorbance.

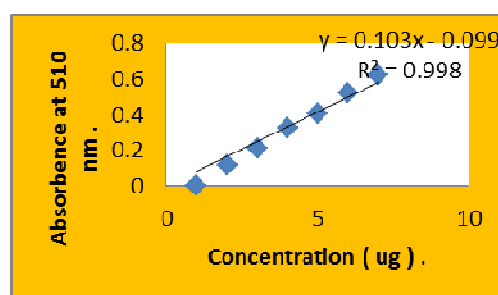


Figure-6 Linearity graph of murraya paniculata

2 ASSAY

The absorbance of standard solution (2 µg/ml) and plant sample solution (2 µg/ml) was measured after adding the reagent as in calibration at 520 nm using against blank and the % assay were calculated by using the formula.

SNO	CONC	ABSORBANCE
1	2	0.475
2	4	0.485
3	6	0.528
4	8	0.585
5	10	0.621
6	Unknown	0.565

Table -1 ASSAY (murraya paniculata)

5.3 METHOD PRECISION

From the sample and stock solution, six replicates of standard and sample solution of 25 ppm were prepared and placed in uv-visible spectro photometer as per test procedure. % RSD is calculated.

SNO	CONC	ABSORBANCE
1	2	0.568
2	4	0.589
3	6	0.621
4	8	0.638
5	10	0.647
6	unknown	0.628
	MEAN	0.6835
	SD	0.009

SNO	CONC	ABSORBANCE
1	2	0.559
2	4	0.627
3	6	0.639
4	8	0.659
5	10	0.679
6	unknown	0.665
	MEAN	0.66
	SD	0.01

Table -2 Method precision (murrayapaniculata), Table-3 Method precision (gliricidiasepium)

5.4 INTERMEDIATE PRECISION

Intermediate precision study was carried out by different analyte, on different instrument and on different instrument days. From sample and stock solutions were prepared.

SNO	CONC	ABSORBANCE
1	2	0.455
2	4	0.465
3	6	0.472
4	8	0.486
5	10	0.495
6	unknown	0.466
	Mean	0.479
	SD	0.0131

Table-4 Intermediate precision (gliricidiasepium)

5.5 Accuracy

A series of solutions were prepared triplicate (6 preparations for 2 to 10 level) by spiking the sample drug substance on placebo in the range about 2 to 10 test concentration and analyzed as per specification level. Triplicate the samples for 80%, 100% and 120%. Individual % recovery, mean % recovery, % RSD, and linearity were calculated at each level

CONC	AMOUNT FOUND (ug/ml)	%RECOVERY
80% 80% 80%	30.317	94%
100% 100% 100%	24.65	96%
120% 120% 120%	31.54	98%

Table-5 Accuracy (gliricidiasepium)

5.6 Robustness

The robustness of proposed method is determined by analysis of aliquots from homogenous slots by differing [physical parameters like wavelength ± 2 nm which may differ but the responses are within the limits

464nm	468nm

0.650	SD=0.002	0.649	SD=0.00115
0.648	RSD=0.3076	0.651	
0.652		0.649	RSD=0.17773

Table-6Robustness(gliricidiasepium)

5.7 LOD&LOQ

Calibration curve was repeated for 3times and the standard deviation (SD)of the intercepts was calculated.The LODwas determined by the formula $LOD=3.3\sigma/S$, $LOQ=10\sigma/S$. The slope s may be estimated from the calibration curve of the analyte. murrayapaniculata0.64 (ug/ml), mimusopselengi: 0.54 (ug/ml), cleomeviscosa 0.32 (ug/ml), milling toniahortensis 0.64 (ug/ml), gliricidiasepium: 0.54(ug/ml).

6. CONCLUSION

A simple, precised, accurate method was developed for the estimation of heavy metals in cleome viscosa, mimusopselangi, milling toniahortensis, gliriciasepium & murraya paniculatamedicinal plants by uv-visible spectrophotometry method. Toxicelements presented plants: milling toniahortensis. Heavy metals presented in plants are: cleome viscosa: iron, lead, calcium, chloride, copper & zincmimusopselngi: iron, lead, calcium , chloride & magnesium murraya paniculata: lead, arsenic, chloride, zinc & iron gliricidiasepium: lead, arsenic, chloride, zinc & ironmillingtoniahortensis: cobalt, lead, zinc, iron & chloride.

7. ACKNOWLEDGEMENT

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FORMULATION AND EVALUATION OF ACECLOFENAC BILAYER TABLETS

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ABSTRACT

The objective of the present research was to develop a bilayer tablet of aceclofenac using sodium starch glycolate (SSG) and croscarmellose sodium (CCS) as super disintegrants for the formulation of immediate release layer whereas polymers such as methocel K15M, Lubrizol 971P were used for the formulation of sustaining layer. The tablets were prepared by direct compression method. The prepared tablets were evaluated for pre-compressed parameters like micromeritic properties and post compressed parameters like weight variation, aceclofenac content and in-vitro dissolution studies. The in-vitro dissolution studies showed about 86.78 % of aceclofenac release from the bilayer tablet indicating that an initial burst release of aceclofenac followed by sustaining action up to 12 h from the sustained layer of the tablets. In-Vitro kinetic data revealed that all the formulations followed the Higuchi model via fickian diffusion as release mechanism after the initial burst release. FT-IR studies revealed that there was no interaction between the drug and polymers used in the study.

KEYWORDS: *Aceclofenac, super disintegrants, sustaining layer, fickian diffusion*

1. INTRODUCTION

Drug delivery systems are designed for maximizing the therapeutic index of the drug and also for a reduction in the side effects ¹. Oral ingestion is the predominant and most preferable route for drug delivery because the low cost of therapy and ease of administration leads to a higher level of patient compliance². Effective oral drug delivery may depend upon factors such as gastric emptying process, the gastrointestinal transit time of dosage form, drug release from the dosage form and site of absorption of drugs ³. Sustained oral drug delivery systems offer several advantages over immediate-release dosage forms and reduced side effects ^{4,5}. Several difficulties are faced in designing sustained release systems for better absorption and enhanced bioavailability. Drug absorption from the gastrointestinal tract is a complex procedure and is subject to many variables ⁶. It is widely acknowledged that the extent of gastrointestinal tract drug absorption is related to contact time with the small intestinal mucosa ⁷. These advantages, explain the need for designing of modified drug delivery system, which will alter the pharmacokinetics and pharmacodynamics of pharmacologically active moiety in a selected route of administration ^{8,9}. Pain is universally understood as a signal of disease, it is the most common symptom that brings a patient to a physician's attention¹⁰. The task of medicine is to preserve and restore health and to relieve suffering. In this context, the most commonly used pain relieving agent is aceclofenac an NSAID. In the present investigation, aceclofenac bilayer tablets were prepared to provide sustain effect for better therapeutic effect.

2. MATERIALS AND METHODS

Aceclofenac was obtained as a gift sample from Acesus Pharma, Ongole, Sodium starch glycolate, croscarmellose, methocel, Lubrizol, were obtained from Capricon, Hyderabad. All other reagents of analytical grade were used.

2.1 Calibration of Aceclofenac

Aceclofenac standard solution was prepared by dissolving 10 mg of aceclofenac in a sufficient amount of ethanol and the volume was made with pH 7.5 phosphate buffers in a 10 mL volumetric flask. The standard solution was subsequently diluted with buffer to prepare 4, 8, 12, 16, and 20 µg/ml solution and the

absorbance was measured at 273nm against blank. Similarly, the calibration of aceclofenac was also performed in pH 1.2 buffers at 269nm.

2.2 Preformulation Studies

Preformulation may be described as a stage of development during which the physicochemical and biopharmaceutical properties of a drug substance are characterized. The drug was tested for organoleptic properties, solubility, and drug – excipient compatibility studies.

2.3 Preparation of Aceclofenac bilayer tablets

2.3.1 Sustained release layer of aceclofenac

Physical mixtures of sustained release layer were prepared by geometric dilution method. After being grinded and sifted, the drug was mixed with methocel and lubrizol on the geometric basis in a mortar and pestle and the mixture was subsequently passed through an 18-mesh screen to blend the ingredients uniformly and then mixed with talc and magnesium stearate for the following tableting process.

2.3.2 Immediate release layer of aceclofenac

The physical mixture of the immediate release layer was prepared by using croscarmellose sodium and sodium starch glycolate by geometric dilution as per the similar procedure described above.

2.3.3 Preparation of bilayer tablets

The bilayer tablets were fabricated with 16 station rotary punch using 12mm punch via double compaction method. Briefly the weighed physical mixture equivalent to 130 mg of aceclofenac of sustained released layer was fed manually in to the die cavity and then compressed in to tablets and sequentially the physical mixture equivalent to 70 mg of aceclofenac of immediate release layer was fed in to the same die cavity containing the sustained release tablet and was compressed ¹¹. Table 1 exhibits the detailed compositions tested for the preparation of bilayer tablets.

2.4 Evaluation of physical mixtures

The physical mixtures prepared were evaluated for precompressed properties, and drug content.

2.4.1 Precompressed properties¹²

The precompressed properties like the angle of repose, bulk density, tapped density, and compressibility index, to determine the flow properties of the prepared physical mixtures.

2.4.2 Drug content

The aceclofenac content was assayed separately for immediate release and sustained release physical mixtures. Physical mixture weighing 100mg of sustained as well as immediate release were transferred and dissolved in 20 ml of ethanol further diluted with pH 7.5 phosphate buffer and make up the volume up to the mark with the buffer in a 100 ml volumetric flask individually. The solution was suitably diluted and the aceclofenac content was measured against blank at 273 nm using UV visible spectrophotometer.

Table 1: Formula of the aceclofenac bilayer tablets

Formulation	ACF (mg)	SSG (mg)	CC (mg)	M K15M (mg)	L 971P (mg)	MS (mg)	Talc (mg)	MCC (mg)
F1	200	5	5	5	5	5	5	250
F2	200	5	5	10	10	5	5	240
F3	200	10	10	20	20	5	5	210
F4	200	15	15	30	30	5	5	180
F5	200	20	20	40	40	5	5	150
F6	200	25	25	50	50	5	5	110

2.5 Evaluation of Bilayer Tablets

2.5.1 Weight Variation

Weight variation was calculated as per the method described in USP. Twenty tablets were selected randomly and their average weight was determined using an electronic balance. The tablets were weighed individually

and compared with average weight¹³.

2.5.2 Friability

Friability was determined using Roche friabilator and calculated as per the method described in USP. Twenty tablets were weighed and placed in the tumbling chamber and rotated at 25 rpm for 5min. Tablets were weighed again and the percentage weight loss was determined using the formula given below⁸.

% Friability = Initial weight – Final weight *100 / Initial weight

2.5.3 Drug content¹¹

The tablet was taken in a 100ml volumetric flask, 20ml ethanol and 20ml of pH 7.5 phosphate buffer were added and the contents were sonicated for 10min and made up to the mark with pH 7.5 phosphate buffer. The solution was suitably diluted with pH 7.5 phosphate buffer and was assayed at 273nm for aceclofenac. The results were given in the table 1.

2.5.4 In-vitro dissolution studies

The dissolution rate testing of aceclofenac bilayered tablets (table) was studied using USP Type I rate testing apparatus (basket type) rotated at 50rpm. The dissolution was performed with 900mL of pH 1.2 buffer up to 2 hours and then replaced with 900 ml pH 7.5, 0.3M Phosphate buffer at 37.0 ± 0.5 °C. At specific time intervals, a 5 ml aliquot of the dissolved medium was withdrawn and was replaced with a fresh quantity of dissolution medium. The samples were suitably diluted with dissolution medium and assayed for aceclofenac content by measuring the absorbance at 269 and 273 nm respectively for pH1.2 and pH7.5 buffers using U.V Spectrophotometer (ELICO SL 159). The results are given in the table. The studies were carried in triplicate.

3.RESULTS AND DISCUSSION

3.1 Preparation of Aceclofenac Bilayer Tablets

The bilayer tablets which were prepared by double compaction technique were used to attain faster onset of action by the loading dose incorporated in the immediate release layer followed by supporting action of aceclofenac from the sustained layer for a prolonged effect. Moreover, methocel K15M and lubrizol 971P were used as sustaining agents; CCS and SSG were used as super disintegrants for the immediate release of aceclofenac.

3.2 Evaluation of Physical Mixtures

3.2.1 FTIR Studies

The physical mixtures were characterized by FTIR to determine the compatibility between the aceclofenac and the excipients used. The FTIR spectra of pure aceclofenac showed characteristic peaks at 3444.6 cm^{-1} , 2919.50 cm^{-1} , 1505.65 cm^{-1} , and 777.36 cm^{-1} due to N-H stretching, C-H stretching, -C=C , and aromatic-Cl respectively. The FTIR spectra of the bilayer tablet (F6) exhibited similar characteristic peaks as similar to the pure aceclofenac indicating that there was not any drug and excipient incompatibility in the designed formulation. The FTIR spectra were shown in Figure 1.

3.2.2 Precompressed Properties

Before compression, the physical mixtures were evaluated for flow properties for better compaction. From the results it is observed that the physical mixture of formulation F6 showed 30.73 ± 1.23 , 0.30 ± 0.28 , 0.32 ± 0.11 , and 0.45 ± 0.10 of angle of repose, bulk density, tapped density and compressibility index respectively indicating an excellent flow indices which may be due to the uniform distribution of all excipients in physical mixtures and a uniform particle size due to the uniform distribution of particles revealing the efficiency of geometric dilution method used for the preparation of physical mixtures. The data was shown in Table 1.

3.2.3 Drug Content

The physical mixtures used for the preparation of the immediate layer and sustained layer of bilayer tablets were assayed separately for aceclofenac content. From the results, it is observed that the drug content was within the limits of 95 – 100% for both immediate and sustained release layers. The data was shown in Table 1.

3.3 Evaluation of Bilayer Tablets

3.3.1 Weight Variation

Weight variation was carried out to ensure that, each tablet containing a specified amount of drug. From the results, it is observed that the maximum weight variation obtained was ± 0.52 , which falls within the acceptable weight variation range of $\pm 5\%$. Hence all the tablets passed the weight variation test. The results were shown in Table 2.

Table 1: Evaluation parameters of Physical Mixtures

Formulation	Angle of Repose	Bulk Density	Tapped Density	Compressibility Index	Drug Content
F1	32 $\pm$ 0.2	0.30 $\pm$ 0.3	0.31 $\pm$ 0.2	0.45 $\pm$ 0.6	98.13 $\pm$ 0.8
F2	28.2 $\pm$ 1.8	0.30 $\pm$ 0.1	0.33 $\pm$ 0.2	0.43 $\pm$ 0.3	100.1 $\pm$ 1.2
F3	32 $\pm$ 0.5	0.31 $\pm$ 0.1	0.33 $\pm$ 0.2	0.46 $\pm$ 0.4	99.5 $\pm$ 1.6
F4	31.4 $\pm$ 0.2	0.31 $\pm$ 0.2	0.33 $\pm$ 0.2	0.45 $\pm$ 0.4	97.5 $\pm$ 1.4
F5	31.8 $\pm$ 0.4	0.32 $\pm$ 0.1	0.33 $\pm$ 0.1	0.43 $\pm$ 0.2	101.5 $\pm$ 1.2
F6	32 $\pm$ 0.2	0.31 $\pm$ 0.1	0.32 $\pm$ 0.3	0.43 $\pm$ 0.2	101.13 $\pm$ 1.2

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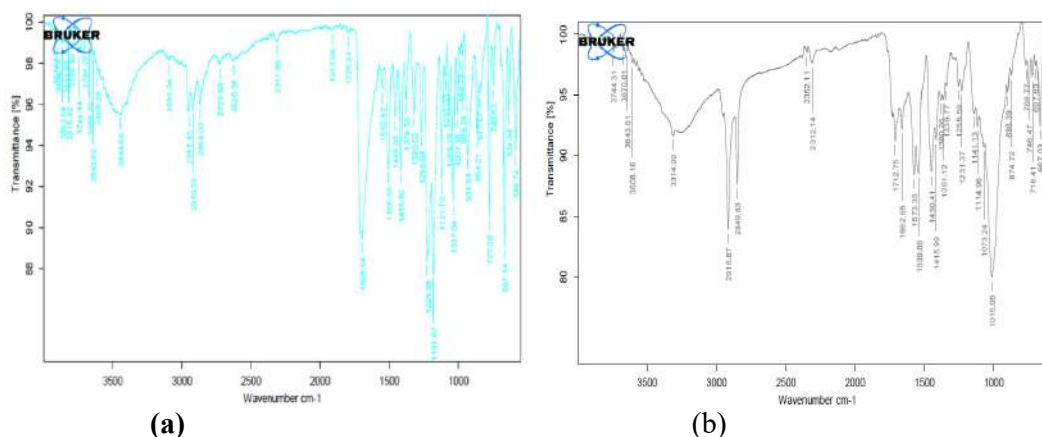


Figure 1: FTIR Spectra of (a) Aceclofenac and (b) Aceclofenac Bilayer Tablet F6

3.3.2 Friability

The physical strength of tablets upon exposure to mechanical shock and attrition was measured by the friability test apparatus. From the results, it is observed that the tablets had not shown a notable effect on the friability of aceclofenac tablets which may be due to the lower amount of magnesium stearate in the formulation. The results were shown in Table 2.

3.3.3 Drug Content

The amount of aceclofenac in the bilayer tablet was assayed to know the dose of the drug in the dosage form. The results indicated that all the formulations exhibited 99-100% of aceclofenac in the dosage forms indicating that the exact amount of immediate release layer and sustain layer was taken for the preparation of bilayer tablets. The results were shown in Table 2.

Table 2: Evaluation Parameters of Aceclofenac Bilayer Tablets

Formulation	Weight Variation % Error (n=3)	Friability	Drug Content (n=3)
F1	1.26 $\pm$ 0.01	0.8	99.13 $\pm$ 1.26
F2	0.59 $\pm$ 0.12	0.6	104.1 $\pm$ 1.10
F3	0.07 $\pm$ 0.10	0.1	101.5 $\pm$ 0.28
F4	0.31 $\pm$ 0.39	0.1	96.5 $\pm$ 0.96
F5	0.55 $\pm$ 1.25	0.3	100.5 $\pm$ 1.15
F6	0.49 $\pm$ 0.79	0.1	99.13 $\pm$ 0.94

3.3.4 In-vitro Dissolution Studies

In the present investigation, aceclofenac release from the bilayer tablets was evaluated using USP Type-II dissolution rate testing apparatus. 900mL of pH 1.2 buffers was selected as a dissolution medium for the first two hours followed by pH7.5 phosphate buffer. The effect of various polymers like L971P and MK15m was studied. The comparative drug release profiles of bilayer tablets were shown in Fig 2. The drug was released rapidly from F1 and F2 within 1hr and 4hrs respectively, but the formulations F3 and F4 showed slow release and lasted for 6hrs and 9hrs respectively. Whereas, the release of aceclofenac from formulations F5 and F6 was much slower and sustained up to 11hrs and 12hrs respectively. From the results, it is observed that the increase in the concentration of SSG and CCS enhanced the release of aceclofenac from the immediate release of bilayer tablets about 86.78% within 5 min indicating a burst release of initial dose for faster onset of action. The increase in the concentration of L971P and MK15M slowed the release of aceclofenac from the sustained layer and the complete release of aceclofenac was observed at 12hrs sustaining the action for a longer period. The sustained effect of aceclofenac in F6 is due to the swelling property of the MK15M and L971P and erosion, so the formulation sustains the drug release to extended time points. To better understand the release profiles obtained with the aceclofenac bilayer tablets, the drug release data obtained at different time points were fitted into kinetic models such as zero order, and first order, to understand the mechanism of drug release from the bilayer tablet the data was fitted to Korsmeyer Peppas model and was shown in Table 3. From the results it is noticed that the K values are less for the formulation F6 compared to other formulation K values of all the kinetic models making a note that the release rate was decreasing from the dosage form due to increase in concentration of MK15M and L971P, the correlation coefficient values are high for Higuchi model compared to other kinetic models ranging about 0.879-0.973 indicating that diffusion as the mechanism of drug release from the bilayer tablet.

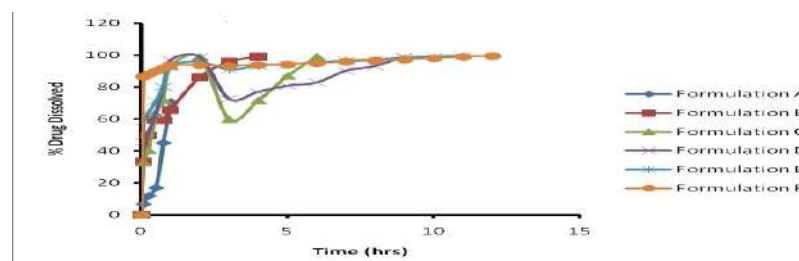


Fig 2: Comparative in-vitro dissolution profile of aceclofenac bilayer tablets

Table 3: Kinetic Data of Aceclofenac Bilayer Tablets

FORMULATION	ZERO ORDER		FIRST ORDER		HIGUCHI		KORSE MEYER	
	K_0	R^2	K_1	R^2	K_H	R^2	K_K	R^2
F1	13.49	0.869	0.484	0.860	64.147	0.879	0.240	0.246
F2	19.077	0.794	0.451	0.980	45.4	0.921	0.914	0.566
F3	7.51	0.648	0.194	0.843	28.52	0.973	0.678	0.306
F4	5.052	0.753	0.105	0.274	19.494	0.923	0.519	0.254
F5	4.10	0.877	0.150	0.712	17.691	0.972	0.854	0.628
F6	2.34	0.878	0.099	0.781	11.149	0.901	0.545	0.409

4. CONCLUSION

Aceclofenac bilayer tablets were successfully prepared by double compaction of immediate release layer and sustained layer physical mixtures which were prepared by geometric dilution method. The polymers MK15M and L971P showed a sustained effect for about 12 hrs and the super disintegrants in the immediate release layer produced an initial burst release and found to be giving effective and safe dissolution profile for 12 hours.

5. Acknowledgments

The authors are thankful to Acesus Pharma, Ongole for providing gift sample of aceclofenac, and the management of Nirmala College of Pharmacy for providing facilities to conduct the research work.

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ASSESSMENT OF ADVERSE DRUG REACTION IN INPATIENT WARD AT A TERTIARY CARE HOSPITAL- A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

The main aim of the study is to evaluate causality, severity, preventability, categories of medication concerned in ADR's, organ system affected, underlying cause and management of adverse drug reaction. An observational prospective study was conducted over a period of six months in a tertiary care hospital using spontaneous reporting system for identifying ADRs. CDSCO-ADR reporting form along with Naranjo algorithm for causality assessment was used as data collection tool for this study. In our study during 6months duration 76 patients were identified with ADRs. All the ADRs were classified as per age group which revealed predominance in adults 60.52% over geriatrics 26.30%. ADR's in females were predominant. Antibiotics were associated with maximum of all the ADR's reported 19 (25%). Gastroenterological ADR'S was found to be maximum 21 (30.26%). Classification and assessment of ADR's showed that most of the reactions 50 (65.7%) were of type A followed by type B 15(19.7%). The suspected ADR's were assessed for their causality using the Naranjo's algorithm probability scale. It was observed that the majority of reports were rated as probable 46 (60.52%) followed by possible 29 (38.15%) and definite 1 (1.31%). About 63 (82.89%) ADR's were Definitely Preventable, 11(14.47%) were Probably Preventable, while remaining 2 (2.63%) were Not Preventable. In 52 (68.42%) cases the drug was withdrawn followed by dose altered in 3 (4%) cases and no change in 21 (28%) cases. It was observed that out of 76 ADR's reports, 26 cases (34.2%) were mild, 49 cases (64.4%) were moderate and 1 case (1.3%) were severe. The results from this prospective, study shows that ADRs in patients may be a Public health issue, impose a big burden on patients by prolongation of hospital keep, increasing the admission rates, health care charge, morbidity, and mortality thus, reporting of ADRs desires continuous stimulation

KEYWORDS: *Adverse Drug Reactions, Preventability, Causality, Severity, Pharmacovigilance.*

INTRODUCTION

Adverse reaction World Health Organization, (1972)

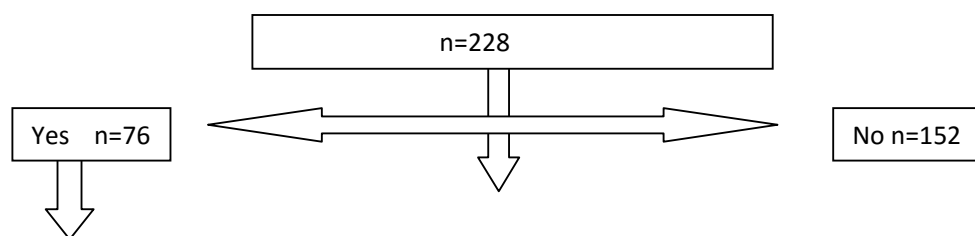
'A response to a drug that is toxic and unintentional, and that occurs at doses usually utilized in man for the prevention, diagnosis, or medical care of disease, or for the modifications of physiological function'.¹ Pharmacovigilance is developed much only from a decade but there were many disasters happened in previous years. Some of the examples like thalidomide disaster in 1960s made the health care professional and pharmaceutical companies alert.² The etiology of adverse drug reaction includes dose related (most common, predictable, drugs with narrow therapeutic index); allergic (not dose related and require prior exposure); idiosyncratic (unexpected, may have pharmacogenetic cause).³ The predisposing factors for developing ADR's are multifactorial. These include drug related factors, patient related factors; disease related factors and social factors.⁴ Adverse drug reactions were originally classified into two subtypes. Type A ADRs are dose-dependent and predictable; they are augmentations of known pharmacologic effects of the drug, such as orthostatic hypotension with antihypertensive medications. Type B ADRs are uncommon and unpredictable, depending on the known pharmacology of the drug; they are independent of dose and hypersensitivity (allergic) reactions to drugs are examples of type B ADRs.⁵ Mechanism of type A includes pharmaceutical causes (drug quality & drug release) and pharmacokinetic causes (ADME) whereas type B occurs mainly due to genetical abnormality.⁶ Adverse drug reaction (ADR) observation involves following steps: distinguishing adverse drug reaction, assessing relation between drug and suspected reaction by victimization numerous algorithms, documentation of ADR in patient's medical records, reporting serious ADRs to Pharmacovigilance centres /ADR regulating

authorities.^{7,8,9} ADR scales were assessed initially. Currently, there are over 170 AMCs in India, with addition of many more annually. The most functions of those AMCs are assortment of adverse events as per the quality procedure, following up to the completeness of the ADR reports and uploading of reports within the Vigiflow software system.¹⁰

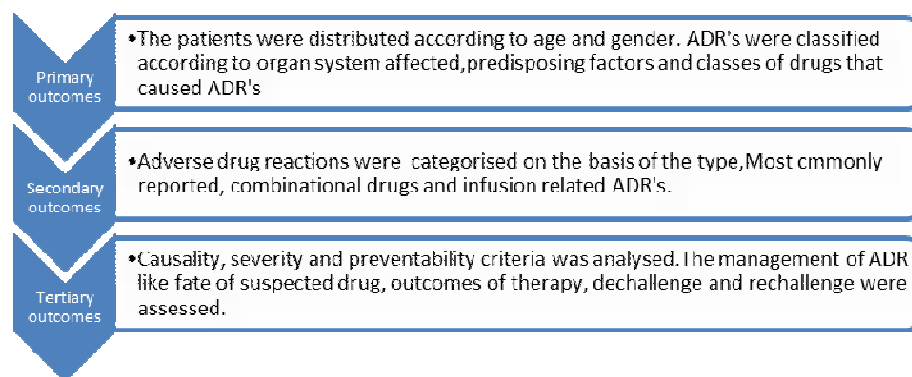
METHODOLOGY

The protocol for the proposed study was approved by the Institutional Review Board (IRB) of Nirmala College of Pharmacy, Atmakuru, Guntur district, Andhra Pradesh. A prospective observational study was conducted in the patient PD004/IRB/NRML/2018-2019 with regarding adverse drug reactions. Here the ADR's were found in all categories of drugs. In addition data on supportive care and co-medication were collected. The study was conducted in a Manipal tertiary care hospital, Andhra Pradesh for a period of six months.

STUDY DESIGN:



The following outcomes were evaluated for the collected cases



1. INCLUSION CRITERIA: All the patients from the inpatient department having any adverse reaction(s) after the commencement of treatment are included in the study.

2. EXCLUSION CRITERIA: Those patients admitted for accidental or intentional poisoning due to drugs are excluded from the study. Pregnancy and Lactation women are also excluded from the study.

RESULTS AND DISCUSSION

Table 1: Causality Assessment By Using Naranjo Algorithm

Score	No.of ADR's	Percentage %
Definite (>9)	1	1.31
Probable (5-8)	46	60.52
Possible (1-4)	29	38.15
Unlikely(<1)	0	0

Table 2: Severity Assessment Using Hartwig And Siegel Severity Scale:

Severity	Female (%)	Male (%)	Total (%)
Mild	12(31.57)	14(36.84)	26 (34.21)
Moderate	25(65.79)	24(63.15)	49(64.47)
Severe	1 (2.63)	-----	1(1.31)

Table 3: Management of ADR's:

Fate of suspected drug	Frequency	%
Drug withdrawn	52	68.42
Dose altered	3	3.94
No change	21	27.63

DISCUSSION

We found that the percentage of Adverse drug reactions were higher in adults than the Geriatrics population with 60.52% and 26.30% respectively. This study is regular with the results of the previous study.^{12, 13} A total of 76 ADR cases were studied during the study period, including 35 (46.05%) were males and 41 (53.94%) were females. This implies ADR's in females were predominant. This may be due to poor immune status and less tolerability against diseases in female patients. Predisposing factors on ADR's showed factors like nil 24 (31.57%), followed by polypharmacy 15 (19.73%), inter current disease 12 (15.78%). The results were appropriate with previous study.¹¹ In this study, the drug class most commonly implicated with ADR's were the class of Antibiotics (25%) following Anticancer drugs (18.42%) and class of drugs on CVS, NSAIDS, Corticosteroids with (11.84%) each. This study is alike with previous study.^{12, 14} Antibiotics were predominant and in contrast, the other study states that Antitubercular and herbal medicines were on high scale.¹³ The most common organ system associated with ADR's were Gastroenterology (30.26%) followed by other organ system (21.05%), Dermatology (17.1%) and then Neurology (9.21%). These findings were parallel with former study which reports ADR's related to Gastrointestinal to be the second highest frequency.^{14, 15} Majority of ADR's in the study population fall within the class of Type A (augmented and predictable) and it was around 50 (65.7%) ADR's and Type B (Bizarre and unpredictable) ADR's were around 15 (19.7%). The findings were related with many other studies most of the ADR's in the study were classified as Type A which are preventable and these reactions are more common in the study because they are dose related, predictable, high morbidity, low mortality and respond to dose reduction. The present study reveals most frequently reported drugs that induced same adverse drug reaction were Tramadol induced vomiting 6 (7.89%), following Dexamethasone induced Hyperglycaemia n=4 (5.26%) and then Doxycycline induced Glossitis and Rituximab induced SOB with n=3 (3.94%) each. To strength and further emphasize the validity of the findings of the study, causality assessment was done by Naranjo Algorithm, this assessment revealed, out of 76 ADR's identified 46 (60.52%) were probable, 29 (38.15%) were possible and 1 (1.31%) were Definite. Severity of ADR's was evaluated by Modified Hartwig severity assessment scale, which showed that most of the ADR's reported in this study were of moderate severity 49 (64.4%) which was reliable with prior study.¹³ In the current study, Dechallenge was done in around 47 (61.84%) patients and Rechallenge was performed in only 9 (11.84%). In 52 patients, the reactions were managed by withdrawing the offending drug and dose was altered in 3 patients. No change was made with the suspected drugs in 21 cases. In our study the symptomatic treatment was given to 21 patients.

CONCLUSION

In order to monitor new ADR's these types of studies are required to conduct regularly in tertiary care hospital. Therefore, reporting of ADRs desires continuous stimulation. It's necessary to attain the event of a positive perspective towards pharmacovigilance among health care professionals, together with pharmacist, in order that ADRs reporting becomes an accepted and understood routine.

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ASSESSMENT AND EVALUATION OF DRUG RELATED PROBLEMS IN PULMONOLOGY DEPARTMENT AT A TERTIARY CARE HOSPITAL

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ABSTRACT

The main aim of the study is to assess and evaluate the drug related problems in the pulmonology department. It was a Prospective Interventional study in PULMONOLOGY DEPARTMENT conducted at Manipal Super Speciality Hospitals, Vijayawada, Andhra Pradesh for a period of twelve (12) Months from February, 2018 to February, 2019. We have collected 115 cases from Pulmonology Department. During the study period, we have collected a total number of 115 cases under pulmonology department. We found that Males are more effected than Females with percentage of 68% (n=78) and 32% (n=37). In the present study 27 DRP's were found in which 10 Adverse Drug Reactions, 4 Untreated conditions, 4 Inappropriate drug according to guidelines, 4 inappropriate timing of administration, 2 Drug dose too high, 2 No Drug treatment inspite of existing indication, 1 prescribed drug not available. The result of the present Interventional study shows that Clinical pharmacy services helps in identifying and resolving drug related problems in consultation with health care professionals. In our study we found 27 DRP's based on PCNE V8.02 classification in which 22 DRP's were informed to the physician, and 5 DRP's were identified by the physician. The study shows that there is a need for Clinical Pharmacist services in healthcare to reduce DRP's by monitoring patient's drug therapy by which Pharm. D (Doctor of Pharmacy) can be suggested as Clinical Pharmacist since they are well versed in the subject areas like Clinical pharmacy, Clinical Pharmacology, Clinical Toxicology and Pharmacotherapeutics.

KEY WORDS: *Drug Related Problems, Pulmonology Department, Adverse Drug Reactions.*

INTRODUCTION

Clinical Pharmacy Practice is concerned with the promotion of effective, safe and economic drug therapy. Pharmacy practice is broad term which includes Clinical Pharmacy and other patient care related activities performed by pharmacists in the hospital and community settings. These include dispensing and drug distribution, drug information, health promotions, patient counselling, Pharmacovigilance, medication reviews, academic detailing and sterile and nonsterile manufacturing. Drug use is a complex process, and there are many drug related problems at various levels, involving prescribers, patients, pharmacists, the pharmaceutical industry and the government¹ According to WHO Chronic respiratory diseases are diseases of the airways and other structures of the lungs. Some of the most common diseases are asthma, bronchitis, chronic obstructive pulmonary disease, pneumonia occupational lung diseases and pulmonary hypertension. Chronic respiratory diseases are not curable. Not only tobacco smoke there are some other risk factors that cause chronic respiratory diseases which include air pollution, industrial chemicals and dusts, and frequent lower respiratory infections during childhood. There are several forms of treatment that can help to dilate major airway passages and improves SOB can help to reduce symptoms and increase quality of life for people with diseases. An Interventional study designs also called as experimental study designs². An Intervention is defined as it the process where a potential or existing drug related problem is identified and addressed by the Clinical pharmacist resulting in a change in patient's therapeutic management and prevention³. Clinical pharmacy interventions are a part of the clinical pharmacist activity. Even though, medications play a major role in the cure, palliation and inhibition of disease, they also expose patients to drug-related problems (DRP's)⁴. A drug related problem is defined as an event or circumstance involving multiple drug therapy that actually or potentially interferes with health outcomes⁵. Classification of DRP's

can serve as a cynosure for establishing a systematic process for pharmacists to put in appreciably to positive patient outcomes⁶. Many ways of classifications are available to code DRP's but all those classifications have not been tested for validity and reproducibility. DRP's can be classified as per different classification systems. These include the American Society of Hospital Pharmacists System, Cipolle et al., Granada consensus, Helper/Strand, Pharmaceutical Care Network Europe (PCNE) classification, problem-intervention documentation (PI-OC), and Westerlund classification. Among all classifications, the most commonly tested were PCNE and Charles and Linda⁷. The PCNE basic classification now has 3 primary domains for problems, 8 primary domains for causes and 5 primary domains for Interventions. In V7 a new section, called 'Acceptance of the Intervention Proposals' was added, including 3 domains. However, on a more detailed level there are 7 grouped sub domains for problems, 35 grouped sub domains for causes and 16 grouped sub domains for interventions, and 10 sub domains for intervention acceptance. Naranjo Adverse Drug Reaction Probability Scale⁸ "It is a method for estimating the probability of ADR". The Centres for Disease Control and Prevention (CDC) says tobacco use is the leading cause of preventable illness and death and produces 480,000 deaths a year (including deaths from second hand smoke). Avoiding sick people and places with a lot of dust or harsh chemicals. "Finally, be kind to yourself and be kind to others. Having that type of outlook will help you avoid illness⁹.

MATERIALS AND METHODS

Study Site, Study Design and Duration of the Study

The study was conducted in medical wards of MANIPAL SUPER SPECIALITY HOSPITAL, with 500 bed capacity. It was a Prospective Interventional study in PULMONOLOGY DEPARTMENT conducted for a period of twelve (12) Months from February, 2018 to February, 2019.

Inclusion Criteria: Patients of both genders are considered. Smokers, alcoholics are also included in this study. Patients with co morbidities are also included in this study.

Approval Number: All procedures performed in this study involving human participants were in accordance with the ethical standards of the Acharya Nagarjuna University Institute Review Board (PD001/IRB/NRML/2018-2019)

Exclusion Criteria: Those are not willing to participate our study. Pregnancy women's and terminally ill are excluded. Unconscious and coma patients were excluded from the study.

Study Procedure

Data collection

Regular pre ward rounds were carried out in all the wards during the study period and all the necessary information from the case sheets of patients was collected using a data collection form.

The data collection form includes the columns for: Patient details like Name, Age, Gender, Inpatient/ Hospital number, Date of admission, Medical condition, Social History, Vitals, Laboratory investigations, Final Diagnosis, Medications prescribed, Types of DRP identified, Intervention, Classification of intervention, Level of significance of DRP.

Data analysis

The collected data from the prescriptions was thoroughly analysed and screened for possible drug related problems using the Lexicomp (Uptodate software) and ADR's from Naranjo scale. And all the DRP's were intervened under the PCNE Guidelines.

RESULTS AND DISCUSSION

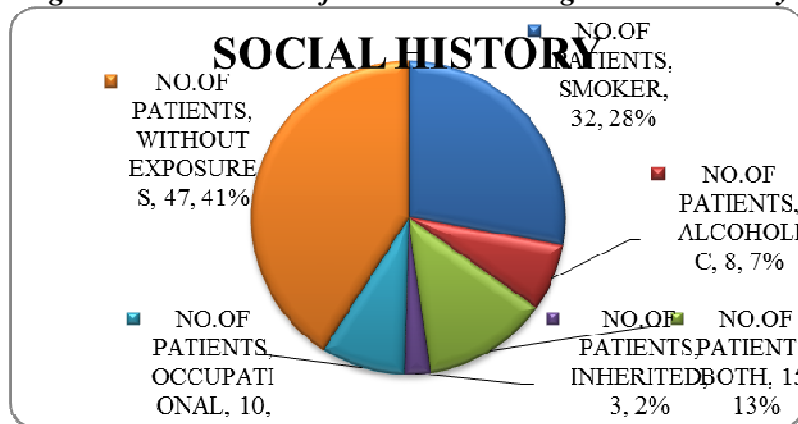
During the study period, we have collected a total number of 115 cases under pulmonology department. Out of which 27 DRP's were found.

Table 1: Distribution of Patients According To Demographic Details, And Past History

AGE	NO. OF PATIENTS	PERCENTAGE
<18	1	0.86%
18-28	10	8.69%
29-38	8	6.95%
39-48	21	18.26%
49-58	23	20%
59-68	25	21.73%
69-78	22	19.13%
79-88	4	3.47%
GENDER	NO.OF PATIENTS	PERCENTAGE (%)
MALE	78	67.82%
FEMALE	37	32.17%
TOTAL	115	100%
COMORBIDITIES	NO.OF PATIENTS	PERCENTAGE%
HYPERTENSION	13	11.30%
DIABETES MELLITUS	11	9.56%
BOTH DM AND HTN	13	11.30%
PULMONORY TUBERULOSIS	9	7.82%
ANEMIA	2	1.73%
HYPERTHYROIDISM	1	0.86%
HYPOTHYROIDISM	6	5.21%
ASTHMA	4	3.47%
HERNIA	3	2.60%
COPD	2	1.73%
RVD POSITIVE	3	2.60%
WITHOUT COMORBIDITIES	48	41.73%

Table -2: Different Types Of Diseases Identified In Respiratory System

TYPES OF DISEASES IDENTIFIED	TOTAL NUMBER (n=115)	PERCENTAGE %
COPD (Chronic Obstructive Pulmonary Disease)	33	28.69%
LRTI(Lower respiratory tract infection)	19	16.52%
PNEUMONIA	14	12.17%
ARDS	10	8.69%
PLUERAL EFFUSION	9	7.82%
PTB(Pulmonary tuberculosis)	9	7.82%
BRONCHIAL ASTHMA	9	7.82%
BRONCHITIS	4	3.73%
DNS	2	1.73%
URTI (Upper respiratory tract infection)	1	0.86%
TYPE 2 RESPIRATORY FAILURE	1	0.86%
PNEUMOTHORAX	1	0.86%
LUNG ABSCESS	1	0.86%
BILATERAL CONSOLIDATION	1	0.86%

Figure-1: Distribution of Patients According to Social History

During the study period, we have collected a total number of 115 cases under pulmonology department. Out of which 27 DRP's were found. We found that most of the people were under the age group of 59-68 years with 21.73% (n=25) and lowest percentage below 18 years with 0.86% (n=1) this observation is same with the adult age group of the study conducted by Koh et. al., and also we found that Males were more effected than Females with percentage of 68% (n=78) and 32% (n=37) this observation is contrasted with the demographic reports of the study conducted by Koh et al.^[10] with the highest co-morbidity disease Hypertension with a number of 26(22.60%), followed by diabetes with a number of 24(20.86%) and the lowest disease is hyperthyroidism with a number of 1(0.86%), this observation is same with the *Yuki Imaizumi et al* shows that has increasingly been recognized that the incidence of cardiovascular events is high in patients with chronic lung disease and the results were tabulated in TABLE 1^[11]. We also found that most of the people were effected with the disease COPD with highest number of 34(29.56%) followed by LRTI, Pneumonia, ARDS, Pleural Effusion, PTB, Asthma, Bronchitis etc and the results were tabulated in Table 2. Considering the etiological factors smoking is the highest possible cause with percentage of 42%, followed by alcoholics 20%, occupational 9%, and inherited 2% this observation is same with the *Yuki Imaizumi et al* which tells that Smoking is an established risk factor for cardiovascular diseases and the results presented in Figure 1.

Table -3: Naranjo Adverse Drug Reaction Probability

DRUG	ADR	SEVERITY	SCORE
Fluconazole	Increased bilirubin level	PROBABLE	07
Budesonide	Decreased potassium level	PROBABLE	05
Hydrocortisone	Decreased potassium level	PROBABLE	05
Amoxicillin / Clavulanic Acid	Hematuria	POSSIBLE	03
Budesonide	Decreased potassium level	PROBABLE	05
Tolvaptan	Abdominal distension	POSSIBLE	02
Doxycycline	Itching	POSSIBLE	04
Doxycycline	Itching	POSSIBLE	04
Clarithromycin	Vomitings	POSSIBLE	03
Aspirin/ Rosuvastatin/ Clopidogrel	Blood in sputum	PROBABLE	05

Table -4: Total No. of Drug Related Problems Identified

DRP	NO OF DRPS
Untreated condition	4
ADR's	10
Inappropriate drug according to guidelines	4
No drug treatment in spite of existing indication	2
Drug dose too high	2
Prescribed drug not available	1
Inappropriate timing of administration	4

In our study we found a total number of 27 Drug Related Problems in which 10 Adverse Drug Reactions, 4 Untreated conditions, 4 Inappropriate drug according to guidelines, 4 inappropriate timing of administration, 2 Drug dose too high, 2 No Drug treatment inspite of existing indication, 1 prescribed drug not available and all these results were tabulated in Table 4.

Table-5: Percentage of the Planned Interventions

Primary Domain	Code	Intervention	NO.OF DRP's	%
No intervention	I0.1	No Intervention	-	-
1. At prescriber level	I1.1	Prescriber informed only	05	18.51%
	I1.2	Prescriber asked for information	-	-
	I1.3	Intervention proposed to prescriber	17	62.96%
	I1.4	Intervention discussed with prescriber	05	18.51%
2. At patient level	-	-	-	-
3. At drug level	-	-	-	-
4. Other intervention or activity	-	-	-	-

Table-6: Percentage of the Acceptance of the Intervention Proposals

Primary Domain	Code	Implementation	No. of DRP's	Percentage
1. Intervention accepted (by prescriber or patient)	A1.1	Intervention accepted and fully implemented	14	51.85%
	A1.2	Intervention accepted, partially implemented		
	A1.3	Intervention accepted but not implemented		
	A1.4	Intervention accepted, implementation unknown		
2. Intervention not accepted (by prescriber or patient)	A2.1	Intervention not accepted: not feasible	03	11.11%
	A2.2	Intervention not accepted: no agreement		
	A2.3	Intervention not accepted: other reason (specify)		
	A2.4	Intervention not accepted: unknown reason		
3. Other (no information on acceptance)	A3.1	Intervention proposed, acceptance unknown		37.03%
	A3.2	Intervention not proposed	10	

CONCLUSION

The result of the present Interventional study shows that Clinical Pharmacy services helps in identifying and resolving drug related problems in consultation with health care professionals. In our study, we found 27 DRP's based on PCNE V8.02 classification in which 22 DRP's were informed to the physician, and 5 DRP's were identified by the physician. The study shows that there is a need for Clinical Pharmacist services in healthcare to reduce DRP's by monitoring patient's drug therapy by which Pharm. D (Doctor of Pharmacy) can be suggested as Clinical Pharmacist since they are well versed in the subject areas like Clinical Pharmacy, Clinical Pharmacology, Clinical Toxicology and Pharmacotherapeutics.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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ASSESEMENT OF DRUG USE PATTREN OF ANTIBIOTICS IN POST OPERATIVE PATIENTS USING WHO INDICATORS AT A TERTIARY CARE HOSPITAL

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ABSTRACT

The aim of this study is to assess the drug use pattern of antibiotics in post-operative patients using WHO prescribing indicators. It was a prospective and observational study was carried out in post-operative patients at Manipal Hospitals, Vijayawada. During the study period, we have collected a total number of 200 cases under surgical department. We found that most of the affected people were females with 56% (n=112). Most of the people were under the age group of 40-60 years with 36% (n=72). The most common disease affected under the department is orthopaedics (29.5%). Drugs prescribed during post-operative stage were Antibiotics (100%), Analgesics (100%). Cephalosporin antibiotics were found to be the most frequently prescribed (89%). In analgesics NSAIDS and Non-Opioid analgesics were found to be the most frequently prescribed drugs with (72%) and (68.5%). In antiemetics Serotonin antagonists 48 (24%) was most frequently prescribed. Under the antiulcer drugs PPI's (69%) followed by H2 blockers (17%), and Ulcer-protective agents (5%). No. of antibiotics per prescription were 3 antibiotics 77(38.5%). Total no of drugs per prescriptions were 4-6(8.5%), followed by 7-9 drugs (70%) etc. A total of 200 post-operative cases were collected. This report is intended to be a step in the broader evaluation safety and efficacy of drug prescription. This study shows the usage of prescription pattern of drugs in post-operative patients. This study shows that poly pharmacy was found to be very common which is mostly observed in case of antibiotics and lack of generic drug prescribing was observed. Drug prescribing from essential drug list (EDL) was low.

KEYWORDS: *Drug Utilization Review, Antibiotics, WHO indicators, Surgical department, Polypharmacy*

1. INTRODUCTION

Drug Utilization Reviews (DUR) in addition named as Drug Utilization Evaluations (DUE) or Medication Utilization Evaluations (MUE), are outlined as an authorized, structured, current review of health care supplier prescribing, pharmacist dispensing, and patient use of medication. DUE's involve a complete review of patients' prescription data before, during, and once dispensing to form positive acceptable medication deciding and positive patient outcomes¹. Irrational use of medicines is a major problem worldwide. WHO estimates that over half all medicines are prescribed, distributed or sold-out unsuitably, which half all patients fail to require them properly. The overuse, underuse or misuse of medicines ends up in wastage of scarce resources and widespread health hazards. For example irrational usage of drugs include: use of multiple drug therapy per prescription; over usage of antibiotics, usually in over dose or under dose quantity, for non-bacterial infections (i.e. viral, parasitic, fungi); over-use of injections once oral formulations would be additional appropriate; failure to speak in accordance with clinical guidelines; inappropriate self-medication, generally of prescription-only medicines; non-adherence to dosing regimes². The principal aim of drug utilization research is to facilitate rational use of drugs in the population. A growing number of pharmaceutical products are available in the world market and there has been an increase both in the consumption of the drugs and in expenditure on them³. The principles of surgical prophylaxis have been established over years. Selection of antibacterial agent for prophylaxis should be based on its activity against anticipated bacteria at the specific surgical site⁴. Properly timed accurate dose of preoperative antibacterial agent reduces the incidence of surgical site infection⁵. Developing countries have limited funds available for healthcare and drugs and it becomes very important to prescribe drug rationally so that the available funds can be utilized optimally⁶. Postoperative utilization of drugs is very much marked. Drugs are prescribed for

the purpose of analgesia, prevention of infection, nausea and vomiting, to maintain the hemodynamic status⁷. Postoperative utilization of drugs is very much marked. Drugs are prescribed for the purpose of analgesia, prevention of infection, nausea and vomiting, to maintain the haemodynamic status⁸. The purpose of antibacterial prophylaxis is to prevent postoperative infections, which are the primary cause of morbidity and mortality in patients undergoing surgery today. Aseptic techniques alone may decrease, but do not completely eliminate bacterial contamination of the surgical field. Therefore, the need for antibacterial agent to supplement aseptic technique becomes more widely accepted⁹. Assessment of drug use patterns with the World Health Organization drug use indicators is becoming more and more necessary to push rational drug use in developing countries^{10,11}. Before activities are started to promote rational drug use, an attempt ought to be created to explain and quantify the situation. Several well-established survey strategies are on the market for this purpose. One assessment methodology may be a prescribing and patient care survey using the World Health Organization hospital drug use indicators. These quantitative indicators are currently wide accepted as a worldwide standard for drawback identification and are utilized in over thirty developing countries¹².

2. METHODOLOGY:

2.1 Study Site& design: A hospital based non-interventional, prospective and observational study was carried out in post-operative patients conducted in Manipal super speciality Hospital, Vijayawada, Andhra Pradesh, India.

2.2 Sample size& study duration: A total of 200 patients from the in-patient wards of department of general surgery over a period of 6 months from June to November 2018

2.3 Source of data: The patient's demographical data, clinical data, therapeutics data and various other relevant and necessary data were obtained every day from the clinical assessment records, including medical records and other relevant information sources are documented, including laboratory investigations.

2.4 Inclusion criteria: 1. All the patients who have been admitted in General surgery wards in Manipal hospital 2. Any age 3. Patients of either sex 4. Patients who had been through a surgery 5. Patients undergoing re-operation

2.5 Exclusion criteria: 1. Any patient who dies post-operatively before being discharged 2. Patients who absconded / discharged against medical advice 3. Patient referred to higher center 4. All pregnant and lactating patients 5. Patients who are not willing to participate in the study.

2.6 Ethical approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the Acharya Nagarjuna university institute review board (PDO11/IRB/NRML/2018-2019)

2.7 Study procedure: A suitable data collection form was designed to collect all the necessary and relevant information. A personal visit was made to all the patients who were included in the study to collect any further information. During the treatment the enrolled patients were evaluated clinically every day to assess the clinical outcome.

2.8 Data analysis

Prescriptions were analysed for WHO- Prescribing Indicators as below:

1. Average number of drugs per prescription (encounter).
2. Percentage of drugs prescribed by generic brand.
3. Percentage of encounters with an antibiotic prescribed.
4. Percentage of encounters with an injection prescribed
5. Percentage of drugs prescribed from essential drug list / formulary

3. RESULTS

Table 1 : Gender Distribution

GENDER	NUMBER OF PATIENT	PERCENTAGE %
MALE	88	44
FEMALE	112	56
TOTAL	200	100

Out of 200 patients studied, the largest proportion of patients were female 112(56%)

Table 2: Age Distribution

Age in years	Number of patients	Percentage %
<20	25	12.5
20-40	64	32
40-60	72	36
60-80	39	19.5
TOTAL	200	100

Figure 2: shows the age wise distribution of patients admitted in surgery ward. In our study, a total of 72 (36%) patients were in the age group of 40-60 years

Table 3: Disease Distribution

DEPARTMENT	NUMBER OF	PERCENTAGE
ORTHOPEDICS	59	29.5
UROLOGY	22	11
ONCOLOGY	18	9
GASTROENTEROLOGY	42	21
NEUROLOGY	14	7
GYNECOLOGY	26	13
ENDOCRINOLOGY	19	9.5
TOTAL	200	100

Figure 3: shows the distribution of various disease patterns of the study population. The most common disease affected department is orthopaedics(29.5%)

Table 4: Pattern Of Usage Drugs In Post-Operative Patients

CLASS OF DRUGS	NUMBER OF	PERCENTAGE
ANTIBIOTICS	200	100
ANALGESICS	200	100
ANTACIDS	188	94
ANTI-EMETICS	54	27
VITAMIN	111	55.5
LAXATIVES	67	33.5
RESPIRATORY	28	14
ELECTROLYTES	22	11
IV FLUIDS	81	40.5

Figure 4: shows the drugs prescribed in the study population during pre-operative stage in the descending order, Antibiotics (100%), Analgesics (100%), Anti-ulcer (94%), Vitamins (55.5%), Anti emetics(27%) and least common prescribed was electrolytes(11%).

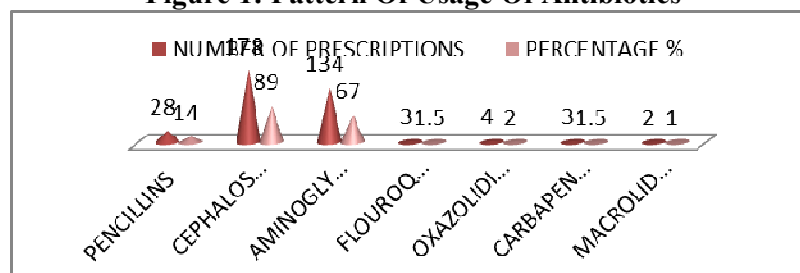
Figure 1: Pattern Of Usage Of Antibiotics

Figure 5: shows that cephalosporin class of antibiotics was found to be the most frequently prescribed (89%) followed by aminoglycosides (67%),

Table 5: Pattern Of Usage Of Analgesics

CLASS OF ANALGESICS	NUMBER OF PRESCRIPTIONS	PERCENTAGE
NSAIDS	94	47
NON OPIOIDS	125	68.5
NSAIDS+NON OPIOIDS	144	72

Figure 6: NSAIDS + Non-Opioid analgesics were found to be the most frequently prescribed (72%), followed by Non-Opioid Analgesics (68.5%)

Figure 2: Pattern Of Usage Of Anti-Emetics

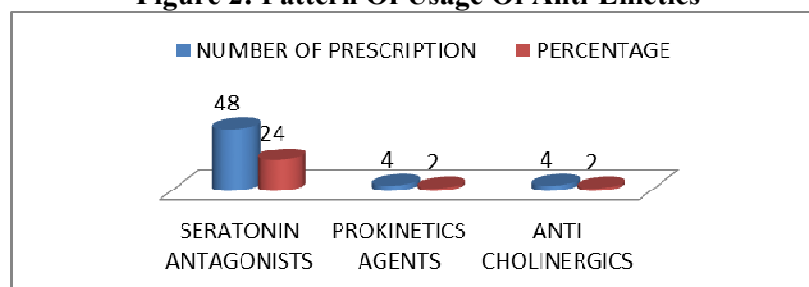


Figure 7: shows the no of different anti-emetic drugs prescribed in the study population. Serotonin antagonists 48 (24%) was most frequently prescribed among which Ondansetron was commonly prescribed.

Table 6 :Pattern Of Usage Of Anti Ulcers

CLASS	NUMBER OF PRESCRIPTIONS	PERCENTAGE
PROTON PUMP INHIBITORS	138	69
H2 RECEPTOR ANTAGONISTS	34	17
ULCER PROTECTIVE	10	5

Figure 3: Pattern Of Usage Of Number Of Antibiotics

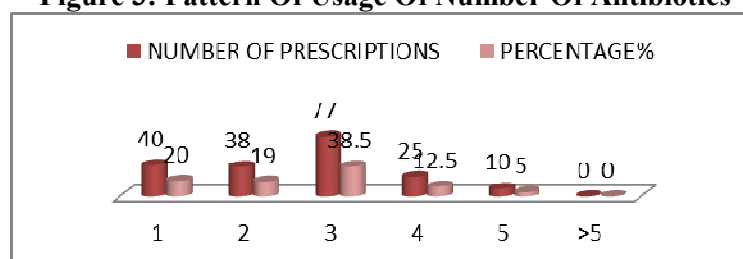


Table 7: Total Number Of Drugs Encounter Per Prescription

NUMBER OF DRUGS	NUMBER OF PRESCRIPTIONS	PERCENTAGE %
4-6drugs	17	8.5
7-9drugs	140	70
10-12drugs	22	11
13-15	17	8.5
15	4	2
TOTAL	200	100

Table 8: Who Indicators

Indicators	Data
Prescribing indicators	
Average number of drugs	1968
Percentage of drugs prescribed by the generic	20.9%
Percentage of encounters with an antibiotic(n=200)	100%
Percentage of encounters with an injection(n=200)	100%
Percentage of drugs prescribed from the essential drug list(n=1.294)	65.75%

DISCUSSION

In general practice, the therapeutic approach for surgical disease conditions is primarily empirical and the main aim of the physicians is to treat as specifically as possible. The present study was focused on evaluating the drug use pattern in post-operative patients of the surgery department. During the study period; we have collected a total number of 200 cases under surgical department. We found that most of the affected people were females with 56% (n= 112) and males with 44% (n=88) which was in accordance with the study done by Ram Nagina Sinha et al¹³. in which 58.5% of patients were female which is almost similar to our data. Most of the people were under the age group of 40-60 years with 36% (n=72) which is comparable to 57.08% in the study done by Bhansali NB et al¹⁴., and also a study done by Bhabhor. P et al¹⁵., shows 49% of patients are in between the age group of 40-60yrs. The most common disease affected under this department was orthopedics (29.5%), followed by gastroenterology (21%), gynecology (13%), urology (11%), endocrinology (9.5%), oncology (9%), neurology (7%). Drugs prescribed in the study population during post-operative stage in the descending order, Antibiotics (100%), Analgesics (100%), Anti-ulcer (94%), Vitamins (55.5%), Anti emetics (27%) and least common prescribed was electrolytes (11%). Cephalosporin class of antibiotics was found to be the most frequently prescribed (89%) followed by amino glycosides (67%). In analgesics NSAIDs and Non-Opioid analgesics were found to be the most frequently prescribed drugs with (72%) and (68.5%). While coming to antiemetics Serotonin antagonists 48 (24%) was most frequently prescribed. Under the anti ulcer classification Proton pump inhibitors (69%) followed by H2 blockers (17%), and Ulcer-protective agents (5%) were the additional drugs prescribed to prevent the adverse effects of analgesics. No of antibiotics per prescription in the study population in descending order, 3 antibiotics 77 (38.5%), 1 antibiotics 40 (20%), 2 antibiotic 38 (19%) and 4 antibiotics 25 (12.5%). Total no of drugs per prescription 4-6 (8.5%) of the study population, followed by 7-9 drugs (70%), 10-12 (11%), 13-15 (8.5%), >15 (2%). In every one of the 200 are prescribed with one or more injections i.e, 100% which was in accordance with the study done by Siddhartha Mondal et al¹⁶. Where percentage of the injections prescribed was 100 %. Among all the drugs prescribed 65.75% were on the WHO model list of essential medicines (EML). This is higher in comparison with the findings of Siddhartha Mondal et al. where it was 54.89 %. In our study total number of drugs prescribed by generic name was 412 (20.90%) which is similar in consistent with the findings of Raj Kumar et al¹⁷ where it was 192 (5%) but there is a wide variation in the study of Siddhartha Mondal et al. where it was 68.51%.

CONCLUSION

In this study a total of 200 case records of post-operative were collected. In this study population male to female proportion was almost equal drug use pattern reflects the common disorders for which surgeries are performed in our hospital. This report is intended to be a step in the broader evaluation safety and efficacy of drug prescription. This study shows the usage of prescription pattern of drugs in post-operative patients. This study shows that polypharmacy was found to be very common which is mostly observed in the case of antibiotics. Lack of generic drug prescribing was observed. Drug prescribing from essential drug list (EDL) was low.

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EVALUATION OF ANTI-HYPERLIPIDEMIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF SETARIA ITALICA SEEDS AND HUSK ON RATS

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ABSTRACT

Setaria italica is commonly known as Foxtail millet (Korralu in Telugu). It belongs to the millet family consisting of high fiber content along with many mineral, vitamins, essential aminoacids, proteins, gluten free and very less quantity of carbohydrates. Due to enormous advantages and was less explored this millet was chosen for the study of antihyperlipidemic activity. To investigate the anti-hyperlipidemic activity of hydroalcoholic extract of seed and husk of *Setaria italica* in atherogenic diet induced hyperlipidemic rats. The hydroalcoholic extract of *Setaria italica* seeds and husk were evaluated for antihyperlipidemic activity in atherogenic diet induced hyperlipidemic rats. The study was performed by comparing the results with that of a standard drug atorvastatin (10mg/kg body wt). The study results were expressed as mean $\pm$ S.E and the data was analysed by using one way analysis (ANOVA). Values with $P < 0.05$ were considered as significant. Oral administration of 500mg/kg body wt of hydroalcoholic extract of *Setaria italica* showed significant reduction in the serum levels of low density lipoproteins (LDL), triglycerides (TG), total cholesterol and an increase in high density lipoproteins (HDL) than the standard control. The Hydroalcoholic extract of seed possess significant activity than hydroalcoholic extract of husk of *Setaria italica*. The hydroalcoholic seed extract of *Setaria italica* produced promising results the millets have to be consumed as a diet as it immensely controls the serum cholesterol levels which plays a vital role in control of cardiovascular diseases.

KEY WORDS: *Setaria italica*, antihyperlipidemic activity, atherogenic diet, Foxtail millet, hydroalcoholic extract.

INTRODUCTION

In this modern era highly sophisticated instruments made the mankind too lazy to work. Lack of physical exercise, sedentary work style, high fat diet, fast foods, irregular time periods of consuming food, diseased states, are being faced by the present generation which is an alarming sign in increasing hyperlipidemia in the present scenario. Hyperlipidemia is a lipid metabolic disorder, which includes abnormal levels of lipids in blood such as elevated levels of total cholesterol (TC), Low density lipoprotein (LDL), Triglycerides (TG) and low levels of High density lipoprotein (HDL).¹ Hyperlipidemia was a modifiable risk factor for the development of atherosclerosis.² This may leads further complications with high morbidity and mortality rate, mainly coronary artery disease and stroke in India most prevalent dyslipidemia was due to borderline and higher levels of LDL-C, low levels of HDL-C (men – 54.9% and women – 64.4%).⁵ Medicinal plants play a major role in hypolipidemic activity and widely used by the majority of population to cure various diseases like hypertension and atherosclerosis and illness and have a high impact on the world's economy. Millets are rich in variable nutrients like fibre, minerals, aminoacids, vitamins, proteins and low content of carbohydrates.^{6,7} foxtail millet, scientific name *Setaria italica* (synonym *Panicum italicum* L.), is an annual grass grown for human food. These are found to be rich sources of phytochemicals such as phenolic acids, flavonoids, catechins, phytic acid, and phytosterols.⁸ Researchers have reported that the presence of dietary fiber and phenolic compounds help in the prevention of many diseases such as diabetes, cardiovascular

diseases, and cataractogenesis.⁹ Due to these tremendous benefits foxtail millet has been chosen for the evaluating the antihyperlipidemic activity.

MATERIALS AND METHODS

Collection of Plant material

The whole grain seeds of *Setaria italica* were purchased from the local market of Mangalagiri, Guntur dist, Andhra Pradesh. They were authenticated by Dr.P.Satyanarayana Raju, Plant Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Preparation of Hydro alcoholic extract

The seeds were shade dried for 3-4 days and grounded to fine powder. The powder was passed through the sieves and husk was separated. The seed powder and husk were taken separately and were macerated in hydro alcoholic solution in the ratio of 3:1 for 3 days and filtered. The procedure was repeated twice and the filterates were combined. The combined filterates were subjected to distillation. The extracts obtained are refrigerated until further studies were carried out.

Materials

Setaria italica whole grains (Local market of Mangalagiri), Albino rats (Mahaveer Enterprises) Formaldehyde (from Sigma Aldrich Chemicals Pvt Ltd, Bangalore, India), Ethanol and chloroform (from Sigma Aldrich Chemicals Pvt Ltd, Bangalore, India), Distilled water.

Atherogenic diet

Experimental hyperlipidemic diet: Experimental diet consists of well pulverized mixture of cholesterol (2%), cholic acid (1%), peanut oil (10%), sucrose (40%) and normal laboratory diet (47%).

Experimental animals

Adult male Albino rats (150-200gm) of swiss strain were obtained from (Mahaveer Enterprises, Hyderabad), for study of antihyperlipidemic activity. The animals were acclimatized to standard laboratory conditions (temp $25 \pm 5^\circ\text{C}$), humidity ($55 \pm 5\%$) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum accordingly as per the instructions given by Institutional Animal Ethical Committee. (CPCSEA)¹¹. (Approval No: 1629/PO/a/12/CPCSEA)

Selection of dose

As per LD₅₀ of both extracts, one tenth of the dose was taken as therapeutic dose to evaluate antihyperlipidemic activity.

Preparation of standard drug

Atorvastatin (10mg/kg) was used as reference standard drug for evaluating the antihyperlipidemic activity which was suspended in distilled water by using 1% tween-80 as suspending agent.

Acute toxicity studies

As per literature review acute toxicity studies for *Setaria italica* seed and husk was found to be safe upto 5000 mg/kg as per OECD guidelines-423.¹⁰

Induction of experimental Hyperlipidemia

High fat hyperlipidemia was induced in experimental animals by giving the high fat atherogenic diet upto 15 days.

Experimental design

Animals were divided into six groups, each containing 6 animals. Group I served as normal control. Group II was fed with high fat diet till 15th day. Group III received standard drug atorvastatin 10 mg/kg. Group IV & V treated with hydro alcoholic extract of the *setaria italica* seed extract at a dose of 100mg/kg and 150 mg/kg body weight. Groups VI received hydro alcoholic extract of the husk of *setaria italica* at a dose of 25 mg/kg body weight.

BIOCHEMICAL ANALYSIS

Serum collection and analysis

On the 15th day blood samples were collected by direct cardiac puncture by using chloroform as anesthesia and were sent to laboratory for analysis to estimate the amount of cholesterol, triglycerides, high density lipoproteins, low density lipoproteins present in the collected samples.

STATISTICAL ANALYSIS

The results of the study were expressed as mean $\pm$ S.E. Data was analyzed by using one way analysis of variance test (ANOVA) followed by Dunnett's t-test for multiple comparisons. Values with $P < 0.05$ were considered as significant.¹¹

RESULTS

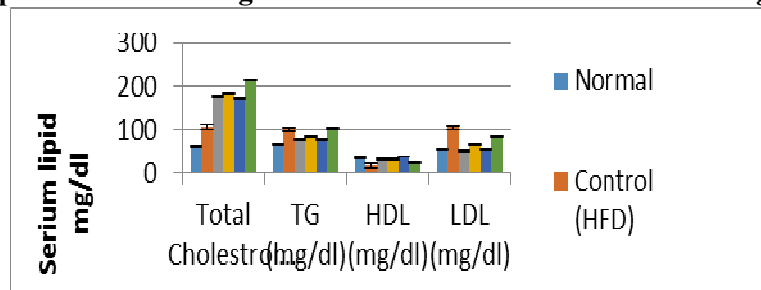
Table 1: Phytochemical constituents of *Setaria italica* Seed and Husk.

Constituent	Hydroalcoholic seed Extract	Hydroalcoholic husk Extract
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	—
Steroids	+	+
Flavonoids	+	—
Saponins	—	—
Tannins	—	—
Terpenes	+	+
Phenols	+	+

Table-2: Effect of *Setaria italica* Seed and Husk extract on Serum lipids mg/dl

S.No.	Treatment	LDL mg/dl	HDL mg/dl	TG mg/dl	Total Cholesterol mg/dl
1	Normal	55.5 $\pm$ 1.23	38.13 $\pm$ 1.83	65.33 $\pm$ 2.14	60.83 $\pm$ 1.82
2	Control (HFD)	105.66 $\pm$ 2.31	18.16 $\pm$ 1.68	101 $\pm$ 3.00	106.5 $\pm$ 4.08
3	Standard Atorvastatin 10mg/kg	49.64 $\pm$ 2.27	32.14 $\pm$ 3.50	76.13 $\pm$ 1.83	174.91 $\pm$ 1.84
4	SIHaSE 250mg	67.45 $\pm$ 2.70	32.44 $\pm$ 2.56	82.45 $\pm$ 2.65	182.45 $\pm$ 1.82
5	SIHaSE 500mg	54.15 $\pm$ 1.87**	38.45 $\pm$ 1.76**	78.48 $\pm$ 1.83**	171.08 $\pm$ 1.69**
6	SIHaHE 25mg	84.26 $\pm$ 1.78	24.56 $\pm$ 1.54	105.30 $\pm$ 1.27	214.12 $\pm$ 1.32

Graph-1: Values were given as mean $\pm$ S.D. for six rats in each group.



Values are statistically significant at ** $p < 0.001$ as compared with standard group

HISTOPATHOLOGICAL STUDY

To confirm the incidence of hyperlipidemias, the animals were sacrificed and their liver was isolated subjected to histopathological studies. Isolated liver were cleaned off extraneous tissue, weighed and rinsed with ice-cold normal saline and sent for histopathological studies.



Fig-1 standard Atorvastatin – 10mg



Fig-2 Setaria italica husk extract--25mg

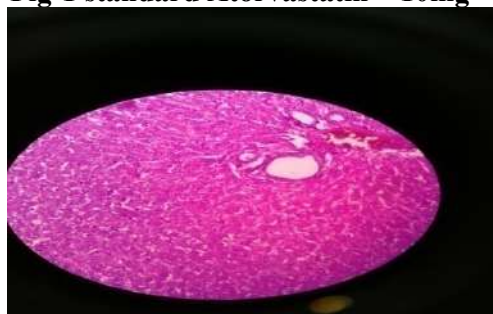


Fig -3 Setaria italica seed extract – 250mg

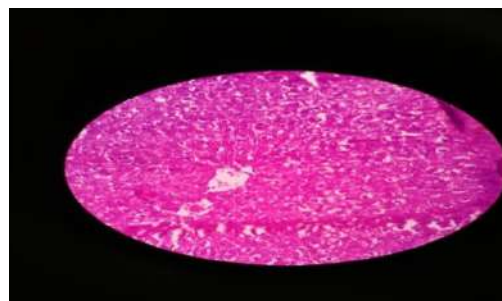


Fig – 4 Setaria italica seed extract – 500mg

From the histopathological studies it is evident that:

Group-III Atrovastatin 10mg shows the multi focal damage in the liver tissue and with extensive damage than treatment groups .

Group-IV Setaria italica seed extract treated with 250mg shows that there is a focal damage in the liver .The damage is limited when compared to standard treated group.

Group-V Setaria italica seed extract treated with 500mg shows very limited and negligible damage in multifocal areas which can be due to hepato-protective activity of test drug administration.

Group-VI Setaria italica husk extract treated with 25mg shows vascular damage which may leads to liver cirrhosis.

DISCUSSION

The antihyperlipidemic activity is evaluated by decrease of LDL levels and increase of HDL levels. The present study reveals that there is a significant increase in HDL levels, which is considered as good cholesterol for the body as HDL removes extrahepatic cholesterol and transports it to the liver for endocytic mediated LDL receptor activity. The hydroalcoholic seed extract of Setaria italic 500 mg has shown a significant effect when compared to that of the standard atorvastatin 10mg. The hydroalcoholic seed extract of 500mg had shown very little and limited damage in multifocal areas which signifies the hepatoprotective activity of the seed extract. The triglyceride levels and total cholesterol levels of seed extract 500mg are nearly equal to that of the standard atorvastatin indicating that the plant extract shows hepatoprotective activity towards hyperlipidemias. The hydroalcoholic husk extract of setaria italic 25mg was found deviating from treating hyperlipidemias. The husk extract has produced a tremendous increase in LDL levels and very low HDL levels indicating that husk doesn't possess flavanoides and phenolic compounds. Researchers have reported that the presence of dietary fiber and phenolic compounds help in the prevention of many diseases such as diabetes, cardiovascular diseases, and cataractogenesis.¹² The triglycerides and total cholesterol levels are very high when compared to that of the standard indicating that husk lack proteins, minerals, fibres, Vit E, etc than that of seed.

CONCLUSION

This study proves that *Setaria italica* seed extract is very useful in treating hyperlipidemias than that of husk. This evidence paves the way to carry out the further study in isolating the chemical constituents and to build up new entities in treating hyperlipidemias.

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THE FUTURE TRENDS OF CRISPR IN GENOME EDITING TECHNOLOGY:A SYSTEMIC REVIEW

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ABSTRACT

In 1987, the first CRISPR was detected 30 years ago in E.coli in the course of analysis of gene responsible for isoenzyme conversion of alkaline phosphatase. The clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) system, a bacterial defence mechanism against phage infection and plasmid transfer in nature, has been repurposed as a dominant RNA-guided DNA targeting platform for genome editing, transcriptional perturbation, epigenetic modulation, and genome imaging. CRISPR–Cas9-mediated DNA interference in bacterial adaptive immunity. Researchers have successfully employed the CRISPR/ Cas9 system to edit targeted genes in many crop plants. Development of a chemically synthesised, 29-nucleotide synthetic CRISPR RNA (scrRNA) suitable for transient, therapeutic delivery. Applications of CRISPR in plants are Research into the function of genes, Modifications of genes for yield traits, Modifications of genes for improved produce quality, Genome editing for abiotic stress tolerance, Genome editing for biotic stress resistance, Regulation of gene transcription or translation, Construction of mutant libraries, Generation of transgene-free, genome-edited crops. CRISPR/Cas9 is a robust tool for effectively editing the genome of a cell. Single-guide RNA (sgRNA)-directed Cas9 activity causes DNA cleavage at specific sites, allowing DNA editing at these sites by subsequent DNA repair in mammalian cells. CRISPR/Cas9 can also be used to repair mutations in proto-oncogenes or to knock out specific genes, which may create opportunities for potential treatments in cancer.

KEYWORDS: *CRISPR, CAS system, genome editing.*

1.INTRODUCTION

1.1CRISPR in microbes

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) –CAS system is well known acquired immunity systems that are widespread in archaea and bacteria. The RNA guided Nucleases from CRISPR-Cas systems currently regard as the most reliable tools for genome editing and engineering ¹. It was undergone first in 1987, an unusual repetitive DNA Sequence which subsequently defined as CRISPR, was discovered in Escherichia coli genome during an analysis of genes involved in Phosphate metabolism. In other bacteria like halophilic archaea, suggesting an essential role for such evolutionarily conserved clusters of repeated sequences. The first CRISPR was discovered 30 years before, in E-COLI in the course of analysis of gene responsible for isoenzyme conversion of alkaline phosphatase ². In 1993 CRISPR were observed in archaea, specifically in Haloferax editorial and subsequently detecting several bacterial and archaeal genomes³. In the early 2000s, the development of sequence comparison between the spacer sections of CRISPR and sequence of bacteriophages archaeal viruses and plasmids finally shed light on the function of CRISPR as an immune system⁴. In similarity, many genes previously introduced to encode DNA repair proteins for hyperthermophilic archaea ⁵ identified as being strictly associated with CRISPR and designated CAS (CRISPR associated) genes ⁶. Comparative genomic analysis suggests that CRISPR and CAS protein work together and constitutes an acquired immunity system to protect the prokaryotic cells against invading viruses and plasmids analogous to the eukaryotic RNA interference (RNAi system)⁷.

1.2CRISPR in Humans

Genome editing with clustered, usually interspaced, short palindromic repeats (CRISPR)-Cas9 nuclease system is a powerful technology for manipulating genomes, including the introduction of gene disruptions or

corrections. Here we tend to develop with chemicals changed, 29 nucleotides synthetic CRISPR RNA (ScrRNA), that together with unadapted transactivating crRNA (TracrRNA) is shown functionally replace the guide polymer within the CRISPR - CAS 9. These findings provide a platform for therapeutic applications, especially for nervous system disease, using successive application of cell membrane, synthetic CRISPR RNAs to activate and then silence Cas9 nuclease activity⁸

2.MECHANISM OF CRISPR IN MICROBES

The clustered frequently interspaced insufficient palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9) system, a bacterial defence mechanism against phage infection and plasmid transfer in nature, has repurposed as a powerful RNA-guided DNA targeting platform for genome editing, transcriptional perturbation, epigenetic modulation, and genome imaging CRISPR–Cas9-mediated DNA interference in bacterial adaptive immunity. (1)(a) A standard CRISPR locus in a type II CRISPR–Cas system contains an array of repeated sequences interspaced by compact ranges of non-repetitive sequences, as well as a set of CRISPR associated (*Cas*) genes. Leading the *cas* operon is the *trans*-activating CRISPR RNA (*tracrRNA*) gene, which encodes a novel noncoding RNA with homology to the repeat arrays. Simultaneous bacteriophage infection, a brand new spacer derived from the invasive genetic elements is included into the CRISPR array by the addition method (*cas1*, *cas2*, and *csn2*). Once combined, the unique spacer is cotranscribed with all other spacers into a long messenger CRISPR RNA (pre-crRNA) including repeats and spacers. The *tracrRNA* is copied individually and then toughens to the pre-crRNA repeats for crRNA maturation by RNase III fracture. Moreover, cutting off the 5' end of the crRNA by foreign nucleases diminishes the length of the guide sequence to 20 nt. When interference, the mature crRNA–*tracrRNA* structure involves Cas9 nuclease and more commands it to separate foreign DNA including a 20-nt crRNA corresponding sequence preceding the PAM array copied separately and then strengthens to the pre-crRNA repeats for crRNA maturation by RNase III division. Further shortening of the 5' end of the crRNA by foreign nucleases decreases the length of the guide sequence to 20 nt. (b) Schematic illustration of the domain organisation of the characteristic Cas9 orthologs from different subtypes. As the hazards denote preserved, essential residues for Cas9-mediated DNA separating activity. Abbreviations: arginine-rich bridge helix (Arg); crRNA, CRISPR RNA; CTD, C-terminal domain; nt, nucleotide; NUC, enzyme lobe; PAM, protospacer adjacent motif; REC, recognition lobe; *tracrRNA*, *trans*-activating CRISPR polymer.⁹ Here technology allows one to accurately manipulate practically any genomic sequence designated by a little stretch of guide RNA¹⁰ according illustration of gene function implicated in disease development and improvements, changing of disease-causing mutations, and inactivation of stimulated oncogenes or activation of deactivated growth suppressor genes when using a smelting protein of nuclease-deficient Cas9 and effector region^{11,12,13,14} Moreover, this programmable endonuclease technology facilitates researchers to explore the function of multiple genes at once by concurrently targeting complicated genomic loci in a unique experiment¹⁵ which considerably stimulates our perception of pathological processes that involve extensive sets of genes or mutations, such as tumour development. Single-guide RNA (sgRNA) libraries, CRISPR-based genome-wide screens can be leveraged to identify drug-target or disease-resistance genes, such as novel tumour suppressors or oncogenes, and to assess drug targets^{16,17} quickly. As such, CRISPR–Cas9-mediated genome construction exists extensive consent to manage or even cure genetic disorders, including various forms of cancer and neurodegeneration, as well as sickle cell anaemia, cystic fibrosis, Duchenne muscular dystrophy, viral infections, immunological disorders, and cardiovascular diseases^{18,19,20,21,22} Despite its benefits and promise, there were some obstacles between CRISPR–Cas9 and its full therapeutic potential^{23,24} Decreasing or evading any undesired off-target mutations at positions with series similarity to on-target sites is predominant for the effective use of CRISPR mediated genome engineering in clinical applications^{25,26} Consequently, how Cas9 establishes specific 20-base-pair (bp) end series within the genomes that are millions to billions of base long and later provokes sequence-specific double-stranded DNA polymer (dsDNA) cleavage may be an essential question not merely in CRISPR biology however within the efforts to develop additional precise and economic Cas9-based tools. Extensive biochemical and structural studies on Cas 9 at different stages of DNA target surveillance as well as several proto spacer adjacent motif (PAM) variants and Cas9 orthologs have contributed significantly to our understanding of CRISPR–Cas9 mechanisms. In this review, we briefly explain the underlying biology CRISPR–Cas9 technology and then focus on the recent structural and mechanistic insights into the molecular mechanisms of RNA-guided DNA targeting and cleavage by the Cas9 enzyme from *Streptococcus pyogenes*, the most well-characterised and widely used Cas9 in genome engineering.

3.MECHANISM IN HUMANS

Development of a chemically synthesised, 29-nucleotide synthetic CRISPR RNA (scrRNA) suitable for transient, therapeutic delivery. Using rational chemical design, we identify scrRNA phosphorothioate (PS) backbone modifications and 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me) and S-constrained ethyl (cEt) substitutions that increase metabolic stability^{27, 28} and binding affinity of scrRNA to tracrRNA while enhancing their ability (relative to unmodified crRNA) to mediate gene editing in human cells. These synthetic CRISPR RNAs provide a platform for therapeutic applications of genome editing, including use of two sc-rRNAs for mediating activation and then silencing of Cas9 nuclease activity in human cells. Production of a scrRNA with Chemical Modifications for Targeted Gene Disruption in Human Cells. Activation of the natural CRISPR-Cas9 system requires hybridisation of a 42-nucleotide crRNA to an 80-nucleotide tracrRNA with both RNAs bound to the Cas9 protein. RNA is highly unstable in vivo as a result of a variety of ribonucleases present in serum and tissues. Specific chemical modifications have significantly increased half-lives and allowed for their use in many therapeutic platforms^{29,30} For the development of a scrRNA, we first tested the activities of Cas9 protein/tracrRNA when combined with crRNA or with a scrRNA in which PS modifications incorporate (i.e., the substitution of sulfur for one of the nonbridging oxygen atoms in the phosphate backbone). The PS modification is known to improve stability to nucleolytic degradation.³¹ We designed synthetic CRISPR RNAs to human low-density lipoprotein receptor (LDLR) as either unmodified (crRNA) or PS backbone-modified (scrRNA-PS). We first compared gene disruption of uniformly PS-modified scrRNA to native RNA. Briefly, cells were transfected with plasmids expressing RNAs encoding Cas9 or tracrRNA. After 24 h, cells were subsequently transfected with either crRNA or scrRNA. CRISPR activity was assessed 48 h later by a Surveyor assay³²⁻³⁶ the genomic DNA from transfected cells was

- (i) used as a template to generate amplicons from the CRISPR target sequence (amplified by PCR);
- (ii) melted;
- (iii) slowly reannealed to allow the formation of homo and heteroduplexes;
- (iv) incubated with the surveyor nuclease that recognises and cleaves DNA mismatches (a result of insertions or deletions from repair of the double-stranded break by NHEJ); and
- (v) Separated using standard electrophoresis. The efficiency of disruption of the LDLR alleles measured by direct comparison with activity measured in a parallel assay 72 h after expression by DNA transfection of both the Cas9 protein and a highly active, 102-base sgRNA. scrRNA with scrRNA-PS showed significant gene disruption (33% of the level of cleavage by Cas9/sgRNA) and a more significant than fourfold activity compared with unmodified crRNA. (We note that this is a relative normalisation to Cas9 activation produced by sgRNA, which is produced by continuous transcription from the active H1 promoter, whereas the scrRNA limited by the amount of modified RNA transfected into cells.) Several modifications at the 2'-position of the sugar ring, such as 2'-O-Me, forces RNA to adopt an energy-favourable confirmation that increases Watson-Crick binding affinity due to the proximity of the 2'-substituent and the 3'-phosphate, thus improving nuclease resistance³⁷ We, therefore, generated scrRNA with five 2'-O-Me-modified nucleotides at both the 5'- and 3'-termini with a fully substituted phosphorothioate backbone (scrRNA-PS-OMe). These combined modifications produced an additional improvement in CRISPR-Cas9-mediated gene disruption activity, yielding a sevenfold increase relative to unmodified crRNA and reaching nearly half (48%) of the activity achieved with sgRNA. As expected, no activity observed in cells in the absence of crRNA/scrRNA or tracrRNA alone.

4.APPLICATIONS OF CRISPR CAS-9 SYSTEM

Microbial engineering directly influences the development of bioindustry. High throughput genome editing tools are useful for breeding economically valuable strains. It is remarkable how quickly practical application of the CRISPR-Cas system has adapted to genome editing in eukaryotic cells. Such rapid success of this technology in eukaryotic cells linked to the fact that eukaryotes employ error-prone nonhomologous end joining (NHEJ) to repair double-strand breaks introduced by the CRISPR-Cas in the target sequence. The use of the CRISPR-Cas technology was not as revolutionary in bacteria, likely because other methods based on homologous recombination (HR) were already available for efficient manipulation of their genomes. Nevertheless, DNA toolkits based on CRISPR-Cas technology for genome editing, gene silencing, and genome-wide screening of essential genes in bacterial and archaeal genomes are Gradually emerging and diversifying³⁸⁻⁴². For instance, a CRISPR-Cas-mediated genome editing technique coupled with

heterologous recombineering using linear single-stranded (single-stranded DNA recombineering [SSDR]) or double-stranded (double-stranded DNA recombineering [DSDR]) DNA templates has developed and successfully applied in *E. coli*⁴³. In archaea, gene silencing has established in *Sulfolobus solfataricus*, *Sulfolobus islandicus*, and *Haloferax volcanii* using the endogenous CRISPR-Cas systems (reviewed in references 41 and 43). More recently, Nayak and Metcalf have harnessed a bacterial Cas9 protein for genome editing in the mesophilic archaeon *Methanosarcina acetivorans*⁴⁴. Hopefully, a thermophilic counterpart of the CRISPR-Cas9 system (or other class 2 systems) will finally be established to perform genome editing in hyperthermophilic species, which are difficult to manipulate genetically. From that perspective, the diversity of CRISPR-Cas systems and mobile genetic elements, which remain to explore fully, is a treasure trove for future exploitation.

5. FUTURE TRENDS

- CRISPR/Cas9 is a robust tool for effectively editing the genome of a cell. Single-guide RNA (sgRNA)-directed Cas9 activity causes DNA cleavage at specific sites, allowing DNA editing at these sites by subsequent DNA repair in mammalian cells⁴⁵.
- CRISPR/Cas9 can also be used to repair mutations in proto-oncogenes or to knock out specific genes, which may create opportunities for potential treatments in cancer. Many studies have shown that using CRISPR/Cas9 to target oncogenic mutations offers a powerful surgical strategy to disrupt oncogenic mutations in cancer treatment⁴⁶. This treatment was regarded as a revolutionary cancer therapy and marked a new era⁴⁷.
- Chimeric antigen receptor T (CAR-T) cell immunotherapy is a T cell immunotherapy approach that involves genetic modification. T cells of patients are modified to 'attack' the tumour. Especially, T cells are modified by CRISPR/Cas9, and the immune system of the patients is mobilising for the treatment of cancer⁴⁸ whereby the antitumor properties of the tumour microenvironment are enhanced to kill the tumour cells.
- The CRISPR/Cas9 system was also used to develop mutants to study the regulatory mechanism of stress/ABA-activated protein kinase2 (SAPK2)-mediated stress tolerance in rice⁴⁹.
- CRISPR/ Cas9 has utilised in tomato to disrupt the promoters of three target genes that control plant architecture, inflorescence branching and fruit size. Generates diverse cis-regulatory alleles, and some promoter variants showed continuous variation for all CRITICAL REVIEWS IN BIOTECHNOLOGY 9 three traits⁵⁰.
- The utility of using CRISPR-Cas to generate an in vitro model of human heart disease was first shown by Liang and colleagues, who obtained hiPSCs from two patients with type 1 Brugada syndrome (BrS) with two different mutations within the sodium voltage-gated channel α subunit 5. BrS is a channelopathy characterised by an elevated Trends in Molecular Medicine 4 Trends in Molecular Medicine, Month 2019, Vol. xx, No. xx precordial ST-segment, ventricular fibrillation, and sudden cardiac death. This study demonstrated that CRISPR-Cas9-mediated correction of the causative variant restored the disease phenotype to health conditions⁵¹.
- Recently, a catalytically inactive variant of Cas9 (dCas9) has exploited to modulate gene expression by fusing it to several effector proteins. For example, Qi and colleagues fused dCas9 to the transcriptional inhibitor Kruppel-associated box (KRAB) and successfully disrupted gene expression in human cells⁵².

6. CONCLUSION

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) cas system is an advanced gene-editing technology worldwide. Which is took from *E. coli* (Gram-negative bacteria) immune system. It has various applications in plants, humans, microbes. A specific mechanism is there in each system; in human beings generate an in vivo model of heart, as per conspiracies of the CRISPR. In future CRISPR is a medicine for every disease and disorder. Moreover, as well might be the best technology in agricultural and biological fields; moreover, CRISPR is a treatment for hereditary disorders. This paper might be useful those who are interested in Biotechnology and life sciences might be useful.

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ASSESSMENT OF PRESCRIBING PATTERN AND PREVALENCE OF VASCULAR COMPLICATIONS IN TYPE II DIABETIC PATIENTS AT SOUTH INDIAN TERTIARY CARE HOSPITAL: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Diabetes mellitus (DM) is a common metabolic disorder. It is associated with complications which will effects the quality of life. Failure to control elevated blood sugar or inadequate treatment of diabetes could cause many complications. A prospective observational study is used to assess the prevalence of diabetic vascular complications in 105 type II diabetic patients. Data was collected regarding patient's demographic and clinical characteristics. Based on our study criteria, males were more when compared to females in getting vascular complications & also Complications were more prominent in the age group of 50-65years. Of all micro-vascular complications, Nephropathy was major whereas in macro-vascular complications, CAD was prominent. Poor glycemic control and a long duration of illness seem to be the most important risk factors for these complications. Physicians play a major role to prescribe anti diabetic medications and Pharmacist plays a keen job to evaluate the prescription pattern in order to achieve successful therapy. The currently anti-diabetic drugs are effective, but a lot of factors such as patient adherence, education related to diabetes, lifestyle modification, cost and type of medication have an association with glycemic control. Commonly prescribed anti-diabetic drug was Insulin. Metformin was mostly preferred drug both as monotherapy and combination therapy. Although poly pharmacy was observed, drug utilization pattern can be rational owing to higher prevalence of complications. Minimization of the occurrence of complications should be encouraged by early diagnosis, intensive blood glucose control and rational drug selections.

KEYWORDS: *Diabetes mellitus, micro-vascular complications, macro-vascular complications.*

1. INTRODUCTION

Diabetes Mellitus (DM) is a common metabolic disorder & is defined as "A metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in the insulin secretion, insulin action, or both" (WHO), which requires life-long medical care and ongoing patient self-management and support to prevent acute complications and to reduce the risk of morbidity and mortality.^{1,2} High blood sugar is the hallmark of diabetes that increases the risk of complications. Failure to control elevated blood sugar or inadequate treatment of diabetes could cause many complications. Generally, the injurious effects of diabetes are separated into macro-vascular complications (coronary artery disease, peripheral arterial disease, and stroke) and micro-vascular complications (diabetic nephropathy, neuropathy, and retinopathy).³ These complications related to DM pose a significant health care burden and affects the overall quality of life.⁴ Effective management of DM requires stringent and sustained glycemic control to lower the risk of macro & micro-vascular complications.⁵ In 2015, more than 415 million adults have DM globally, and this number is estimated to increase to 642 million by 2040.⁶ India is one of the epicenters of the global DM epidemic and has the second-highest number of people with the disease in the world ~69 million individuals as of 2015.⁷ The micro-vascular and macro-vascular complications of DM account for most of the morbidity and mortality associated with the disease. Studies done in Chennai (2000-2008) revealed that the prevalence of diabetic retinopathy (DR) was 17.6%, micro-albuminuria was 26.9%, neuropathy was 26.1%, coronary artery disease (CAD) was 21.4% and peripheral vascular disease (PVD) was 6.3%.⁸⁻¹² Poor glycemic control and a long duration of illness seem to be the most important risk factors for these complications.¹³ Also, these long term complications develop gradually as the age advances making the elderly more prone. According to the

International Diabetes Federation, Four out of every five people with diabetes now live in developing countries, with most affected men and women of working age. Diabetes and its risk factors increase the risk of myocardial infarction by 3-6 times. Similarly, it increases the risk of heart failure by 2-3 times, risk of amputation 45 times, blindness 10-25 times and Kidney failure 15-20 times.¹⁴ The currently anti-diabetic drugs are effective, but a lot of factors such as patient adherence, education related to diabetes, lifestyle modification, and cost and type of medication have an association with glycemic control.¹⁵⁻¹⁷ Medication costs, regimen complexity, and irrational prescribing are the challenges for patient compliance and therapy adherence that consequences will lead to poor glycemic control and increase the morbidity and mortality. Study on anti-diabetic prescribing patterns provides useful insights into the current prescribing evaluation, and it eventually leads to achieving rational drug therapy, optimal glycemic control and reduces the health-care cost for patients and society in a large scale.

2. MATERIALS AND METHODS: (IRB Ref. No: PD007/IRB/NRML/19-20)

2.1 Study type:

This was the Multi-Centre, prospective observational study. All the patients who meet inclusion criteria were taken as the study population.

2.2 Study site:

This study was carried out in Manipal tertiary care Hospital, Vijayawada.

2.3 Study Population and Inclusion and Exclusion Criteria:

The study population of this study was all diabetic patients had complete medical records. The patients who met the inclusion criteria were enrolled in the study. The inclusion and exclusion criteria were as follows

2.3.2 Exclusion criteria:

- Patients with Type 1 Diabetes.
- Mentally retarded and psychiatric patients.
- Patients below 35 and above 85 years of age.

2.3.1 Inclusion criteria:

- Patients aged ≥ 35 years of both the genders.
- Patients with Type II Diabetes Mellitus.
- Patients with co-morbid conditions and complications

2.4 Sample Size:

The study population is of 105 patients.

2.5 Study duration:

From July 1st to November 1st 2019

2.6 Data Collection:

Data collection form was developed to collect the data which includes demographic details and medication list.

2.7 Data Entry and Analysis:

We choose paired *t*-test, Co-relation and regression, and anova test. These all statistical procedures analyzed by Statistical program for the Social and sciences (SPSS-26version)

3. RESULTS AND DISCUSSION

Based on the study criteria, 105 cases were selected and taken for the study. Of the total patient majority were male (58.09 N: 61) in comparison to female (41.90, N: 44) and 49 (46.6%) were belonged to the age group 50-65 years and 45 (42.8%) were in the age group >65 . According to the body mass index (BMI), 10 (9.5%) of the patient has normal weight followed by 41 (39.04%) were overweight and 54 (51.4%) patients were obese. Of the 105 patients, 16(15.23%) were suffering from diabetes for <5 years followed by 52(49.5%) and 37(35.2%) had type 2 diabetes mellitus for 5-10 years and >10 years respectively. The glycosylated haemoglobin (HbA1c) level was mostly observed in the study population was between 7.1 – 8.5. Among comorbidities hypertension 59(56.19%) is most prevalent among cardiovascular complication.

AGE

- The Mean value for age is 62.16 and Standard Deviation (SD) is 12.193 and Standard Error Mean (SEM) is 1.561 i.e., $(\text{Mean} \pm \text{SD}) = (62.16 \pm 12.193)$
- T value for age is 39.819 and 95%CI is 59.04 lower, 65.29 upper

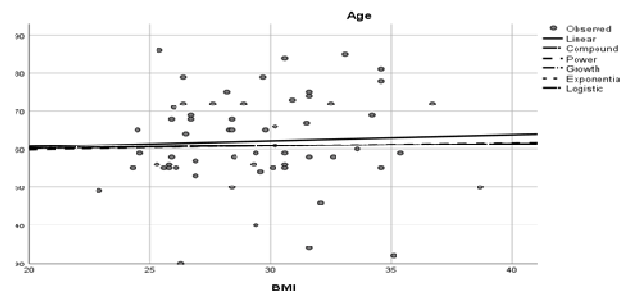


Figure 1. This graph represents the relation between the Age and BMI of the patients

RBS & HbA1c

- RBS: Mean value is 230.82, Standard Deviation (SD) is 114.536, and Standard Error Mean (SEM) is 14.787
- HbA1c- mean value is 7.08, Standard Deviation (SD) is 1.672, and Standard Error Mean (SEM) is 0.216
- RBS & HbA1c Correlation value is 0.263, Sig. is 0.042
- RBS, HbA1c mean is 223.737; Standard Deviation (SD) is 114.107; 95% Confidence Interval (CI) is 194.260 lower; 253.214 upper

3.2 Age wise distribution of Diabetic Vascular Complications

COMPLICATIONS	AGE		
	35-50yrs	50-65yrs	>65yrs
Micro-vascular	4	22	23
Macro-vascular	2	26	23
None	1	6	6

Table 1 Shows that, age wise distribution of diabetic vascular complications. From that table, Age group >65 years were having more micro-vascular complications, Whereas the age group 50-65 years were having more macro-vascular complications.

3.3. Gender wise distribution of Diabetic Vascular Complications:

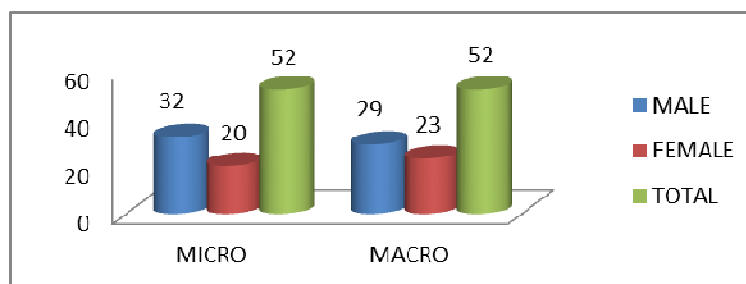


Figure 2. Gender wise distribution of Micro & Macrovascular Complications.

Fig.2 shows gender-wise diabetic vascular complications. Out of 52 Micro-vascular complications 32 were male, 20 were female patients. Whereas from 52 Macro-vascular complications 29 were male, 23 were female patients. This shows that males were very prone to having Diabetic vascular complications.

3.4. Overall distribution of micro-vascular complications

3.5.

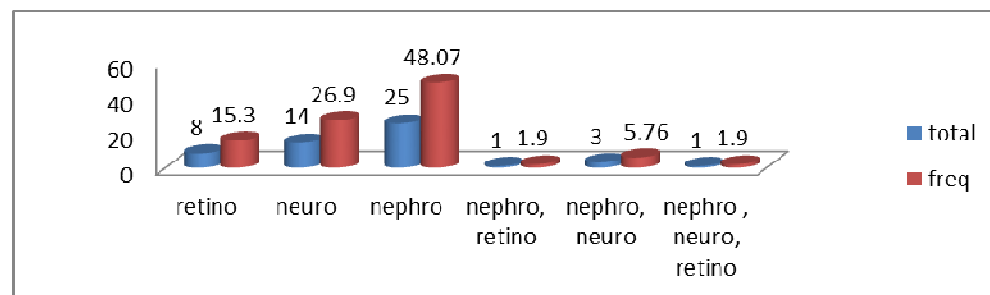


Figure 3 Overall distribution of micro-vascular complications

Fig.3 shows the overall distribution of micro-vascular complications. Of all 52 micro-vascular complications, Nephropathy (n=25, 48.07%) complication was major followed by Neuropathy (n=14, 26.9%), Retinopathy (n=8, 15.3%), Nephropathy & Neuropathy (n=3, 5.76%), Nephropathy & Retinopathy (n=1, 1.9%), and Nephropathy, Neuropathy & Retinopathy (n=1, 1.9%)

3.6. Macro-vascular complications include Stroke, CAD, and Peripheral vascular disease

Macro-vascular Complications	Total	Frequency (%)
Stroke	8	15.3
CAD	30	57.69
PVD	8	15.3
CAD, PVD	3	5.76
CAD, Stroke	3	5.76

Table 3.5 shows the overall distribution of macro-vascular complications. Of all 52 macro-vascular complications CAD (n=30, 57.6%) were major, followed by Stroke (n=8, 15.38%), PVD (n= 8, 15.3%), CAD, PVD (n=3, 5.76%), and CAD, Stroke (n=3, 5.76%)

3.6. Types of therapies

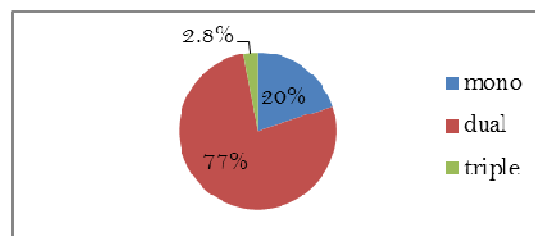


Figure 4. Mono, Dual & Triple therapies of Diabetic drugs used

Fig 4 shows different therapies used by type II diabetic patients in our study. Of all these therapies, dual therapy usage was mostly identified.

3.7. Type of Hypoglycemic Agents Prescribed (n=105)

Types of Hypoglycemic agents	Frequency (%)
Insulin	54(51.4)

Sulphonyl ureas	31(29.5)
Biguanides	78(74.2)
Sitagliptin	7(6.6)
Teneligliptin	5(4.7)

Table 2 shows the overall usage of Hypoglycemic agents. Of all them, Insulin (n=54, 51.4%) were mostly used.

3.8. Combinational usage pattern of Anti-Diabetic agents

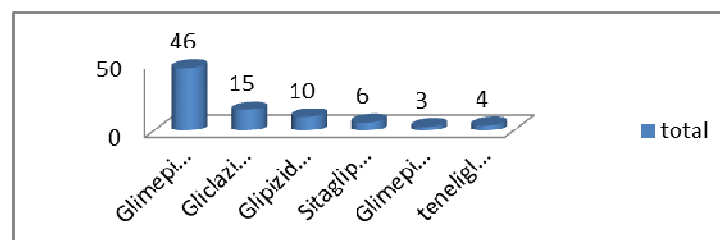


Figure.5 Combinational drugs used by diabetic patients in this study.

Fig.5 shows the combinational usage of drugs prescribed for type II diabetic patients. of all them, Glimepiride + Metformin (n=46, 43.8%) was mostly commonly prescribed, followed by Gliclazide + Metformin (n=15, 14.28); Glipizide + Metformin (n=10, 9.52); Sitagliptin + Metformin (n=6, 5.7); Teneligliptin + Metformin (n=4, 3.8); Glimepiride + Metformin + Voglibose (n=3, 2.8)

3.9. Overall Utilization Pattern of Drugs (n=438)

DRUGS	FREQUENCY (%)
Anti-Diabetic	175(39.9)
Anti-Hypertensive	77(17.5)
Anti-Platelets	59(13.4)
Statins	32(7.3)
Analgesics	25(5.7)
GI drugs	75(17.1)
Vitamins & Minerals	17(3.8)
Others	21(4.79)
Levothyroxine	17(3.8)

Table 3 shows the overall utilization pattern of drugs. Of all them, Anti-diabetic drugs accounted for almost (n=175, 40%) followed by Anti-hypertensive medications, which might be due to higher prevalence of hypertension (n=77, 17.57%) among diabetic patients. The utilization of anti-platelets and lipid lowering drugs were (n=59, 13.4%) and (n=32, 7.3%) respectively. Furthermore, the utilization patterns of drugs used for pain were (n=25, 5.7%) and GI drugs were (n=75, 17%).

4. CONCLUSION

As per the findings of the study, type II DM was more prominent among male gender and was highly prevalent on age group over 50 years of age. At least one chronic complication was found in 65 (61.9%) of the study population. The reasons for higher prevalence of complications might be longer duration of diabetes, poor glycemic control as observed in the study. The observed diabetic complications were as follows viz. retinopathy (n=8, 15.3%), nephropathy (n=25, 48.07%), neuropathy (n=14, 26.9%), CAD (n=30, 57.6%), Stroke (n=8, 15.38%), PVD (n= 8, 15.3%). The overall utilization pattern of drugs were as follows viz. anti-diabetics (n=175, 40%), anti-hypertensive (n=77, 17.57%), anti-platelets (n=59, 13.4%),

lipid lowering agents (n=32, 7.3%). Among anti-diabetic drugs, metformin (n=37, 35.2%) was the most preferred agent both as mono-therapy and combination therapy. Although poly pharmacy was observed, drug utilization pattern can be rational owing to higher prevalence of complications. Minimization of the occurrence of complications should be encouraged by early diagnosis, intensive blood glucose control and rational drug selections.

5. ACKNOWLEDGEMENTS

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CHEMOMETRIC ASSISTED NEW STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF PARACETAMOL, DICLOFENAC POTASSIUM AND SERRATIOPEPTIDASE IN COMBINED DOSAGE FORM ACCORDING TO ICH GUIDELINES.

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ABSTRACT

A new chemometric assisted by high-performance liquid chromatography (HPLC) with UV-Visible detection was implemented for the coincidental determination of tablet dosage form. Two chemometric calibration techniques, principle least squares analysis (PCA) and partial least squares (PLS) were applied to the peak area at 226 nm of PDA detector responses. The method was carried out on a Luna C18, 250 mm x 4.6 mm, 5 µm column with a mobile phase consisting of Acetonitrile and Buffer in the ratio of (10:90 v/v) and flow rate of 1.0 ml/min. The retention time for Paracetamol, nonsteroidal anti-inflammatory drug, and Serratiopeptidase were found to be 3.02, 7.16 and 13.45 min severally. The strategy was valid per the ICH pointers for specificity, LOD, LOQ, precision, accuracy, dimensionality and robustness. The method showed smart reproducibility and recovery with %RSD less than 2. Therefore the proposed method was found to be simple, specific, precise, accurate and linear. The 'UNSCRAMBLER (camo)' software was used for the numerical calculations. All of the two-chemometric analysis methods in this study in this satisfactorily applied for the measurement of Paracetamol, diclofenac potassium, and Serratiopeptidase in pharmaceutical tablet dosage form.

KEYWORDS: *Paracetamol, Diclofenac potassium, Serratiopeptidase, RP-HPLC, unscramble software, PLS, PCA.*

INTRODUCTION

The chemometric methods are one type of multivariate analysis that is considering more than one variable at a particular time.¹ Thus, it does not exist in one dimensional data.² The science of chemometric can be briefly described as the interaction of certain mathematical and statistical methods to chemical problems. It has developed as a consequence of a change in the data obtained with the chemistry with the emergence of the new analytical techniques as well as microprocessors³. The applications of using chemometric techniques in analytical chemistry are now numerous and applications have been revealed in spectroscopy, chromatography and other disciplines of analytical chemistry². Diclofenac potassium (DCL) is (Benzene acetic acid, 2-[(2, 6 dichlorophenyl) amino]-monopotassium salt)⁴ non-steroidal anti-inflammatory drug which inhibits leukocyte migration and the enzyme cyclooxygenase (COX-1 and COX-2)⁵⁻⁸. Paracetamol (PCM) (4-hydroxyacetanilide)⁹ non-steroidal anti-inflammatory drug which inhibits isoforms of cyclooxygenase, COX-1, COX2 and COX-3 enzymes involved in prostaglandin (PG) synthesis¹⁰⁻¹³ and Serratiopeptidase (SER) is Proteolytic enzyme¹⁴ which produce hydrolysis of histamine, bradykinin and serotonin, thus produce anti-inflammatory effect¹⁵.

MATERIALS AND EQUIPMENT

The developed RP-HPLC method for the estimation of Paracetamol, Diclofenac potassium, and Serratiopeptidase was carried out on Luna C18, 250 mm x 4.6 mm, 5 µm column using mobile phase composition of a mixture Acetonitrile and buffer in the ratio of (10:90 V/V). adjusted to P^H 2.5 with flow rate of 1.0 ml/min at 226 nm.

MATERIALS

Instruments used-HPLC, Empower version 2.0 software, UV-Visible detector, Shimadzu Analytical balance.

Chemicals and Reagents: HPLC grade Acetonitrile, HPLC grade Water, Ortho Phosphoric acid, Triethyl amine.

Drugs-Paracetamol, Diclofenac potassium and Serratiopeptidase

METHODOLOGY

METHOD DEVELOPMENT In the present investigation, we have developed a simple and sensitive RP-HPLC method for quantitative estimation of Paracetamol, Diclofenac potassium, and Serratiopeptidase in bulk drug and Pharmaceutical dosage forms.

OPTIMIZED METHOD

Preparation of Buffer solution: 1ml Ortho Tri ethyl amine was mixed in 1litre water and filtered through 0.45µm nylon membrane filter.

Mobile Phase A mixture of buffer and Acetonitrile in the ratio of 10:90%v/v was sonicated and filtered through 0.45µm nylon membrane filter.

Chromatographic conditions

Diluent : Acetonitrile: Buffer(10:90v/v)

Column : Luna C18 (250×4.6mm, 5µ)

Mobile phase : Acetonitrile:Buffer(10:90v/v)

Flow rate : 1.0mL/min

Detection wavelength : 226nm

Injection volume : 10 µl

Temperature : Ambient

Run time : 16min

Retention time of Paracetamol, diclofenac potassium and Serratiopeptidase is about 3.119 min, 7.168 min and 13.452 min.

Preparation of standard stock solution

Accurately weighed about 9.75 mg of Paracetamol working standard and 1.5 mg of diclofenac potassium and 0.3 mg of streptopeptidase working standard into a 10 ml volumetric flask. 7 ml of the diluent was added and sonicate to dissolve and dilute to volume and then dilute 1 ml of the above solution to 10 ml of the diluent. Further dilute 1 ml of the above solution to 10 ml of the diluent.

Preparation of Sample solution

Accurately weighed about 23.04 mg of sample taken into 10 ml volumetric flask. 7 ml of diluent was added and sonicated to dissolve and dilute to volumetric diluent. Further dilute 0.1 ml to 10 ml with the diluent. Filter through 0.45µm nylon syringe filter

METHOD VALIDATION

A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely. There are several parameters that are considered in the method validation process as per International Conference of Harmonization (ICH) guidelines and the values for these parameters are given in Table 02.

STABILITY STUDIES

The purpose of stability testing is to provide evidences on how the quality of the drug substance or the drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light and to establish a re-test period for the drug substance or a period for the drug product and recommended storage conditions.

CHEMOMETRIC ANALYSIS

In this chemometrics assisted HPLC study PCA, PLS calibrations were used to analyse the drugs of Paracetamol, diclofenac potassium, and Serratiopeptidase at 227nm by using UV detector. The data obtained from analysed drugs were stored in computer having required software to perform chemometric analysis.

Table:1 Assay Calculations

DRUG	AREA AU	LABELLED AMOUNT(mg)	AMOUNT PRESENT(mg)	%ASSAY
PARACETAMOL	1504429	10	10.02	100.6
DICLOFENAC POTASSIUM	1910844	50	50.3	100.7
SERRATIOPEPTIDASE	751576	325	325	100.1

Figure1: Overlay spectrum of Paracetamol, diclofenac potassium and Serratiopeptidase

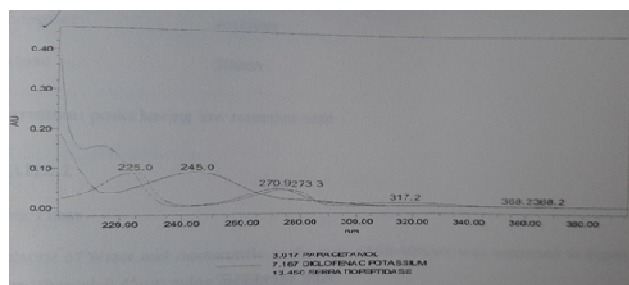


Figure 02: A Representative chromatograph of blank

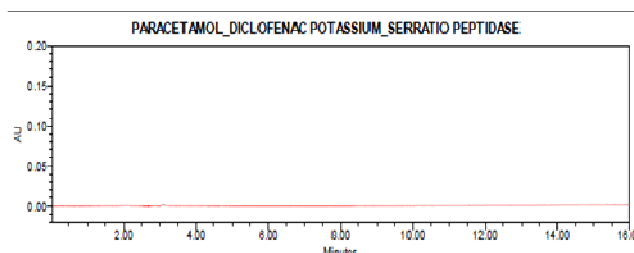


Figure03: A Representative chromatograph of Standard

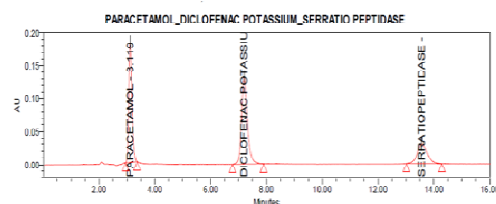


Figure04: A Representative chromatograph of sample

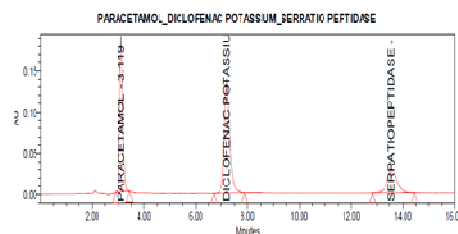


Table:2 Validation parameters for Paracetamol, Diclofenac potassium and Serratiopeptidase

PARAMETER	ACCEPTANCE CRITERIA	PARACETAMOL	DICLOFENAC POTASSIUM	SERRATIOPEPTIDASE
Linearity Range Correlation Coefficient	Correlation coefficient $r^2 > 0.999$	$r^2 = 0.999$	$r^2 = 0.9992$	$r^2 = 0.9994$

System Precision	RSD < 2%	%RSD = 0.173	%RSD = 0.134	%RSD = 0.354
Intermediate Precision	RSD < 2%	%RSD = 0.12	%RSD = 0.34	%RSD = 0.37
Method precision	RSD < 2%	%RSD = 0.4	%RSD = 0.25	%RSD = 0.41
Accuracy	Recovery 98-102% (individual)	% recovery=100.5	% recovery=100.2	% recovery=100.4
Solution Stability	> 12 hour	Stable up to 24 hour	Stable up to 24 hour	Stable up to 24 hour %RSD = 0.761
Robustness	RSD NMT 2% in modified condition Flow minus Flow plus Organic plus Organic minus	Complies %RSD= 0.49 %RSD= 0.38 %RSD=0.32 %RSD=0.15	Complies %RSD= 0.49 %RSD= 0.38 %RSD=0.32 %RSD=0.15	Complies %RSD= 0.26 %RSD= 0.35 %RSD=0.15 %RSD=0.35

Table:03 Results of forced degradation for Paracetamol

S.NO	STRESS CONDITIONS	Rt	Peak area	Plate count	USP Tailing	%degraded	Purity angle	Purity Threshold
1	CONTROL	3.029	1656807	3329	1.46	-0.1	0.778	5.119
2	ACID	3.027	1161216	2761	1.43	20.1	0.541	5.106
3	ALKALI	3.025	1165376	2964	1.49	23.6	0.817	5.106
4	PEROXIDE	3.029	1205381	3440	1.39	27.6	0.721	5.11
5	REDUCTION	3.029	1213247	3398	1.40	27.4	0.861	5.10
6	THERMAL	3.028	119118	3699	1.31	25.8	0.369	5.112
7	PHOTO	3.029	1203915	3443	1.37	25.6	0.0321	5.14
8	HYDROLYSIS	3.021	1210172	2955	1.44	27	0.336	5.412

Table:04 Results of forced degradation for Diclofenac potassium

S.NO	STRESS CONDITIONS	Rt	Peak area	Plate count	USP Tailing	%degraded	Purity angle	Purity Threshold
1	CONTROL	0.172	1983014	6251	1.28	-0.8	0.081	5.084
2	ACID	7.171	609538	5527	1.31	28.5	0.073	5.074
3	ALKALI	7.163	607169	5907	1.32	28	0.079	5.076
4	PEROXIDE	7.168	618624	6103	1.26	27.4	0.082	5.078
5	REDUCTION	7.172	620524	6342	1.28	27.9	0.078	5.078
6	THERMAL	7.171	624893	6493	1.28	25.9	0.073	5.07
7	PHOTO	7.168	596247	6214	1.26	27.9	0.067	5.064
8	HYDROLYSIS	7.173	619938	5830	1.28	28	0.08	5.079

Table: 05 Results of forced degradation for Serratiopeptidase

S.NO	STRESS CONDITIONS	Rt	Peak area	Plate count	USP Tailing	%degraded	Purity angle	Purity Threshold
1	CONTROL	13.465	2687463	3329	1.46	-0.4	0.108	5.108
2	ACID	13.456	2087536	6798	1.12	24.2	0.121	5.117
3	ALKALI	13.439	2116474	7031	1.15	24	0.131	6.132

4	PEROXIDE	13.456	2174040	7175	1.12	22.4	0.121	5.122
5	REDUCTION	13.464	2172942	7429	1.14	21.7	0.12	5.121
6	THERMAL	13.456	2170502	7385	1.11	23	0.135	5.133
7	PHOTO	13.481	2067109	7368	1.08	28	0.105	5.106
8	HYDROLYSIS	13.465	2168798	6929	1.12	26.3	0.151	5.148

Figure 05: PLS accuracy spectra data of diclofenac potassium
Paracetamol

Figure 06: PLS accuracy spectra data of

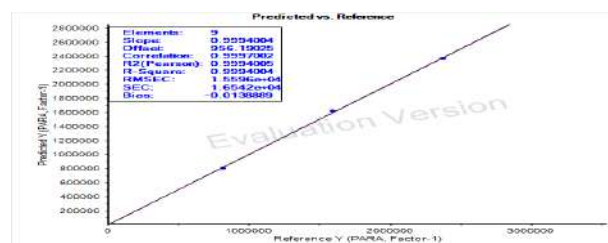
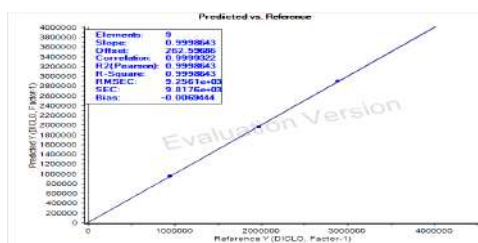
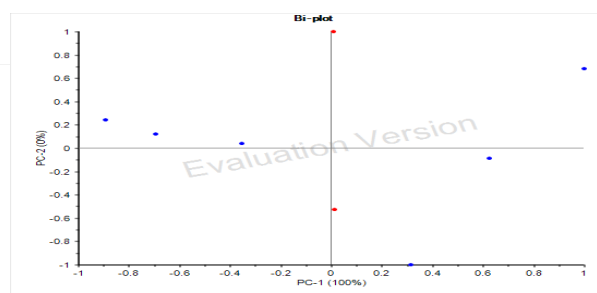
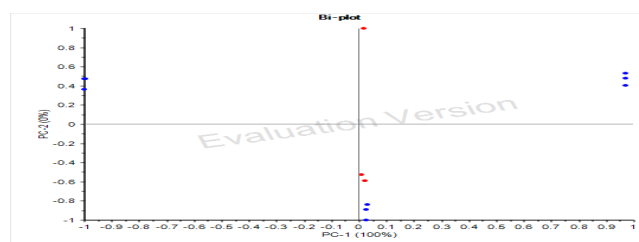


Figure 07: PLS accuracy spectra data of Serratiopeptidase



Figure 08: PCA accuracy spectral data of PARA+DICLO+SERR
data of PARA+DICLO+SERR

Figure 08: PCA Linearity spectral



CONCLUSION

In the present investigation new analytical methods have been developed for the simultaneous estimation of the potent drug Paracetamol, diclofenac potassium and Serratiopeptidase. This study contains evaluation of HPLC data for the chemometric techniques of PCA and PLS. These chemometric methods could be applied with great success for the simultaneous determination of Paracetamol, diclofenac potassium and Serratiopeptidase in the pharmaceutical formulation without the interference of each other. The two chemometric method that i.e. PCA and PLS are found to be simple, precise, accurate, rapid and economical method for their simultaneous determination. The methods were successfully validated and found suitable for quality control laboratories. It is concluded that it is a novel stability indicating method for the determination of drugs in combined dosage form for Paracetamol, diclofenac potassium, and Serratiopeptidase according to ICH guidelines and it can be used for meeting the regulatory guidelines for above drugs.

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STABILITY INDICATING AND EVALUATION OF PRESERVATIVES IN COCOS NUCIFERA WATER USING HPTLC AND OTHER ANALYTICAL METHODS

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ABSTRACT

This study deals with the stability and effective use of preservatives in the coconut water. The fresh coconut water was filtered in Aseptic area through whatman filter paper and equal quantities of fresh coconut water are packed in self sealable covers. Propylparaben and Benzoic acid are used as preservatives of different strengths. These samples are stored for about 1 to 12 weeks at room temperature and refrigerated condition at 25°C. Samples are tested by different analytical methods like pH, Turbidimetry, Titrimetry, Flame photometry, and HPTLC the colour change of samples are also observed. The change in the fresh coconut water and samples with the preservatives are measured for pH at regular time intervals such that the pH are maintained in between 5.5 - 6 and to determine the microbial growth by using Turbidimetry, % of magnesium and % of ascorbic acid by Titrimetry. Sodium Potassium ions are determined by using flame photometry and to determine the sugars by using HPTLC. The colour, odour of the coconut water samples are remained unchanged throughout the test period. The addition of the preservatives improved the transparency of the samples and the effect of preservatives on the Magnesium, Sodium, Potassium, Ascorbic acid, sugars are very less such that the stored coconut watersamples can be packed in a self sealable covers.

KEY WORDS: *Propylparaben, Benzoic acid, turbidimetry, Titrimetry, Flame photometry, HPTLC*

INTRODUCTION

The coconut palm (*Cocos nucifera*) is a member of the family arecaceae (palm family). It's the only accepted species within the genus *Cocos*. Coconut L. is a crucial angiospermous tree within the world, providing nutrients to several individuals, significantly in the wet and semitropical provinces; because of its numerous usages it's frequently called the "tree of life"²⁻³. The plant is native to tropical eastern regions and is also grown in Asian continent (India, Ceylon, and Indonesia) and in Central and South America (Mexico, Brazil). In Africa, the largest producing countries are Mozambique, Tanzania and Ghana.⁴ Several studies have been conducted to identify the active molecules in coconut and their possible pharmacological and biological activities. Various extracts, fractions, and isolated compounds from different parts of the coconut fruit were tested, showing different activities, including antihypertensive; analgesia; vasodilation; protection of kidney, heart, and liver functions; protection against ulcers; and anti-inflammatory, anti-oxidant, anti-osteoporosis, antidiabetes, antineoplastic, bactericidal, antihelminthic, antimalarial, leishmanicidal, antifungal, and antiviral activities⁵⁻⁹. The main objectives of the present study is to collect fresh coconut water and to estimate the nutrient values using different analytical techniques and to add preservatives into the coconut water at different concentrations and compare their physical properties of the fresh coconut water and the samples with the preservatives. To compare the % of Metal ions and % of Ascorbic acid in *cocos nucifera* water by Flame photometry and Titrimetry and to compare the sugars in *cocos nucifera* sample.

MATERIALS AND EQUIPMENT

The stability indicating and evaluation of effective use of preservatives in *cocos nucifera* water are determined by using different analytical methods like pH, Turbidimetry, and Titrimetry and flame photometer.

Instruments used

Elico pH meter, Elico Turbidimetry, Titrimetry, Elico Flame photometer, Shimadzu 0.1mg Digital balance, Aetron HPTLC.

Chemicals used and sample: Coconut water, Propylparaben, benzoic acid, methanol, Acetonitrile, Pentanol, Water, sulphuric acid.

METHODOLOGY

- In the present investigation, we have determined the stability of coconut water by using pH meter for about 1-12 weeks.
- Firstly the fresh coconut water of equal quantities were taken in a self sealing packs for the samples the pH is determined and the first sample was stored at room temperature and the other at refrigeration (4°C to 10°C).
- The samples of fresh coconut water were observed for colour, pH, clarity, turbidity and microbial load at an interval of 1-12 weeks.
- By observing the results of fresh coconut water samples it reveals that there is a change in colour, pH, clarity, turbidity, and microbial load upon storage of 4 weeks at room temperature and refrigerated conditions
- To get better results preservatives like benzoic acid and propylparaben of different concentrations were added
- The fresh coconut water of equal quantities were taken in a self sealing packs by adding the preservatives on one set of samples are store at room temperature and the other set of samples at refrigeration (4°C-10°C). Each set contains two benzoic acid concentrations i.e.; 10mg, 50mg and 3propylparaben concentrations i.e.; 5 mg, 10 mg and 50 mg.

Procedure For Determination Ascorbic Acid

This method used to determine the amount of vitamin C in a solution is by a redox titration using Iodine. The blue – black starch- iodine complex is formed when all the ascorbic acid molecules have been oxidized; the excess iodine is free to react with starch indicator. This is the end point of the titration.

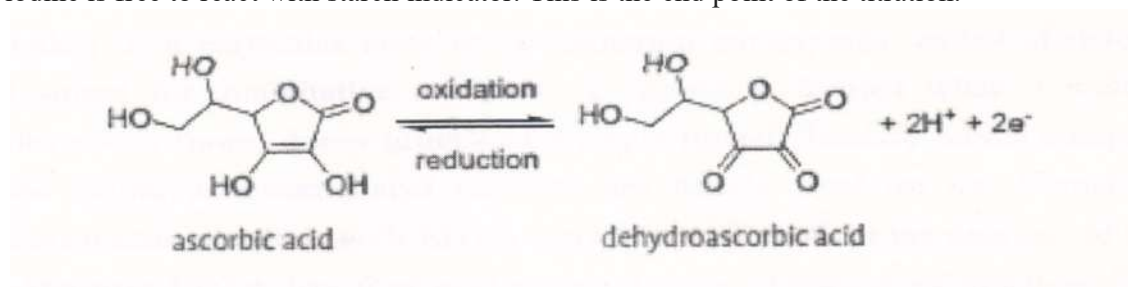


Figure 1. Redox Reaction Of Ascorbic Acid

Preparation of reagents

Iodine solutions (0.005 mol / L): weighed 2gms of potassium iodide and transferred into a 100 ml beaker. Then weighed 1.3 gm of iodine and added it into the same beaker. Then few ml of distilled water was added and swirled for a few minutes to dissolve iodine. Then iodine solution was transferred into a 1lit volumetric flask. Make up the volume up to the 1lit mark with distilled water.

Starch indicator solution (0.5%): 0.25 gm of soluble starch was weighed and 50 ml of boiling water was added in 100 ml conical flask. Then it was stirred and cooled before using.

Procedure For Determination Of Ascorbic Acid By Titration Method:

Determination of ascorbic acid by using Titrimetry method was done by weighing 20 mg of sample and then added 150 ml of water to it. It was titrated against 0.05M iodine solution, using starch as indicator. The end point was determined by change in colour to dark blue black. The titration was repeated for further aliquots of sample solution until concordant results were obtained.

□ Equivalent weight of ascorbic acid $\approx$ 0.008806 gm

Procedure For Determination Of Magnesium By Titration Method:

Determination of magnesium by using Titrimetry method was done by weighing 45 mg of sample and make up to 100 ml from the above solution. 50 ml was pipetted out into a conical flask and to that solution 5 ml ammonia was added. The sample was titrated against 0.05 M EDTA, using erichrome black-T as indicator. The end point was determined by change in colour to deep blue. The results were report as percent magnesium (% w/v).

- Equivalent weight of ascorbic acid ≈ 0.008806

Procedure For Determination Of Sodium And Potasium By Flame Photometry:

Potassium (K) is the major cation found inside of cells. The normal blood potassium level is 3.5-5.0 mille moles/liter (mol/L). The cationic solution of sodium and potassium was sprayed into the flame, this causes the solvent to evaporate and ions were converted into atomic state. The small fractions of atoms were excited in the heat of flame (temp about 1800°C) (Na: 589 nm, K: 766 nm). The concentration of particular atoms in flame decides the intensity of the emitted light. Flame photometer instrument is used to measure the concentration of Na and K.

The flame photometer was made to warm for 5-10min. Then Distilled water was feed into the instrument. Then the reading was adjusted to 0 using the knobs. The most concentrated standard solution was aspirated and readout was adjusted to approximately 90 (on upper most scale) using the big knobs. Then distilled water was aspirated and the instrument read as 0. The standard solution number 1, 2, 3, test solution, and then standards 4, 5, 6 were aspirated and the results were recorded.

- Stock solutions of Na^+ and K^+ con. = 1 mg/ml.

Procedure For Determination Of Sugars By Hptlc

Preparation of CW –I solution: 10 ml of fresh cocos nucifera water was measured and transferred without adding any preservative.

Preparation of CW –II solution: 10 mg of propylparaben was weighed and transferred into a 10 ml volumetric flask and fresh cocos nucifera water was added and made up to the mark and shaken well.

Preparation of standard coconut water for HPTLC: 300 mg of sample was weighed and transferred into a 10 ml volumetric flask and the volume was made up to the mark with methanol and sonicated to dissolve in methanol. The solution was filtered into auto injector vial.

Preparation of standard sugar solution: 10 mg of sugar was weighed and dissolved in 10 ml of distilled water.

Chromatogram layers: TLC and HPTLC plates' silica gel 60 F₂₅₄ (20×10 cm)

Sample application: Manually with disposable micropipettes of 15 micro liter volume.

Spraying reagent: 10% of H₂SO₄ spray reagent

HPTLC Conditions

Mobile phase - Acetonitrile: pentanol : water (4:1:1)

Stationary phase - Silica gel 60F₂₅₄ (20X10 cm)

Wavelength - 500 nm

Diluent - Methanol

Application volume - 1.5 μL

Spraying agent - 10% H₂SO₄

Table 01 - Ph Evaluation Of Coconut Water For About 3 Months

Days	Propylparaben 5 mg		Propylparaben 10 mg		Propylparaben 50 mg		Benzoic acid 5 mg		Benzoic acid 10 mg		Control	
	Room temp	Refrigerator	Room temp	Refrigerator	Room temp	Refrigerator	Room temp	Refrigerator	Room temp	Refrigerator	Room temp	Refrigerator
Day 1	5.38	5.47	5.45	5.46	5.45	5.47	5.12	5.16	4.90	4.85	5.27	5.38
Day 10	5.25	5.44	5.44	5.42	5.33	5.41	5.05	5.08	4.57	4.51	4.95	5.27
Day 20	4.91	5.40	5.36	5.30	5.30	5.39	4.91	4.86	4.46	4.68	4.87	4.95
Day 30	4.90	5.39	5.30	5.28	5.28	5.37	4.80	4.76	4.34	4.30	4.38	4.58

Day 40	4.88	5.37	5.29	5.24	5.24	5.30	4.75	4.52	4.20	4.18	3.90	3.99
Day 50	4.85	5.31	5.25	5.20	5.22	5.28	4.54	4.49	4.01	3.98	3.50	3.20
Day 60	4.60	5.20	5.17	5.01	5.10	5.20	4.44	4.20	3.89	3.50	3.01	3.40
Day 90	4.20	4.90	5.00	4.81	4.98	5.01	4.10	3.99	3.78	3.28	2.50	3.70

Table 02 - Physical Evaluation Of Coconut Water In Presence Of Preservatives

Evaluation Parameters	Room temperature					Refrigeration				
	Benzoic acid		Propylparaben			Benzoic acid		Propylparaben		
	5 mg	10 mg	5 mg	10 mg	50 mg	5 mg	10 mg	5 mg	10 mg	50 mg
Colour	Colour less	Colour less	Colour less	Colour less	Colour less	Colour less	Colour less	Colour less	Colour less	Colour less
Odour	Odour less	Odour less	Odour less	Odour less	Odour less	Odour less	Odour less	Odour less	Odour less	Odour less
pH	5.12	4.85	4.50	4.56	4.66	5.33	5.11	5.69	5.82	5.86
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Turbidity (NTU)	17.0	16.8	18.7	18.4	18.2	17.5	16.8	18.9	18.2	17.5

Table No.03- Determination Of % Magnesium And Ascorbic Acid By Titrimetry

SAMPLES WITH PRESERVATIVES	MAGNESIUM		ASCORBIC ACID	
	Mg	%	mg	%
Fresh coconut water sample	43mg	100	26mg	100
Sample with Propylparaben(10mg)	42mg	97.6	24mg	92.3
Sample with Propylparaben (50mg)	40mg	93	20mg	76.9
Sample with benzoic acid (5mg)	26mg	60	15mg	57.6

Table 04 - Estimation Of %Sodium And Potassium Ions Present In Fresh

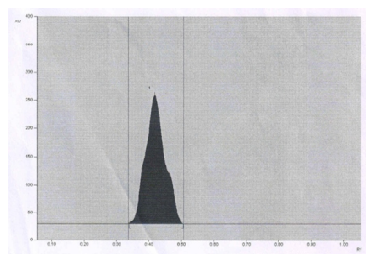
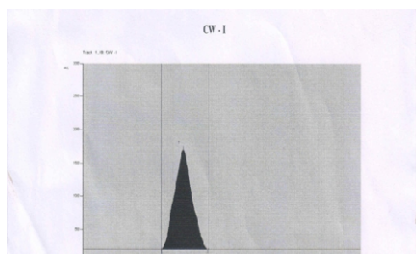
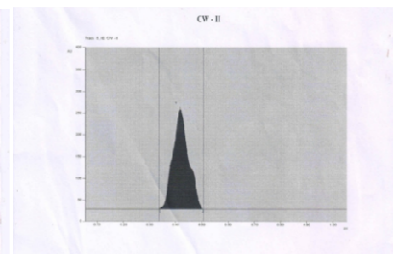
SAMPLES WITH PRESERVATIVES	SODIUM		POTASSIUM	
	PPM	%	PPM	%
Fresh coconut water reference	90	100	100	100
Sample with Propylparaben(10mg)	81	90	92	92
Sample with Propylparaben (50mg)	76	85	86	86
Sample with benzoic acid (5mg)	65	73	74	74

Table 05 - Determination Of Sugars By Using Hptlc

SAMPLE WITH PRESERVATIVE	AU(Absorbance Unit)	Percentage
Standard sugar solution	250	100%
Fresh coconut water	210	84%
Sample with Propylparaben(10mg)	215	86%

Table 06- Determination Of Sugars By Using Hptlc

SAMPLE WITH PRESERVATIVE	AU(Absorbance Unit)	Percentage
Standard sugar solution	250	100%
Fresh coconut water	210	84%
Sample with Propylparaben(10mg)	215	86%

FIGURE 02- CHROMATOGRAM FOR STANDARD SUGAR SOLUTION**FIGURE 03- CHROMATOGRAM FOR PURE COCONUT WATER SOLUTION****FIGURE 04- CHROMATOGRAM FOR COCONUT WATER WITH PRESERVATIVE SOLUTION**

CONCLUSION

In the present study the stability evaluation and indication of different preservatives in different concentrations in *Cocos nucifera* water was done and compared between the two preservatives i.e., Benzoic acid and propylparaben. Among these two propylparaben have shown better results. The pH, Metal ions like sodium, potassium, magnesium, ascorbic acid and sugars present in coconut water were determined by using pH meter, Titrimetry, HPTLC, Flame photometry are within the limits. Finally it is concluded that the following observations can be helpful for commercialization of coconut water in presence of propylparaben at 10 mg concentration. Thus we are planning for commercialisation of coconut water using propyl paraben preservative in tetra packs.

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METHOD OF DEVELOPMENT AND VALIDATION OF NIRAPARIB IN PLASMA SAMPLES BY LC-MS/MS

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ABSTRACT

The aim of the present study is to develop and validate a rapid and efficient liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the analysis of Niraparib in plasma samples. Niraparib was separated using X-Bridge C18, 50 x 4.6 mm, 5 μ m column with mobile phase composed of 10 mM Ammonium formate and Methanol in the proportion of (20:80 v/v). Liquid-Liquid extraction was utilized in the extraction of analytes from human plasma. MRM positive mode was used to identify the Niraparib at 321.5@195.4. The method is validated over a linear concentration scope of 5.0-5000.0 pg/mL with a Correlation coefficient (r) of ≥ 0.9994 . This method illustrates intra and inter-day precision surrounded by 0.7 to 2.0 and 0.7 to 2.7 % and accuracy within 101.4 to 102.4 and 99.5 to 104.8 %. Niraparib was observed stable throughout Freeze-thawing cycles, benchtop, and postoperative stability studies.

KEYWORDS: *Niraparib, Liquid Chromatography-Tandem Mass Spectrometry, Liquid Liquid Extractions.*

1. INTRODUCTION

Ovarian cancer is the principal reason for death from gynecological Malignancy. Germline mutations in BRCA1 and 2, two genes associated with the mechanisms of reparation of DNA impairment, are appeared to be connected with the incidence of breast and malignant ovarian growth, both irregular and familiar. PARP is a group of enzymes engaged with the base excision repair (BER) system. The presentation of inhibitors of PARP in patients with BRCA-transformed ovarian malignant growth is associated with the concept of synthetic lethality¹⁻⁴. Niraparib (NR) is an inhibitor of poly (ADP-ribose) polymerase (PARP) enzymes, PARP-1, and PARP-2, which play a role in DNA restoration. In vitro investigations have demonstrated that Niraparib-induced cytotoxicity may involve inhibition of PARP enzymatic activity and expanded the development of PARP-DNA complexes resulting in DNA damage, apoptosis, and cell death. Increased Niraparib-induced cytotoxicity was seen in tumor cell lines with or without lacks in BRCA1/2. Niraparib diminished tumor development in mouse xenograft models of human malignant growth cell lines with deficiencies in BRCA1/2 and in human patient-inferred xenograft tumor models with homologous recombination deficiency that had either mutated or wild type BRCA1/2⁵⁻⁷. The chemical name for Niraparib tosylate monohydrate is 2-{4-[(3S)- piperidin-3-yl]phenyl}-2H indazole 7-carboxamide 4-methyl benzenesulfonate hydrate (1:1:1). The Molecular Formula is C₂₆H₃₀N₄O₅S, and it has a Molecular weight of 510.61 amu⁸ and the chemical structure of Niraparib and Niraparib-D4 was shown in (Figure.1).

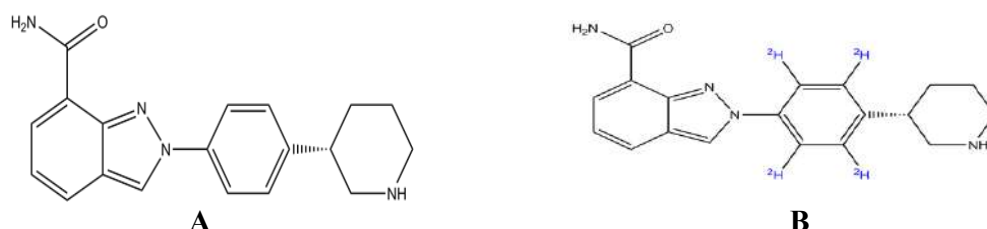


Figure.1: Chemical structures of A) Niraparib B) Niraparib-D4

The overview of the literature survey reveals that few methods were accounted for quantification of Niraparib in human plasma by using LC-MS/MS⁹. We observed such vast numbers of challenges with the revealed strategies regarding reproducibility and stability for long-run analysis. It is crucial to build up the tremendous bioanalytical method with appropriate Deuterated or analog-based internal standard in terms of matrix effect and reproducibility. The objective of the current analysis is to develop and validate the bio-analytical method to measure Niraparib in human plasma test samples by LC-MS/MS compare with respective deuterated internal standard according to US-FDA guidelines¹⁰⁻¹². Moreover, it must be developed a simple extraction method, with a highly sensitive, linear method with the small amount of plasma usage. The proposed analytical method is five-folds higher sensitive than the reported method.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Niraparib (NR) (AbbVie, Inc. (North Chicago, IL), Niraparib-D4 (NRD4)(Champchem, China), diethyl ether, methanol (J.T. Baker USA), Potassium dihydrogen phosphate, ammonium acetate (Merck Limited, Worli, Mumbai). HPLC grade water, Biological matrix (Plasma), Doctors pathological lab (Hyderabad, A.P).

2.2. Instrumentation

HPLC system (Agilent Technologies). Mass spectrometric detection was performed on an HPLC-MS/MS (Applied biosciences).

2.3. Detection

The mass transitions were selected as m/z (amu) 321.5→195.4 for NR, 325.4→195.4 for NRD4, respectively (Table-1).

2.4. Chromatographic conditions

Zorbax SB-C18, 4.6 x 75 mm, 3.5 μ m 80 Å analytical column, mobile phase composition of 10mM Ammonium formate and Methanol in the ratio of (20:80 v/v) with a flow rate of 0.7 mL.min⁻¹. The column was placed at a temperature of 40°C. 20 μ L of sample was injected into the LC-MS/MS System. And NRD4 was eluted at 0.9 $\pm$ 0.2 min approximately with a total run time of 3 min for each sample.

2.5. Calibration standards and quality control Samples

Standard stock solutions of NR (100.0 μ g/mL) and NRD4 (IS) (100.0 μ g/mL) was prepared in methanol. The IS spiking solution (10.0 ng/mL) prepared in reconstitution solution (10mM ammonium formate and methanol in the proportion of (20:80 v/v)) from IS stock solution. Standard stock solutions and IS spiking solutions stored refrigerator conditions 2-8°C until investigation. Standard stock solutions of NR (100.0 μ g/mL) was added to the drug free screened human plasma to acquire standards calibration concentrations (5.0, 10.0, 200.0, 800.0, 1400.0, 2000.0, 3000.0, 4000.0 and 5000.0 pg/mL) and Quality control standards of (5.0, 15.0, 2500.0 and 3500.0 pg/mL) were stored at -30°C. The neat standards were prepared in the mobile phase (10mM ammonium formate and Methanol in the ratio of (20:80 v/v) for validation exercises and stored in the refrigerator at 2-8°C until analysis. Liquid-Liquid extraction method was used to isolate NR and NRD4 from human plasma this, 100 μ L of NRD4 (10 ng/mL) and 400 μ L of plasma sample (respective concentration) were added into marked polypropylene tubes and vortexed briefly about 5 minutes followed by, 100 μ L of 0.1N NaOH solution and 3 mL of extraction solvent (ethyl acetate: dichloromethane 80:20v/v) was added and vortexed about 10 minutes. Then the samples were centrifuged at 4000 rpm for approximately 5min at ambient temperature. From this, the supernatant sample was transferred into marked polypropylene tubes and evaporated to dryness at 40°C briefly, and then reconstituted with reconstituting solution (10 mM ammonium formate and Methanol in the ratio of (20:80 v/v), vortexed and transferred the sample into auto sampler vials for injection.

2.6. Selectivity and specificity

Six lots were screened to identify the possible interferences at the retention times of analyte and internal standard. The peak area of NR in blank samples should not be more than 20% of the mean peak area of LOQ

of NR. Similarly, the peak area of NRD4 in a blank sample should not be more than 5% of the mean peak area of LOQ of NRD4.

2.7. Precision and Accuracy

Precision and accuracy were determined by replicate analysis of quality control samples ($n = 6$) at LQC (low-quality control), MQC (medium quality control) and HQC (high-quality control) levels. The relative standard deviation NMT 15%, and for LLOQ NMT 20%.

2.8. Matrix effect

It was performed by with mid-quality control concentration levels in spiked human plasma using six different plasma lots. The relative standard deviation of NMT is 15%.

2.9. Recovery

Six replicate concentrations of LQC, MQC, and HQC and internal standard and % recovery were evaluated.

2.10. Limit of detection and quantification (LOD and LOQ)

The Limit of quantification was performed by with known concentration (5pg/mL) prepared in the mobile phase and calculated through analyst software 1.4.1.

2.11. Stability (Freeze-thaw, Autosampler, Room temperature, Long term)

Stock solution stability: Stability in-stock solution was performed by comparing the area response of analyte and internal standard in the stability sample, with the area response of sample prepared from the fresh stock solution. Stability studies in plasma: Stability in plasma samples were performed at the LQC and HQC concentration level using six replicates at each level. The analyte was considered stable if the % Change is less than 15% as per US-FDA guidelines¹⁷. Low-quality control and high-quality control samples ($n=6$) were retrieved from the deep freezer after three freeze-thaw cycles according to the clinical protocol. Samples were stored at -30°C in three cycles of 24, 36, and 48 h. Also, the long-term stability of NR in quality control samples was also evaluated by analysis after 105 days of storage at -30°C . Spiked stability samples were processed and extracted along with the freshly spiked calibration curve standards. The precision and accuracy for the stability samples must be within ≤ 15 and ± 15 % respectively of their nominal concentration.

3. RESULTS AND DISCUSSION

3.1. Method development and validation:

The objective of this work is to develop and validate a simple, rapid, and sensitive assay method to measure the quantitative determination of NR from plasma samples. LC-MS/MS has been used as a standout amongst the most dominant scientific devices in clinical pharmacokinetics for its selectivity, affectability, and reproducibility. The MS optimization was performed by direct infusion of solutions of NR and NRD4 into the ESI source of the mass spectrometer. The crucial parameters like ionization type, temperature, voltage, gas parameters, such as, nebulizer and heater gases, compound parameters like DP, EP, FP, CE, and CXP were upgraded to get a better spray, shape, and ionization to frame the respective productions from the protonated NR and NRD4 molecules (Figure. 2 and 3) (Table.1). A satisfactory separation and elution were achieved using 10mM Ammonium formate and Methanol in the ratio of (20:80 v/v) as the mobile phase, at a flow rate of 0.7 mL/minutes. The flow rate of 0.7mL/min without splitter was utilized and reduced the run time to 2.5 min both Drug and IS were eluted with shorter time at 0.9 ± 0.2 min for NR and NRD4 (Figure.2 & 3).

3.2. Linearity

The calibration curve was plotted as the peak area ratio (NR/NRD4) versus (NR) concentration. Calibration was found to be linear with a correlation coefficient higher than 0.9997. The results were depicted in Table 2.

3.3. Selectivity

Interference peaks were not observed at the respective retention time of NR and NRD4. The results were depicted in Fig.4 & 5.

3.4. Precision and Accuracy

Within-run Precision and Accuracy were between 1.6 to 2.8 and 96.3 to 103.43.7% for. Similarly, Between-run Precision and Accuracy were between 2.1 to 3.4 and 98.0 to 100.4%. The results were depicted in Table.3

3.5. Matrix effect

The ion suppression/enhancement in the signal at MQC level was found % CV 1.34 for NR and 1.41 for NRD4. These results are indicating that there is no effect on ion suppression and ion enhancement.

3.6. Recovery

The recovery was calculated by comparing the peak area ratios of NR in plasma samples with the peak area ratios of solvent samples and was estimated at control levels of NR.

The recovery of NR was determined at three different concentrations 15.0, 2500.0 and 3500.0 ng/mL, were found to be 89.6, 90.2, and 91.2 %, respectively. The overall average recovery of NR and NRD4 was found to be 90.3 and 92.5% respectively.

3.7. Limits of Detection and Quantification (LOD&LOQ):

The limit of quantification was determined at five pg/ml. The limit of detection was determined at 50 fg/10 μ L injection volume.

3.8. Stability (Freeze-thaw, Autosampler, Room temperature, Long term):

Quantification of the NR in plasma subjected to 3 freeze-thaw (-30°C to room temperature) cycles showed the stability of the analyte. The concentrations ranged from 99.3 to 98.5% of the theoretical values. No significant degradation of the NR was observed even after 53 h storage period in the autosampler tray, and the final concentration of NR was between 97.3 to 103.2 % of the theoretical values. Room temperature stability at 24.5h was between 98.0 to 99.4 % of the theoretical values. Also, the long-term stability of NR in QC samples after 105 days of storage at -30°C was also evaluated. The concentrations ranged from 94.6 to 101.8% of the theoretical values. These results confirmed the stability of NR in human plasma for at least 105 days at -30°C (Table 4).

Table.1: Optimized mass parameters of Niraparib and Niraparib -D4

Molecular ion to production transitions used for quantification								
Compound	Molecular ion (m/z)				Product ion (m/z)			
Niraparib	868.12 [M+H] ⁺				321.54 ⁺			
Niraparib -D4	876.90 [M+H] ⁺				329.70 ⁺			
	Source dependent parameters (psi)				Compound dependent parameters (Volts)			
	CUR gas	CAD gas	Nebulizer gas	Heater gas	EP	DP	CE	CXP
Niraparib	20	4	20	40	10	30	35	12
Niraparib-D4	20	4	20	40	10	28	30	12
Common mass parameters for NR, NRD4								
Ion spray voltage	5500 volts							
Temperature	400°C							
Scan type	MRM							
Dwell time	500 msec							
Type of ionization	Electro spray ionization (ESI)							

Table 2: Calibration curve details of Niraparib

Spiked plasma concentration (pg/mL)	Concentration measured(mean) (pg/mL)	SD	(%) CV (n = 5)	Accuracy %
5.0	5.1	0.1	1.9	102.0
10.0	9.76	0.3	3.0	97.6
200.0	199.6	6.4	3.2	99.8

800.0	817.2	15.2	1.8	102.2
1400.0	1389.5	19.5	1.4	99.3
2000.0	2006.6	23.1	1.1	100.3
3000.0	2999.7	19.6	0.6	100.0
4000.0	3968.9	75.1	1.8	99.2
5000.0	5026.8	91.6	1.8	100.5

Table 3: Precision and accuracy (analysis with spiked plasma samples at three different concentrations)

Spiked plasma concentration (pg/mL)	Within-run			Between-run		
	Concentration measured (n=6) (Pg/mL) (mean $\pm$ S.D.)	(%)CV	Accuracy %	Concentration measured (n=30) (Pg/mL) (mean $\pm$ S.D.)	(%)CV	Accuracy %
5.0	5.12 $\pm$ 0.1	2.0	102.4	5.24 $\pm$ 0.1	1.9	104.8
15.0	15.28 $\pm$ 0.3	2.0	101.9	14.92 $\pm$ 0.3	2.0	99.5
2500.0	2535 $\pm$ 21.4	0.8	101.4	2495.2 $\pm$ 25.1	1.0	99.8
3500.0	3577.1 $\pm$ 25.2	0.7	102.2	3550.7 $\pm$ 25.5	0.7	101.4

Table 4: Stability of Niraparib in human plasma samples

Spiked plasma concentration (Pg/mL)	Room temperature stability		Autosampler sample stability		Long term stability		Freeze and thaw stability	
	24.5 h		53 h		105 days		Cycle 3 (48 h)	
	Concentration measured (n=6) (Pg/mL) (mean $\pm$ S.D)	(%)CV (n=6)	Concentration measured (n=6) (Pg/mL) (mean $\pm$ S.D)	(%)CV (n=6)	Concentration measured (n=6) (Pg/mL) (mean $\pm$ S.D)	(%)CV (n=6)	Concentration measured (n=6) (Pg/mL) (mean $\pm$ S.D)	(%)CV (n=6)
15.0	14.7 $\pm$ 0.5	3.4	14.6 $\pm$ 0.5	3.4	14.2 $\pm$ 0.5	3.5	14.9 $\pm$.2	1.3
3500.0	3480.3 $\pm$ 43.1	1.2	3614.4 $\pm$ 256.6	7.1	3563.7 $\pm$ 63.4	1.8	3450.2 $\pm$ 39.1	1.1

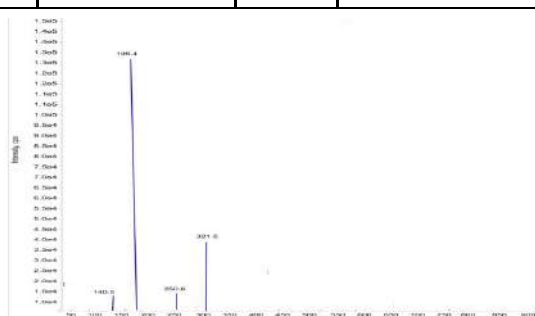


Figure.2. Parent and product ion mass spectra (Q1& Q3) of Niraparib

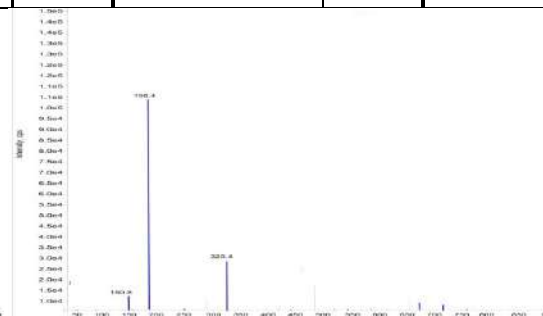


Figure.3. Parent ion and product ion mass spectra (Q1 & Q3) of Niraparib-D4

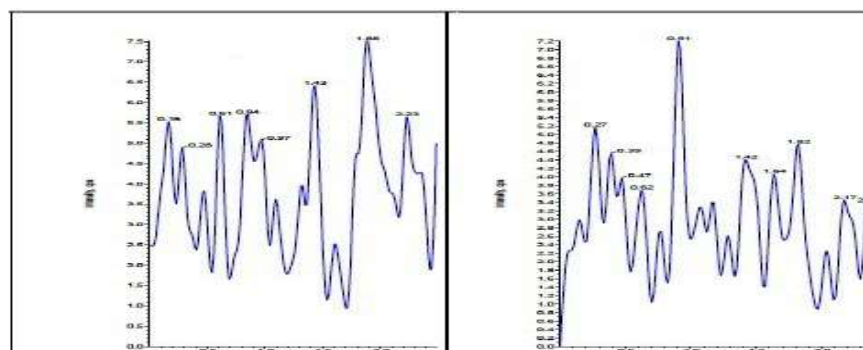


Figure.4: Blank plasma chromatogram of interference-free Niraparib and Niraparib -D4

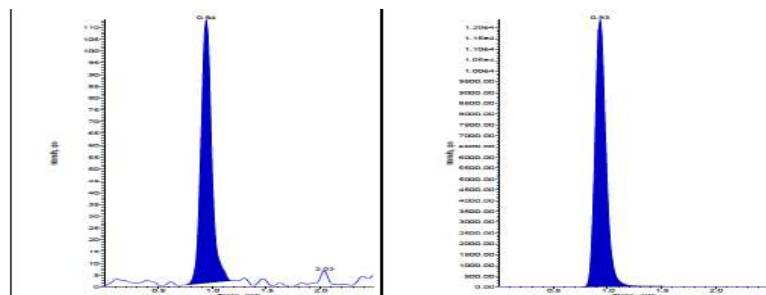


Figure.5: LOQ sample (Niraparib and Niraparib -D4)

4. CONCLUSION

The proposed research work is exceedingly sensitive because of the tandem mass spectrometry and has significant advantages over other depicted methods in the previous quantification of Niraparib was compared with respective isotope marked internal standard. The affectability of the assay is adequate to follow the pharmacokinetics of Niraparib accurately. Hence this method has significant advantages over recently revealed method regarding selectivity, affectability, linearity and reproducibility.

5. Acknowledgments

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FORMULATION AND EVALUATION OF METOPROLOL MUCOADHESIVE TABLETS BY USING NATURAL AND SYNTHETIC POLYMERS

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ABSTRACT

Nature has provided us a wide variety of materials to help improve and sustain the health of all living things either directly or indirectly. In recent years there has been an important development in different dosage forms for existing and newly designed drugs and natural products, and semi-synthetic as well as synthetic excipients often need to be used for variety of purposes [1]. Gums and mucilage's are widely used natural materials for conventional and novel dosage forms. With the increasing interest in polymers of natural origin, the pharmaceutical world has compliance to use most of them in their formulations. Buccal mucosa is preferred for the site for both systemic and local drug action. The mucosa has a rich blood supply and it relatively permeable. Buccal transmucosal delivery helps to bypass first- pass metabolism by allowing direct access to the systemic circulation through the internal jugular vein [2]. This article describes about Buccal Adhesive Delivery of Drugs, Bio Adhesion, Buccal Adhesive Polymers, and Bio Adhesive Strength etc.

KEYWORDS: *Natural polymer's, Muco adhesion, Theories, Factors effecting Muco adhesion, Bio adhesion, Buccal Delivery System*

INTRODUCTION

A large number of plant-based pharmaceutical excipients are available today. Many researchers have explored the usefulness of plant-based materials as pharmaceutical excipients. The reason for increase in importance of natural plant based material is that plant resources are renewable and if cultivated or harvested in a sustainable manner can provide a constant supply of raw material. Mucilages are naturally occurring, high molecular weight, polyurinoide consisting of sugar and uronic acid units. These are esters of sulphuric acid, wherein ester group is a polysaccharide complex. Chemically, mucilages resemble gums and pectins but differ in their physical properties. Gums swell in water to form sticky, colloidal dispersions and pectins gelatinise in water, while mucilage's form slippery, aqueous colloidal dispersions [4]. Bio adhesion may be defined as the state in which two materials, at least one of which is of biological nature, are held together for extended periods of time by the interfacial forces. For drug delivery system, the term bioadhesion implies attachment of a drug carrier system to a specific biological location [5]. If the biological source is epithelial tissue or the mucous coat, the phenomenon is referred to as mucoadhesion. Mucoadhesion is believed to occur in three stages: wetting, interpenetration and mechanical interlocking between mucin and polymer.

MATERIALS AND METHODS

Buccal drug delivery was introduced by Orabase in 1947, when gum tragacanth was mixed with dental adhesive powder to supply penicillin to the oral mucosa. Buccal route of drug delivery is a good alternative, amongst the various routes of drug delivery. Oral route is perhaps the most preferred for the patients. Within the oral mucosal cavity, the buccal region offers an attractive route of administration for systemic drug delivery. However, oral administration of drugs has disadvantages such as hepatic first pass effect, avoidance of pre-systemic elimination within the GI tract; these factors make the oral mucosal cavity a very attractive and feasible site for systemic drug delivery. Considering the low patient compliance of rectal, vaginal, sublingual and nasal drug delivery for controlled release, the buccal mucosa has rich blood supply and it is relatively permeable [7]. The buccal mucosa lines the inner cheek and buccal formulations are placed in the mouth between the upper gingival (gums) and cheek to treat local and systemic conditions.

ISOLATION OF MUCILAGE

The mucilage was isolated from the branches and stem by washing properly with distilled water to remove any dust and adhered particles. After that bark of stem and branches was peeled off and was cut into small pieces. These pieces were soaked in distilled water for 24 h. After 24 h, material was squeezed through 8 fold muslin cloth to separate the mure from filtrate [8]. Then acetone was added to filtrate in a ratio (1:2) to precipitate the mucilage. The precipitated mucilage was separated by decantation and washed several times with acetone. The mucilage was dried in hot air oven at 40°C and powdered. The powdered mucilage was passed through British standard sieve (BSS) 80 and kept in a desiccator for the further studies.

PERMEABILITY

The permeability of the buccal mucosa is estimated to be 4-4000 times greater than the skin. In general, the permeabilities of the oral mucosa decrease in the order of sublingual greater than buccal and buccal greater than palatal. This rank order is based on the relative thickness and degree of keratinization of these tissues, with the sublingual mucosa being relatively thin and non-keratinized, the buccal thicker and non-keratinized, and the palatal intermediate in thickness but keratinized [9]. The permeability barrier property of the oral mucosa is predominantly due to intracellular materials derived from the so called "membrane coating granules"(MCGS). Recent evidence has shown that passive diffusion is the primary mechanism for the transport of drugs across the buccal mucosa while carrier mediated transport has been reported to have a small role. In buccal mucosa two routes of passive transport are found one involves the transport of compounds through the intercellular space between the cells (paracellular) and other involves passage into and across the cells (transcellular). Another barrier to drug permeability across buccal epithelium is enzymatic degradation.

ROLE OF SALIVA

- Protective fluid for all tissues of the oral cavity.
- Continuous mineralization / demineralization of the tooth enamel.
- To hydrate oral mucosal dosage forms.

ROLE OF MUCUS

- Made up of proteins and carbohydrates.
- Cell-cell adhesion
- Lubrication
- Bioadhesion of mucoadhesive drug delivery systems.

Buccal Drug Delivery and Mucoadhesive property

For the development of buccal drug delivery systems, mucoadhesion of the device is the important criteria. For proper and good mucoadhesion, mucoadhesive polymer have been utilized in many different dosages form such as tablets, patches, tapes, films, semisolids and powders[10]. Many studies showed that addition of various polymers to drug delivery systems such as gums increased the duration of attachment of the formulations to the mucous surface and also increased the efficacy. The polymers should possess following general physiochemical features so as to serve as mucoadhesive polymers

- Predominantly anionic hydrophilicity with numerous hydrogen bond-forming groups.
- Polymer and its degradation products should be non-toxic, non-irritant and free from leachable impurities
- Should have good spreadability, wetting, swelling and solubility and biodegradability properties.
- pH should be biocompatible and should possess good viscoelastic properties.
- Should possess peel, tensile and shear strengths at the bioadhesive range.

MECHANISM OF MUCOADHESION

Mucoadhesion has the following mechanism,

- intimate contact between a bioadhesive and a membrane (wetting or swelling phenomenon)
- penetration of the bioadhesive into the tissue or into the surface of the mucous membrane i.e. interpenetration.

FACTORS AFFECTING MUCOADHESION

The mucoadhesion of a drug carrier system to the mucous membrane depends on the following mentioned factors.

POLYMER BASED FACTORS

a. Molecular weight of the polymer

Low molecular weight (less than 100 dalton) can easily penetrate through the oral mucosa.

b. Concentration of polymer

An optimum concentration of a mucoadhesive polymer is required to produce maximum bioadhesion.

c. Flexibility of polymer chains

It is critical for interpenetration and entanglement. Due to the cross-linking of water-soluble polymers, the mobility of character polymer chains decrease and thus the valuable length of the chain that can penetrate into the mucus layer also decreases.

d. Swelling factor

Swelling characteristics are related to the mucoadhesive polymer and its environment. Swelling depends on polymer concentration, ionic strength, and presence of water.

e. Stereochemistry of polymer

Spatial conformation of a molecule is also important factor which can affect the mucoadhesion.

Physical factors

a. pH of polymer-Substrate interface

pH can influence the formal charge on the surface of the mucus as well as certain ionizable mucoadhesive

b. Applied strength

To place a solid mucoadhesive system, it is necessary to apply a defined strength depending on the type of polymer.

c. Contact time

Contact time between the mucoadhesive and mucus layer determines the extent of swelling and interpenetration of the mucoadhesive polymer chains. More mucoadhesive strength increases as the initial contact time increases [11].

Limitations of Buccal Drug Delivery System

a. Drugs which irritate oral mucosa or have bitter taste, or cause allergic reactions, discoloration of teeth cannot be formulated.

b. If formulation contains antimicrobial agents, affects the natural microbes in the buccal cavity.

c. The patient cannot eat/drink/speak.

d. Only those drugs which are absorbed by passive diffusion can be administered by this route.

e. Drugs which are unstable at buccal pH cannot be administered by this route.

f. Swallowing of saliva can also potentially lead to the loss of dissolved or suspended drug.

g. Low permeability of the buccal membrane, specifically when compared to the sublingual membrane.

Formulation of Buccal Drug Delivery System

Formulation design

a. General criteria for selection of drug candidate

* Buccal adhesive drug delivery systems with the size 1–3 cm² and a daily dose of 25 mg or less are preferable.

*The maximal duration of buccal delivery is approximately 4–8 hr

* Drug must undergo first pass effect or it should have local effect in oral cavity.

*Drugs with biological half life 2-8 hr will in general be good candidates for sustained release dosage forms.

*Local drug irritation caused at the site of application is to be considered while selecting the drug.

b. Pharmaceutical considerations

Great care needs to be exercised while developing a safe and effective buccal adhesive drug delivery device. Factors influencing drug release and penetration through buccal mucosa, organoleptic factors, and effects of additives used to improve drug release pattern and absorption, the effects of local drug irritation caused at the site of application are to be considered while designing a formulation¹².

c. Buccal adhesive polymers

Polymer is a generic term used to describe a very long molecule consisting of structural units and repeating units connected by covalent chemical bonds. The term is derived from the Greek words: polys meaning many, and meros meaning parts. The key feature that distinguishes polymers from other molecules is the repetition of many identical, similar, or complementary molecular subunits in these chains. These subunits,

the monomers, are small molecules of low to moderate molecular weight, and are linked to each other during a chemical reaction called polymerization.

d. Ideal characteristics

- * Polymer and its degradation products should be non-toxic, non-irritant and free from leachable impurities.
- * Should have good spreadability, wetting, swelling and solubility and biodegradability properties.
- * pH should be biocompatible and should possess good viscoelastic properties.
- * Should adhere quickly to buccal mucosa and should possess sufficient mechanical strength.
- * Should possess peel, tensile and shear strengths at the bioadhesive range.
- * Polymer must be easily available and its cost should not be high.
- * Should show bioadhesive properties in both dry and liquid state.
- * Should demonstrate local enzyme inhibition and penetration enhancement properties.
- * Should demonstrate acceptable shelf life.
- * Should have optimum molecular weight.

Buccal mucoadhesive dosage forms may be classified into three types:

- * A single layer device with multidirectional drug release.
- * An dosage form with impermeable backing layer which is superimposed on top of an drug loaded bioadhesive layer, creating a double layered device and preventing loss from the top surface of the dosage form into the oral cavity.
- * Unidirectional release device, the drug is released only from the side adjacent to the buccal mucosa.

EVALUATION OF TABLETS [99]

Tablets formulated were studied for Weight variation, Hardness, Thickness, friability and disintegration time in water, 0.1N HCl and pH 6.8 .

Weight Variation: Weight variation was tested by using Balance in comparison with average weight of tablets.

Hardness: Hardness of the tablets was tested by using a Monsanto hardness tester.

Thickness: Thickness was tested by using Vernier callipers.

Friability:

The friability test was carried out in Roche Friabilator. Ten tablets were weighed (wo) initially and put in a rotating drum. Then, they were subjected to 100 revolutions. After completion of rotations, the tablets were again weighed (w).

The percent loss in weight or friability (f) was calculated by the given formula.

$$F = (1 - W/W_0) \times 100$$

Disintegration

Disintegration times were determined in a disintegration test Apparatus. The prepared tablets were studied for swelling index and buccaladhesive strength, the swelling ability of the polymer shows impact on the drug release hence it has to be studied. The swelling behavior of a dosage was measured by studying its weight gain. The swelling index of tablets was determined by placing the tablets in dissolution medium (6.8 pH buffer for Metoprolol). The swollen weight of the tablet was determined at predefined time intervals over a specific period.

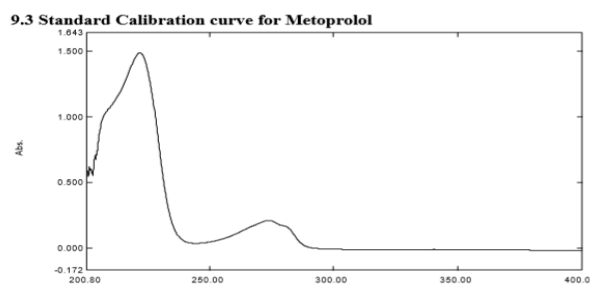
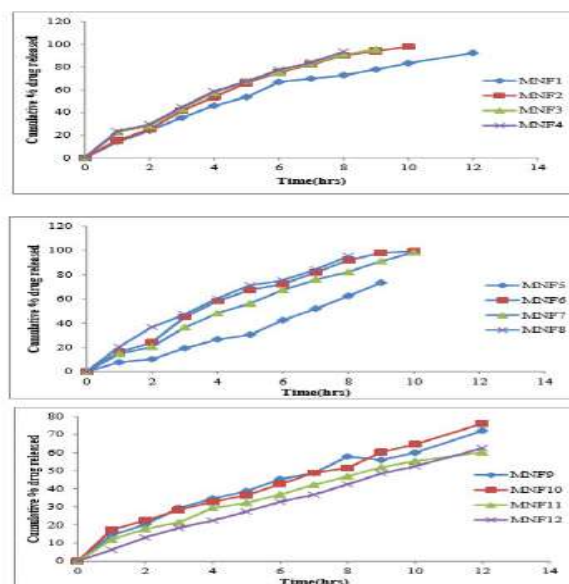


Fig. 9.1: UV Spectra of Metoprolol in 6.8 pH buffer

Formulation Code	Weight (mg) ±SD*(n=20)	Friability (%)±SD* (n=10)	Hardness (Kg/Cm ²)±SD* (n=3)	Thickness (mm) ±SD* (n=3)	Drug Content(%) ±SD* (n=10)	Disintegrating Time(Min)
MSF1	200±0.19	0.16 ± 0.01	4.50 ± 0.74	3.3± 0.04	91.90 ± 0.42	45min
MSF2	199±0.42	0.24± 0.18	3.67 ± 0.21	3.4± 0.02	93.61 ± 0.70	43min
MSF3	200±0.27	0.29 ± 0.02	3.69 ± 0.95	3.2± 0.03	93.22 ± 0.57	42min
MSF4	201±0.63	0.21 ± 0.06	4.33 ± 0.61	3.1± 0.14	96.02 ± 0.33	40min
MSF5	200±0.39	0.23 ± 0.07	3.50 ± 0.12	3.3± 0.14	97.27 ± 0.21	38min
MSF6	199±0.27	0.25 ± 0.12	4.41 ± 0.57	3.1± 0.16	99.14 ± 0.17	36min
MSF7	199±0.91	0.16 ± 0.04	4.21 ± 0.84	3.2± 0.13	99.47 ± 0.58	35min
MSF8	198±0.22	0.25 ± 0.02	3.45 ± 0.17	3.1± 0.12	98.13 ± 0.14	34min
MSF9	200±0.67	0.24 ± 0.13	4.65 ± 0.29	3.3± 0.11	99.28 ± 0.78	33min

*Each value represents the mean ± standard deviation of 3 trails



Graphs Showing In Spectroscopy Values

CONCLUSION

It is concluded that particular work was planned to prepare a buccal adhesive tablets by using natural and synthetic gums, the usage of natural gums were more safe when compared to synthetic, the reason behind this was since they were natural and easily compactable to the body nature. Several formulations were prepared; it was clearly observed that the formulations prepared by using NBA01 were most suited for Metoprolol.

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SP-117

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCL, GLIMEPIRIDE, ATORVASTATINE BY RP – HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Reverse phase chromatographic techniques are used for analysing drugs. It is a advanced analytical instrumentation method used for analysis of drugs. A New simple, precise, accurate and selective RP- HPLC method has been developed and validated for stability indicating RP – HPLC method for simultaneous estimation of Metformin HCL, Glimepiride and Atorvastatin in bulk and pharmaceutical dosage form by RP – HPLC method. The method was carried out on a Luna C 18, 250mm × 4.6 mm, 15 µm. Column with a mobile phase consisting of buffer and acetonitrile in the ratio of (85:15 v/v) and flow rate of 1.0ml/ min. The detection was carried out at 258 nm. The retention time for metformin HCL, Atorvastatin and Glimepiride were found to be 3.266, 5.237 and 8.457 respectively. The method validated accordingly to the ICH guidelines for specificity, LOD, LOQ, precision, accuracy, linearity and robustness. The method showed good reproducibility and recovery with % RSD less than 2. The proposed method was found to be simple, specific, precise, accurate and linear.

KEYWORDS : *Rp- Hplc, Metformin, Glimepiride, Atorvastatine.*

INTRODUCTION

A simple, precise, rapid, and reproducible reversed – phase high – performance liquid chromatography method is developed for the simultaneous estimation of metformin hydrochloride (MET), pioglitazone hydrochloride (PIO), and glimepiride (GLP) present in multicomponent dosage forms. Chromatography is carried out isocratically at 25°C ± 0.5 °C on an Inertsil- ODS-3 (C- 18) Column (250 × 4.60 mm, 5 µm) with a mobile phase composed of methanol- phosphate buffer (pH 4.3) in the ratio of 75 : 25 v/v at a flow rate of 1 ml/min. Detection is carried out using a UV-PDA detector at 258 nm. Parameters such as linearity, precision, accuracy, recovery, specificity, and ruggedness are studied as reported in the International Conference on Harmonization guidelines. The retention times for MET, PIO, and GLP are 2.66 + 0.5 min, 7.12 + 0.5 min, and 10.17 + 0.5 min, respectively. The linearity range and percentage recoveries for MET, PIO, and GLP are 10-5000, 10-150, and 1-10 µg/ml and 100.4%, 100.06 %, and 100.2 % respectively. The correlation coefficients for all components are close to 1 . The relative standard derivations for three replicate measurements in three concentrations of samples in tablets are always less than 2 %.

MATERIALS AND METHODS:

Chemicals: HPLC grade Acetonitrile, HPLC grade Water, are used as solvents. Ortho phosphoric acid of Analytical grade was used in the buffer preparation.

Drug Samples: Metformin Hcl, Glimepiride, And Atorvastatin.

MATERIALS

Instrument	Specifications
HPLC	Water, 2695 separation module
Software	Empower, version 2.0
Detector	UV- Visible detector
Analytical balance	Shimadzu

Table 1: Table showing the specification of instrument.

METHOD DEVELOPMENT

The present investigation was designed to develop a simple and sensitive RP- HPLC method for quantitative estimation of Metformin Hcl, Glimepiride and Atorvastatin in bulk drug and Pharmaceutical dosage forms. Trials performed for HPLC method development of Metformin Hcl, Glimepiride, and Atorvastatin.

SELECTION OF WAVE LENGTH (FOR DETECTION)

The choice of detection wave length was based on the scanned absorption for Metformin Hcl, Glimepiride, and Atorvastatin. The UV- Spectrum of Metformin Hcl, Glimepiride and Atorvastatin was obtained separately by scanning the sample over the wave length range 200-400nm against blank as methanol. After thorough examination of the spectra, the wave length 253 nm was selected for further analysis.

OPTIMIZED METHOD

PREPARATION OF BUFFER SOLUTION: Mix 1ml of ortho phosphoric acid in 1 litre water, filtered through 0.45µm nylon membrane filter.

MOBILE PHASE: A Mixture of Acetonitrile and 0.1% Ortho phosphoric acid in the ratio of 15 : 85 % v/v was sonicated to degas and filtered through 0.45µm nylon membrane filter.

PREPARATION OF DILUENT: Acetonitrile: Buffer (15 : 85 v/v)

CHROMATOGRAPHIC CONDITIONS

Column	:	Zodiac C18 (250 × 4.6 mm, 5 µ)
Mobile Phase	:	Buffer: Acetonitrile (85 : 15 v/v)
Flow rate	:	1.0 ml / min
Detection wave length	:	258 nm
Injection volume	:	10µl
Temperature	:	Room temperature
Run time	:	20 min

Retention time for Metformin HCL is about 3.066

Retention time for Atorvastatin is about 5.237

Retention time for Glimepiride is about 8.457

OBSERVATION : The method is suitable for validation.

PREPARATION OF STANDARD STOCK SOLUTION

Solution A: Atorvastatin:

Accurately 10.1 mg of Atorvastatin was weighed and transferred into 100ml volumetric flask that is considered as working standard solution. Add 80 ml of diluent sonicate to dissolve and dilute to volume with diluents.

Solution B : Metformin :

To prepare working standard solution of metformin 50.9 mg was weighed accurately and transferred into a 100 ml volumetric flask. Add 80 ml of diluent, sonicate to dissolve and dilute the volume with diluents.

Solution C: Glimepiride:

Weigh accurately about 10.2 mg of Glimepiride working standard into a 100ml volumetric flask. Add 80 ml of diluent sonicate to dissolve and dilute to volume with diluents.

Further dilute 1ml of the above solution to 10 ml with diluents. Further dilute each 1 ml of solution- A, B & C to 100 ml with diluent.

PREPARATION OF SAMPLE SOLUTION

Weigh accurately about 82.49mg of sample into a 100 ml volumetric flask. Add 80 ml of diluent sonicate to dissolve and dilute to volume with diluent. Further dilute 5 ml of above solution to 50 ml with diluent. Filter through 0.45µ Nylon syringe filter.

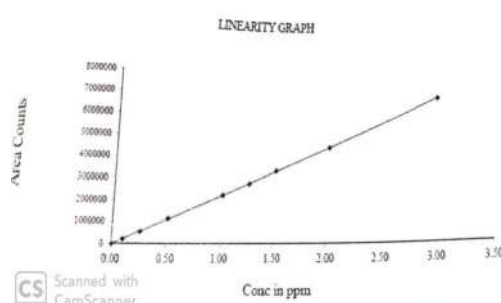
PROCEDURE

Inject 5 µl of standard preparation five times and sample preparation in the Chromatograph. Record the Chromatograms and measure the peak responses of Metformin Hcl, Atorvastatin and Glimepiride. The system suitability parameters should be met. From the peak responses, calculate the content of Metformin Hcl, Atorvastatin and Glimepiride in the sample.

METHOD VALIDATIONS

LINEARITY

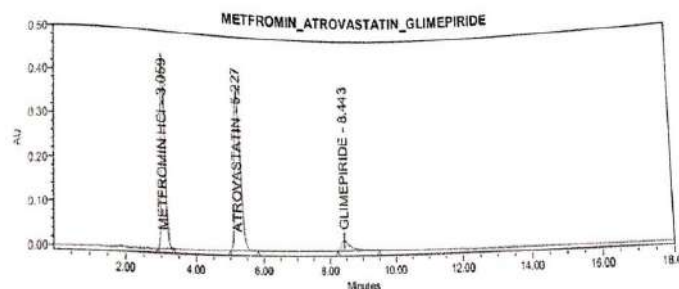
Linearity of detector responses for metformin, glimepiride and atorvastatin was established. 6 concentrations ranging from 0 µg/ mL to 14 µg/ml ,1µg/ml to 28µg/ml, and 1µg/ml to 17µg/ml of the target concentration were prepared. The final concentration of each solution in µg/ml was calculated and plotted against area response. The slope, y-intercept, correlation coefficient (R) were calculated.



Graph 1: Graph representing Linearity of results obtained.

ACCURACY

A study of accuracy was conducted for Metformin Hcl, Glimepiride and Atorvastatin in triplicate (50%, 100%, and 150%) as per test method with equivalent amount of drug containing into each volumetric flask for each spike level to get the concentration of Metformin, Glimepiride and Atorvastatin equivalent to 50%, 100% and 150% of the labelled amount.



Graph 2: Graph showing accuracy obtained for drugs

ROBUSTNESS

The robustness of the proposed method was determined by analysis of aliquots from the homogenous lots by different physical parameters like flow rate, mobile phase, composition, wave length which may differ but the responses are within the specified limits of the assay.

LOD AND LOQ

LOD and LOQ were calculated by the method based on the standard deviation (σ) and slope of the calibration curve, using the formula

$$\text{LOD} = 3.3 \sigma/S$$

$$\text{LOQ} = 10 \sigma/S$$

Where,

σ = the standard deviation of the response

s = the slope of the calibration curve.

Table showing results obtained for LOD and LOQ values.

SAMPLE	LOD	LOQ
Metformin Hcl	10.1	10.1
Atorvastatin	50.2	50.2
Glimepiride	10.2	10.1

Table 2: Table showing LOD and LOQ Values

Solution stability

The solution stability of Metformin, Glimepiride, and Atorvastatin diluents were determined by storing sample solutions in a tightly capped volumetric flask at room temperature for 24 hrs. The amount of Metformin Hcl, Glimepiride, and Atorvastatin were measured at different time intervals at 6, 12, 18, and 24 hrs and results obtained were compared with freshly prepared solutions of Metformin Hcl, Glimepiride, and Atorvastatin.

Forced degradation studies

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, thermal and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient. Regulatory guidance in ICH Q2A, Q2B, Q3B, and FDA 21 CFR section 211 requires the development and validation of stability indicating potency assays.

DISCUSSION

In HPLC method, the conditions were optimized to obtain adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to separate compounds. Mobile phase and flow rate selection was based on peak parameters, run time, resolution. The system with buffer acetonitrile (85:15 v/v) with 1.0 mL/ min flow rate is quite robust. The optimum wave length for detection was 258 nm at which better detector response for all the drugs was obtained. The retention times for Metformin Hcl, Glimepiride, and Atorvastatin was found to be 3.066, 8.457 and 5.237 min respectively. The linearity of Atorvastatin, Glimepiride and Metformin Hcl was carried out at different concentrations ranging from 0-3.03 µg/mL and 0 – 150.60 µg/ml. Correlation coefficient was found to be 0.9999, 0.99967, 0.99964 which indicates that the concentration had given good linearity. The % RSD values of Atorvastatin, Glimepiride and Metformin Hcl for system precision and Method precision was found to be 0.225, 1.527, 0.261 and 0.283, 0.765, 0.669 respectively. The results are within the acceptance limits of less than 2 % indicates that the proposed method has good reproducibility. The Robustness of the developed method was validated by changing the flow rate, mobile phase composition, and changing the wave length. The % RSD was found within the limits i.e, should not be more than 2.0.

CONCLUSION

The present investigation led to the development of new analytical method for the estimation of potent drugs Atorvastatin, Glimepiride and Metformin Hcl .All the methods developed provides a wide choice for the routine determination of Atorvastatin, Glimepiride and Metformin Hcl in bulk and pharmaceutical dosage forms.

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SP-118

ANTI-HISTAMINIC AND ANTI-CHOLINERGIC ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF PUNICA GRANATUM PITH

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ABSTRACT

Pomegranate a fruit of promise for its therapeutic purpose and considered as holy fruit since antiquity. The pomegranate is topical fruit, which thrives well under arid and semi arid conditions. A vast literature is available regarding the health beneficial properties of pomegranate fruit. This research is focused to characterize the bio active molecules from pith and carpellary membrane of pomegranate fruit. This research summarizes the Anti-Histaminic and Anti - Cholinergic activity of hydro alcoholic extract of pomegranate.

KEYWORDS: *Bio active molecules, carpellary membrane, Anti-histaminic, Anti-cholinergic.*

INTRODUCTION

Pomegranate is probably originated in Iran and Afghanistan and has been cultivated since ancient times throughout the Mediterranean basin to India, where it is still a fruit of commerce. It was considered as a holy fruit since antiquity by many religions. The plant is used in many ways like juice, dyes, inks, tannins for leather (bark) and in treating a variety of remedies for various ailments. The fruit has a fairly long shelf life at room temperature, and hence was carried on long journeys through desert climates as a source of water and nourishment. A vast literature is available regarding the health beneficial properties of pomegranate fruit. The major functional properties include antioxidant, antibacterial, antitumor, antiplatelet aggregation, antimutagenic, estrogenic property and many more. Pomegranate fruit contains variety of phenolic molecules like anthocyanins, ellagitannins, flavanoids etc. All the parts of pomegranate reported to have one or the other functional attribute towards human health. A clinical trial on healthy male volunteers by Aviram et al (2000) regarding the health beneficial properties of pomegranate fruit juice revealed that, in humans, pomegranate juice consumption decreased LDL susceptibility to aggregation and retention and increased the activity of serum paraoxonase (an esterase that can protect against lipid peroxidation) by 20%. In this present research the pith of pomegranate is screened for anti histaminic and anticholinergic activity.

HISTAMINE

Histamine is synthesized locally from the amino acid histidine and degraded rapidly by MAO and COMT. Histamine, meaning tissue amine (histo-tissue) is almost ubiquitously present in animal tissues and in certain plants, e.g. stinging nettle. As the pith of pomegranate fruit contains flavanoids the hydroalcoholic extract of pomegranate pith is employed in the study.

CHOLINERGIC SYSTEM

Acetylcholine is a major neuro transmitter autonomic, somatic as well as central sites. Acetylcholine is synthesized locally in cholinergic nerve endings and acts on respective receptors namely muscarinic and nicotinic. Based upon the review literature the pith of the fruit is selected for screening of anti histaminic and anticholinergic activity.

MATERIALS AND METHODS

PREPARATION OF EXTRACT

The pith of pomegranate was collected, shade dried and made into fine powder. Extraction is done by using continuous hot percolation method. 20 gm of powder was packed carefully in unit and (1:1) ration of hydroalcoholic solvent was used for extraction. Extraction is continued for 3-4 days at 70 °C and the solvent was filtered to free the marc. The solvate was heated in water bath to make solvent free and the resultant extract was collected and packed in a air tight container that is stored at 5-10 °C until usage.

ANTI HISTAMINIC ACTIVITY

Anti histaminic activity was screened using guinea pig (*Dunkin hartley*).

ANIMALS USED

Type : Guniea pig
Species : Dunkin hartley
Gender : Both genders
Weight : 300-500gms

Animals were supplied from Mahaveer enterprises, Hyderabad. Animals were housed cages at constant temperature (26+or -2⁰C) and 12-h light/12-h dark (8am-8pm). The guniea pigs were allowed to acclimatize to the laboratory environment 10 days before the study. In this period the animals are fed with standard pellet diet and water ad libitum. Experimental protocols were approved by the institutional animal ethics committee.

ANTI HISTAMINC PROCEDURE

The hydroalcoholic pomegranate pith extract was evaluated for their anti histaminic and anti muscarinic activity using guinea pig iluem. Standard procedure for setting up isolator tissue was used. The non-cumulative dose dependent response due to hiatamine was recorded. Dose response curve of histamine is recoreded by using guinea pig ileum at different doses (10, 20, 40, 60, 80, 100µgm/ml) . At a particular selected dose of histamine the response were recorded for the test dose 20,40,60,80 and 100 mg/ml concentration. The percentage of inhibition was calculated.

ANTICHOLINERGIC ACTIVITY

Anti cholinergic activity was screened using frog (*Rana tigrina*)

ANIMALS

Type : Frog
Species : Rana tigrina
Gender : Both genders
Weight : 300-500gm

Animal was produced from Mahaveera enterprises, Hyderabad. Animals were housed in cages at constant temperature (26 + or - 2⁰C) and at 12-h light/12-h dark (8:00 AM to 8:00 PM). Experimental protocol was approved by the institutional animal ethics committee.

ANTI CHOLINERGIC ACTIVITY

Standard procedure for setting up isolated tissue was used. The contractile response with increasing doses of acetylcholine in a sequence 10,30,100,300,1000 µgm/ml was recorded; so that same test of study in the presence of test compounds Punica granatum pith extract instead of vehicle was repeated. He potentiating orinhibiting effects of the test compunds on acetylcholine response was noted and the relative IC₅₀ values were calculated. The antagonism effect of the extract was measured in terms of the % inhibition concentration higher the dose ratio (>1) represents more specific antagonist effect of the extract.

RESULTS

ANTI HISTAMINIC ACTIVITY

Tables showing variation in height of responses observed when histamine is given at different

concentrations to guinea pig ileum and also percentage inhibition in response observed in presence of extracts is given in table 1.

CONCENTRATION ($\mu\text{g/ml}$)	HEIGHT OF RESPONSE (cm)	%INHIBITION
10	0.8	-
20	2.2	-
40	1.3	-
60	1	-
100	1	-
100+20	0.3	72%
20	0.2	81%
20	0.2	81%
20	0.2	81%

Table 1: variation in response when standard and extracts are given to tissue.

Formula for calculating percentage inhibition

Percentage inhibition was calculated for the following doses by using the equation

$$\% \text{Inhibition} = a - b / a \times 100$$

a=height of the histaminic response (in cm)

b=height of the test (or) standard response (in cm)

ANTICHOLINERGIC ACTIVITY

FORMULA

Effect of hydroalcoholic pith extracts on Cumulative Dose Response Curve (CDRC) of Acetylcholine (ACh 100 micro gm/ml) using frog rectus abdominis muscle and percentage inhibition in response observed in presence of extracts is as shown in the table 2:

CONCENTRATION	HEIGHT OF RESPONSE (cm)	%INHIBITION
Ach100 $\mu\text{gm/ml}$	4.7	-
20mg/ml	3.7	-
40mg/ml	4.1	12.76%
60mg/ml	4.5	4.25%
80mg/ml	4.7	-

Table 2: Height in response of acetyl choline obtained with standard and in presence of extracts

Formula for calculating percentage inhibition

Percentage inhibition was calculated for the following doses by using the equation

$$\% \text{ Inhibition} = a - b / a \times 100$$

a = height of the anticholinergic response (in cm)

b = height of the test (or) standard response (in cm)

DISCUSSION

Anti histaminic activity was done by using guinea pig ileum which is rich with histamine receptors. Responses were recorded after stabilising the tissue for one hour. Histamine was added at concentrations of 10, 20, 40, 60 and 100 $\mu\text{gm/ml}$ and the corresponding responses obtained were 0.8, 2.3, 1.3, 1.1 were ceiling effect is observed. When the extract at dose of 20 $\mu\text{gm/ml}$ was added to the tissue along with the standard histamine dose inhibition was observed initially at 72% but after subsequent washing and repetition of responses inhibition was found to increase to 81% where the repetition of response is also observed. Anti cholinergic activity was performed using frog rectus abdominus muscle which is with muscarinic receptors. The rectus abdominus muscle was stabilized for half an hour and responses were recorded. Dose response

curve for acetyl choline was recorded at doses of 20, 40, 60, 80 and 100 $\mu\text{g}/\text{ml}$ and responses observed were 3.7, 4.1, 4.5, 4.7, 4.7 respectively. Ceiling effect was observed at 80 $\mu\text{g}/\text{ml}$. The prepared extract was added at dose of 20 $\mu\text{g}/\text{ml}$ to all doses and inhibition was observed only at two doses namely 40 and 60 mg/ml . The percentage inhibition observed at 40 and 60 mg/ml was found to be 12.76 and 4.25 respectively. The extract could not show inhibition at other doses.

CONCLUSION

Pomegranate a fruit bearing deciduous shrub belonging to the family lythraceae. It is a fruit of high availability in India. Many researchers posted their article on different activities on fruit of pomegranate. The focus on pith of pomegranate was very less. Hence in this present research we made an attempt on focusing anti histaminic and anti cholinergic activity of pith of pomegranate using guinea pig and frog by using student organ bath. Anti histaminic activity was performed using guinea pig ileum taking histamine as standard drug. Observed at 20 $\mu\text{g}/\text{ml}$ where 81% inhibition was observed in response with the standard drug response which proves the efficiency in acting against histaminic receptors. The anti-histaminic activity may be reported due to presence of punicalagin which is a flavanoid. Anti cholinergic activity was performed using frog rectus abdominus muscle using acetyl choline as standard drug. The inhibition was response observed was deficient compared with histaminic response though inhibition was observed at 12.76% at dose of 40 $\mu\text{g}/\text{ml}$. According to the literature review the activity may also be due to the presence of punicalgin. The results may be more accurate and efficient if the drug was isolated purely by chromatographic method and further screening was performed.

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A SYSTEMATIC REVIEW ARTICLE ON CANCER CAUSING CHEMICALS PRESENT IN PLASTIC

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ABSTACT

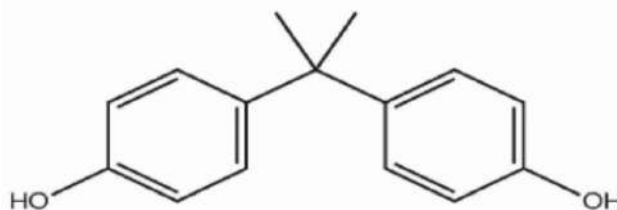
Plastics are widely used everywhere in our life and without plastic, modern civilization would indeed look very diverse. This study focuses on the toxic effects of plastic on human health and environment and possible consequences of health risk assessment in Bangladesh. Plastics are essential materials in modern civilization, many products manufactured from plastics and in numerous cases; they promote risk to human health and the environment. Plastics are contained many chemical and hazardous substances such as Bisphenol A(BPA), thalates, antinitroxide, brominated flame retardants, and polyfluorinated chemicals etc. which are a serious risk factor for human health and environment. Plastics are being used by Bangladeshi people without knowing the toxic effects of plastics on human health problems like irritation in the eyes, vision failure, breathing difficulties, respiratory diseases, liver dysfunction, cancers, skin diseases, lung problems, headache, dizziness, birth effect, reproductive, cardio vascular, genotoxic, and gastrointestinal causes for using toxic plastics. Plastics occurs serious environment pollution such as soil pollution, water pollution, air pollution. Applications of proper rules and regulations for the production and use of plastics can reduce the toxic effects of plastics on human health and environment.

KEYWORDS: *Plastics, Bisphenol A, thalates, antinitroxide, skin diseases, lung problems*

INTRODUCTION

When you expose plastic bottles to regular wear and tear, plastics release chemicals like BPA(Bisphenol A) or BPS(Bisphenol s). Even small amounts when ingested mimic estrogen, which can change the way your endocrine system functions. Prolonged exposures have been linked like chronic diseases, including diabetes, asthma and cancer. Plastic bottles are made with chemicals known as plasticizers. Their purpose is to make plastic strong and flexible. There are two main forms of plastic that make up plastic water bottles; polyvinyl chloride plastics (PVC) and polycarbonate plastics. PVC contains the most common used commercial plasticizers known as phthalates. Polycarbonate plastics contain a chemical called bisphenol-A (BPA). Both phthalates and bisphenol-A are known hormone disrupting chemicals, often called hormone mimicking compounds. Studies show that both phthalates and BPA have adverse health effects in humans and are linked to infertility, prematured puberty, asthma, allergies, menstrual cycle's irregularities and breast cancer and prostate cancer. Disposable water bottles can leach their hormones disrupting chemicals right into the water you drink from them. Heat or light exposure can speed up this process as can the amount of water spends in the bottle.

Bisphenol [BPA]



Bisphenol A [BPA] is an industrial chemical that has been used to make certain plastics and resins since the 1960s. BPA is found in polycarbonate plastics are often used in the containers that store food and beverages, such as water bottles. They may also be used in other consumer goods. Epoxy resins are used to coat the

inside of metal products, such as food cans, bottle tops and water supply lines. Some dental sealants and composites also may contain BPA.

HEALTH RISK OF EXPOSURE TO BISPHENOL A [BPA]

The chemical, Bisphenol A, or BPA, is used in the manufacture of hard, polycarbonate plastic for baby bottles, microwave cook wares and other consumer goods, and it has been detected in nearly every human body tested. Scientists and health experts have theorised for more than a decade that chemicals in the environment and in the consumer products mimic estrogens and may be contributing to male and female reproductive diseases, particularly prostate cancer. BPA is commonly used as monomer in polycarbonate synthesis, plasticizer in the production of epoxy resins, as well as an additive for the elimination of surfactant of HCl during the polyvinyl chloride (PVC) production. BPA is not only used in the production of plastics intended to a direct contact with food, including plastic package and kitchen ware, but also in inner coatings of can and jars. The BPA is metabolised in the liver to form Bisphenol A glucuronide and mostly in this form is excreted with urine. Therefore, BPA has been shown to play a role in the pathogenesis of several endocrine disorders including male and female infertility, precocious puberty, hormone dependent tumours such as breast and prostate cancer and several metabolic disorders including polycystic ovarian syndrome (PCOS). Because of constant, daily exposure and its tendency to bioaccumulation

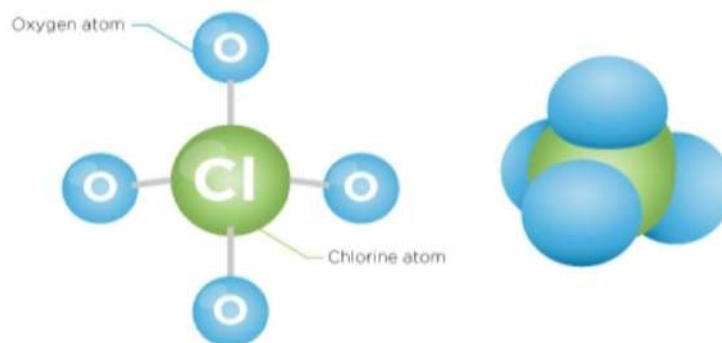
POLYVINYL CHLORIDE [PVC]

Polyvinyl chloride (PVC) a synthetic resin made from polymerisation of vinyl chloride. Second only to polyethylene among the plastics in production and from raincoats and used in plastic bottles, shower curtains to window frames and indoor plumbing. A light weight, rigid plastic in its pure form, it is also manufactured in a flexible "plasticised form". It does not matter if it is water or soda or juice that is inside. Plastic bottles are made with chemicals known as plasticizers. Their purpose is to make plastic strong and flexible. There are 2 main forms of plastic that make up plastic water bottles are Poly vinyl chloride plastics (PVC) and Poly carbonate plastics. Poly vinyl chloride exposure is associated with an increased risk of rare form of liver cancer, as well as brain and lung cancers, lymphoma and leukaemia.

POLYCARBONATE

Polycarbonate is a lightweight plastic with a unique combination of attributes that make it an ideal material for use in a wide variety of applications. Included are a number of home and kitchen applications involving direct contact with food and beverages that take advantage of polycarbonates inherent shatter-resistance, optical clarity, and heat resistance. Common examples include reusable 5- gallon water bottles, baby bottles, tableware such as plates and cups, and containers for storing food and reheating in a microwave oven. The primary building block used to make polycarbonate plastic is BPA. Many researchers have studied the potential for trace levels of BPA to migrate from polycarbonate into food and beverages under conditions typical for uses of polycarbonate products. There is a potential that certain types of polycarbonate could be hazardous in food contact situations due to the release of BPA during hydrolysis. The most commonly made types of polycarbonate are created by the combination of BPA and COCl_2 . Due to the BPA present in polycarbonate there is a high risk of leaching in to the food content and when the BPA leached water or food is consumed at high rate it may lead to cancer, it is also an endocrine disruptor.

PERCHLORATE



The packaging material can be used for fines or raw materials before or during processing. Even if the final product is a liquid, raw material such as rice, wheat, sugar, starch, or spice may have contacted the perchlorate-laden plastic. The petition asked FDA to reverse its 2005 decision and ban use of perchlorate. The salt within perchlorate is a toxin, but there is disagreement about how much is bad for you when ingested. The U.S Environmental protection Agency is creating drinking water standards for the pollutant. There is general agreement that exposure to perchlorate is a big risk. It interferes with the way the body takes iodide into the thyroid and can disrupt the glands regulation of metabolism. In adults, the thyroid helps to regulate metabolism. Impairment of thyroid function in expectant mothers may impact the foetus and new born and result in effects including changes in behaviour, delayed development and decreased learning capability. Changes in thyroid hormones levels may also result in thyroid gland tumours.

ALTERNATIVES FOR PLASTIC WATER BOTTLES

One of the worst culprits of single-use plastic waste is plastic water bottles that are discarded as soon as the drink has been finished. According to campaigners, Ban the Bottle, plastic water bottles can take between 400 and 1,000 years to decompose. Furthermore, it takes three times the amount of water to produce a plastic bottle than it does to fill one. The plastic bottles which are in the market which says BPA free bottles are also having some harmful chemicals which leaches in the food and give any toxic effects to the body.

PAPER WATER BOTTLES



The paper water bottle is made up of biodegradable pulp mixture of wheat straw, bamboo, husks, sugarcane, and bulrush. The shape is completely customizable. It is important that the design and prototype is the first generation of paper water bottle. Our eventual goal for paper water bottle is to attain a package that is Backyard Compostable. The paper water bottle is currently available in 500ml size (17 oz), and alter, other sizes 70, 250, 750, 1000 and 2000ml will be added. The material can be printed using soy and other eco-friendly links.

GLASS WATER BOTTLES

GLASS WATER BOTTLES



Glass is 100% recyclable and it can be recycled over and over again without losing its quality. For this reason, glass is known as one of the eco-friendly materials. A glass reusable bottle is also much easier to keep clean since it can however, it is heavier and more expensive than other eco-conscious options, so keep this in mind when choosing the right reusable bottle for your lifestyle. Glass water bottles are the best choice for safe, toxin free, clean tasting drinks. Find the best glass water bottle range of Biome. Lifefactory, Camelbak, BBYO and Full Circle high quality glass drink bottles with colourful protective silicone sleeves. Glass is free of harmful chemicals found in many plastic bottles. No chemical leach from the glass,

so they are very safe and good for you. Glass water bottle are also the best for juices, smoothies, milk and tea.

COPPER WATER BOTTLES



Copper is found to possess a plethora of health benefits, including weight loss. It has the potential to kill the microorganisms present in the water that are harmful to the body. When you store water in a copper vessel, the copper gently leaches into the water and lends all its positive properties, thereby creating a natural purification process. Copper bottles are great alternatives to plastic bottles. It is good for health and great for the environment. For ages, people in India have used copper pots to consume water. There are a lot of benefits in using copper bottle. It helps in improving overall immunity and health. It is a metal present in trace levels in the body and is needed for many key metabolic activities. Copper does not contains any type of chemicals which are harmful. Copper fight against the cancer and also copper has an antimicrobialproperties. Copper is an essential nutrient for the body and at any point in time, the human body has about 0.9mg of copper within it. This is required for the body as it helps make and repair red blood cells and is responsible for the health of the nerve cells. It is also required in the upkeep of the immune system. Thus, it is always advisable to store drinking water in copper bottles or vessels as it will be far more beneficial than drinking water from plastic bottles.

CONCLUSION

The research has highlighted the range of chemicals that we could be exposed to from packaging. Many of them come from groups of related chemicals of concern such as bisphenols and phthalates. Often one or two chemicals in a group have been banned or partially banned, but others are still in use. Alongside this, even for chemicals for which hazards have been identified, there is lack of harmonised toxicology information. Both of these issues need to be addressed urgently for the purpose of identifying and removing hazardous chemicals from plastic packaging to protect human health and the environment. Regulators and industry need to stop moving from one chemical to another within the same group, and instead move to safe alternatives.

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FORMULATION AND EVALUATION OF PRAVASTATIN ORAL THIN FILMS

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ABSTRACT

In the present study oral thin films of Pravastatin were developed to have a faster on set of action. The oral thin films were developed by using polymers HPMC E5, HPMC E 15 and PVP K90. Oral thin films were prepared by employing solvent casting method. Propylene glycol was selected as permeation enhancer and plasticizer. FTIR was used to perform drug excipient compatibility studies, it was observed that there were no interactions. Varying concentrations of polymers ranging from F1-F6 were used for preparing the formulations. Among all the 6 formulations F1 formulation which contain HPMC E15 200mg and shown 97.2. % cumulative drug release within 30 min. And compared to HPMC E15, HPMC E5 and PVP K90, HPMC E 15 showed better drug release profile.

KEYWORDS: *Pravastatin, oral thin films, solvent casting, HPMC E15, HPMC E5, PVPK90.*

INTRODUCTION

Oral thin films for oral administration are used for the patients who experience difficulty in swallowing tablets and capsules. Geriatric and pediatric patients find it difficult to swallow solid formulations. For such patients to overcome this problem oral thin films are of major use. Formulation of oral thin films started in early 1970's ⁽¹⁾. Oral thin films do not require water for its administration. They get hydrated when come in contact with water. They adhere to the mucosa and dissolve within one minute. Patients compliance is more ⁽²⁾. Oral route of drug administration is the most common and popular route of drug delivery. Almost 60% of all dosage forms available in the market are oral solid dosage forms, but the effectiveness of such drugs get reduced when administered through the per-oral route. In the present experiment pravastatin is used. Pravastatin is structurally similar to HMG a substrate of the endogenous substrate of HMG Co-A reductase. It acts by reversible inhibition of HMG Co-A reductase activity; it effects modest reductions in intra cellular pools of cholesterol which increases the number of LDL receptors on cell surface and enhance receptor mediated catabolism and clearance of circulating LDL. Pravastatin also inhibits LDL production by inhibiting hepatic synthesis of VLDL, the LDL precursor ³.

MATERIALS AND METHODS

Pravastatin was procured from TCI Chemicals, Chennai. HPMC Polymer, PVP K90, Propylene Glycol was procured from Bross scientifics, Tirupati. Aspartame, citric acid was procured from S.D. Fine Chem Ltd., Mumbai.

METHOD OF PREPARATION

DEVELOPMENT OF ORAL THIN FILMS

SOLVENT CASTING METHOD

Solvent casting method is the method used for the preparation of oral thin films. Film forming polymers like HPMC E5 and HPMC E15 were selected and weighed in required ratios and they were then dissolved in water (cold water) as solvent. Pravastatin (100 mg), propylene glycol was added to the above dispersion under continuous stirring. The uniform dispersion was poured in the petri plate. The rate of evaporation of solvent was controlled by inverting cut funnel over the thin films. After 24 hours, the dried thin film were taken out and stored in desiccators, and the formulations prepared were shown in table no 1.

Table no.1: Formulation of pravastatin oral thin films

S.No	Ingredients	F1	F2	F3	F4	F5	F6
1	Drug(mg)	100	100	100	100	100	100
2	HPMC E 15 (mg)	200	250	---	---	---	---
3	HPMC E 5 (mg)	---	---	200	250	---	---
4	PVP K90	---	---	---	---	200	250
5	Propylene glycol(ml)	0.3	0.3	0.3	0.3	0.3	0.3
6	Citric Acid	0.1	0.1	0.1	0.1	0.1	0.1
7	Aspartame	0.1	0.1	0.1	0.1	0.1	0.1
8	Water	15ml	15ml	15ml	15ml	15ml	15ml

All the ingredients were taken in mg.

EVALUATION^{4,5}

Organoleptic evaluation

For evaluation of psychophysical evaluation of the product, special controlled human taste panels are used. In-vitro methods for utilizing the taste sensors, especially designed apparatus and drug release by modified pharmacopoeial methods are being used for this purpose. These assessment apparatus and methodologies are well suited for high-throughput taste screening of oral pharmaceutical formulations.

Thickness measurement

Thickness of the film is measured using a dial gauge tester and results are shown in table no 3.

Disintegration time

It is the time at which the film begins to break down when brought into contact with water. It is determined by using Petri dish methods.

Measurement of folding endurance

The strip of film is repeatedly folded at the same place until it breaks. The number of times the film is folded at the same place prior to breaking gives the folding endurance and are shown in table no 3.

Assay

The assay was performed in order to ensure the loading of drug into each film. This test was performed by dissolving a 6 cm² 6.8 phosphate buffer with stirring. The resultant solution was filtered using a whatman filter paper, and the filtrate was diluted to 100 ml with the same buffer in a volumetric flask. Then 1 ml of the filtrate was further diluted to 10ml with buffer. This solution was analyzed using a spectrophotometer at 259 nm⁽⁶⁾ and results are shown in table no 3.

Content uniformity

The content uniformity test was used to ensure that every film contains the intended amount of drug substance with little variation among films area of film in 50 ml of a patch. Three pieces, each 4 cm²cm), were cut from the whole patch, and assayed for drug content. Same procedure was repeated for all the nine batches.

In vitro dissolution

The in vitro drug release study of film was carried out using a USP 23 type 2 rotating paddle dissolution test apparatus. 250ml of phosphate buffer (pH 6.8) was used, and maintained at 37±5°C at 50 rpm. A film sample of 4 cm sq was fixed onto the specially designed SS disk with the help of cyanoacrylate adhesive. The disk was put at the bottom of the dissolution vessel so that the patch remained on the upper side of the disk. Five milliliters of samples were taken at a interval of 60 sec., and the same amount was replaced with fresh buffer. The withdrawn samples were filtered through whatman filter paper and then 1ml of the filtered

sample was further diluted to 10ml of the same medium and analyzed using a spectrophotometer at a wavelength of 259 nm. The cumulative percentage release for different formulations was calculated. The relationship between time and percentage release were plotted in figure no 1.

Moisture uptake ⁷

The test is done to determine the percentage of moisture uptake. Percentage of moisture uptake can be calculated as follows and results are shown in table no 3.

Percentage of moisture uptake = $\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$.

Moisture content

Percentage of moisture content can be calculated as follows, and results are shown in table no 3.

Percentage of moisture content = $\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$

Tensile strength

Tensile strength of the film is determined by using a tensile testing machine like the Instron or Monsanto tester. It is calculated by the equation below.

Tensile strength = $\frac{\text{Load Failure}}{\text{Area}} \times 100$

Swelling Property

The following formula is used to calculate the degree of swelling.

$$\text{Swelling Index (SI)} = \frac{(W_t - W_o) \times 100}{W_o}$$

W_t is the weight of the film at time "t" and W_o= weight of the film at t = 0.

Percent elongation

It is determined by using the following equation

Percentage of Elongation = $\frac{\text{Increase in length of strip}}{\text{Initial length of strip}} \times 100$

Tear resistance

Tear resistance of film is the intricate function of its ultimate resistance to rupture. Maximum force required to tear the film is measured as tear resistance value. This test is typically attributed to plastic industry. The rate of loading employed is 2 in/min which is planned to determine the magnitude of force required to initiate tearing in the film specimen. The maximum amount of force necessary for tearing is generally found near the tearing onset which is ranked as tear resistance value.

Drug excipients interaction studies

FT-IR spectrum interpretation : IR spectral analysis was carried out using FT-IR by the KBr disc method. The sample and KBr were triturated and compressed to get the discs. The samples of pure drug, dummy formulation and optimized formulation were analyzed between wave numbers 4000.0 and 400.0 cm⁻¹.and results are shown in figure no 2 & 3.

RESULTS AND DISCUSSION

Standard Calibration curve of Pravastatin

It was found that the estimation of Pravastatin by UV spectrophotometric method at λ_{max} 259 nm in 6.8 pH saline phosphate buffer and had good reproducibility and this method was used in the study. The correlation coefficient for the standard curve was found to be closer to 1, at the concentration range, 2-12 µg/ml and results are shown in table no 2 .

Table2. Calibration curve of pravastatin in pH 6.8

S. No.	Concentration (µg/ml)	Absorbance (at 259 nm)
1	2	0.128
2	4	0.267
3	6	0.456

4	8	0.589
5	10	0.762
6	12	0.963

Evaluation of Pravastatin oral thin films

Physical appearance

All the Oral thin films were visually inspected for colour, clarity, flexibility⁽⁸⁾. All the Oral thin films were found to be flat without any foam and results are shown in table no 3. The folding endurance is more for HPMC E15 followed by HPMC E5 and PVP K 30. The tear resistance, transparency, percentage elongation, drug content and tear resistance is more for the F1 formulation when compared to other formulation. The swelling index of all the formulations is lies between 93-102.

Table 3: Evaluation of Oral thin films by using Physical methods

Formulation	Thickness (mm)	Folding endurance	Drug content (%)	Moisture uptake (%)	Moisture content (%)	Weight variation
F1	0.3569	20	95	7.98	3.77	27.46
F2	0.3520	25	65	5.05	9.2	32.57
F3	0.3470	27	87.5	3.09	5.16	29.21
F4	0.3496	24	80	5.63	5.66	33.65
F5	0.3460	30	72.5	3.73	4.87	28.39
F6	0.3517	32	92.5	9.65	12.67	35.53

Tensile strength

The patches (10 samples of each) were dried at 60°C for 24 hrs. Then they were placed in an isometric transducer and the force required for their rupture was measured by an oscillograph. The tensile strength of the patch was found to be 1.63 gm/cm².

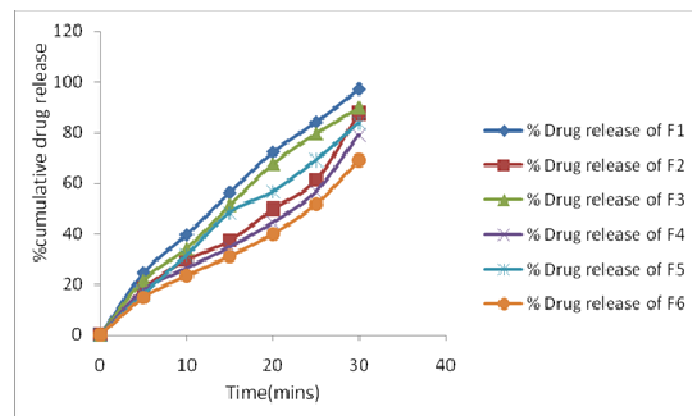


Figure 1: Dissolution graph of all formulations (F1-F6)

FTIR

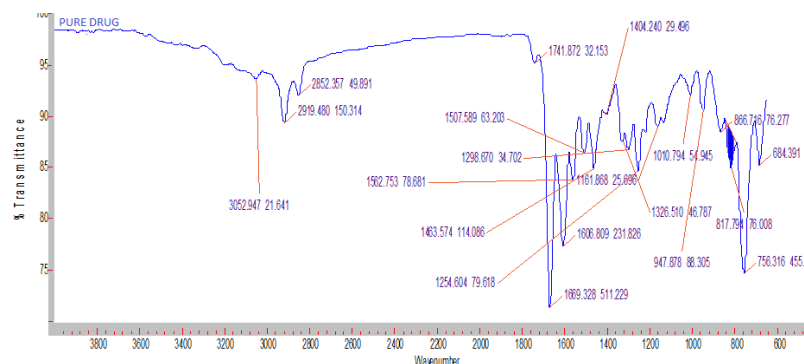


Figure 2: FTIR spectrum of pure drug

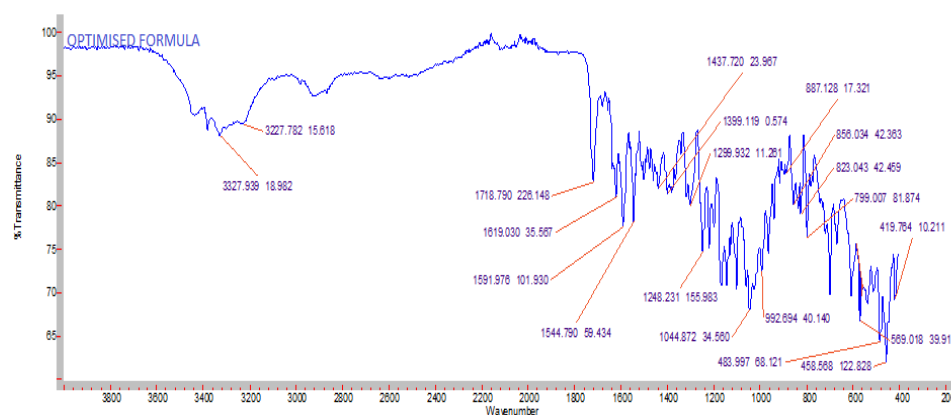


Figure 3: FTIR spectrum of optimised drug

CONCLUSION

In present study oral thin films of Pravastatin were developed to have a faster on set of action. The oral thin films were developed by using polymers HPMC E5, HPMC E 15 and PVP K90. Oral thin films were prepared by employing solvent casting method. Propylene glycol was selected as permeation enhancer and plasticizer. FTIR was used for drug excipient compatibility studies and it was observed that there were no interactions. Formulations were prepared with the varying concentrations polymers ranging from F1-F6, and all the formulations were evaluated for various physical parameters Physical appearance, Weight variation, Thickness, Folding endurance, Tensile strength, Drug content, Moisture uptake, Moisture content and all the results were found to be with in the pharmacopeial limits, invitro drug release studies by using dialysis membrane. Among all the 6 formulations F1 formulation which contain HPMC E15 200mg and shown 97.2. % cumulative drug release within 30 min. And compared to HPMC E15, HPMC E5 and PVP K90, HPMC E 15 showed better drug release profile.

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ASSOCIATION BETWEEN TYPE-II DIABETES MELLITUS AND ABO AND RHESUS BLOOD GROUPS: A CASE-CONTROL STUDY

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects of insulin secretion and/or insulin cellular resistance and is the leading cause of morbidity and mortality. The ABO blood groups often associated with several diseases with one blood group more often seen with the patients of a particular disease. The aim of this study was to determine the relationship between ABO & Rhesus blood groups and Type-2 diabetes mellitus in the local population of Visakhapatnam. The present study was a hospital based cross-sectional, case-control study, conducted over a period of six months in out-patient units of endocrinology department of a tertiary care hospital. Patient demographics were obtained from various sources and ABO blood grouping for both the patient groups was determined by using blood grouping Anti-Sera kit and was documented in a suitably designed data collection form. A total of 332 patients with Type-II DM and 200 age and sex matched healthy controls were included. In cases group, out of 332 patients 174(52.4%) were males (vs 113(56.5%) controls) and 158(47.5%) were females (vs 87(43.5%) controls) and about 289(87.04%) patients were found to be Rh+ve type (vs 178(89%) controls). About 91(27.4%) were found to be B-positive (vs 28(14%) controls), 87(26.2%) were O-positive (vs 69(34.5%) controls), and 73(22%) are A-positive (vs 62(31%) controls), 38(11.45%) are AB-positive (vs 19(9.5%) controls), 14(4.2%) are O-negative(vs 3(1.5%) controls), 11(3.3%) are A-negative(vs 10(5%) controls), 10(3.0%) are AB-negative(vs 3(1.5%) controls), 8(2.4%) are B-negative(vs 6(3%) controls). There is an association between ABO antigens and Rhesus blood groups with Type-II DM. DM are more common in patients with B blood group and Rh-Positive.

KEYWORDS: *ABO & Rh blood groups, Type-2 diabetes mellitus, cases and control groups*

1. INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disorder characterized by increased levels of glucose in blood due to defective production of insulin. Type-II DM is considered to be the most predominant form of diabetes accounting for about 90 percent of all cases globally.¹⁻³ India is the country with largest number of diabetic people with around 32 million in 2015 and is predicted to be increased to 80 million by the year 2030.² Type-II DM results in failure of multiple organ systems thus leading to morbidity and mortality imposing a major burden on the patient and the health care team^{1&2}. Many studies were conducted to discover a possible association between ABO and Rh blood groups and Type-II DM and reported that there is a strong relation between Type-II DM and ABO blood groups.⁴ However, very little research was conducted in India and therefore, our study aims to determine the frequency and distribution of blood groups in patients with Type-II DM in Visakhapatnam.

OBJECTIVES OF THE STUDY

Major objective: To investigate any association between ABO and Rhesus blood groups and Type-2 Diabetes mellitus (D.M) in the population of Visakhapatnam city visiting the study site

Minor objective: To determine & compare the frequency & patterns of distribution of ABO & Rh blood groups in patients with Type-2 DM (cases) and in those without Type-2 DM (Controls)

2. MATERIALS & METHODS

Study site: The study conducted in the Department of Endocrinology, King George Hospital, Visakhapatnam.

Study population: In our study, we included the out-patients diagnosed with Type-II diabetes mellitus who attended the department of endocrinology, King George Hospital, Visakhapatnam.

Study duration Study was conducted for a period of six months i.e., from November 2018 to April 2019.

Sample size: 332 cases (Type-II Diabetic patients) and 200 healthy control subjects were selected for this study to compare distribution of ABO blood groups.

Study criteria

Inclusion criteria

Subjects of either sex (Males & Females) diagnosed with Type-II DM (age group of 20-60 years) who are attending the out-patient wards of endocrinology department of KGH hospital were included in our study.

Exclusion criteria

1. In-patients and patients with other forms of diabetes i.e., Type-1 DM as defined by ADA, gestational DM.
2. Patients who are aged below 20 yrs & elderly (>60 yrs) and patients with a history of diabetic ketoacidosis (DKA)

Study design: The present study was a prospective; hospital based cross-sectional case-control study.

Study approval: Prior approval for the study was obtained from the Institutional Human Ethical Committee (IHEC), Andhra University, Visakhapatnam on 05-11-2018, and Approval No: IEC/Pharm.D 07

Study Procedure

Patient data of both the study groups (cases and controls) i.e., demographics, blood group, laboratory investigations, diagnosis of type-II DM (for cases) was obtained from patients medical records, and by interviewing the patients and their care takers.

Upon physician's advice, Blood grouping was done by using Anti-Sera kit (Mediclone-B; Mediclone-A; Spanclone-Anti-D Monoclonal). Following this, blood grouping data of controls were taken from Patients who attends out patient departments of Endocrinology and KGH-Blood bank. Data analysis was done after data was entered into excel sheet and double checked for errors.

3. RESULTS AND DISCUSSIONS

A total of 532 people were included into the study out of which 332 patients who met our study criteria with type-2 diabetes mellitus were included into the study as case population and 200 Non-diabetic people were included as control population. Gender wise distribution of the overall study population has been tabulated in Table-1. Age-wise distribution of study population has been tabulated in table-2. Association of ABO & Rhesus blood groups in patients with Type-II diabetes mellitus in Case-control population has been tabulated in table-3&4 respectively. Frequency distribution of blood groups in both cases and control groups has been tabulated in table-5. Prevalence of blood groups in Males; females and overall study population has been shown in figure-1; 2&3 respectively. Results of this study indicated that Rh positive is high frequent in diabetes in which AB blood group is less frequent while those with blood groups B & O are more frequent and blood group O and B were more likely to get diabetes and people with AB group were less likely to get diabetes. In controls those with blood groups O & A are more frequent and those with blood group AB is less frequent in non diabetic population.

Demographics of overall study population

Table-I: Gender-wise distribution of the overall study population (cases and control groups)

Study groups	Males n (%)	Females n(%)	Total n (%)
Cases group	174 (52.4)	158 (47.5)	332 (62.4)

Control group	113 (56.5)	87 (43.5)	200 (37.6)
Total	287	245	532

Table-II: Age-wise distribution of study population.

Age (years)	Cases group n(%) =332	Control group n (%)=200	Total
20-39	73 (21.98)	127 (63.5)	200
40-59	223 (67.16)	71 (35.5)	294
60-79	36 (10.84)	02 (1)	38

Table III: Association of ABO & Rhesus blood groups in patients with Type-II diabetes mellitus (cases)

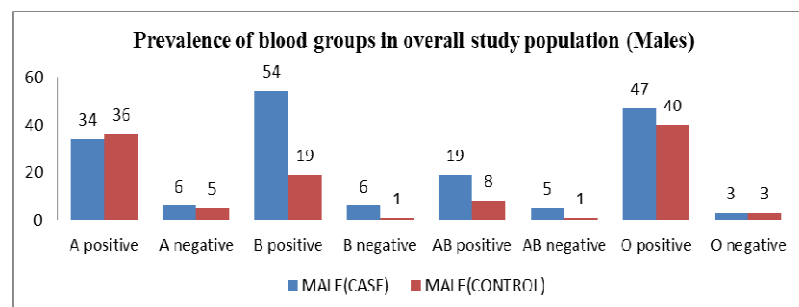
BLOOD GROUP	RHESUS FACTOR		Total
	Rhesus positive(+ve)	Rhesus -ve	
O	87	14	101
B	91	8	99
A	73	11	84
AB	38	10	48
TOTAL	289	43	332

Table -IV: Association of ABO & Rhesus blood groups in patients without Type-II diabetes mellitus (controls)

BLOOD GROUP	RHESUS FACTOR		Total	
	Rhesus positive(+ve)	Rhesus -ve		
O	69		3	72
B	28		6	34
A	62		10	72
AB	19		3	22
TOTAL	178		22	200

Table -V: Frequency distribution of blood groups in both cases and control groups

S.No	Blood group	Cases n (%)	Controls n (%)	Row total
1.	B positive	91 (27.4)	69 (34.5)	160
2.	O positive	87 (26.2)	62 (31)	149
3.	A positive	73 (22)	28 (14)	101
4.	AB positive	38 (11.45)	19 (9.5)	57
5.	O negative	14 (4.2)	10 (5)	24
6.	A negative	11 (3.3)	6 (3)	17
7.	AB negative	10 (3)	3 (1.5)	13
8.	B negative	8 (2.4)	3 (1.5)	11
	Total	332	200	532

**Figure 1: Prevalence of blood groups overall study population (Males)**

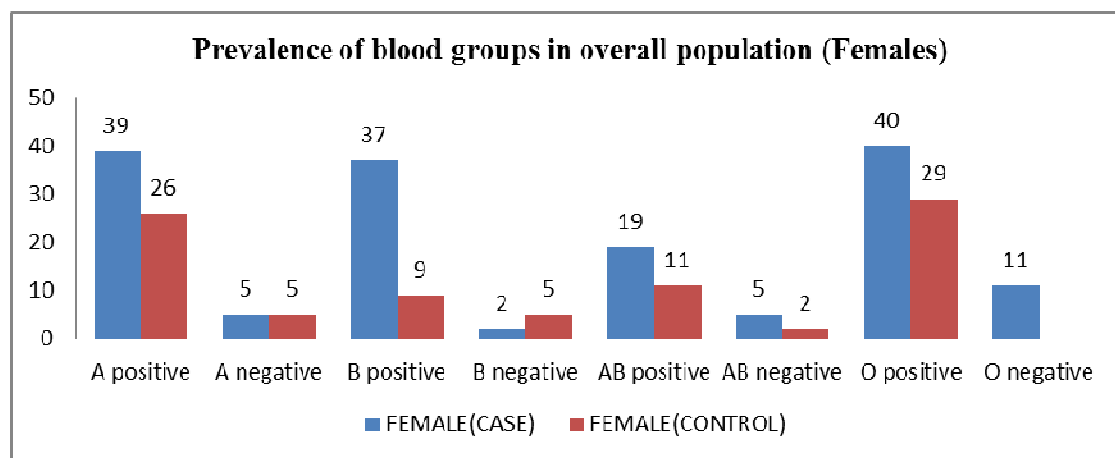


Figure II: Prevalence of blood groups in overall population (Females)

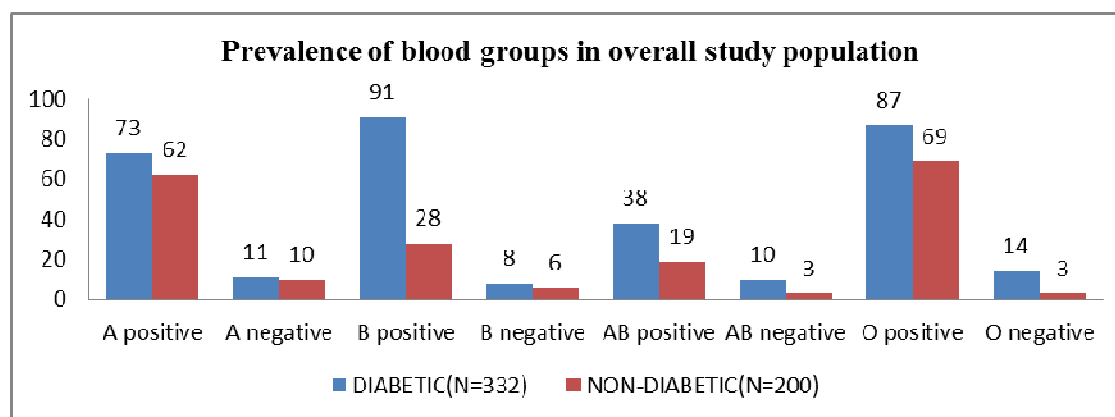


Figure III: Prevalence of blood groups in overall study population.

CONCLUSION

We found that in Type-II Diabetes Mellitus patients, B positive blood group is more frequent than other blood groups, whereas in Non-diabetic patients (control group) O positive blood group was found to be more frequent. Also, Rhesus +ve were found to be more predominant in both cases and controls (87.04% and 89%) respectively. The findings of our study suggest that ABO antigens are associated with Type-II DM. Diabetes mellitus is more common in individuals with B blood group and Rh-positive (B-Positive).

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A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND LOSARTAN POTASSIUM IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A specified, accurate and exact RP-HPLC method was developed for the estimation of Hydrochlorothiazide and Losartan Potassium in pharmaceutical dosage form. The novel method was developed by make use of waters HPLC System on a Zodiac C18 (250 cm x 4.6 mm, 5 µm) column. The Mixture of 0.01% Ortho Phosphoric Acid: Acetonitrile 70:30 v/v mobile phase is used at a flow rate of 1.5 ml/min. The injection volume selected about 20 µl. The wavelength detection is carried out at 220 nm. The retention time of Hydrochlorothiazide and Losartan were found to be 3.1 min and 7.2 min respectively. The developed RP-HPLC method was validated as per ICH guidelines for the estimation of both the drugs in bulk as well as in pharmaceutical dosage forms. The developed method was validated with regard to Accuracy, Precision, Linearity, Robustness and Ruggedness. Hence, the proposed method was successfully used for the simultaneous determination of Hydrochlorothiazide and Losartan Potassium.

KEYWORDS: *RP-HPLC, Losartan Potassium (LOS) and Hydrochlorothiazide (HCTZ)*

INTRODUCTION

Hydrochlorothiazide¹⁻³ Is A Diuretic Medication Often Used To Treat High Blood Pressure. For High Blood Pressure It Is Considered As First-Line Treatment, Although Chlortalidone Is Effective With A Similar Rate Of Adverse Effects. HCTZ Is Combined With Other Blood Pressure Medication As A Single Pill To Increase Effectiveness. It Is Frequently Used For The Treatment Of Hypertension, Congestive Heart Failure, Symptomatic Edema, Diabetes Insipidus, Renal Tubular Acidosis. The Thiazide-Type Diuretics (Including Hydrochlorothiazide) Are Less Effective Than The Thiazide-Like Diuretics (Chlortalidone And Indapamide) For Reducing The Risk Of Heart Attack, Stroke, And Heart Failure In Persons With High Blood Pressure. HCTZ Is Also Sometimes Used To Prevent Osteopenia And For Treatment Of Hypoparathyroidism, Hypercalciuria. Thiazides Are Also Used In The Treatment Of Osteoporosis. It Decreases Mineral Bone Loss By Promoting Calcium Retention In The Kidney, And By Directly Stimulating Osteoblast Differentiation And Bone Mineral Formation. It May Be Given With Other Antihypertensive Agents In Combination Preparation, Such As In LOS/HCTZ. Losartan Potassium Is An Orally Active, Nonpeptide Atii Receptor Antagonist. The Novel Agent Bind Competitive And Selective To The Aii Subtype 1 Receptor, Hence Blocking Aii-Induced Receptor. An Active Metabolite - E3174, Contributes Antihypertensive Effect. Hence, Present Aim Of Our Work Is To Develop A Specific, Precise, Accurate, Linear, Simple, Rapid And Cost Effective Stability Indicating HPLC Method⁴⁻¹⁰. The Scope Of Our Work Also Extends To Validation For The Development Methods.

MATERIALS AND METHODS

Chemicals

Zorbax Cn, Hypersil Silica, Acetonitrile (HPLC Grade), Triethylamine Buffer, Sodium Acetate, Dil. Sodium Hydroxide, Hydrochlorothiazide, Losartan Potassium, Dis. Water (Distilled Grade).

Equipments

Waters HPLC With Empower – 2 Software, Zodiac C18(250 X 4.6mm, 5µm) Column. The Mixture of 0.01% OPA : Acetonitrile 70:30 V/V At A Flow Rate 1.5 ml / Min Are Used As A Mobile Phase. The

Injection Volume Was 20 μ l. The Run Time Was 10min.

Preparation Of Standard Stock Solution

The Standard Stock Solution Was Prepared By Adding 20 Mg Of HCTZ And 80 Mg Of Losartan Potassium Were Accurately Weighed And Were Taken Into 100 Ml Volumetric Flask. 70 Ml Of Diluent Were Added And Was Sonicated To Dissolve. It Was Cooled To Room Temperature And The Volume Was Made Upto 100 Ml With Diluent.

Preparation of Standard solution (further dilution)

The standard solution was prepared by adding 4ml of standard stock solution was pipetted out into 100 ml volumetric flask and the volume was made upto the mark with diluent.

Preparation of Sample solution

Weighed accurately about five tablets and transferred into a 250 ml volumetric flask. To that 200 ml of diluent is added and it was sonicated for 30 min with vigorous shaking. Then it was cooled to room temperature and the volume was made-up with diluents upto 100 ml. From that 4 ml of the solution was taken and diluted to 100 ml with diluents and mixed. The obtained solution was filtered through 0.45 μ nylon filter.

Validation procedure

The standard preparation of 20 μ l for 5 times and also the sample preparation were injected into the chromatographic system. Chromatograms were read and peak responses for HCTZ + Losartan potassium were measured. The system suitability parameters were met from the peak responses the content of HCTZ + Losartan potassium in the sample was calculated.

System Suitability

The system suitability parameters were evaluated from standard chromatograms obtained. The parameters include retention times, tailing factor, theoretical plates and % RSD are calculated and found to be in limits.

Linearity and Range

Linearity means an analytical method in which the obtain results are directly proportional to the concentration of the analyte in the sample within a range. The 6 series of standard solutions are selected for the estimation of linearity range. The calibration curve was plotted by using a peak area (vs) concentration of the standard solution and the regression equation is calculated.

Precision

An analytical method is the degree of reproducibility among individual test results is known as Precision. It was measured by the analysis of multiple sampling of homogeneous sample. The precision of the present method was calculated in terms of repeatability, intraday and interday variations. It was estimated by analysing the samples at various time intervals of the same day as well as on other days.

Accuracy

The closeness of the test results obtained by the method to the true value is known as Accuracy. The accuracy is measured by the recovery studies at three different concentration levels. In each level, a minimum of three injections are given and the amount of the drug present, % of recovery and related standard deviation (Sd) were calculated.

Specificity

Specificity is defined as the ability to assess unmistakable the analyte in the presence of other components such as impurities, degraded products (or) excepients. These components may be expected to present in the test sample and standard solution. It was observed by checking the chromatograms of blank sample.

Robustness

Robustness is defined as an analytical procedure is a measure of ability to remain unaffected by deliberate changes in method parameters and provides an indication of its reliability during normal conditions. This

study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions that is flow rate (± 0.2 ml/min), wavelength (± 5 nm), and change of pH (± 0.5), mobile phase ($\pm 2\%$). The retention time and peak asymmetry are calculated by determining the effect of the obtained parameters.

LOD and LOQ

In a sample solution the lowest amount of analyte can be detected as LOD and where as LOQ is the lowest amount of analyte in a sample solution that can be quantified can be determined. LOD and LOQ were separately estimated based on the calibration curves. The LOD and LOQ were calculated as 3.3 Sd/s and 10 Sd/s respectively as per ICH guidelines, where Sd/s indicates standard deviation and slope method.

Statistical Analysis

The data obtained were analysed by chromatograms. The data is subjected to regression analysis to obtain the line of equation in linearity studies.

RESULTS AND DISCUSSION

Method validation

in this method system suitability, linearity, precision, accuracy, lod, loq, robustness are validated. The proposed method is having blank solution, standard solution and sample solution chromatograms are shown in the figures.

System suitability

The HPLC system was stabilized for 30 min by following the chromatographic conditions to get a stable base line. A blank solution followed by 6 replicates of a standard solution is injected to perform the system suitability. The system suitability parameters were determined from standard chromatograms obtained, by calculating the retention times, tailing factors, theoretical plates and % RSD of peak areas.

Linearity

Series of solutions are prepared using Hydrochlorthiazide and Losartan working standard at a concentration levels from 10 – 300 $\mu\text{g/ml}$ and 10 – 300 $\mu\text{g/ml}$ respectively. The solutions are injected into the HPLC system as per procedure. Measured the peak area of the injected solution. A calibration curve was plotted with peak area in the y axis and concentration of standard solution in the x axis. The linearity of both the drug solutions are estimated by calculating the correlation co-efficient. The data of the calibration curve was given in table 1. The equation of calibration curve and correlation coefficient is shown in figure x linearity plot for Hydrochlorothiazide, figure x linearity plot for Losartan potassium. The correlation coefficient values of these analytes were not less than 0.990.

Limit of Detection and Quantification

Limit of detection and quantification were determined from standard deviation and slope method as per ICH guidelines. LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve. LOD of HCTZ and LSR were found to be 1.14 $\mu\text{g/ml}$ and 1.34 $\mu\text{g/ml}$. LOQ of HCTZ and LSR were found to be 3.414 $\mu\text{g/ml}$ and 4.066 $\mu\text{g/ml}$.

Precision

System precision was carried out using 6 replicates of the same standard concentration (8 $\mu\text{g/ml}$ of HCTZ and 32 $\mu\text{g/ml}$ of LSR). The data of the system precision was given in table 2. Method precision was carried out using 6 different sample preparations from same homogenous blend of marketed sample (8 $\mu\text{g/ml}$ of HCTZ and 32 $\mu\text{g/ml}$ of LSR). The data of the method precision was given in table 2. The data of the intermediate precision was also given in the table 2.

Accuracy

Accuracy of developed method was calculated by assay and recovery procedures. The recovery studies are carried out at three different levels. The pre analysed sample solution was spiked with 50%, 100%, 150 % of mixed standard solution. The prepared mixture of solutions were analysed by the proposed method. The

study was carried out in triplicate. Then the obtained recovery results are given in the table 3.

Specificity

The effect of wide range of excipients and other additives usually present in the combination of HCTZ and LSR in the determinations under optimum conditions were investigated.

Placebo solutions, sample and standard solutions were analysed individually as per the method to examine interference. Chromatograms of placebo, standard and sample as in the figures.

Robustness

To demonstrate the robustness of the method, prepare standard solution as per test method and injected at different variable conditions like using different flow rates and change in the mobile phase composition in the mobile phase. The obtained method was robust and it was enough to withstand the slight changes and allowed the routine analysis of the samples. The observed data was given in table 4.

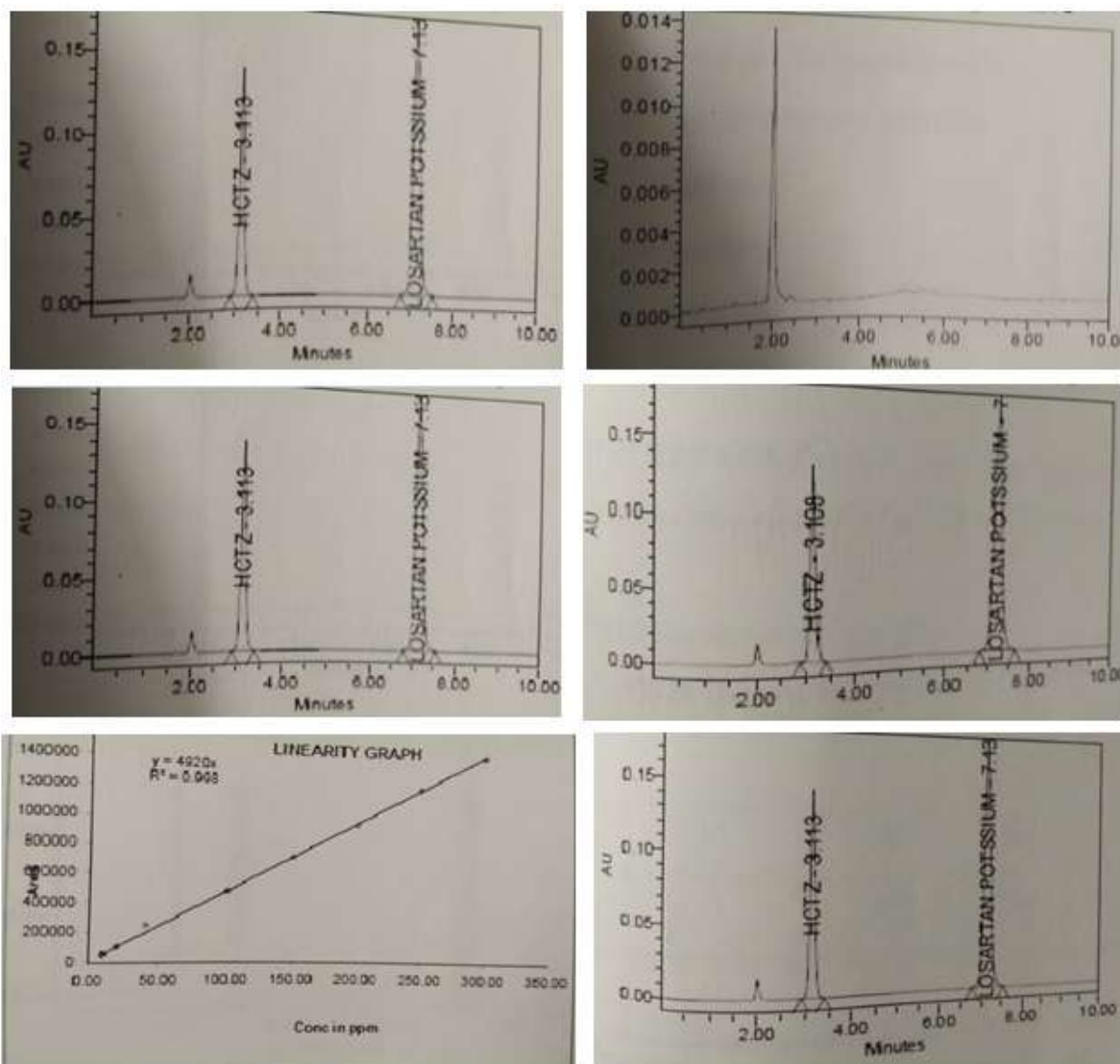
LOD and LOQ

Formula

$$\text{LOD} = 3.3 (S_d/s)$$

$$\text{LOQ} = 10 (S_d/s)$$

The Limit of Detection and Limit of Quantification are estimated from standard deviation and slope method as per ICH guidelines. LOD were found to be 1.14 $\mu\text{g/ml}$ and 1.34 $\mu\text{g/ml}$ for HCTZ and LSR. LOQ were found to be 3.414 $\mu\text{g/ml}$ and 4.066 $\mu\text{g/ml}$ for HCTZ and LSR.



Chromatographs of Optimized conditions of HCTZ & LSR

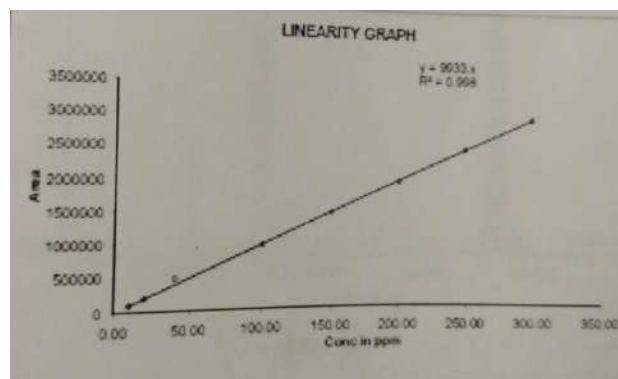
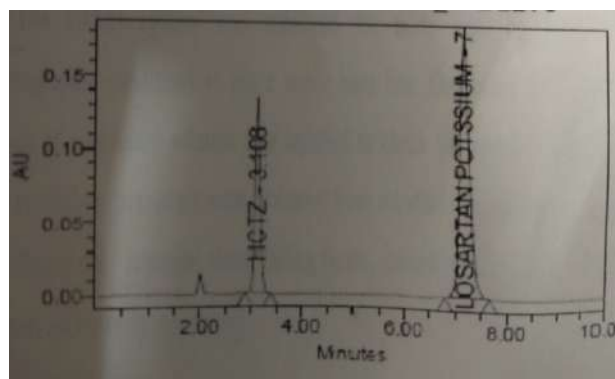


TABLE1: FOR LINEARITY

Analyte	Linearity Range	Equation of Calibration Curve	Correlation Coefficient
HCTZ	10-300 $\mu\text{g/ml}$	$Y=4920x$	NLT 0.990
LSR	10-300 $\mu\text{g/ml}$	$Y=9033x$	NLT 0.990

TABLE 2: FOR PRECISION (SYSTEM, METHOD, INTERMEDIATE)

Analyte	Amount Present	% RSD
Hydrochlorothiazide	12.50 Mg	0.29
HCTZ	12.50 Mg	0.657
HCTZ	12.50 Mg	0.31
Losartan Potassium	50 Mg	0.37
LSR	50 Mg	0.515
LSR	50 Mg	0.23

TABLE 3: FOR ACCURACY

% of Target Conc.	Hydrochlorothiazide (% Recovery)	Losartan Potassium (% Recovery)
50	100.1	100.0
100	100.7	100.2
150	100.3	100.8
Mean	100.5	100.3

TABLE 4: FOR ROBUSTNESS

Drug Name	Flow Plus	Flow Minus	Org Plus	Org Minus
HCTZ	1.7	1.3	65:35	75:25
LSR	1.7	1.3	65:35	75:25

CONCLUSION

This chapter deals with development of simultaneous RP-HPLC method for the estimation of Hydrochlorothiazide and Losartan potassium in bulk and pharmaceutical dosage form. The results obtained with proposed methods confirm the suitability of these methods for pharmaceutical dosage forms. The other active ingredients & excipients usually present in the pharmaceutical dosage forms did not interference in

the estimation. The accuracy of the methods was confirmed by the recovery studies. These methods can be used for the simultaneous estimation of Hydrochlorothiazide and Losartan potassium by RP-HPLC method in both bulk drug & Pharmaceutical formulations.

Acknowledgement

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Conflict of Interest

Conflict of Interest is declared as none.

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A REVIEW ON VARIOUS VEIN FINDERS AND ITS APPLICATIONS

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ABSTRACT

The most common methods of diagnosis in the medical laboratory testing is done by using venous blood as a sample. This invasive method requires a proper vein selection. The phlebotomist can easily locate the vein by using these vein finders hence preventing possible pre-analytical errors in the specimen collection and reduces the discomfort and pain to the patient. Frequent and excessive punctures in the vein cause anxiety, pain in patients. In this present study, through a detailed literature review we would like to highlight the details about various veinfinders. Depending on the earlier studies, based on the economical and feasible near-infrared systems, there is much possibility in development of cost-effective vein detection systems. However, their limitations are restricting their applicability to clinical practice. Finally, based on the current findings, we outline the future approaches of veinfinders in the present article.

KEYWORDS: *Veinfinder, Vein Punctures, Applications.*

INTRODUCTION

A vein finder is a medical device that helps healthcare professionals find the veins quickly. A vein finder device allows you for quick access to the veins in an emergency for every second count. The patient may have health issues that can complicate the search for veins. Any medical professional who performs venipuncture and cannulation – knows that some people have veins that are hard to detect. The ratio of difficult venipuncture cases is higher in the case of infants aged less than six months, dark skin color and obese patient's to perform quick and accurate venipuncture, because of veins in children are not always visible due to the fat tissue¹. They are also challenging to locate in the elderly, in people with dark skin or obese patients. In these cases, vein finder is useful for patients and health professionals. They use the reflection of near-infrared light to draw a map of the veins.² The visibility of cannulated veins is increased for all those who use an infrared vein finder device. One of the most important challenging aspects of taking blood is finding an adequate blood vessel.³ Some patient's veins are tiny and deep and prevent healthcare professionals from finding it quickly and easily. The repeated needle is painful for patients. These devices can be important in different tasks, such as non-invasive visualization of superficial veins and obtaining a map of veins that, after image processing can be useful in the diagnosis of some disease. These tools use LEDs with near-infrared wavelengths to illuminate the whole area. The veins appear as dark bands because they absorb the light spectrum more than the surrounding tissue. An infrared vein emits infrared rays through LED lamps. The oxygen and hemoglobin molecules quickly absorb infrared light.⁴ Luminetx launched a device called VeinViewer in 2006, and Accuvein launched a product called Accuvein in 2008.

Benefits of Vein Finders

At least 25% of all patients go through more sticks, which is painful, stressful and reduces the safety of the procedure without vein finder or vein viewers. Here are the obvious benefits of using a simple tool like the infrared finder (vein Finder):

Proven increased visibility of veins, The infrared vein light device is noninvasive and painless (as is infrared light therapy and its amazing uses), Avoiding unnecessary pain and stress for the patient, Reducing waste of needles, syringes, PICC and midline trays and time-saving process.



Vein Visualization

Vein visualization is an innovative technology that assists healthcare professionals with finding the best veins for IV starts and blood draws. Also known as vein mapping, this technology creates a real-time visual “map” of the veins on the surface of the skin.

Types of Vein Finders

There are two types of vein finders; active and passive devices.⁵ Active devices use infrared and red light to shine the hemoglobin in the blood. On the other hand, passive vein finders change the amount of visible light entering your eye so that you can see the veins easily. Some important vein finder or vein viewers are used in medical professionals who perform vein visualization or venipuncture and cannulation are following : AccuVein Vein Finder, Venoscope II Transilluminator Adult/Baby Vein Finder, Respiroics Wee Sight Transilluminator Vein Finder, O2Amp Vein Finder Glasses, Veinlite EMS for adults and children, Veinlite LEDX Adult Transilluminator Vein Finder, UMTEC Vein Finder.

Accuvein Vein Finder

The Accuvein is an important vein finder that is easy to use and is quickly becoming an essential tool for all health care professionals who perform venipuncture procedures.

Method

Vein visualization (also known as vein illumination) uses Near-infrared (NIR) imaging for detecting veins. The AccuVein employs two safe barcode-scanner class lasers: an invisible infrared and a visible red. The two lasers work in tandem to provide a real-time image of the subcutaneous vasculature up to 10mm deep.⁶ First, Hemoglobin in the blood absorbs infrared light. When the device is held above the skin, veins appear noticeably different than the surrounding tissue. The vasculature shows up clearly on the skin’s surface, help in vein location for access and avoidance.

Applications

In general surgery the device can be used to locate and avoid veins to minimize bleeding. In vascular surgery the device can be used to locate veins, find the source or feeder veins. Used in IV, phlebotomy, blood donation, cosmetic procedures, sclerotherapy. This device for preventing complications from accidental venipuncture when administering dermal filler injections.⁷ Portable, comfortable and easy to use.. It consist rechargeable battery.⁸ AV400, AV500 and AV300⁹ vein viewing systems are latest in vein visualizations from AccuVein.

Venoscope Transilluminator II

The Venoscope II is a lightweight, portable high intensity LED vein light (near-infrared), allows the physician to easily locate veins that are difficult to find, it works for both adults and children's. It is also effective for the obese and dark-skinned as well.



Method

Venoscope 2 Transilluminator acts by using High intensity light directing towards the subcutaneous tissue which is made up of adipocytes and forms an area of orangish reflection of the fatty tissue. At first this light rays falls on surface of the skin and by moving device around, at a particular point you will able to see a dark line in the arm then you came across a vein. This dark line represents the vein because deoxygenated blood in the veins absorbs the light where as the fatty tissue reflects the light.

Applications

This device is used in IV Therapy, IV Infiltrations, Hemophilia and emergency Care. It works with 3 AA batteries. This device is used to find difficult veins in dark skinned people obese and also for baby vein visualization.

Respironics Wee Sight Transilluminator Vein Finder

This device is useful for both infants and children who are under 2 year's. it is designed in such a way that it can be easily positioned around babies tiny limbs. This device uses IR light - 629 nm (LED bulbs) to light up veins. This high intensity LEDs does not harm the babies, it is safer for babies to find veins. Its actions are same as venoscope 2 Transilluminator.



Applications

A pediatric vein transilluminator is an amazing tool to decrease babies and toddlers pain when drawing blood or starting IV's. It can also be used for thin-skinned adults, IV therapy and blood with drawl. This device Works with 2 AA batteries. Relatively expensive, but works as promised. The curved shape helps to stabilize the infant hand/foot, LEDs run for years with no problem occurs. The LEDs do not heat up and will not hurt babies and toddlers tender skin.¹⁰

Vein Finder Glasses

The vein finder glasses are mainly of two types. They are used to protect from UV rays of the sun. They are Oxy-Iso Paramedic vein glasses and O2Amp Vein Finder Glasses. They look like a nice pair of sunglasses. These vein glasses protect the user from the UV rays of the sun, and while they filter UV rays, most serve 2 major purposes: Enhancing veins, Correcting color blindness (improving the ability to see greens and reds)

Method

The specially tinted lenses filter light in a way that it makes veins appear to fluoresce or glow and amplify the ability to see blood oxygenation by hindering perception of blood concentration variations.

Applications

This device is used to identify superficial vessels, detect oxygen levels in the blood, detect rashes and determine if the patient has cyanosis. The large lenses protect from blood splatter. Relatively low-cost. These glasses worn by paramedics can be worn daily. They are not harmful to the wearer's eyes, even if they are worn all day. They are shock-resistant, antistatic, anti-fog and scratch-resistant. If a person works at night, it is better to have a powerful flashlight or a portable lamp. Squeeze it for a few minutes before you need it, so your eyes adjust. If the person is not comfortable to use over prescription glasses, they have uncut Rx lenses are available to use.¹¹

Veinlite EMS for adults and children:

It is one of acceptable device for health care provider and EMS technicians. This Veinlite EMS is used to find the vein in the scalp and limbs. It is very easy to handle and carry and also useful for both adults and children.

Applications

This device was proven to increase the quality of care in pediatric and neonatal cases. This device Works great in low light situations.

It is used find veins in the limbs and scalp and also find veins in pediatric patient. No battery charger required.

Disadvantages

It is not effective for dark skinned patient, deep vein and obese patients. This can be minimised by using Veinlite LEDX Adult Transilluminator Vein Finder.

Veinlite LEDX Adult Transilluminator Vein Finder

The Veinlite LEDX is very useful to find vein quickly and efficient IV access especially in emergency cases and it is easy to use.

Method

The powerful LEDX consists of 32 bright LEDs in which 24 are orange lights and 8 red lights. This orange and red colour combination provide an excellent vein visualization of deeper vein even if the patient with thick skin (obesity) and dark skinned.

Applications

It is used in IV access in emergency treatment, and for seeing deeper veins even if the skin is thick or dark-colored. This device also provides the highest contrast imaging for sclerotherapy treatments, Safe for eyes (comes with light shield), Works great in low light situations, No need of battery charger.

UMTEC Vein Finder

UMTEC is portable, adjustable and acceptable device especially for diabetes patients and some other conditions because it is useful find the subcutaneous vein quickly for administration of insulin and hormones etc to improve the success rate of injections. It uses cold light rays which are safe. By the transmissions of LED red lights through the surface of the skin it is easy to identify the vein at back of the hand. It is extensively used in hospitals, homes as well as any other places.

Applications

Widely used in clinics or hospitals for IV Phlebotomy especially suitable for baby children or obesity patients. Suitable for dark Skin, Hairy Skin, infants or kids and also in Plastic Surgery, UMTEC vein finder will reduce 39% injecting pain, less pain; better successful rate will definitely improve patient's satisfaction, Used Medical Cold Light. It is safe for your eyes. It does not cause eye fatigue, Provides views of blood vessels to a subcutaneous depth of 10mm.



Precautions

Certain precautions must be taken before using these devices. Any medical professional who performs venipuncture and cannulation, they should carry hand wash, gloves, bandages, preparation pads, disposable Tourniquet's (cleanliness), disposable containers, disposable wipes and disposable covers (to cover the vein finder to prevent contamination and spread of germs from one person to other).¹³⁻¹⁶

CONCLUSION

The vein finders are one of the best medical devices to be used when the task at hand is providing top-quality health care to the patients. It is used by the health professionals to find out the vein quickly in certain emergency conditions. Veins in children are not always visible due to the fat tissue and also it is really challenging to locate in people with dark skin or obese patients. In these cases, vein finder is a useful tool for finding the veins easily in patients and thus more helpful for the health professionals. It helps nurses and other people who need to look for veins for different purposes especially in cases like surgeries and blood drawing procedures for diagnostic purposes. By using vein finders, we can avoid unnecessary pain and stress for the patients. It also helps in reducing the wastage of needles, syringes, PICC and midline trays.

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STEM CELL THERAPY FOR CARDIOVASCULAR DISEASE

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ABSTRACT

Cardiovascular disease is the primary cause of mortality and morbidity worldwide. Therapeutic approach, for the problem of cardiac tissue loss is by heart transplantation. Alternative options, are stem cell therapy, has emerged as a novel and promising approach. Stem cell treatment is a therapeutic option to regenerate myocardium and improve cardiac function. Bone marrow precursor cells were the first cell type used in clinical studies. Stem cells are multipotent, and capable of multiplication and differentiation. Several different stem cells include bone marrow-derived mononuclear cells, mesenchymal stromal cells, endothelial progenitor cells, adipose-derived mesenchymal stromal cells (ASCs) and stem cells from placenta and umbilical cord. This review presents about the state of research on stem cell therapy for cardiovascular disease

KEYWORDS: *stem cells, cardiovascular disorder, myocardial infraction, bone marrow cells*

1. INTRODUCTION

Cardiovascular disease refers to a group of diseases that affect the heart and blood vessels and contributes to approximately 30% of global mortality.¹ Coronary artery disease (CAD) is a major cause of death and disability, including in the young adult population.² Regenerative therapies using stem cells for the repair of heart tissue have been widely used in preclinical and clinical studies during the past 16 years.³ Cardiovascular disorders are multi-faceted, a fundamental problem is the presence of scar tissue, and the irreversible loss of cardiomyocytes, which contribute to the contractility and the relaxation of ventricles. Stem cell therapy has become a new focal point for the treatment of cardiovascular disorders. The bone marrow-derived mono-nuclear cells (BMMNCs) can repair myocardial damage and improve heart function by favoring myocardial regeneration or reduction of ventricular remodelling has generated great expectations.⁴

2. SPECTRUM OF STEM CELLS INVESTIGATED

Since the first study reported in 1998 indicating the ability of skeletal muscle to repair the heart (figure-1).

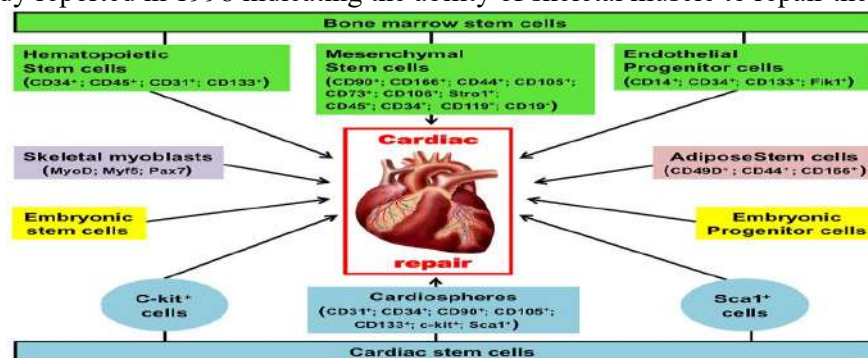


Figure 1: Spectrum of stem cells investigated for cardiac repair.

These cells include skeletal myoblasts, whole bone marrow cells or bone marrow-derived HSCs, bone marrow-derived MSCs, adipose-derived stem cells, adult cardiac resident stem cells, and ESCs including their differentiated progeny.

2.1. Skeletal Myoblasts (SMs)

SMs have sufficient plasticity to give rise to cardiac muscle, specimens of SMs obtained from muscle biopsies were originally used for cardiac regeneration.⁵ These cells have the ability to derive autologous cells, thus eliminating the need for immunosuppression.^{6,7} In addition, SMs have a high proliferative ability at a later stage of differentiation and are resistant to ischemia.⁵⁻⁷

2.2. Bone Marrow-Derived Stem Cells

Bone marrow stem cells include mesenchymal and hematopoietic stem cells which are used in tissue regeneration.

2.2.1. BMMNCs

In acute myocardial infarction (AMI), BMMNCs have been used and were found to contribute to the regeneration of cardiomyocytes and endothelial cells were injected into infarcted myocardium.^{4,8} The first clinical trial of BMMNCs in AMI, carried out in 2002 by Strauer et al⁹; revealed that these cells improved global and regional ventricular function and enhanced myocardial perfusion. Similar results were obtained more recently in two separate trials of BMMNCs in AMI.¹⁰ However, a double-blind investigation of BMMNC transplantation in the placebo-controlled late time trial, at 2–3 weeks after myocardial infarction (MI) revealed no improvement in regional or global cardiac function.¹¹

2.2.2. Mesenchymal stem cells (MSCs)

MSCs are non-hematopoietic cells that have the potential to differentiate into a variety of cell types. They have initially identified in bone marrow, but are also found in umbilical cord blood, adipose tissue, and the heart. MSCs can express CD44, CD29, CD73, CD90 and CD105, as well as CD106, CD166, and ICAM-1, but not the hematopoietic markers CD45, CD34, CD14, CD79a, CD11b and HLA-DR.¹² Intracoronary infusion of autologous bone marrow-derived MSCs given to patients after MI resulted in improved LV function and myocardial perfusion.

2.2.3. HSCs and EPCs

HSCs present in the bone marrow have the potential to differentiate into myeloid as well as lymphoid cell lineages. Whereas, EPCs are found in peripheral blood and they can differentiate into endothelial cells to promote neovascularisation in response to ischemic injury. Injection of CD34+ cells into the peri-infarct during coronary artery bypass grafting (CABG) surgery in patients with ischemic cardiomyopathy led to better improvement of contractile function as compared to CABG alone.¹³

2.3. Adipose-Derived MSCs

Miyahara et al were the first investigators to practice transplantation of adipose-derived MSCs into scarred myocardium in a rat model of chronic MI, and reported that this intervention led to better cardiac function, which was associated with reversal of wall thinning in the scar area.¹⁴

2.4. Cardiac Stem Cells (CSCs)

These cells were found to express markers of both mesenchymal stem cells (CD90, CD105), embryonic stem cells (Rex1, Nanog, Sox2) and also early markers of cardiogenesis (platelet derived growth factor receptor- α).¹⁵ There are multiple different types of cardiac stem cells (CSCs) including ckit+ cells, Isl 1+ cells, cardiac mesoangioblasts, cardiosphere derived cells and epicardial progenitors which all express slightly different but overlapping surface markers.¹⁶

2.5. Embryonic Stem Cells (ESCs)

ESCs are considered to be promising therapeutic candidate stem cells. Human ESCs form contracting areas and embryoid bodies, which are positive for cardiomyocyte markers such as myosin heavy chain, α -actin, desmin and tropin I.¹⁷

3. MODES OF STEM CELL DELIVERY

The present stem cell delivery approaches are transvascular and direct injection to ventricular wall.

3.1. Transvascular Approaches

3.1.1. Intracoronary delivery: Cells are infused inside the coronary artery, by coronary occlusion caused by inflating a balloon at the tip of the catheter.

3.1.2. Intravenous infusion: This is not injected in post-AMI patient.

3.1.3. Mobilization of stem cells: Stem cells by cytokines provide a non-invasive approach for cardiac regeneration.

3.2. Direct Injection Into The Ventricular Wall

3.2.1. Transepicardial injection: It is an adjunct to CABG during cardiac surgery.

3.2.2. Transendocardial injection: Stem cells are delivered directly inside the left ventricular wall.

3.2.3. Transcoronary vein injection: In this, cells are delivered parallel to the ventricular wall and deep into the injured myocardium.

4. THERAPEUTIC MECHANISM OF STEM CELLS

Stem cell has ability to repair myocardium and improve cardiac function by direct and indirect mechanisms. It includes cardiac cells, vascular cells, paracrine effect and cell fusion (figure.2).

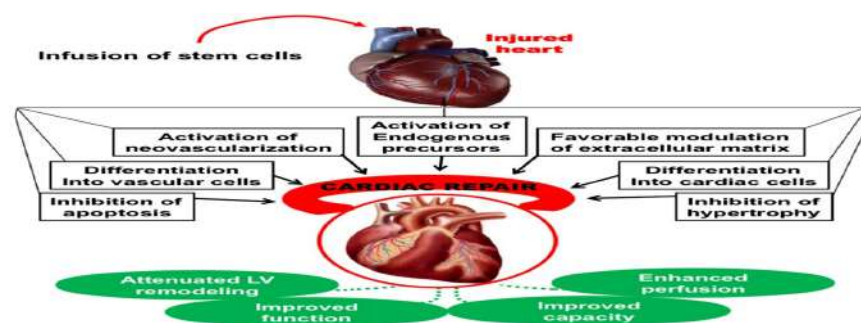


Figure 2: Therapeutic mechanisms of stem cells.

Infusion of stem cells to injured heart repairs myocardium by direct and indirect mechanism including cardiac and vascular cells, paracrine effect leads to activation of precursors, neovascularization and inhibition of apoptosis and hypertrophy.

4.1. Stem Cells Into Cardiac Cells

Stem cell therapy has improved left ventricular function and vascular remodelling.

4.2. Formation of New Blood Vessels

Ischemic heart disease improved cardiac function, formation of new blood vessels from stem cells.

4.3. Paracrine Effect

Paracrine effect is about the therapeutic effects of transplanted stem cells to injured tissues.

4.4. Cell Fusion

Cell fusion is a cellular mechanism with mononuclear cells combine to form a syncytium.

5. CONCLUSION

Cardiovascular diseases are leading cause of death worldwide. Therapeutic option of stem cell has capacity to regenerate damaged myocardium and its function. Stem cell has capability of differentiating cardiomyocytes and endothelial cells. The stem cell therapy was encouraging and safe.

6. ACKNOWLEDGEMENTS

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REVIEW ON CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM

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ABSTRACT

With the advancement of the technologies in the pharmaceutical field, drug delivery systems have drawn an increasing interest over the last few days. Nowadays, the importance of pharmaceutical galenic research is turned towards chronopharmaceutical drug delivery systems (CDDS) as they deliver the drug at the right site of action at the right time and in the right amount based on circadian rhythms, thus providing spatial and temporal delivery and increasing patient compliance. Sustained and controlled drug delivery system release the drug at a substantially steady rate of release per unit of time. However, there are instances where maintaining a constant blood level of a drug is not desirable. In such cases a pulsatile drug delivery may be more advantageous. A pulse has to be designed in such a way that a complete and rapid drug release is achieved after the lag time. The aim of this current review is to describe, the diseases requiring CDDS, methodologies involved for the existing systems and more focus on the time inced and stimuli induced methodologies, recent update and CDDS product currently available in the market.

KEYWORDS: *Chronopharmaceuticals, Pulsatile drug release, lag time, circadian rhythm, Sustained and controlled drug delivery.*

INTRODUCTION

The term "chrono" basically refers to the observation that every metabolic event undergoes rhythmic changes in time. Researchers have recently concluded that both disease states and drug therapy are affected by a multitude of rhythmic changes that occur within the human body ¹ulsatile drug delivery systems are gaining a lot of interest and attention these days. These systems have a peculiar mechanism of delivering the drug rapidly and completely after a "lag time," i.e., a period of "no drug release."² Though most delivery systems are designed for constant drug release over a prolonged period of time, pulsatile delivery systems are characterized by a programmed drug release, as constant blood levels of a drug may not always be desirable Figure - 1. However, only a few such orally applicable pulsatile release systems are available due to potential limitations of the dosage form size, and/or polymeric materials and their compositions used for producing such dosage forms³. Such a novel drug delivery has been attempted for the following diseases listed in Table 1⁴.

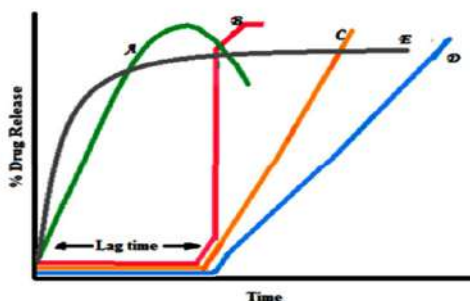


Figure: 1.1 Drug Release Profile of Pulsatile Drug Delivery System

Pulsatile devices may have many applications in areas of other medicine where a constant rate of drug release does not match the physiological requirements of the body. This is often the case when treatments

involve hormone based drugs. Secretion of many hormones exhibits pulsatile patterns comprising frequent pulses over periods from hours to weeks ⁵, so it is more effective to mimic this with a synthetic delivery system. Current research in the field of drug delivery devices, by which triggered and/or pulsatile release is achieved, has been intensified.

NEED FOR CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM⁶

There are many conditions and diseases where sustained release formulations don't show good efficacy.

The shift from conventional sustained release approach to modern pulsatile delivery of drugs can be credited to the following reason(s):

- First pass metabolism
- Biological tolerance
- Special chronopharmacological needs
- Local therapeutic need
- Gastric irritation or drug instability in gastric fluid
- Drug absorption differences in various gastrointestinal segments

Table 1: Diseases requiring Pulsatile Drug Delivery

Disease	Chronological behavior	Drugs used
Arthritis	Pain in the morning and more pain at night	NSAIDs, Glucocorticoids
Asthma	Precipitation of attacks during night or at early morning hour	β 2agonist, Antihistaminics
Attention deficit syndrome	Increase in DOPA level in afternoon	Methylphenidate
Cardiovascular diseases	BP is at its lowest during the sleep cycle and rises steeply during the early morning awakening period	Nitroglycerin, Calcium channel blocker, ACE inhibitors etc.
Diabetes mellitus	Increase in the blood sugar level after meal	Sulfonylurea, Insulin, Biguanide
Neoplastic	It has been demonstrated that "susceptibility rhythms" to drugs may differ between healthy tissue and cancerous tissue	Alkylating agents, Antimetabolites, Vinca alkaloids, antibiotics etc.
Peptic ulcer	Acid secretion is high in the afternoon and at night	H ₂ blocker
Hypercholesterolemia	Cholesterol synthesis is generally higher during night than during day time	HMG CoA reductase inhibitors

METHODOLOGIES FOR PULSATILE DRUG DELIVERY

Methodologies for the pulsatile drug delivery system can be broadly classified into three classes;

1. Time controlled
2. Stimuli induced
3. Externally regulated

1.1 Pulsatile System Based On Osmosis⁷

- Osmotic system consists of capsule coated with the semipermeable membrane. Inside the capsule was an insoluble plug consisting of osmotically active agent and the drug formulation.
- When this capsule came in contact with the dissolution fluid, the semipermeable membrane allowed the entry of water, which caused the pressure to develop and the insoluble plug expelled after a lag time.
- Such a system was utilized to deliver methylphenidate used in the treatment of attention deficit hyperactivity disorder as the pulsatile port system^{8,9}

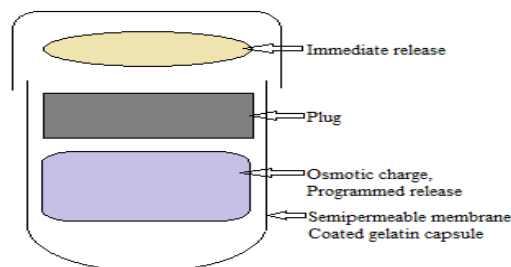


Fig.2 Schematic diagram of osmosis system

1.2 Capsule Shaped System Provided With Release Controlling Plug¹⁰

- Single-unit systems are mostly developed in capsule form. The lag time is controlled by a plug, which gets pushed away by swelling or erosion, and the drug is released as a “pulse” from the insoluble capsule form the capsule body.
- This dosage form consists of an insoluble capsule body containing a drug and swellable and degradable plugs made of substances such as hydrophilic polymers or lipids.
- The lag time can be controlled by manipulating the dimension and the position of the plug.
 - Polymers used for designing of the hydro gel plug includes:
- Insoluble but permeable and swellable polymers(eg: polymethacrylate)
- Erodible compressed polymers(eg: hydroxypropylmethyl cellulose, polyvinyl alcohol, polyethylene oxide)
- Congealed melted polymers(eg: saturated polyglycolated glycerides, glyceryl monooleate)
- Enzymatically controlled erodible polymer (eg: pectin)

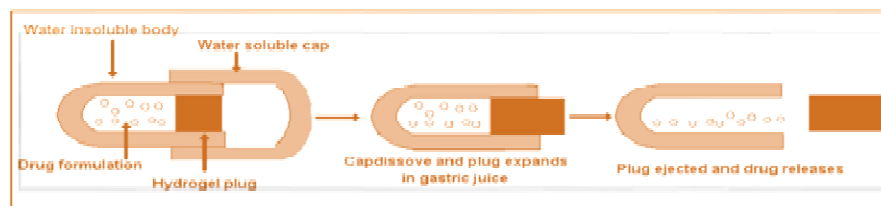


Figure :3 Schematic diagram of release of drug from capsule

1.3 Delivery Systems with Rupturable Coating Layer¹¹

Factors In place of swelling or eroding, these systems are dependent on the disintegration of the coating for the release of drug. The pressure necessary for the rupture of the coating be achieved by the swelling, disintegrants, effervescent, excipients, or osmotic pressure. Water permeation and mechanical resistance of the membrane are major affecting the lag time.

Eg: BuflomediHcl is used for treatment of peripheral.

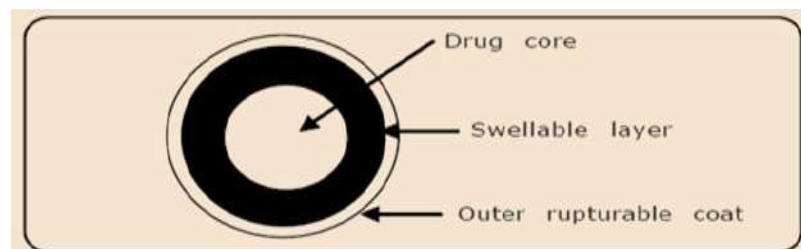


Fig:4-Schematic diagram of drug delivery with rupturable coating layer

1.4 Delivery System With Erodible Coating Layers¹²

In such systems, the core containing drug is coated with the soluble or erodible polymer as outer coat and the drug release is controlled by the dissolution or erosion of the outer coat.

Time dependent release of the drug can be obtained by optimizing the thickness of the outer coat as shown in fig 5.

Ex: The time clock system and the chronotropic system.

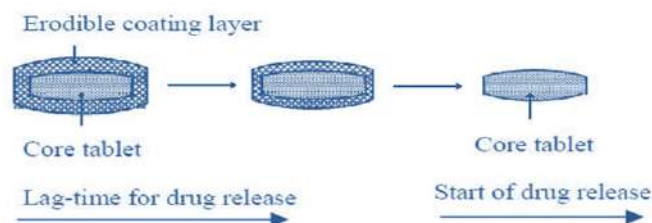


Fig: 5 Schematic diagram of drug delivery with erodible coating layer

2. STIMULI INDUCED PULSATILE RELEASE SYSTEM ¹³

In these systems there is release of the drug after stimulation by any biological factor like temperature, or any other chemical stimuli ¹⁴. These systems are further classified as:

2.1 Temperature Induced Systems

The temperature induced pulsatile/triggered drug delivery systems utilize various polymer properties, including the thermally reversible coil/globule transition of polymer molecules swelling change of networks, glass transition and crystalline melting. ¹⁵

2.1.1 Thermo Responsive Hydrogel and Polymer Systems

Thermally responsive hydrogels and membranes have been extensively exploited as platforms for the pulsatile drug delivery.

In these systems the polymer undergoes swelling or de-swelling phase in response to the temperature which modulate drug release in swollen state ¹⁶.

Eg: Y.H. Bae et al developed indomethacin pulsatile release pattern in the temperature ranges between 200C and 300C by using reversible swelling properties of copolymers of N-isopropylacrylamide and butyrylacrylamide ¹⁷

2.2 Chemical Stimuli Induced Pulsatile System

2.2.1 Glucose-responsive release devices ¹⁸

In a glucose-rich environment, such as the blood stream after a meal, the oxidation of glucose to gluconic acid catalysed by glucose oxidase can lower the pH to approximately 5.8. This pH change includes swelling of the polymer which results in insulin release. Insulin by virtue of its action reduces blood glucose level and consequently gluconic acid level also gets decreased and system turns to the deswelling mode thereby decreasing the insulin release. Examples of the pH sensitive polymers include N, N-Dimethylaminoethyl methacrylate, chitosan, polyol etc.

2.2.2 Inflammation-Induced Pulsatile Release

During inflammation, hydroxyl radicals are produced from these information-responsive cells. Degradation via hydroxyl radicals however, is usually predominant and rapid when hyaluronic acid gel is injected at inflammatory sites. Thus, it is possible to treat patients with inflammatory diseases like rheumatoid arthritis; using anti-inflammatory drug incorporated HA gels as new implantable drug delivery systems. ¹⁹

2.2.3 Drug Release From Intelligent Gels Responding To Antibody Concentration

There are great in number of bioactive compounds which exist in the body. Recently, novel gels were developed which responded to the change in concentration of bioactive compounds to alter their swelling/deswelling characteristics. Special attention was given to antigen-antibody complex formation as the cross-linking units in the gel, since such interaction is very specific ²⁰. Utilising the difference in association constant's between polymerised antibodies and naturally derived antibodies towards specific antigens, reversible gel swelling/deswelling and drug permeation changes occurs. ²¹

2.2.4 pH Sensitive Drug Delivery System

This type of PDDS contains two components. The first is fast release type while the other is pulsed release which releases the drug in response to change in pH. In case of PH dependent system, advantage has been taken of the fact that there exists different pH environment at different parts of the gastrointestinal tract ^[22]. By selecting the pH dependent polymers drug release at specific location can be obtained. Examples of pH

dependent polymers include cellulose acetatephthalate, polyacrylates, and sodium carboxy methyl cellulose. These polymers are used as enteric coating materials so as to provide release of drug in the small intestine. Eg: Eudragit in colon target systems.



Figure 6. Schematic diagram of pH dependent drug delivery system

3. EXTERNALLY REGULATED SYSTEMS

The externally regulated systems in which drug release is programmed by external stimuli like magnetism, ultrasound, electrical effect and irradiation in order to release the drug in a pulsatile manner²³

ADVANTAGES OF CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM²⁴

- Predictable, reproducible and short gastric residence time.
- Bioavailability is increased.
- Not only less inter but also intra-subject availability.
- Reduce side effects, reduced dosage frequency, Reduction in dose size and patient compliance.
- Increases absorption and bioavailability than conventional immediate release or sustained released drugs.
- Lower daily cost to patient due to fewer dosage units are required.
- Drug adapts to suit cardiac rhythms to body function or disease.

LIMITATIONS OF CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM²⁵

- Lack of manufacturing reproducibility and efficacy.
- Technologies employed and the equipment's used are complicated.
- Multiple manufacturing steps and large number of process variables.
- In vivo variability in single unit pulsatile drug delivery system.
- Homogeneity coated barrier is mandatory to assure the predictability of lag time.
- Higher cost of production as need of advanced technology.
- Immediate withdrawal of drug is not possible.

RECENT ADVANCES IN THE CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM²⁶

NAME	ACTIVE INGREDIENT	DISEASE	MECHANISM
PULSYS R	Amoxicillin	Pharyngitis/tonsillitis	Time controlled system
UNIPHYL	Theophylline	Asthma	Externally regulated system
RITALIN	Methyl phenidate	Attention deficit hyperactive disorder	Osmotically regulated system
CODAS	Verapamil Hcl	Hypertension	Multiparticulate pH dependent system
DIFFUCAS	Verapamil Hcl Propranolol Hcl	Hypertension	Multiparticulate system

CONCLUSION

It can be concluded that there is a constant need for new delivery systems that can provide increased therapeutic benefits to the patients. Chronopharmaceutical drug delivery system offers a solution for delivery of drugs exhibiting chronopharmacological behavior, extensive first pass metabolism, necessity of night time dosing at right time, right place and in right amounts. These systems can effectively treat diseases with non-constant drug therapies such as diabetes, asthma, arthritis etc. These considerations should ensure that

the current high level of interest in this area would stretch well in to future and ensure in the betterment of quality of life.

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A COMPREHENSIVE REVIEW ON THREE-DIMENSIONAL PRINTING (3DP) OF MEDICINES - THE PHARMACY OF THE FUTURE

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ABSTRACT

Three-dimensional printing could become part of the drug production line. 3D printing technology helps pharmaceutical and medical companies to generate more specific drugs. The FDA has recently approved the potential applications of 3D printing (3DP) technology in pharmaceutical drug delivery systems. USFDA for the first 3D printed antiepileptic drug, the interest has now moved to the personalized medicine as to provide essential comfort to patients who require medications that possess better result. The present achievements include multi-useful drug delivery complex with accelerated release attribute, adjustable and customized dosage forms, transplant, and phantoms corresponding to individual patient anatomy and also cell-based materials for revitalization medicine. Many types of research have attempted to utilize computer-aided design (C.A.D), computer-aided manufacturing (CAM) to notice a scaffold that generates a three-dimensional (3D) nature for reanimation of tissues and organs.

KEYWORDS: *3D printing, personalized medicine, Drug delivery, USFDA*

1. INTRODUCTION

3D printing (3DP) is a unique technology that was first reported by Charles Hull in 1986. It was first progress in 1996 as a favorable candidate to restore conventional tableting techniques. 3D printing uses computer-aided drafting technology and programming to manufacture three-dimensional objects by layering substances onto a substrate. The process requires 3D proto-typing of layer-by-layer creation (via computer-aided design models) to formulate drug materials into the desired dosage form. The achievement either may be the multi-purpose drug delivery systems with accelerated release characteristics, adjustable and personalized dosage forms, implants and, phantoms corresponding to specific patient anatomy as well as cell-based materials for regenerative medicine.¹ The term 3D Pharming encloses the various 3D printing and 2D content, driving out technologies that are used to generate personalized pharmaceutical dosage forms.²

2. HISTORY OF 3D PRINTING

3D printing technology first suits to visible in 1884, when Charles Hull design Stereolithography. He moved on to become the co-founder of 3D Systems. Personalized medicine in the 1990s. There are many techniques developed in 3D printing, and some are discussed in Figure 1, FDA's Center for Device and Radiological Health (CDRH) has analyzed and cleared 3DP medical devices. In 1994 Model Mark's wax printer was delivered. Aeromet in 1997 invents laser 3D printing. In 2013 World's 1st 3D printed gun was designed, which shoot successfully. In 2015 there was a development in the Pharmaceutical field.³

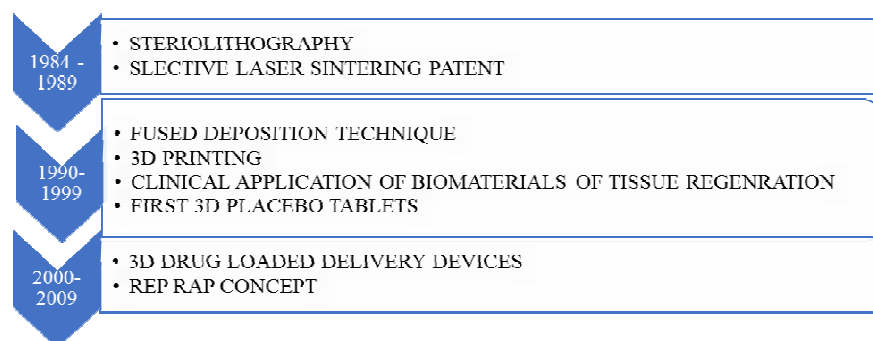


Figure 1. History of 3D printing

3. TECHNIQUES IN 3D PRINTING

3D printing comprises a wide variety of manufacturing techniques, which showed in Figure 2, which is based on digitally-controlled depositing of materials (layer-by-layer) to produce freeform geometries.

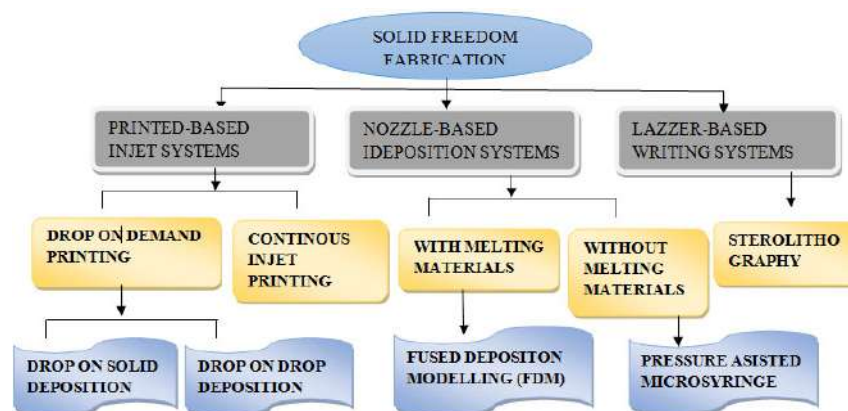


Figure 2. Schematic view of different 3D printing techniques

3.1. Inkjet printing

Inkjet printing also called as 'mask-less' or 'tool-less' approach because of the formation of the desired structure. In this technique, the Ink is set down onto a substrate that appears as Continuous Inkjet printing (CIJ) or Drops on Demand (DoD) printing.⁴ The two main printing types employed under inkjet printing are thermal inkjet printers and piezoelectric inkjet printers.

3.1.1. Thermal Ink-Jet Printing

This technique used in the preparation of drug-loaded biodegradable microspheres, drug-loaded liposomes, patterning microelectrode arrays coating, and loading drug-eluting stents.⁵

3.1.2. Piezoelectric inkjet printing

A piezoelectric crystal source is a liquid to flow in sequence to break into drops at a certain speed and size and at regular intervals of time. For estimating the electrostatic repulsion, The droplets are charged and separated by "droplets of the guard." Due to the electrostatic field, the charged droplets are set down to the substrate.⁶

3.1.3. Printing-Based Inkjet Systems

3.1.3.a. Continuous inkjet printing (CIJ)

In continuous inkjet printing, the liquid ink administered through an orifice of 50-80 μm diameter generating a continuous ink flow. The liquid is caused to flow and break into drops at a specified speed and size at regular intervals using a piezoelectric crystal. It works with a counter mechanism.⁷

3.1.3.b. Drop-on-demand (DOD) printing

The DOD technique includes multiple heads (100–1000) that can use two types of translators, either a thermal head or a piezoelectric crystal. The DOD technique divided into two subtypes: drop-on-drop

deposition and drop-on solid deposition. In the drop-on-drop deposition system, the drop disruption produces the different layers of the RP. In contrast, the drop-on-solid deposition, known as "powder bed fusion," is based on the projection of drops directly on a solid material.⁸

3.2. Nozzle-Based Deposition Systems

3.2.1. Fused Deposition Modelling (FDM)

FDM 3D printing helps in manufacturing delayed-release print lets without an outer enteric coating, and also provides personalized dose medicines. FDM printing works based on the use of thermoplastic polymers such as polylactic acid (PLA), acrylonitrile. Drug loading in the filament achieved through incubation in organic solvents and reduced drug loading may limit its use to low dosed drugs. Xuyu Chai et al. used fusion deposition modeling 3D printing to prepare intragastric floating sustained-release tablets of domperidone.⁹

3.2.2. PAM Technology

The PAM technology finds use in the syringe extruder that retains a semi-liquid material, under pressurized environment of air piston geometry. It has advantages over other processes, as it can work with a continuous flow and at room temperature and presents disadvantages, like the use of solvents, which are often toxic. Its major applications include 3D tissue printing of substitutes or scaffolds prepared for soft tissues and the design and manufacture of intricate drug delivery systems.

3.3. Laser-Based Writing System

3.3.1. Stereolithography

The principle involved is photopolymerization, in which free radicals leave after the interchange between the photoinitiator and UV light beam, in the form of a laser, which transfers the energy into a liquid photopolymerizable resin. The process is repeated layer after layer to build the entire parts of the object in the first layer, occurs as unreacted functional groups on the solidified structure polymerizes with illuminated resin in the next layer providing adhesion and, therefore, layer formation. SLA has advantages over other types of 3DP, mainly, it is a remarkable resolution, and the avoidance of thermal processes can be detrimental for specific drug molecules.¹⁰ In Table 1, the drugs prepared by SLA are given.

3.3.2. Selective laser sintering

SLS utilizes a laser to bind together the powder particles from the powder bed. The laser is directed to draw an individual pattern onto the surface of the powder bed, thereby creating a 3D structure during the printing process.

3.4. Extrusion of 3D printing

In this technique, the material extruded from the automatic nozzle on to the substrate, and it does not require any higher support material. Layers of the dosage form are not hardening; it may not be able to bear the weight of the subsequent layers leading to collapsing of the delivery system during the printing. The materials that can extrude are molten polymers, suspensions, semisolids, pastes.¹¹

3.5. Hot-melt extrusion (HME)

Hot-melt extrusion (HME) is the process of melting the polymer, and for blending the drug should have a high temperature, the pressure is applied in the instrument continuously. The drug release should be evaluated using a dissolution apparatus or Franz diffusion cells.¹²

3.6. Zip dose

Zip dose is the world's first, FDA-validated, commercial-scale 3DP in new therapeutic areas for drug manufacturers. It helps in overcoming a difficulty in swallowing. Spritam® (Antiepilepsy drug) is an orodispersible tablet, marketed by Aprelia Pharmaceuticals based on powder bed fusion by the layer-by-layer production system. Zip dose technology enables the incorporation of large doses up to 1000 mg in a single dosage form. Zip dose technology integrates the drug formulation science with specialized manufacturing capabilities of 3D printing.¹³ All the above-listed techniques examples had been mentioned in Table 1.

Table 1. A different 3D printing technique prepares a number of the drugs

3DP Technology	Drug	Dosage form	Application
Ink-jet printer	Insulin	Microneedle	Anti-hyperglycemic
Thermal inkjet printer	Folic acid	Nano suspension	Anemia
Piezoelectric inkjet printer	Paclitaxel	Microparticles	Anti-cancer
Drop on drop	Ropinirole HCl	Tablets	Anti-parkinsonism
Drop on solid	Isoniazid	Implant	Anti-tuberculosis
Multi-nozzle 3D printer	Acetaminophen & Caffeine	Capsule	Analgesic
FDM	Ibuprofen	Tablet	NSAID
PAM	Naproxen sodium	Tablet	NSAID
Stereolithography (SLA)	Riboflavin	Hydrogel	Tissue engineering
Extrusion 3D printing	Glipizide	Tablet	Anti-diabetic
HME printer	Indomethacin	Subcutaneous rods	NSAID
Zip dose	Spritam	Tablet	Anti –epileptic
3D printer	Nitrofurantoin	Biofilm disk	Urinary tract infections

4.0 INTELLIMEDICINE PROTOTYPE PRINTER-PERSONALIZED PILLS

If the doctor or pharmacist had this information integrated into their workflow augmented with AI or intelligence augmentation to know of the 20,000 or more approved drugs, which either the right dose or combination for you. A polypill is the integration of multiple medications into a single pill. Ex: Common over the counter cold and flu remedies. Combination of statins, BP medications, aspirin, is giving those which is randomized studies have been shown to dramatically reduce risk (heart attack risk reduces to 70 %, and stroke risk is reduced to 64 %), compared to placebo to improve our health experience globally. But these polypills were not personalized; are not optimized to the individual. What if we could optimize your personalized polypill? So it would be built for you based on you, it could adapt to you even every single day. We are now in the era of 3D printing. So it would be easier to take, improve adherence, and potentially it could even integrate into supplements like vitamin D. When we hit print, and then print your combination of medications that might be needed by you on any particular day. We could write the name of the patient, the date, the day of the week, a QR code. The main advantage is we can potentially print our meds based on us. Instead of taking six pills, we can now be looking at our dosing. Smartwatch is looking at our blood pressure. We can adjust our blood pressure medicines. If the blood is too thin so we can lower our micro med dose of coumadin. So this could be smartly adapted day to day programmed by our physician or cardiologist. But it could finally merge and shrink to small ones that could be in your home with integrated cartridges like this that are delivered by drone could print our personalized polypill every day.

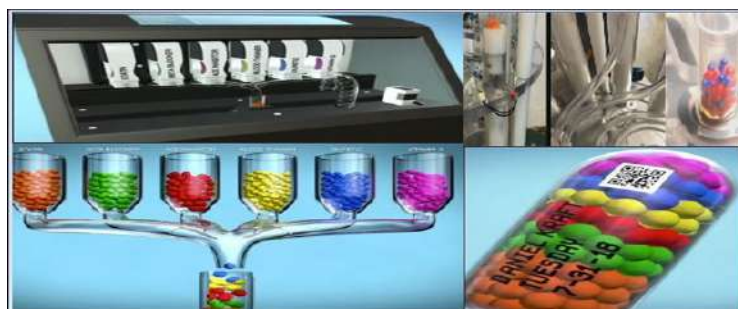


Figure 4. IntelliMedicine prototype printer (Source: Daniel kraft personalized pills)

CONCLUSION

Individualized medications fabricated through 3DP may offer a vital advantage to patients. Printable dosage forms on paper may be easier to deliver to the patient than powder-based printed types. Control surface tension and viscosity of binder solution is essential to achieve stable droplet formation. Further advancements in the process of performance are required to overcome these issues where 3DP technology

can be a success. If you believe the hype, 3D printing is intended to renovate each part of our lives at home, our labs, and in hospital.

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A REVIEW ON IMMUNIZATION AND ROLE OF PHARMACIST IN IMMUNIZATION

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ABSTRACT

Immunization is considered as an important role in the health of an individual. The endemic diseases, outbreaks can be prevented by the immunization programmes. The key role in immunization is played by "vaccines", injection of a killed microbe to stimulate the immune system against the microbe; thereby preventing disease is called "vaccination". After a study of decades to promote and enhance the human immune system the vaccines were discovered. The history of vaccines in India was started since 1802 and it's still developing, implementing newer immunization plans and promoting public health, the use of vaccines for infants, pregnant women, adults' overall impacts on the welfare of society and country's health system. The health care professionals responsible for the vaccination are the physicians, paediatricians, nurses, vaccinators and pharmacists. While providing immunization the national guidelines and schedules must be followed strictly.

KEYWORDS: *immunization, vaccine, vaccination, pharmacist, immunization programme.*

INTRODUCTION

Immunization is the process whereby a person is made immune or resistant to an infectious disease, typically by the administration of a vaccine. Vaccination is proven and is a cost-effective child survival intervention. In the world about all the countries have their immunization programmes to provide selected vaccines to the individuals especially focusing on pregnant women, infants, children who are at risk of diseases preventable by vaccines. There are at least 27 causative agents against which vaccines are available and many more are in development ¹. Vaccine safety gets more public attention than vaccination effectiveness but independent experts and WHO have shown vaccines are far safer than therapeutic medicines ². The number and type of vaccines in the immunization programmes varies from country to country; however, few selected vaccines are used in the immunization programme those are:

1. BCG vaccine (Bacille Calmette Guerin)
2. Poliovaccine
3. Hepatitis B vaccine
4. Haemophilus influenza B vaccine (HiB)
5. Pneumococcal vaccine
6. Typhoid vaccine
7. Diphtheria, Tetanus, pertussis vaccines (DTP)
8. Varicella vaccine
9. Measles, Mumps, Rubella (MMR) vaccine
10. Hepatitis A vaccine
11. Rotavirus vaccine
12. Human papillomavirus (HPV) vaccine
13. Rabies vaccine
14. Combination vaccines ²

HISTORY OF VACCINATION IN

INDIA: The history of vaccine and vaccination starts with the first effort to prevent diseases in society. Due to the spreading of several unknown diseases and loss of people the physicians, many researchers

started working on the remedies for the diseases. Though the records show many epidemic diseases the reasons for them were unknown back then so, later due to a thorough study and research the vaccines were discovered, the governments for all the countries were made aware of the immunization and implemented the immunization programmes depending on the public need for vaccination. History of vaccination started since 900 AD and it's been developing until providing and implementing immunizations programmes the biggest and latest achievement was in the year 2012 which is discussed below. Abu Bakr- 900AD gave the initial account of distinguishing measles and smallpox. Smallpox was well known since ancient times and its believing to have originated in India and Egypt. He was the first to mention the endemic diseases that are leading to the death of several people. Draft comprehensive multiyear strategic plan (MYP) for UIP (2012-2017) Ready, declared as a year of intensification of routine immunization in India. WHO (World Health Organization) removed India from the list of polio-endemic countries.¹

INFORMATION ON VACCINES THAT ARE IN NEED FOR AWARENESS IN INDIA:

VACCINES	USE	DOSING SCHEDULE	ADVERSE EFFECTS
BCG VACCINE The original strain of mycobacterium bovis. BCG was developed in 1921 at the Pasteur institute. ³	Tuberculosis meningitis, military disease Efficacy: 60-80%	Routine dose: At birth or 6 weeks of age Catch-up dose: Up to 5 years Dose: 0.1ml (0.05ml until 1 month of age). Route: Intradermal (I/D) Site: left upper arm	Injection site reaction characterized by papule may be red, tender and indurated. Lymphadenitis, skin lesions, ocular lesions, osteitis, osteomyelitis.
HEPATITIS B VACCINE⁶ Hepatitis 'B' vaccines contain one of the proteins from the surface of hepatitis B virus (HBsAg). This protein is made by inserting the genetic code into yeast cells, which removes any risk of viral DNA getting into the final product. The entire process called recombinant DNA technology	Uses: Used to prevent acute and chronic hepatitis 'B' virus infection. Efficacy: 90% (adults); 95% (children)	Doses: 1 st dose- at birth - If at birth missed then before 4 weeks of age 2 nd dose- at 6 weeks - Next dose after 1 month followed by a third after 6 months of 1 st dose (0, 1, 6 month). Dose: 0.5ml Route: IM Site: Anterolateral side of mid-thigh (LEFT)	soreness at site of injection, fever Major events: fever, diarrhea, feeling dizzy, low blood pressure
HiB VACCINE (Hemophilus influenza type B) Several HiB conjugate vaccine is available from different manufacturers but currently available is purification and conjugation of the capsular polysaccharide <i>Polyribosyl-Ribitol-Phosphate (PRP)</i> to tetanus toxoid results in the Hemophilus influenza type B	used to prevent epiglottitis, septicemia, osteomyelitis, cellulitis, pericarditis in children caused by HiB. Efficacy: 95% after 2 or 3 primary doses	Routine dose: 3 doses at 6, 10 and 14 weeks of age Booster dose at age 12-18 m Catch-up dose: <12 months age- 2 doses at 4 weeks interval with a booster dose at age of 12-18 months 12-15 months age- 1	redness, warmth or swelling at the injection site, fever no major events can be seen in the patients

vaccine. ¹⁸		dose followed by a booster dose after 4 weeks Above 15 months age- 1 dose No dose after 5 yrs.	

		Dose: 0.5ml Route: IM Site: anterolateral side of mid thigh- LEFT	
DIPHTHERIA, TETANUS AND PERTUSSIS (DTP) Diphtheria toxoid, tetanus toxoid, and pertussis toxoids are prepared by inactivation of <i>diphtheria toxin</i> with help of formalin and absorbed on aluminium hydroxide. Now -a- days these three toxoids are available in combination forms. ⁸	Uses : DPT vaccine mainly used to prevent diseases associated with diphtheria, tetanus, and pertussis viruses. (8) Efficacy: the efficacy of DPT vaccine is about 97% against Diphtheria, 100% against tetanus, 73% against pertussis. ⁸	DTaP (Diphtheria, Tetanus toxoids, and acellular pertussis): doses : at 2, 4, 6, 15 to 18 months age and 4 to 6 years, time gap for 3 rd to 4 th dose is about 6 months. Dose : 0.5 ml Route : Intramuscular (IM) Site: Anterolateral side of the left thigh ⁹ Tdap: Routine dose: First dose at 11 to 12 years, for pregnant women at 27 to 36 gestational weeks	Local reactions like erythema, injection site pain, swelling. Systemic reactions like fever malaise, shivering, general aches and headache. Major events: Persistent crying (>1 hour), seizures, Encephalopathy, Dravet's syndrome, anaphylaxis and hypotonic-hyporesponsive episode (HHE).
VARICELLA VACCINE (CHICKEN VACCINE) It is a live weakened form of the natural varicella virus. The virus is weakened by cell-culture adaptation process. ¹⁰	Uses: Varicella vaccine mainly used to prevent the chickenpox disease which is caused by the varicella-zoster virus. 17 Efficacy: 98% effective to prevent severe varicella.	Dosing schedule: dose: First dose at 12 to 15 months, the second dose at 4 to 6 years Catch up dose: Children of 7 to 12 years, 2 doses a minimum gap of 3 months, a person with age ≥ 13 years two doses a minimum gap of 4 weeks. Dose: 0.5 ml Route: SC	Minor events: Injection site pain, redness, rashes at the site of injection and fever. Major events: Rashes all over the body, seizures, infection to lungs and meninges of the brain and spinal cord.

HEPATITIS 'A' VACCINE Hepatitis-A- the virus is grown in cell culture and cultivate in human fibroblast or human diploid cell cultures, purified from cell lysates, inactivated with formalin and adsorbed on an aluminium hydroxide adjuvant. ¹¹	Use: Hepatitis A vaccine mainly used to prevent hepatitis. It is an inactivated vaccine. Efficacy: 95% of adults protective against hepatitis A within 4 weeks.	Routine dose: First dose at 12 to 13 months age, the second dose at 18 months age or a minimum gap of 6 months from the first dose. Dose : 0.5 ml, Route : Intramuscular (IM)	Adverse events: Minor events: Injection site pain, low-grade fever, headache, and tiredness. Major events: severe allergic reactions.

ROTAVIRUS The origin of the first vaccine from <i>bovine (cow) or rhesus (monkey)</i> ¹²	Uses: The Rotavirus vaccine mainly given to prevent diarrhoea caused by rotavirus. Efficacy: 91% is effective against rotavirus	dose: Three doses at the age of 6, 10, 14 weeks. Dose: 5 drops Route: Orally	Fever, diarrhoea, Intussusception (it is a type of bowel blockage)
HUMAN PAPILLOMA VIRUS (HPV) VACCINE (20) The HPV vaccine is prepared by virus-like particles (VLPs) produced by <i>recombinant technology</i> . Purified <i>L1 protein</i> self-assembled to form shells which resemble HPV VLPs. ²¹	Uses: Human papillomavirus vaccine can be given to prevent cancers associated with human papillomavirus. Efficacy: After the administration the HPV infections in teen girl reduced by 56%.	Routine dose: 2 doses of HPV vaccines (HPV2/ HPV4) of 9 to 14 years the minimum dosing interval should be 6 months. Catch up dose: Vaccination can be given until 26 years of age and maintains a minimum interval between the doses. Dose : 0.5 ml Route : Intramuscular (IM).	Minor events: Injection site pain, redness, swelling, mild (100 ⁰ F) to moderate (102 ⁰ F) fever. Major events: Severe allergic reactions.

14. COMBINATION VACCINES:

Different types of combination vaccines are now available. These are mainly used to protect against multiple pathogens. ²³ The combination of vaccines are:

- Diphtheria, Tetanus and Pertussis(DTP) combinations.
- DTP with other vaccine antigen combinations

ROLE OF PHARMACIST IN IMMUNIZATION:

As health care provider pharmacist plays an important role in disease prevention by advocating and administering immunizations. Earlier, pharmacists were only confined to the duty of providing medications i.e. compounding, dispensing of drugs, patient counselling, TDM (therapeutic drug monitoring) but to improve the public health, pharmacist involving in the immunization program is very beneficial. The pharmacist can administer vaccines or host other health care professionals who can administer vaccines. Pharmacist holding a pharmacy degree generally has the duties of patient-centred care, population-based care, manage human; human medical information and technological resources,

- The epidemiology of populations identifying who are at risk for vaccine- preventable diseases.
- MMR vaccine
- DTaP and IPV (polio) combination

- DTaP, IPV AND Hibcombination.

Manage medication use system, promoting the availability of effective health and disease prevention services and health policy. Though they are having the following duties in India their duties are yet to be made aware in the society, hospitals, and different communities. But pharmacists, if trained in immunization program appropriately, promote country's health system, physician view towards the pharmacist role in immunization has to be changed and should actively involve pharmacist in advocacy and administering vaccines, to track doses, maintain records and implementing the appropriate immunization schedule. The pharmacy degree holders after they graduate can be provided with immunization programs based on their community settings. The following areas pharmacist must be trained in the immunization program:

- Public health goals for immunization
- Vaccine safety
- Screening for contraindications and precautions of a vaccine in each patient.
- Vaccine stability, transportation and storage capacity.
- Immunogenic drug interactions
- Vaccine dosing schedule
- Proper dose preparations and injection technique

TYPES OF VACCINATION PRACTISING METHODS:

There are different systems involved in practising vaccines but the most commonly used are:

- An outsourced approach
- In house approach.

In the outsourcing model pharmacies may contact other health care professionals notably nurses to provide immunization services within the pharmacy. Using an outsourced mechanism does not require much commitment from the hosting pharmacy beyond providing space in the pharmacy for the contracted immunization provider to work. Outsourced immunization services also require minimal pharmacy staff participation, allow for the trail of immunization services within the pharmacy and are less likely to disrupt the dispensing activities. The outsourced approach can be practised in India if the pharmacy store has an immunization trained pharmacist to carry out these activities.¹⁶ In house approach where pharmacists are immunizers, improve vaccination rates and increase convenience for patients. In this approach, the delivery and workflow vary from pharmacy to pharmacy. The immunization services can be provided by appointment on a walk-in basis or a combination of both. Appointment based services tend to be less disruptive to the pharmacy's normal workflow, a patient volume is planned and expected, however, it can limit the number of patients who can be vaccinated. Walk-in based services are more convenient for patients but may cause disturbances to the normal pharmacy workflow to accommodate the patients¹⁶. This kind of approach if implemented in India that may not be effective as not all population of the community have the access of making appointment neither they tend to remember their dosing schedule to visit the pharmacy for a vaccine dose.

PATIENT COUNSELLING REGARDING VACCINATION:

- Signs and symptoms of ADRs to vaccines, reporting ADR, emergency procedure and advanced cardiac life support.
- Documentation
- Reporting to the primary health care provider or local health department and
- Billing¹⁷

Patients in need of vaccination have to be identified and should be advised of their infection risk and encouraged to accept the immunizations they need, guided about the vaccine safety, efficacy to improve their health also protect from the risk of vaccine-preventable diseases¹⁶. Patients after their vaccine are given the advice to be remained seated for 15 minutes to observe if any allergic reactions are observed and also before they leave the pharmacy to have to be informed about the upcoming doses they have to take and next visits for immunization. The person should also be informed and advised about the adverse events that may be seen after a vaccine if observed telling them to immediately attend the nearest physician or pharmacist for treatment. By following the above recommendations by a pharmacist and patient promotes immunization awareness in the community and country.

CONCLUSION

Vaccines are important preventive medicines for primary health care, area critical component of the nation's health security. Although international agencies such as WHO and United Nations Children Fund (UNICEF) promote global immunization drive policies for the success of an immunization programme India still needs to make aware of the immunization through all possible community pharmacies. The history of vaccination in India denotes efforts for the development of newer vaccines. Awareness of immunization is very limited in certain areas of the country especially in the rural areas even in urban areas the importance of vaccines is known when some health professional tells them so the pharmacist in that community can be given the role of implementing and improving immunization in those areas this also leads to reduce the burden of the physicians and other health care professionals. The training in the immunization program for a pharmacist is also very beneficial for the future public health.

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A REVIEW ON CARDIAC IMPLANTS TYPES, COMPLICATIONS AND PREVENTIVE MEASURES

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ABSTRACT

Heart is having a special property of automaticity by using specialized myocardial cells (Sinoatrial cells) to generate electrical impulses which help in contraction and relaxation of the heart muscles. This process was under the control of sympathetic and parasympathetic outflow. In some diseases, like arrhythmias, obstruction to coronary blood flow, problems with heart valves heart is unable to contract properly, and results in decrease the function of heart. Various types of cardiac implants were used to overcome these situations mainly coronary stents to remove plaques in blood vessels, pace makers to generate impulses at atria's, implantable cardiac defibrillators to treat arrhythmias in ventricles, CRT (Cardiac resynchronization therapy) device used in heart failure, heart valves to overcome regurgitation, stenosis and atresia. This paper mainly deals with when the first cardiac implants were discovered what are different types of cardiac implants, what are the different conditions and how the implants should be placed and what care should be taken before and after the insertion of implants to the cardiac patients, what are the complications associate with these implants and if occur how these to betreated.

KEYWORDS: *cardiac implants, types, complications, prevention, heartfailure*

INTRODUCTION

Heart is truly remarkable organ which beats more than forty million times a year and having a capacity to pump about 7500 liters of blood per day¹. It composed of three types of cardiac muscles; those are atrial muscle, ventricular muscle and specialized excitatory and conductive muscle fibers which help in conduction of heart². This conductive system was disturbed by some cardiac diseases like arrhythmias, complete heart block, atrial fibrillation, valvular stenosis coronary artery diseases and heart failure, etc.³. Heart failure is a worldwide health problem with high prevalence rate is about 23 millions. Heart failure is characterized by inability of heart to pump an adequate amount of blood to achieve the demand of different organs of the body and caused by irreversible damage from coronary artery diseases sometimes from viral infections, toxins, genetic factors⁴. To overcome these problems and to maintain a normal heart function cardiac implants are used. The implantation of cardiac implantable electronic devices (CIEDs) which include pacemakers, implantable cardioverter defibrillators (ICDs), and cardiac resynchronization therapy (CRT) has increased substantially over the past six decades to reduce the complications. Recent reports show that worldwide implantation rates (CIEDs) estimated to be 1,250,000 pacemakers and 4, 100, 00 cardioverter defibrillators per year with an annual increase about 5%⁵. Current ESC guidelines separates the heart failure into heart failure with preserved ejection fraction (LVEF >50%), mild range ejection fraction (LVEF 40 to 49%), reduced ejection fraction (LVEF < 40%). The cause of death in patients with heart failure is not only from haemodynamic failure but also unexpected or sudden ventricular disturbance such as ventricular tachycardia and ventricular fibrillation. In those conditions implantable cardiac defibrillators (ICD) proven to prevent the sudden cardiac death due to ventricular rhythm disturbances. In addition to this cardiac resynchronising therapy (CRT) shown as treatment choice to improve bothmortalityand quality of life. The automatic implantable cardioverter defibrillator (AICD) is the most effective treatment for people with life threatening ventricular tachycardia (VT) or ventricular fibrillation⁶.

CARDIAC IMPLANTS TYPES

Cardiac implants are the following types

1. Coronarystents
2. Pacemakers
3. Implantable cardioverter defibrillators(ICDs)
4. Cardiac resynchronization therapy(CRT)
5. Heart valves(23)

SL. NO	IMPLANT TYPE	PROCEDURE	PRECAUTIONS
1.	Coronary stents	Coronary stenting procedure was done in catheterization laboratory; a sedative medication was given to the patient. A small puncture is made in artery of arm or groin region, a guide catheter and guide wire is inserted in the artery and moved up to the coronary artery. This entire process was guided with the help of x- rays ⁷ . The diseased artery need to be enlarged first to make the area for placing the stent for this process a small, deflated balloon with the help of guide wire through the catheter move up to the blocked coronary artery when the balloon is in correct position it is inflated and make the artery to restore blood flow. After that, the balloon was deflated, a small metal mesh tube (stent) sends through catheter up to blocked region of coronary artery expanded against the wall of the artery and make sure the stent is in better contact with the artery ^{8,9} .	Proper sanitisation must be maintained to reduce the infections.
2.	Pacemakers	Local anaesthetic medication was given at the incision, it will block the sharp pain but the patient may feel some pressure while implanting the device. A small cut was made in the upper chest and locates the vein; a small puncture was made in the vein the leads placed in the heart this process can be monitored by fluoroscope (a type of moving X ray picture). The leads should be placed at the right position for better results this can be checked after the placement of leads and advised to take a deep breath or coughing vigorously which helps to assess the stability of leads. After completing this procedure a small pocket was generated beneath the skin by separating the skin underlying muscles to place the pulse generator (can be placed after the leads connected to generator). Once the all procedure was done incision area will be closed ¹⁰⁻¹² .	Most of home appliances do not interfere with the pacemaker activity. It can work with the medical equipment's like X-rays, diagnostic ultrasound, mammography. Patient should avoid the (EMI) electromagnetic interference produced by electrical appliances in poor conditions and not grounded correctly, arc-welders, industrial generators, specific medical equipment, and radio transmitters. These are mainly disturbing theprogramming of generator.
			Cell phones also send electromagnetic signals which may interfere with device operation, do not carry the pocket over the pacemaker. It is important to avoid hitting or bumping near the area of pacemaker ^{11,12} .

3.	Implantable cardiac defibrillators (ICD's)	A local anaesthetic is given at the insertion site, a sterile towel and sheath placed around the insertion site (usually collarbone). A small incision made at the insertion site, a introducer (like a plastic tube) is inserted into the blood vessel, through this introducer an ICD lead wire will be inserted into the blood vessel send it up to the heart and is tested to verify proper location where it to be work. Depending on the type of device there may be one, two or three lead wires inserted. The entire process was guided with the help of fluoroscopy. For subcutaneous ICD's one or two small incisions were made near the top and bottom of the breast bone (sternum bone). The ICD generator will be placed under the skin through the incision after the lead wire is attached to generator (for traditional ICD just below the collarbone and for subcutaneous ICD (S-ICD) left side of the chest. If the patient was right handed the device will be placed in the upper left chest and S-ICD will be on the left side of the chest near to the heart. If the patient was left handed ICD can be placed in the upper right chest, monitored with ECG. The skin incision is closed with sutures, special glue or adhesive strips and a sterile bandage or by dressing ^{13,14} .	Anti-theft system or electronic article surveillance (EAS) used in the department stores may interfere the ICD. Avoid to working in the large magnetic fields such as power generating sites and industrial sites which are used large magnets. Do not use diathermy (use of heat in physical therapy to treat muscles) and don't use the heating pads directly over the ICD. Avoid transcutaneous electrical nerve stimulation (TENS) therapy. Avoid high voltage machinery, radar machinery such as television, radio transmitters, and electric arc welders, high tension wires. Mobile phones are generally safer to use but keep a distance of at least six inches, avoid to carrying mobiles phones in the breast pocket over the ICD ^{14,15} .
4.	Cardiac Resynchronization therapy (CRT)	Cardiac resynchronizing therapy (CRT) requires a minor surgical procedure to implant the device in chest. A tiny wire (Leads) implanted through the vein (under the collarbone) in to the ventricle and into the coronary sinus vein to regulate the left ventricle, a lead also implanted in the right atrium which helps to beat the heart in a balanced way. The tip of the lead is attached to the generator which is placed under the skin of upper chest. This type of approach is known as Endocardial or transvenous approach. In epicardial approach the tip of the leads attached to outside of the heart and other end attached to pulse generator which is placed under the skin of abdomen. These two processes were guided by fluoroscope machine. CRT is indicated for the patients with left ventricular ejection fraction less than or equal to 30% ¹⁶ .	Patient should avoid magnetic and electromagnetic radiation sources. Do not place transthoracic defibrillation paddles directly on to the device. Biventricular pacemakers improves the symptoms of heart failure about 50% of people who have been treated with medication still have significant heart failure symptoms ^{16,24} .

5.	Heart valves	surgical options for heart valve replacement includes the following Mechanical valve: It is a long-lasting valve made up of double material Tissue valve: It consist of animal or human donor tissue Ross procedure: borrowing the healthy valve and replacing them with damaged valve TAVI/ TAVR: Transcatheter aortic valve implantation/ replacement is a technique in which a bio-prosthetic valve is introduced into a catheter and implanted at the injured aortic valve. TAVI is the treatment choice for the patient with symptomatic and severe aortic stenosis. It raises the patient survival and quality of life ¹⁷.	Mitral valve replacement can often provide a normal life without the need of blood thinners and other supportive medications associated with valve replacement whereas aortic valve replacement may require on-going medications. The patient post-operative medication mainly depends upon the condition of the patient and risk factors ^{17,18}.

COMPLICATIONS ASSOCIATED WITH CARDIAC IMPLANTS

The complications associated with cardiovascular implantable electronic devices (CIEDs) is due to surgical, software, hardware or device malfunctions, failure to sense, failure of pulse generator, pacemaker mediated tachycardia etc. The cardiac device infections (CDI) can be classified as isolated pocket infections, cardiac device related endocarditis (CDRIE) and blood stream infections. Isolated pocket infections are most frequently occur among the cardiac device infections. More than one half infections are due to isolated pocket infections. Patients with implanted cardiac pacemaker the most common point for the bacteria entry is subcutaneously placed generator pocket. Eighty percent of infections associated with pacemakers are mainly caused by methicillin resistant staphylococcus aureus (MRSA) and coagulase-negative staphylococci, Gram negative bacilli such as *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Fungi such as *Torulopsis glabrata*, *Candida albicans* and *Aspergillus* species. Around half of all are due to Staphylococcus infections. These infections are associated with severe mortality and morbidity, long hospital stay and high treatment cost. Among the all cardiac device infections the pocket infections are more challenging infections, these pocket infections further leads to systemic infections ¹⁹. Endocarditis related to pacemaker lead infection is a serious complication of permanent transvenous pacing but rare. The CIED infections varied clinical presentation. They can present as febrile illness, discharge from the wound, pacemaker pocket inflammation, abscess, erosion of the part of pacemaker through the skin, tricuspid valve endocarditis with regurgitation, endocarditis of the leads, rarely pulmonary embolism. Atrial fibrillation is also very common in patients with cardiac electronic implantable devices. During the lifetime of device the incidence of atrial fibrillation may be high as 50 % but mostly asymptomatic. Early detection, early reaction (device reprogramming, drug therapy or electrical cardioversion) may prevent the atrial remodelling and severe adverse events related to atrial fibrillation ¹⁹. To diagnose the CIED infections requires clinical awareness, precise physical examination, careful patient history assessment, blood cultures, transthoracic and trans-oesophageal electrocardiography. Use of C-reactive protein (CRP) and procalcitonin assessment can help the diagnosis if the blood cultures are negative. Patients with less than six months from the implantation are frequently expressed with local pocket infections whereas patients with more than six months from the time of implantation may experience with systemic pocket infections. Once the CIED infection is suspected it is essential to obtain at least two sets of blood cultures. This should be done prior to the initiation of antibiotic therapy. In general most patients are started on antibiotic therapy for suspected cases prior to blood cultures in these cases most of the blood cultures is seems to be negative ^{19,20}.

Management

For the superficial or incisional infections that do not involve hardware there is no need to remove the device. 7 to 10 days oral antibiotics are adequate for the treatment. Once the diagnosis was made the hardware should be removed to identify whether the infection is systemic or localised. Prior to the removal

after the blood cultures intravenous (IV) antibiotics should be initiated. About 50% of the infections due to Methicillin resistant staphylococcus aureus (MRSA) so Vancomycin is administered intravenously to all systemic and pocket infections. Daptomycin are an alternative for Vancomycin. In general American heart association updated a statement that recommends for CIED infections 10 to 14 days of antibiotic therapy. In case of device endocarditis or complicated bacteraemia requires 4 to 6 weeks of antibiotic therapy. In case of pocket infections complete debridement of the pocket is necessary. If the complete debridement is performed irrigate vigorously with antibiotic solution and primary closure was helpful in most cases. In patients with extensive erosion primary closure may not be possible in this case after the complete debridement and irrigation a wound vac is good option to promote healing. For the pacemaker dependent patients a semi-permanent temporary pacemaker is placed^{2f,22}.

PREVENTION

The most important step in the treatment of CEID infections is prevention. Prevention is mainly achieved with good antiseptic and surgical techniques at the time of implantation. It is also important that physician recognises the risk factors associated with CIED infections. The risk factors can be divided into two main groups one is patient related (diabetes mellitus, congestive heart failure, chronic renal failure, long term use of corticosteroids) and another one is procedure related (longer procedure time, operative inexperience, use of temporary pacing leads, lack of antibiotic prophylaxis and development of post-operative haematomas). The staff must be trained appropriately and the operating room should have quality ventilation, careful setup of tables, care must be taken while preparing the patient for surgery, administration of prophylactic antibiotics shown to be lower the infections rate. During the implantation care should be taken to achieve complete haemostasis in the pocket, avoiding pocket haematoma is a crucial part of infection prevention. Use of anticoagulants is considered as risk factor for infections. Coronary stent infections are very rare post percutaneous coronary intervention complication. They can be prevented by taking some preventive measures those are removal of hair from the puncture area, application of antiseptic to the skin, use of prophylactic antibiotics in immunodeficiency patient. By using the above preventive measures the infection rate should be controlled²¹.

CONCLUSION

Cardiac implants mainly used to reduce the risk associated with cardiac diseases. In recent years the usage of cardiac implants is being improved to reduce the mortality and morbidity. These implants may help to improve the patient quality of life. At present coronary stents were highly recommended to prevent the risk associated with coronary artery diseases. Dual chamber pacemakers, implantable cardioverter defibrillators (ICDs) shown to improve the patients from sudden cardiac death. The ICDs and cardiac resynchronization therapy (CRT) are play a major role in the modern treatment of heart failure. Before going through the procedures care must be taken and proper sanitization should be maintained to prevent the post-operative infections. Management of cardiac device infections can be challenging, before the initiation of therapy proper diagnosis should be made by using the blood cultures.

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A REVIEW ON INSULIN RESISTANCE IN GESTATIONAL DIABETES MELLITUS

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Guntur, Andhra Pradesh (AP) - 522601***ABSTRACT**

Diabetes mellitus [DM] is a disease defined as the increased glucose level in blood, Gestational diabetes mellitus [GDM] is defined as the increased blood sugar level in pregnant woman usually during 2nd and 3rd trimester of pregnancy, the increased blood glucose level is due to the hormones produced by placenta generally called as placental hormones helps in developing baby, but these hormones also blocks the action of insulin & gains resistance to insulin responsible for raising blood glucose level in mother leads to problems during delivery and after the birth of baby may also cause type-2 DM to mother and may cause birth defects in baby. Gestational diabetes is a treatable condition and women who have adequate control of glucose levels can effectively decrease risks. In this review article we briefly presented about the mechanism of placental hormones in causing insulin resistance, complications, diet plan and management.

KEY WORDS: Gestational diabetes mellitus, placental hormones, insulin resistance**INTRODUCTION**

Gestational Diabetes Mellitus (GDM) refers to clinical presence of diabetes mellitus in pregnant women which may lead to the termination of delivery. Thus the elevated levels of serum glucose levels in a pregnant women who has no any evidence of diabetes in the past years is termed as gestational diabetes mellitus. According to the American Diabetes Association (ADA), GDM was defined as any degree of glucose intolerance that was first recognized during pregnancy^{1,2}. Generally the most common forms of diabetes are type-1 (diminished production of insulin) in some conditions it may also consider as autoimmune diabetes and type-2 diabetes (effect of insulin is impaired (or) abnormality of β -cells). Both type-1 & type-2 diabetes leads to hyperglycemia as major condition³. "Prediabetes" is the term used for individuals whose glucose levels do not meet the criteria for diabetes but is too high to be considered normal. Gestational diabetes is a condition that is observed in pregnancy. Pregnancy induces series of progressive changes in maternal carbohydrate metabolism and glucose tolerance. As pregnancy advances insulin resistance and diabetogenic stress due to action of placental hormones in maternal blood may necessitate compensatory raise in insulin secretion and due to resistance of insulin pregnant women develops Gestational Diabetes Mellitus [GDM]^{4,5}.

PREGNANCY-PLACENTAL HORMONES:

Several physiological and metabolic changes occur in women during pregnancy condition. During pregnancy, the mother must adapt her body systems to support nutrient and oxygen supply for growth of the baby in uterus and during the subsequent lactation. These include changes in the cardiovascular, pulmonary, immune and metabolic systems of the mother. Failure to appropriately adjust maternal physiology to the pregnant state may result in pregnancy complications, including gestational diabetes and abnormal birth weight, which can further lead to a range of medically significant complications for the mother and baby [Guyton & Hall-10th edition]. Pregnancy lasts for 9 months fetal growth is divided into 3-trimesters^{10,11,12}. The placenta, which forms the functional interface separating the maternal and fetal circulations, is important for mediating adaptations in maternal physiology. GDM can be effected to the mother in 2nd or 3rd trimester due to the effect of placental hormones produced by placenta that are responsible for growth of fetus.

The hormones produced by placenta are

1. Prolactin-growth hormone family [human placental lactogen (hPL), human growth hormone

- (hGH),proliferins&proliferinlikeproteins
2. Steroids [Estrogen, Progesterone,Cortisol],
 3. Neuropeptides [Melatonin,Serotonin,Oxytocin],
 4. Other neuroactive hormones [kisspeptin, Thyrotropin-releasing hormoneTRH]Human Chorionic Gonadotropin hormone[hCG]-generally released by trophoblast in veryearly gestation and considered as 1stplacental hormone,it is used to maintain corpus luteum in early pregnancy.
 5. Activins&relaxins
 6. Parathyroid hormone-related protein [PTHrp]-a key hormone involved in bonemetabolism.
 7. Leptin- it is an abundant circulating hormone involved in regulatingappetite.

8. PLACENTAL HORMONES - INSULIN RESISTANCE: (1)INSULIN

Insulin is a polypeptide hormone consisting of 2-polypeptide chains with 51 aminoacids that are connected each other by disulphide linkages.it is synthesized as precursor(pro-insulin) that undergoes proteolytic cleavage to form INSULIN and peptide C.⁶ Insulin is synthesized in β -cells by the usual cell machinery for protein synthesis,beginning with the translation of the insulin RNA by ribosomes attached to the endoplasmic reticulum to form an *insulin preprohormone*,it is then cleaved in the endoplasmic reticulum to form a proinsulin.[Guyton&hall-10thedition] later undergoes cleavage and forms 2-polypeptide insulin that releases in response to various stimuli like glucose, arginine, and sulphonylureas although physiologically glucose is the major stimulant. Glucose is taken up by β -cells through, GLUT-2 receptors. After entering the beta cell, glucose is oxidized by glucokinase, which acts as a glucose sensor.⁷ that leads to release ofinsulin.

2. INSULIN RESISTANCE

Insulin resistance is defined as the decreased sensitivity of insulin response. Majorly insulin resistance is main reason for causing gestational diabetes in pregnant womens. Insulin resistance is caused by several mechanisms. Insulin resistance increases as pregnancy progresses, insulin sensitivity falls by ~50% in late Pregnancy, Increased insulin resistance due to increased maternal adiposity and effects of placental hormones, Hormones produced by the placenta: human placental Growth Hormone(hGH), human Placental Lactogen(hPL), Progesterone, Estrogen. As pregnancy progresses and placenta grows, production of hormones increases thus leading to increased insulin-resistance. GDM occurs when insulin secretion is not sufficient to offset the decrease in insulin sensitivity^{13,18}.

(2.1)Role of human Placental Lactogen (hPL)

Placental lactogen is a hormone from PRL-GH family produced by placenta.several studies prove that placental lactogenincreasesupto 30-folds throughout pregnancy plays important role in secreting insulin. PRL, PRL-like proteins and PL, through PRL receptor, induce β -cell mass expansion by both increasing β -cell proliferation and reducing apoptosis of islets in-vivo and in-vitro.⁸ PRL and PL also increase insulin secretion during pregnancy, particularly in response to glucose, by enhancing the expression of glucose sensors (glucokinase, hexokinase and glucose transporter-2) and activating the serotonin biosynthesis pathway in pancreatic islets. several studies show that hPL can cause PERIPHERAL INSULIN RESISTANCE.^{9,13} because it acts as one of the signaling and cell cycle regulators that regulates the molecular path way involved in lactogens transduction signal [JAK/STAT,IRS-1&2,PI3-kinase,MAPKs] known as Signal transductionmechanisms.⁹

(2.2)Role of human Placental Growth Hormone (hPGH)

PGH is the product of the GH-V gene specifically expressed in the syncytiotrophoblast layer of the human placenta. PGH differs from pituitary growth hormone by 13 aminoacids. It has high somatogenic and low lactogenic activities.PGH is produced by the placenta and found predominantly in the maternal circulation. It progressively replaces pituitary growth hormone (hGH) in the human maternal circulation from mid-gestation (20-weeks) onwards, peaking towards term of pregnancy the molecular mechanisms of insulin resistance in skeletal muscle in response to elevated hPL or hPGH. Recent evidence has shown that an important effect of hPGH is to specifically increase the expression of the p85 sub-unit of phosphatidylinositol (PI) 3-kinase in skeletal muscle increases in the p85sub-unit of PI3-kinase acts as a dominant-negative competitor to forming a PI 3-kinase hetero dimer with the p110 sub-unit, thereby inhibiting PI 3-kinase activity and preventing further insulin signaling downstream ^{13,20}. Barbour et al demonstrated a unique mechanism of insulin resistance in non-pregnant transgenic mice and suggested that

human placental growth hormone (hPGH) may contribute to the insulin resistance of normal pregnancy secondary to its effect on p85 α expression and its interference with PI 3-kinase activity in skeletal muscle. Placental GH reduces insulin receptor expression and signaling, as well as, diminishes the abundance of the insulin-sensitive glucose-transporter, GLUT-4, in the skeletal muscle¹⁴.

(2.3) Role of adipose tissue in causing insulin resistance

(2.3.1) Adipokines

In past studies placental hormones have been considered as the primary mediators of insulin resistance during gestation. Over the past decade, adipose tissue has been shown to produce numerous factors (adipocytokines/adipokines), most of them act as hormones. These adipocyte-derived hormones have been implicated in the regulation of maternal metabolism and **gestational insulin resistance**. Adipocytokines, includes- leptin, adiponectin, tumor necrosis factor(TNF- α), interleukin-6.^{13,9} the role of adipocytokines and elevated lipid concentrations in pregnancy have been correlated with the longitudinal changes in insulin sensitivity in non-pregnant well as in pregnant women. Evidence suggests that one or more of these adipokines (as TNF- α and leptin) could impair insulin signaling and cause insulin resistance.

(2.3.1.1) Adiponectin

Adiponectin is a hormone that is primarily secreted by adipocytes. However, plasma adiponectin concentrations are inversely proportional to adipose tissue mass, with low concentrations in obese individuals. GDM is similarly associated with decreased adiponectin. There is a stronger association of adiponectin with insulin resistance than with adiposity. This suggests that adiponectin plays an important role in the pathogenesis of GDM, independent of obesity^{13,20}. Adiponectin enhances insulin signaling and fatty acid oxidation, and it inhibits gluconeogenesis by activating AMP-activated protein-kinase (AMPK) with in insulin-sensitive cells, which facilitates the action of IRS-1, and by activating the transcription factor peroxisome proliferator-activated receptor alpha (PPAR α) in the liver. Furthermore, adiponectin stimulates insulin secretion, by up regulating insulin gene expression and exocytosis of insulin granules from β -cells. Several studies & evidences suggests adiponectin impairs insulin signaling and amino acid transport across the placenta, limiting fetal growth or causes fetal macrosomia.

(2.3.1.2) Tumor Necrosis Factor (TNF- α)

Tumor Necrosis Factor (TNF) which is released from macrophages and induces tumor necrosis. Increased circulating TNF- α level have been associated with insulin resistance in obesity, aging, sepsis, muscle damage, and burn patients. Obese animals and humans show a positive correlation between TNF- α levels and BMI and hyperinsulinemia reported that TNF- α is a significant predictor of insulin resistance during pregnancy. Together with a small additive contribution from leptin and cortisol, TNF- α exerted a significant influence on insulin-mediated glucose disposal. Circulating TNF- α showed a reduced levels during early pregnancy and increased during the third trimester, revealing the reduced insulin sensitivity. TNF- α correlates inversely with insulin secretion in normal pregnancy and was significantly higher in GDM group²³. Infusion of TNF- α results in increased insulin resistance in rat and human skeletal muscle cells incubated in culture⁹. Although the concentration of TNF- α in plasma of obese patients is much lower compared with that found in burn patients and patients with cachexia (Wasting syndrome) some evidence suggests that local skeletal muscle TNF- α may act in a paracrine fashion to contribute to skeletal muscle insulin resistance. TNF- α impairs insulin signaling by increasing serine phosphorylation of insulin receptor substrate (IRS)-1 and diminishing insulin receptor (IR) tyrosine kinase activity. Studies in pregnancy have reported that changes in insulin sensitivity from early (22–24 weeks) to late (34–36 weeks) gestation correlate with plasma TNF- α and that circulating TNF- α may be produced by the placenta and skeletal muscle to induce or exacerbate insulin resistance. In maternal obesity, the level of TNF- α is increased in the placenta compared with the non-obese pregnant women¹⁶. Further studies has been shown that placenta and subcutaneous adipose tissues obtained from women with GDM release greater amount of TNF- α in response to high glucose compared with glucose levels in normal patient. The increased TNF- α levels in pregnancy fall rapidly after delivery, which is consistent with the idea that the increase in circulating TNF- α during late pregnancy is mainly due to placental secretion. These findings may also help to explain the rapid reversal of insulin resistance after delivery, since maternal levels of TNF- α decrease substantially after delivery of the placenta²³. TNF- α and other pro inflammatory mediators suppress the transcription of adiponectin in adipocytes that explains the lower levels of serum adiponectin in individuals with GDM.

(2.4) Mechanism of insulin resistance

Insulin resistance occurs when cells no longer adequately respond to insulin. The insulin resistance occurs during the second trimester of pregnancy, when placenta produces hormones (cortisol, placental lactogen, placental growth hormone, estrogen) preserving pregnancy and blocking action of insulin – the “counter-insulin” effect, and which becomes apparent between 20-24 weeks of pregnancy till the 35th week when the growth of placenta stops.²⁰ At the molecular level, insulin resistance is usually a failure of insulin signaling, resulting in inadequate plasma membrane translocation of GLUT4 the primary transporter that is responsible for bringing glucose into the cell to use as energy. The action of insulin to increase glucose uptake is controlled initially by the transport of glucose across the cell membrane, which takes place by facilitated diffusion through glucose transporters. The reduction in glucose transport occurs in the absence of any detectable change in total GLUT4 transport abundance in skeletal muscle leads to reduced insulin action^{9,13}. The action of insulin begins with binding to the insulin receptor; insulin stimulates tyrosine phosphorylation of the β -subunit of the receptor on at least six tyrosine residues. The initial step in insulin receptor signaling is defective in GDM patients who have significantly less maximal tyrosine phosphorylation of the IR compared with women with normal glucose tolerance during pregnancy with no change in receptor binding or numbers. This indicates that in GDM condition there is an intrinsic defect in IR tyrosine phosphorylation per receptor protein that could be due to an endogenous inhibitory pathway for receptor signaling. The auto-phosphorylation of these tyrosine residues activates the receptor to bind with intracellular substrates (IRS-1 to IRS-6). IRS-1 is the major binding protein in human skeletal muscle. After IRS-1 is phosphorylated on tyrosine residues, which triggers the PI3-kinase pathway which is a crucial event required for stimulating glucose transport. Tyrosine phosphorylation of IR and IRS proteins is balanced by de-phosphorylation reactions carried out by cellular and membrane-bound protein tyrosine phosphatases. Friedmann et al., 2007 stated that several studies indicate that protein tyrosine phosphatase activity can serve as a negative regulator of insulin receptor phosphorylation to alter reduced sensitivity in human and animal models.⁹ In the condition of reduced adiponectin levels and reduced lipolysis by the action of placental growth hormone along with the inflammatory mediators or adipocytokines that regulate or down stream the insulin signaling by blocking the mechanism as showed in the figure:1, the regulation of IR and IRS-1 by blocking serine/tyrosine phosphorylation which clearly regulates the IRS-1 protein to act on insulin binding to the insulin receptor (IR) that regulates the PI3-kinase pathway which is a major mechanism in glucose metabolism as mentioned above that is blocked by increase the expression of the p85 sub-unit of phosphatidylinositol (PI) 3-kinase in skeletal muscle increase in the p85 sub-unit of PI3-kinase acts as a dominant-negative competitor on forming a PI 3-kinase hetero dimer with the p110 sub-unit, thereby inhibiting PI 3-kinase activity by blocking Akt-2 which is responsible for GLUT4 translocation of glucose uptake there by reduces the uptake of glucose and along with down streaming of insulin response leads to hyperglycemia causing GDM. Insulin binds to the insulin receptor (IR) and activates IRS-1. reduced Adiponectin and action of TNF- α , IL-6 and other inflammatory mediators blocks the action of IRS-1 even after binding of insulin to IR, if IRS-1 is activated later due to action of placental hormones that regulates the phosphatidylinositol kinase-3 (PI3K) activated by IRS-1 in which p85 subunit binds with p110 sub unit and forms a heterodimer which blocks the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-bisphosphate (PIP3) that leads to blockage of Akt-2 which acts on GLUT4 translocation and reduces the glucose uptake.

3. COMPLICATIONS IN GDM

Gestational diabetes is not a serious condition and it is considered as temporary which lasts till pregnancy and later decreases, but in some conditions it may cause the permanent defects to baby and to mother respectively,

(3.1) Maternal complications

Several studies and evidences had proved that women with history of GDM are very prone in effecting with cardiovascular diseases, T2-DM & obesity after delivery. The majority develop type-2 DM in the first decade after delivery, especially those who show impaired glucose tolerance after postpartum. Women who have had GDM have a significant higher risk to develop type 2 DM. These women must be accompanied with oral glucose tolerance test (OGTT) from 2 to 3 months after childbirth, and they must receive annual monitoring with OGTT and yearly be tested for fasting glucose.^{3,13,20} Complications may be severe at the time of delivery of baby if mother has GDM or hyperglycemia. Gestational diabetes mellitus is not an

absolute indication of cesarean birth, it may leads to death of mother.

The most common maternal complications are:

- 1.risk of DM recurrence in future pregnancies, possibility of Type 2-DM, 2.polyhydramnios,
- 3.pregnancytoxaemia, 4.urinary tract infection, 5.candidiasis,
- 6.higher incidence of premature childbirth, 7.cesarean delivery

(3.2)Fetal complications:

- 1.According to thrifty phenotype hypothesis proposed by Hales et al.,& barker et al., 1992 stated that babies who grow less well due to starvation in uterus were more likely to become overweight and develop T2DM and cardiovascular diseases in adulthood.along with they also stated that "A genetic interpretation of concordance rates of Type 2 diabetes in identical twins may not be justified since identical twins share a common early nutritional environment in uterus of mother". The familial pattern of Type 2 diabetes may have a similar explanation. Family members share a similar socio-economic environment, which is known to be linked to the incidence and prevalence of Type 2 diabetes . Poor maternal nutrition may be the key influence associated with low socio-economic status may be a specific reason in causing T2 DM in children.^{19,20}
- 2.the leading cause of perinatal mortality is major congenital anomalies that occur in 9%-14% of infants born to mothers having DM, the major malformations observed include NTD(Neural tube defects) and other anomalies involving cardiac, renal & GI system defects,rarely chances of Caudal regressionsyndrome.
- 3.The most common fetal complications are macrosomia, spontaneous abortion, congenital malformation, intrauterine death.
- 4.The most common neonatal complications include hypoglycemia, hypocalcemia, polycythemia, jaundice requiring phototherapy, trauma (shoulder dystocia) and respiratory distress syndrome.^{3,20}

(4)MANAGEMENT

The aim of the treatment approach is maintainance of normal blood glucose levels through pregnancy, and avoidextreme fluctuations in blood sugar levels avoiding the adverse events caused byhyperglycemia

(4.1)Non-pharmacological treatment

A diet with the intake of dietary fiber, mainly cereals and fruit reduce the risk of developing GDM. each 5gms of fruit and cereal fiber decreases the risk of GDM at a range of 23% and 26%. The intake of dietary fiber reduces appetite and thus reduces the total energy consumption that delays gastric emptying, and decreases glucose uptake. The ideal diet is that includes chicken, fish, fruits, vegetables and green leaves. Exercise has important role on glycemic control and thus may prevent, reduce or delay the need for insulin. Exercise is responsible for improving glycemic control in women with GDM, but also plays an important role on preventing the development ofGDM. ^{13,20} After GDM diagnosis, all patients should start lifestyle modifications on the diet and the physical exercises. Studies proven that 70% to 85% of cases of GDM can be controlled with changes in the lifestyle. If glycemic control is not achieved within 2 weeks, pharmacotherapy should be started. ^{13,20,22}

(4.2)Pharmacological treatment:

Insulin is the first-line therapy for treatment of GDM according to most Guidelines, as insulin does not cross the placental barrier, providing safety to the fetus. For patients who are resistant to insulin, use metformin and glibenclamide as an alternative or second line therapy, as they are considered safe and effective. Glibenclamide is a second-generation sulfonylurea, which acts by binding to the pancreatic β -cell receptors, blocking the potassium channel and opening calcium channels, stimulates the release of insulin. As it reduces hepatic glucose production, it contributes to fasting hyperglycemia. The transfer of glibenclamide by the placenta barrier exists, but it is minor. Glibenclamide is an acceptable alternative medication for pregnant women who cannot control blood glucose with diet and resistant to insulin. (22,24) Metformin is the drug used in treatment of type 2 diabetes in non-pregnant, and appears to be a great oral hypoglycemic agent for the treatment of GDM. Metformin acts by decreasing hepatic gluconeogenesis and enhancing peripheral absorption of glucose.The use of metformin in pregnant women is still controversial in guidelines, but there are increasing strong evidences on the effectiveness of metformin during pregnancy, and it has been suggested that metformin is a safe alternative both in mono-therapy and associated with the use of insulin.

Metformin exhibits beneficial effects for the treatment of patients who have gestational diabetes mellitus & appears to be safe if used in a short period.²⁵

(5)CONCLUSION

Gestational diabetes mellitus is associated with serious consequences both to the mother and the fetus. During screening of the disease, it is detrimental to identify the type of diabetes. Most of the women condition becomes normal after delivery, suggesting that the placenta is the major contributing organ in the development of GDM. The main cause of insulin resistance during GDM is post-cellular defect manifested by a decreased phosphorylation of tyrosine residues in insulin receptors and insulin receptor substrate-1, while tyrosine/serine phosphorylation is increased which inhibit insulin signaling from activating GLUT4 translocation. The women who are diagnosed with GDM in early pregnancy are clearly monitored until the delivery of baby as to provide quality life care for mother and baby.

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF SORAFENIB (A NOVEL KINASE INHIBITOR DRUG) IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate, highly sensitive, and reproducible high-performance liquid chromatographic method was developed for the determination of Sorafenib in bulk and tablet dosage form. Good quality chromatographic separation of Sorafenib Tosylate was carried out using thermo-scientific model C₁₈ column (4.6 mm i.d. X 250 mm, 5µm particle size) (based on 99.999% ultra-high purity silica) using mobile phase that containing acetonitrile: methanol (40:60 % v/v) at a flow rate of 1.0 mL/minute. The λ_{max} of Sorafenib in the mobile phase was found to be 265.5 nm. It had a retention time of 3.223 min. The calibration curve was in the linear function of the drug in the concentration range of 2-10 µg/mL ($r^2 = 0.999$) for the HPLC method. The regression equation for Sorafenib was found to be $Y = 68228x + 8071$ for HPLC. The Detection Limit (DL) & Quantitation Limit (QL) results of Sorafenib were found to be 0.5263 µg/mL and 1.5948 µg/mL for the HPLC method, respectively. The developed methods are validated in pursuance of ICH Q2 (R1) guidelines. The method was linear, precise, accurate with recoveries in the range of 98-102 %, and minimum values of % RSD indicate the accuracy of the method. The detailed quantitative results of the study show that this method is precise, accurate, cost-effective and robust. Thus, the developed gradient RP-HPLC method can be successfully practiced for the routine quality control analysis of Sorafenib in a pharmaceutical formulation.

KEYWORDS: *Sorafenib, RP-HPLC, Pharmaceutical Formulation, Validation.*

INTRODUCTION

The chemical name of Sorafenib is 4-[4-({[4-chloro-3-(trifluoromethyl) phenyl] carbamoyl} amino) phenoxy]-N-methyl pyridine-2-carboxamide. Its molecular formula is C₂₁H₁₆ClF₃N₄O₃. Sorafenib is an anti-cancer drug that is a protein kinase inhibitor. A thorough literature survey reveals that few analytical methods are reported for the determination of Sorafenib in bulk & pharmaceutical preparations and in biological fluids which include, UV spectrophotometric method¹⁻², RP-HPLC³⁻⁶, HPTLC⁷, High-Performance liquid chromatography with tandem mass spectroscopy⁸⁻¹⁰. However, most of the available methods have limitations such as arduous sample preparations, increased solvent consumption, long run times for biological samples, low sensitivity, uneconomical, and also have reduced symmetry. Keeping in view of these tests, we have decided to develop a simple, accurate, precise and reliable UV-spectrophotometric and Gradient RP-HPLC methods for the estimation of Sorafenib in the pharmaceutical dosage form. The chemical structure of Sorafenib is shown in Figure 1.

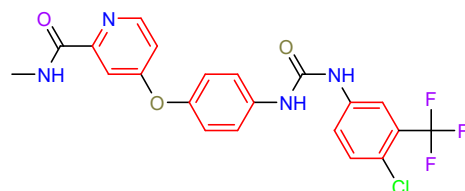


Figure1. Chemical structure of sorafenib

MATERIALS AND METHODS

Chemicals and Reagents

Sample of Sorafenib was gifted from Hetero Labs Ltd., Hyderabad, India. Soranib (Sorafenib 200 mg, Cipla Ltd.) was purchased from a local pharmacy. HPLC grade Methanol, HPLC grade Acetonitrile, HPLC grade Water and AR grade HCl, AR grade Sodium Hydroxide, AR grade Hydrogen Peroxide were purchased from Merck specialties Pvt. Ltd., Mumbai, India. Other excipients were prepared in our laboratory.

Instrumentation

For UV detection of the sample, ELICO SL-210 UV spectrophotometer with 1 cm matched quartz cells was used for all spectral and absorbance measurements and solutions were prepared in methanol. For HPLC, the chromatographic system consists of Agilent technologies -1260 series with G1311C Quat pump VL, Thermo scientific C₁₈ column, 1260 series with G11511D DAD VL detector was used. For homogenizing the solution prepared, Ultra-sonicator of Spectral labs, model UCB 40, was used. For the weighing of the sample and excipients, ESSAE VIBRA AJ (0.001g), ESSAE-Teraoka Ltd., was used. For measuring the pH of the prepared solutions pH meter, Systronics model – 802 was used. The data was acquired and processed by utilizing EZ chrome elite software.

Method development and optimization of chromatographic conditions

For HPLC development, various mobile phases containing HPLC grade water, acetonitrile, methanol in different ratios with or without buffers, and also various flow rates were performed. A good symmetrical peak was found when the mobile phase comprising a mixture of acetonitrile: methanol (40:60 % v/v). Thermo-scientific model C₁₈ column (4.6 mm i.d. X 250 mm, 5µm particle size) (based on 99.999 % ultra-high purity silica). Column temperature of about 25 °C was maintained. Adjust the flow rate to 1.0 mL/min so that the retention time of Sorafenib is about 3.223 min. The mobile phase was prepared by mixing acetonitrile and methanol in the proportion of 40: 60 v/v. The prepared mobile phase was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Selection of detection wavelength

To estimate the maximum λ_{max} , Sorafenib 10 µg/ml of working standard solution was prepared and scanned in a UV wavelength range of 200 - 400 nm utilizing as a blank. It was observed that the drug showed maximum absorbance at 265 nm which was chosen as the detection wavelength for the determination of Sorafenib.

Preparation of Stock and Working standard solution

For RP-HPLC method: Precisely weighted 100 mg of Sorafenib was transferred into 100 mL volumetric flask, dissolved and diluted up to the mark with mobile phase to get stock solution containing 1.0 mg/mL of Sorafenib. Aliquots of stock solution were diluted with mobile phase to attain the calibration standard solutions over the range of 2, 4, 6, 8 and 10 µg/mL.

RECOMMENDED PROCEDURE

Method for RP-HPLC: For the assay of pharmaceutical formulation, 20 tablets of Soranib marketed formulation (Sorafenib 200 mg) were weighed, the average weight was calculated, and a quantity of tablet powder equivalent to 100 mg of Sorafenib was accurately weighed and transferred into a 100 mL volumetric flask containing 30 mL of mobile phase. The solution was ultra - sonicated for about 15 minutes, filtered through a Whatman filter paper (0.45 µm) nylon filter, and the filtrate was made up to volume with mobile phase. The concentration was 1mg/mL. Transfer 1 ml of the filtered sample solution to 10 mL volumetric flask and made up to volume with mobile phase to get 100 µg/mL. This is used as working solution for the preparation of the assay. Then 0.2 ml of this solution is transferred into a 10 ml volumetric flask and made up to volume to obtain 2 µg/mL, which is used for the assay.

METHOD VALIDATION

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its pre-determined specifications and quality characteristics. The method was validated as per ICH guidelines¹¹⁻¹³.

RESULTS

Table 1. System suitability parameters

System suitability parameters	Limits	Sorafenib
Tailing factor (T)	≤ 2.0	1.122
Number of theoretical plates	NLT 2000	8768
Theoretical plates per meter (N)*	-	1,66,592
Retention time*	-	3.223 minutes
SD for peak area and RT		0.0228
% RSD	NMT 2.0	0.5628

* Average of five determinations, SD = Standard deviation, RSD = relative standard deviation.

Table 2. The summary output of ANOVA study of sorafenib

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.999505293							
R Square	0.999010831							
Adjusted R Square	0.998763539							
Standard Error	8981.168063							
Observations	6							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	3.25856E+11	3.25856E+11	4039.798238	3.6704E-07			
Residual	4	322645519.1	80661379.78					
Total	5	3.26178E+11						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	8071.047619	6500.092225	1.241682016	0.282188065	-9976.1016	26118.197	-9976.1016	26118.197
X Variable 1	68228.15714	1073.454901	63.55940716	3.67042E-07	65247.7685	71208.546	65247.7685	71208.546

Table 3. Summary of the RP- HPLC of Sorafenib validation parameters

Validation Parameters		Results
System suitability		Complies
Specificity		No interference observed, peak purity complies
Precision	System precision	% RSD - 0.029675
	Method precision	% RSD - 0.540332
	Intermediate precision	% RSD-0.6054, Cumulative % RSD-1.1457
	Precision at different levels	50 % - 0.2541, 100% - 0.2129, 150%- 0.1353
Linearity (r^2 should not less than 0.997).		0.999
Accuracy (mean % should be 98-102 %)		50 % = 99.4 %, 100 % = 99 %, 150 % = 99.6 %
Robustness		Complies
Limit of Detection (LOD) $\mu\text{g/ml}$		0.5263 $\mu\text{g/mL}$
Limit of Quantification (LOQ) $\mu\text{g/ml}$		1.5948 $\mu\text{g/mL}$

Table 4. Assay results of sorafenib by HPLC

S. No.	Formulation	Labeled claim	Amount found*	Mean % recovery $\pm$ SD	% RSD
1	Sorafib tablets	200 mg/tablet	199.9 mg/tablet	99.95 $\pm$ 12	1.23

* Average of six determinations, SD = standard deviation, RSD = % relative standard deviation

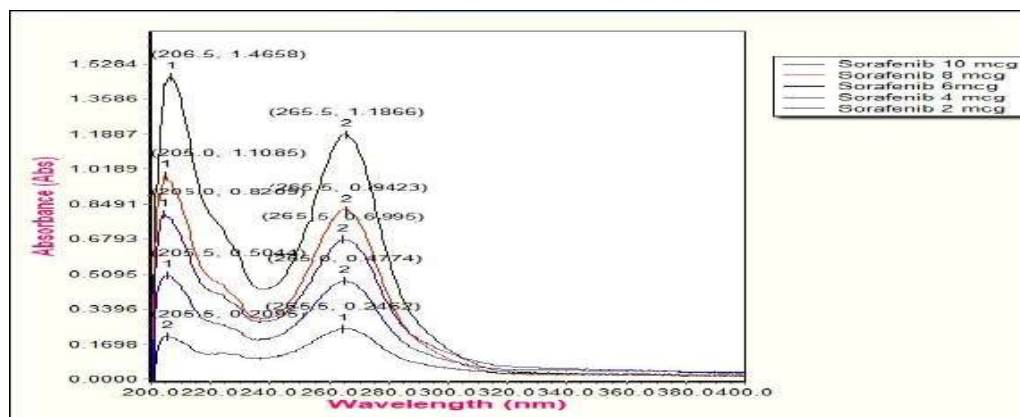


Figure 2. Overlay Spectrum of Sorafenib for UV method

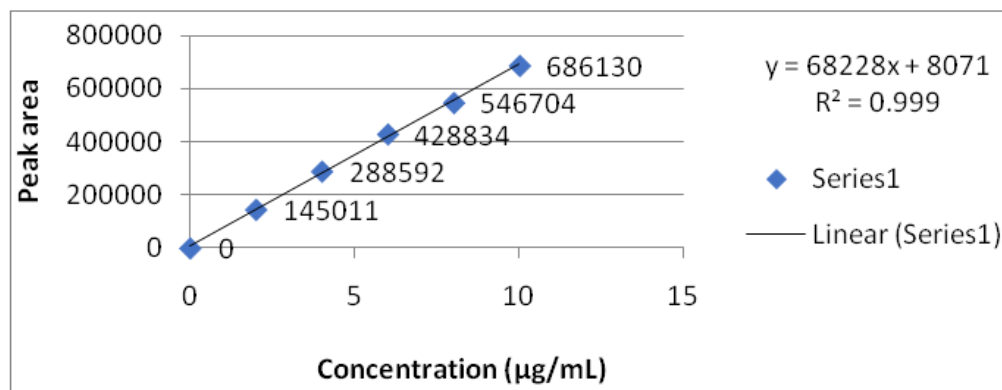


Figure 3. Calibration graph of Sorafenib by RP- HPLC

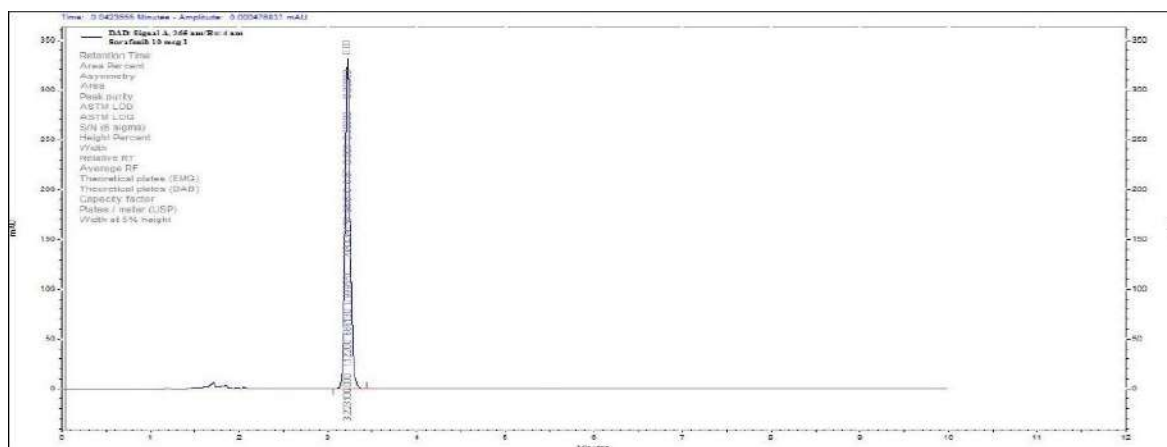


Figure 4. Standard chromatogram of Sorafenib (10 µg/mL)

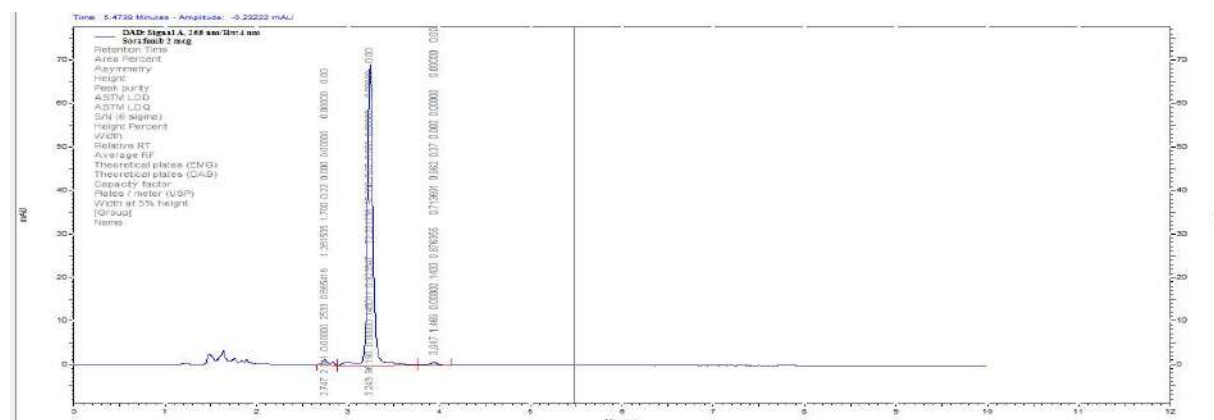


Figure 5. Sorafenib sample chromatogram

DISCUSSION

Since the above mentioned Sorafenib is relatively polar, an RP-HPLC method was used. The column for the separation was a C₁₈ column that has an internal diameter of 4.6 mm, length of 250 mm and 5µm particle size. Multiple numbers of trials were performed using various buffer solutions with various compositions of methanol, ethanol, acetonitrile, and HPLC grade water and variable flow rates. Eventually, the optimum separation obtained with a mixture of acetonitrile and methanol (40:60 v/v). The mobile phase flow rate adjusted at 1mL/min, and the detection wavelength set at 265.5 nm. The overlay spectrum is shown in Figure 2. System suitability conducted as per the methodology system suitability solution, and six replicate of standard preparation was injected into HPLC. The tailing factor was found to be 1.122. The number of theoretical plates was 8768; the number of theoretical plates per meter was 1,66,592. The retention time was found out to be 3.223 minutes, and the % RSD calculated to 0.5628. The results were well within the acceptance criteria, and the study concludes the suitability of the analytical system for analysis. System suitability parameters represented in Table 1. For specificity studies, blank, standard, system suitability, placebo, test preparations, individual impurities, placebo spiked with the analyte, and spiked test preparations were analyzed to examine the interference of blank and placebo with Sorafenib peaks. The purity angle for standard preparation is 0.139, for placebo with analyte preparation is 0.138, for test preparation is 0.132 and spiked test preparation is 0.137. No interference observed in analyte peaks, and peak purity values comply, thus proving the specificity of the method. The precision of the method examined by using System Precision Method and Intermediate precisions. Various levels of concentration were taken in six replicate samples. For Method and Intermediate precisions, the % RSD was found to be 98.1667 and 0.605449. The %RSD of the System precision was found to be 0.029675. The results are well within the acceptance criteria, and the %RSD observed for the replicate injections indicates the precision of the HPLC used, assay values indicates the precision of the method. The linearity of Sorafenib was determined in the concentration range of 2 µg/mL to 10µg/mL of the test concentration. The squared correlation coefficient value was found to be 0.999010, which is well within the limit. The summary output of ANOVA study of sorafenib is shown in Table 2. The calibration graph is shown in Figure 3. To determine the accuracy of the Sorafenib, the drug was spiked with placebo at three different levels in triplicate preparations. The mean % recovery at each level was found out to be within limits, i.e., 98.0% to 102.0 %. The robustness of the HPLC was determined for the suitability and assay value under multiple variable conditions like Flow rate change, Wavelength change, and change in mobile phase composition. The Limit of Detection and Limit of Quantitation of Sorafenib were found out to be 0.5263 µg/mL and 1.5948 µg/mL, respectively. The summary of validation parameters of sorafenib is mentioned in Table 3. The assay results are presented in Table 4. The representative standard and sample chromatograms of Sorafenib are shown in Figures 4 and 5 respectively.

CONCLUSION

The present study demonstrated a validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of Sorafenib available as the tablet dosage form. The method was validated entirely and showed satisfactory results. The method was free from the interference of the other active ingredients and additives used in the formulation. The RP-HPLC method for the estimation of Sorafenib has various advantages like less solvent consumption, low retention time, good peak symmetry, precise, accurate, and robust. The results of the study indicate that the developed method was found to be accurate, precise, linear, sensitive, simple, economical, and reproducible, which has a short run time, which makes the method rapid. Hence it can be concluded that this method may be employed for the routine quality control analysis of Sorafenib in active pharmaceutical preparations.

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ADVANCING DRUG DISCOVERY VIA ARTIFICIAL INTELLIGENCE

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ABSTRACT

Artificial intelligence(AI) is the stimulation of human intelligence processes by machines, especially computer systems. Most of the pharmaceutical industries are beginning to invest in artificial intelligence (AI), partnering with AI start-ups in 2017, to develop better diagnostics or biomarkers, in identifying drug targets and designing new drugs. AI is playing an increasingly important role in rational drug design, screening, and identification of candidate molecules; study on quantitative structure-activity relationships (QSAR) and docking. While using AI techniques, issues to be considered are the complexity of the model, as roughly defined by the discriminatory function and number of free parameters to be optimized, design of appropriate training and control sets, and careful validation of the results. Prediction methods developed in bioinformatics are important for drug design & discovery and those are integrated with modeling and docking techniques.

KEYWORDS: *New drug discovery, drug docking, patient safety, rational drug design, human intervention.*

INTRODUCTION

1. ARTIFICIAL INTELLIGENCE (AI)

AI is the stimulation of human intelligence processes by machines, especially computer systems.

1.1 TRADITIONAL DRUG DISCOVERY

This method is target-driven, a known target is used to screen for small molecules that either interrelated damage their role in the cell. All these approaches work well for easily druggable targets that have a well-defined structure and whose interactions inside the cell understood in detail. However, these methods extremely limited due to the complex nature of cellular interactions as well as limited knowledge of intricate cellular pathways.

1.2 AI BASED DRUG DISCOVERY

AI can overcome these challenges by identifying novel interactions and inferring the functional importance of different components of cellular pathways. Computer automated structure evaluation of biological activity of organic molecules¹. AI helps to extract meaningful information from a large dataset by using AI-based algorithms and machine learning. Use of artificial intelligence in the design of small peptide antibiotics effective against a broad spectrum of highly antibiotic resistant superbugs². The RNA sequencing dataset is used to identify the genes whose expression correlates with a given cellular condition. Applications in pharmaceutical research³. AI is also used to identify the compounds that bind to 'undruggable target,' i.e., proteins whose structures are not defined. Artificial intelligence in healthcare⁴ and medicine⁵. Stroke biomarkers: progress and challenges for diagnosis, prognosis, differentiation, and treatment⁶.

1.2.1 BENEFITS OF APPLYING AI IN DRUG DISCOVERY

The application of AI drug discovery has the potential to revolutionize the current time and scope in discovering the drug. Applications of machine learning in drug discovery and development⁷. Artificial intelligence within pharmacovigilance: A means to identify cognitive services and the framework for their validation⁸. AI uses advances in biology and computing to develop state-of-the-art algorithms for drug discovery. By using AI in pharma industries many startups started investing on it⁹. With the fast- increasing

process power and decreasing process cost, AI has the power to maintain the playing field in drug development¹⁰. AI has a high predictive power to define meaningful interactions in a drug screen. AI is a novel approach in drug discovery¹¹. Most importantly, AI has the potential to move drug screening from bench to a virtual lab, where results of a screen can obtain with higher speed, and promising targets can shortlist without the need for extensive experimental input and human resources. Artificial intelligence for clinical trial design¹².

1.2.2 DRAWBACKS OF APPLYING AI IN DRUG DISCOVERY

AI is affected by algorithm bias, where the creator's own bias manifests in the way that information is developed to generate the predictions. Therefore, the process is not entirely objective. Ultimately, predictions made by a computer have to be verified to make sure to valid.

1.3 COMPANIES USING AI IN DRUG DISCOVERY¹³

Table 1 shows companies using AI in drug discovery.

Table 1. Companies using AI in drug discovery

S.NO:	COMPANIES	Partnerships, memberships & Investments
1.	AbbVie	Aicure, Atomwise
2.	Amgen	GNS Health care, Machine Learning Ledger Orchestration for Drug Discovery (MELLODDY), Owkin.
3.	Astellas	Biovista, MELLODDY, NuMedii
4.	AstraZeneca	BenevolentAI, Berg, MELLODDY, ProteinQure, Schrodinger
5.	BASF	Machine Learning for Pharmaceutical Discovery & Synthesis Consortium (MLDPS), Nuritas
6.	Bayer	Alliance for AI in Health Care (AAIH), Cyclica, Genpact, MELLODDY, MLDPS, Sensyne Health
7.	Boehringer Ingelheim	MELLODDY, Nanna Therapeutics, Numerate
8.	Bristol-Myers Squibb	Concerto HealthAI, PathAI, Sirenas
9.	Celgene	Exscientia, GNS Health Care
10.	Eli Lilly	Atomwise, MLDPS, Strateos
11.	Evotec	Exscientia, University of Oxford
12.	GSK	ATOM, AAIH, Cloud Pharmaceuticals, Exscientia, Google, Insilico Medicine, MELLODDY, University of Nottingham, University of Strathclyde
13.	Genentech	GNS Healthcare
14.	Gilead	Glympse Bio, Insitro
15.	Ipsean	Owkin
16.	Janssen	AAIH, BenevolentAI, Celsius Therapeutics, Iktos, MELLODDY, WinterLight Labs
17.	Merck Group	Atomwise, Cyclica, Iktos, MELLODDY, Numerate, PathAI
18.	Mitsubishi Tanabe Pharma	Hitachi
19.	Nestle	Nuritas
20.	Novartis	BenevolentAI, IBM, Intel, MELLODDY, MLDPS, Microsoft, Oxford Big Data Institute, QuantumBlack
21.	Novo Nordisk	e-Therapeutics
22.	Ono Pharmaceuticals	TwoXAR
23.	Pfizer	Atomwise, Concerto HealthAI, CytoReason, IBM, MLDPS, Xtalpi
24.	Roche	Owkin
25.	SK Biopharmaceuticals	TwoXAR
26.	Sanofi	Berg, Exscientia, Google, Recursion Pharmaceuticals,

		Researchably
27.	Santen	TwoXAR
28.	Servier	Iktos, MELLODDY, Numerate
29.	Sumitomo Dainippon Pharma	Exscientia
30.	Sunovion	Exscientia, MLDPS, PsychoGenics
31.	Takeda	Numerate, Recursion Pharmaceuticals
32.	Wave Life Sciences	Deep Genomics
33.	Yuhan Corp	Cyclica
34.	Zambon Pharma	GNS Health Care

1.3 STARTUPS USING ARTIFICIAL INTELLIGENCE IN DRUG DISCOVERY¹⁴

167 Startups using artificial intelligence had evolved. AI in drug discovery startups by category is shown in Figure 1.

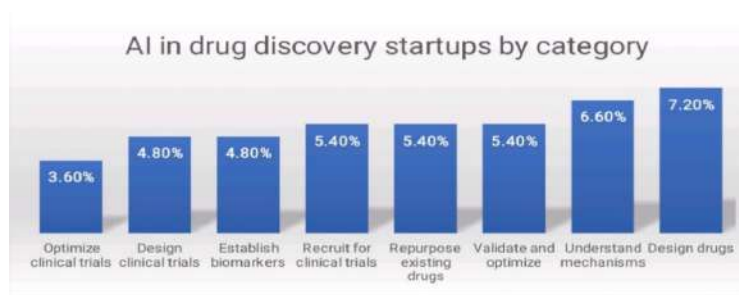


Figure 1. Startups by category

1.4.1. CATEGORIES

- Aggregate and synthesize information
- Understand mechanisms of disease
- Establish biomarkers
- Generation of models and data
- Repurpose existing drugs
- Generate novel drug candidates
- Validate and optimize drug candidates
- Design drugs
- Design preclinical experiments
- Run preclinical experiments
- Design clinical trials
- Recruit for clinical trials
- Optimize clinical trials
- Data publication
- Analyze real world evidence.

1.5 CHALLENGES OF USING AI IN DRUG DISCOVERY¹⁵

1.5.1. ETHICAL ISSUES & REGULATIONS

AI algorithms used today are not only to optimized but optimized to do a method and task. A supervised learning algorithm is designed to verify the statistical patterns in a provided dataset. If this training dataset reflects the prevailing biases against minority class when a dataset is unbalanced, the algorithm incorporates these biases that lead to less profitable decisions for these minority group classes.

1.5.2. TECHNICAL OBSTACLES

The biggest hindrance in using AI is to predict targets and to convert the traditional basic research held in labs around the world into a language that computer can understand easily. Machine learning programs depend on data present in a format where the patterns are to be crossed. This process requires a sophisticated experimental design where human error is minimum.

CONCLUSION

AI has the potential to reform drug discovery. A branch of AI (machine learning) can predict the physical and chemical properties of small molecules at quantum mechanics level accuracy with much lower expenditure and time. It is also helpful of searching correlations between molecular presentation, biological and toxicological properties. The pathways of synthesis of novel drug candidates that are efficiently developed by using algorithms based on AI. In the grouping of AI with robotic platforms, the chemical space for the innovative reactions from automated analysis of reaction feasibility has increased. New drug finding and discovery with development are among the most significant science activities that contribute to human health and wellbeing of society. However, the traditional method of new drug development is an affluent and a long process. The most challenging and urgent question is to decrease the cost and speed up new drug discovery and AI combined with experimental technologies. It is expected to make the hunt for new pharmaceuticals quicker, cheaper, and more effective.

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FORMULATION AND EVALUATION OF HERBAL OINTMENT CONTAINING NICOTINE EXTRACT

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ABSTRACT

Plant derived substances have recently attracted the great interest towards their versatile application as medicinal plants are the richest source of bioactive compounds used in traditional and modern medicine. The present work is to formulate and evaluate the ointment of Nicotine Extract. The ethanolic extracts were prepared by using maceration method. The ointment was prepared by levigation method. And the formulation was evaluated for its stability at various temperature conditions which shows no change in the irritancy, spreadability and diffusion characteristics.

KEYWORDS: *Maceration, Levigation, Extrudability, Spreadability, nicotine extract.*

INTRODUCTION

Tobacco is a product prepared from the leaves of the tobacco plant by curing them. The plant is part of the genus *Nicotiana* and of the Solanaceae (nightshade) family. While more than 70 species of tobacco are known, the chief commercial crop is *N. tabacum*. The more potent variant *N. rustica* is also used around the world. Certain European and oriental countries have been exploring the use of herbs and have been in practice since the centuries. With the techno-savvy lifestyle in 21st century human sufferings are coming out with different names. The basic herbs have the answer with no side effects and effective remedies and the golden fact is use of herbal treatment is independent of any age group. When two or more herbs are used in the formulation they are known as polyherbal formulations¹. Nicotine is the major alkaloid found in tobacco, but it can be found in lower concentrations in plants of the same family, potatoes, tomatoes or eggplants. Humans have always been curious to find plants that can have pleasurable or medicinal effects. Tobacco has been used in South America from pre-Colombian times to the present for magico-religious, medicinal and recreational purposes. Over the centuries, tobacco use became common in most parts of the world. The ability of nicotine to regulate mood and improve cognitive functioning, and acting as a strong reinforcer of tobacco dependence, is probably the motivation for its widespread use. All these positive aspects of nicotine use have been reviewed 15 years ago (9), but little progress has been made to explore further these potential beneficial effects of nicotine². Renewed interest in nicotine science, linked with the recent development of e-cigarettes, might inspire new investigations into the positive effects of nicotine including its potential role in disease prevention and treatment. Along with other dosage forms herbal drugs are also available in the form of ointment which is semisolid preparation used topically for several purposes e.g. as protectants, antiseptics, antibacterial and antifungal, Alzheimer's disease³.

Collection of Plant material

Leaves of neem were collected from the local area of Guntur

Preparation of Nicotine extract

Leaves of the plant were collected and washed thoroughly with distilled water and shade dried for 10 days. Dried leaves were ground into powder form. 100gm powder was imbibed with 350ml of 90% ethanol for 3hrs. and transferred to percolator with addition of 150ml of 90% ethanol for maceration for 7 days with occasional stirring. Finally ethanolic extract was collected and concentrated to get blackish green residue. The extract was stored in the airtight container at cool and dark place⁴. The extract with brown colour was obtained and stored at cool and dark place in air tight container.

MATERIALS AND METHOD

Table 1: Formulation of ointment base

S.NO	Name of Ingradient	Quantity to be taken
1.	Wool fat	0.5gm
2.	Cetostearyl alcohol	0.5gm
3.	Hard paraffin	0.5gm
4.	Yellow soft paraffin	8.5gm

Table 2: Formulation of Herbal ointment

S.No	Name of Ingradient	Quantity to be taken
1.	Prepared nicotine extract	0.06gm
2.	Ointment base q.s.	10gms

Procedure for preparation of herbal ointment

- Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base
- Herbal ointment was prepared by mixing accurately weighed Nicotine extract to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more bases until to form homogeneous ointment, finally transferred in a suitable container.

Evaluation Colour and Odour

Physical parameters like colour and odour were examined by visual examination.

Consistency

Smooth and no greediness are observed.

PH

PH of prepared herbal ointment was measured by using digital PH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. PH was determined in triplicate for the solution and average value was calculated ^{5,6}.

Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as Spreadability ⁷. Lesser the time taken for separation of two slides results better spreadability. Spreadability was calculated by following formula

$$S=M \times L/T$$

Where,

S= Spreadability M= Weight tide to the upper slide L= Length of glass slide T= Time taken to separate the slides

Extrudability

The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium. A whole board at the center of medium and ointment was placed in it. The time taken by ointment to get diffused through was noted. (after 60 minutes)

LOD

LOD was determined by placing the formulation in petri-dish on water bath and dried for the temperature 105°C⁸.

Solubility

Soluble in boiling water, miscible with alcohol, ether, chloroform.

Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

Non irritancy Test

Herbal ointment prepared was applied to the skin of human being and observed for the effect⁹. **Stability**

Study

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2°C, 25°C and 37°C. The herbal ointment was found to be physically stable at different temperature i.e. 2°C, 25°C, 37°C within four weeks.

RESULT AND DISCUSSION

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using simple maceration process to obtain a good yield of extract and there was no any harm to the chemical constituents and their activity. The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occurred which was stable during the storage. The physicochemical properties were studied which shows satisfactory results for spreadability, extrudability, washability, solubility, loss on drying and others. Also the formulation was placed for a stability study at different temperature conditions like 20°C, 25°C and 37°C within four weeks. There were no changes observed in spreading ability, diffusion study as well as irritant effect.

Physicochemical parameters	Observation
Colour	Yellow
Odour	Characteristic
Consistency	Smooth
PH	5.4
Spreadability(seconds)	7.5
Extrudability	0.4 gm
Diffusion study (after 60 min)	0.7 cm
Loss on drying	30%
Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform
Washability	Good
Non irritancy	Non irritant
Stability study (20°C, 25°C, 37°C)	Stable
Loss on drying	30%
Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform
Washability	Good
Non irritancy	Non irritant

CONCLUSION

From the ancient time Nicotine extract is used for their various medicinal properties like antibacterial, antifungal, antiseptic activity etc. Thus this ointment could become a media to use these medicinal properties effectively and easily as a simple dosage form.

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SP-133

DETECTION AND ASSESSMENT OF MEDICATION RELATED ISSUES IN ELDERLY PATIENTS

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ABSTRACT

Clinical pharmacy services are the services provided by the pharmacists to promote patient care, optimizes medication therapy, promote health and disease prevention. This prospective cross sectional study was conducted in tertiary care hospital over a period of 6 months. This collected data is checked for their appropriateness of any prescription related errors and DRPs were identified. Results obtained were assessed to determine the influence of Clinical pharmacist services. Majority of the prescriptions were with 5-9(62.5%) drugs. The majority of co morbidities among 125 enrolled patients in age group of 60-70, 55 patients were with 3-4 co morbidities. Among 125 prescriptions around 121 prescriptions were identified with 622 drug interactions. Among 125 patients 2 (0.277) adverse drug reactions were observed and according to naranjo 's probability assessment scale these adverse drug reactions were mild and 15(2.08%) dispensing errors ,10(1.386%) prescription errors where majority of prescription errors are due to missed written frequencies in the prescriptions.5(0.693%) administration errors,5 (0.693%)untreated indications were observed.Presence of clinical pharmacist in hospital settings can reduce drug related problems and they can assist other staff in improving patient care.

KEYWORDS: *Clinical pharmacy services, drug related problems, fixed drug combinations, drug interactions untreated indications.*

INTRODUCTION

- The advancement of medicine and its research and the development of medicines stilted with pharmacological Research have geometrically doubled quadrupled and forth so alleviated problems to mankind. This has led to exponential increased in number of surviving people.
- This has lead to increase in the number of surviving people; this also has lead to an increase in elderly population. Compounded, with its attendant problems elderly care has grown from a bud to at all tree, as time progressed ¹.

ALTERED PHARMAKINETICS IN ELDERLY^{2,3}

Ageing results in many physiological changes that could theoretically affect absorption, first-pass metabolism, protein binding, distribution and elimination of drugs. Age-related changes in the gastro-intestinal tract, liver and kidneys are

- Decreased gastro-intestinal motility
- Reduced total surface area of absorption
- Reduced liver blood flow
- Reduced glomerular filtration
- Reduced gastric acid secretion
- Reduced liver size
- Reduced splanchnic blood flow
- Reduced renal tubular filtration.

ALTEREDPHARMACODYNAMICS IN ELDERLY^{4,5}

There is some evidence of altered drug response or "sensitivity" in older adults. Four possible mechanisms have been suggested:

- (a) changes in receptor numbers,
- (b) Changes in receptor affinity,

- (c) Post receptor alterations, and
- (d) Age-related impairment of homeostatic mechanisms.

MEDICATION RELATED ISSUES OR PROBLRMS:^{6,7,8}

- The major drug related issues are
- ADRS
- Drug interactions
- Medication non adherence
- Inappropriate prescribing
- Medication errors(dispensing or administration or prescription error)
- Untreated indication
- Missed dose ,over dose, sub therapeutic dose
- Drug use without indication

AIMS

- To assess the drug related issues in elderly patients with poly pharmacy.
- To improve the health outcomes of geriatric patients.

OBJECTIVES

- To enhance the role of a clinical pharmacist in optimizing drug therapy and preventing drug-related problems in elderly patients.
- To provide better patient compliance.
- To improve effectiveness and safety of treatment and quality of life of elderly patients.
- To reduce the morbidity and mortality
- To decrease the hospital stay of elderly patients.

METHODOLOGY

This was a prospective, cross sectional study conducted in various departments of a tertiary care hospital for a period of 6 months by collecting patient information in accordance with institutional standards of PIPER/EC/PHARM.D/2019-9 in well structured data collection forms after taking written informed consent from patients in both out-patient and in-patient elderly individuals attending the hospital for any medical conditions and assessment of medication related issues were done. All the studies were done according to the ICMR guidelines.

STUDY PERIOD: The study is being conducted october 2017-march 2018.

STUDY DESIGN: A prospective cross sectional study.

INCLUSION CRITERIA:

- Patients of either sex of 60 years old and above in both out-patient and in-patient departments.
- Patients who are willing to give consent.
- Patients having more than 5 prescribed drugs.

EXCLUSION CRITERIA:

- Patients who are not willing to participate in the study.
- Patients with life threatening and end stage cancers.
- Patients who are too weak to participate in the study.
- Patients aged below 60 years.

METHOD OF COLLECTION OF DATA

Data was collected from patient case sheets, medication charts, lab reports and through structured interviews with patient or their care takers

STUDY METHOD

The study was conducted in the hospital settings by taking the samples randomly.

For all identified patients meeting study eligibility criteria, data was collected by using well structured data collection forms and questionnaires and assess the MRPs

- Poly pharmacy
- Noncompliance
- ADRs
- Drug-drug interaction

Ethical clearance

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Priyadarshini Institute of Pharmaceutical Education And Research (PIPER/EC/PHARM D/2019-9)

RESULTS

GENDER WISE DISTRIBUTION

The chi-square value for gender wise distribution to be 4.05. Degree of freedom alpha=1 or 0.5 at the level of a significance $p=0.015$

TABLE 1 GENDER WISE DISTRIBUTION:

Number of patients	In patient	Percentage	Outpatient	Percentage	Total
Male	29	59.2%	35	46.05%	64(51.2%)
Female	20	40.8%	41	53.94%	61(48.8%)
Total	49	100%	76	100%	125(100%)

CO MORBIDITIES

Among 125 enrolled patients majority 98 (31%) patients had hypertension followed by 61 (19%) patients had diabetes and 37 (12%) patients had CAD, 27 (9%) patients had CVA problems followed by patients with different co morbidities are presented in figure 1

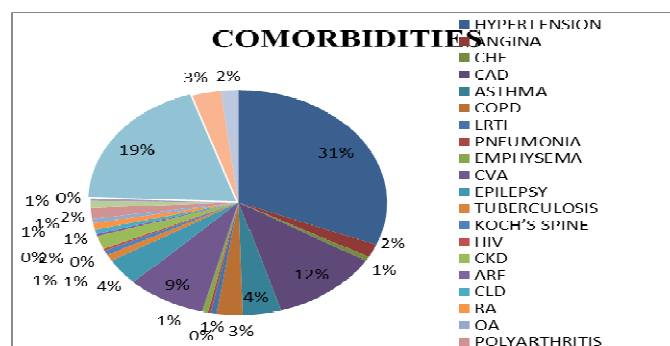


Figure -1

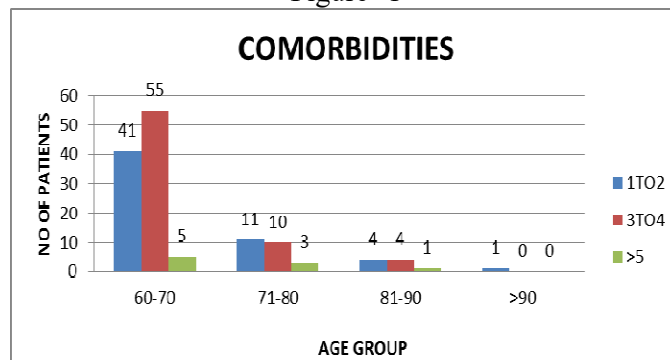


Figure 2 Number Of Comorbidities In Different Age Groups

NUMBER OF DRUGS IN EACH PRESCRIPTION

Among the enrolled patient minimum of 5 and a maximum of >14 drugs were prescribed per prescription. Majority prescriptions 76 (60.8%) contained 5-9 drugs per prescription followed by 41 (32.8%) prescriptions

10-14 drugs, 8(6.4%) prescriptions >14 drugs, the chi-square statistic for relation to poly pharmacy increased with age is 9.0289. the p value is 0.002657. This result is significant at $p < 0.05$ Results are presented in table 2.

Table 2 Numbers Of Drugs In Each Prescription

No of drugs in prescription	Patient age				Total no of drugs
	60-70yrs	71-80yrs	81-90yrs	>91yrs	
5-9	60(62.5%)	12(54.54%)	4(66.67%)	0	76(60.8%)
10-14	33(34.3%)	6(27.27%)	2(33.34)	0	41(32.8%)
>14	3(3.125%)	4(18.18%)	0	1	8(6.4%)
TOTAL	96	22	6	1	125(100%)

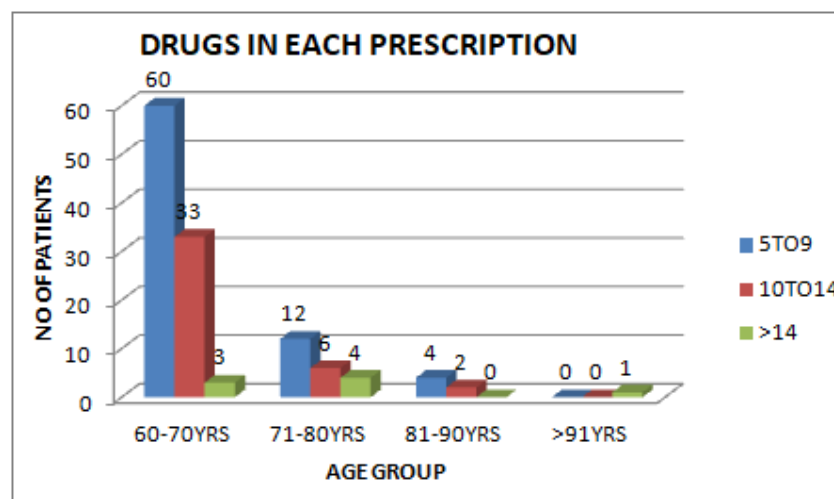


Figure 3 Drugs In Each Prescription

DRUG WISE DISTRIBUTION IN EACH CATEGORY

In a total of 1158 drugs, Majority of drugs prescribed are anti hypertensive's 170(14.68%) followed by antimicrobials 113(9.758%), 103(8.894%) were statins, 84(7.25%) prescriptions had oral hypoglycemic, followed by PPI's 62(5.354%), analgesics 60(5.181%) and least prescribed are smooth muscle relaxants, laxatives, anti virals with 3(0.259%).

DRUG INTERACTIONS PER PRESCRIPTION

Among 125 prescriptions around 121 prescriptions were identified with 622 drug interactions. Among those 121 prescriptions, 100 prescriptions were having 1-8 drug interactions followed by 18(14.4%) prescriptions having 9-16 drug interactions, 2 (1.6%) prescriptions 17-32 drug interactions, 1(0.8%) prescriptions having >32 drug interactions and 4(3.2%) prescriptions with no drug interactions.

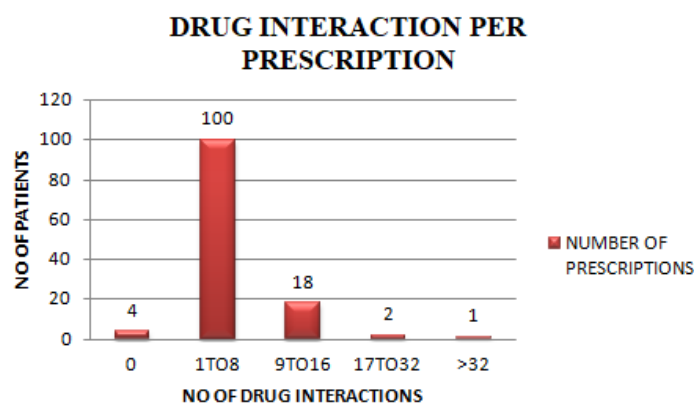


Figure 4. Drug Interactions per prescription

TYPE OF DRUG INTERACTIONS

Among 622 drug interactions in 125 prescriptions, 53(8.52) were severe interactions followed by 398(63.98) were moderate interactions and 171(27.4%) were minor drug interactions.

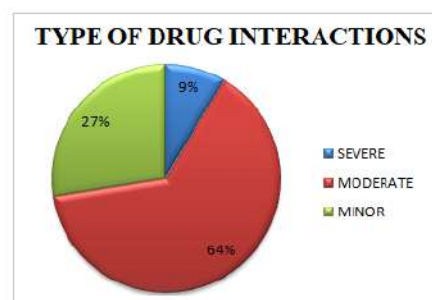


Figure -5 Type of Drug Interactions

Table 3 Some Examples Of Severe Drug Interactions

Drugs	Type of interaction	Effect
Telmisartran+perindopril	Severe	Increases toxicity of other by pharmacodynamicsynergism,contraindicated combination
Escitalopram+domperidone	Severe	Both increase qt prolongation
Levosulpride+chlorthalidone	Severe	Increase qt prolongation cause dangerous arrhythmias

MEDICATION ADHERENCE

Among 125 enrolled patients, majority of patients 60(48%) with low adherence followed by36(28.8%) with medium adherence and 29(23.2%) with high adherence .

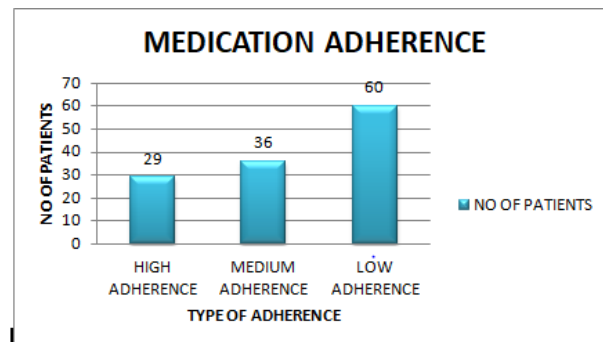


Figure 6 Medication Adherences

MEDICATION RELATED PROBLEMS IDENTIFIED

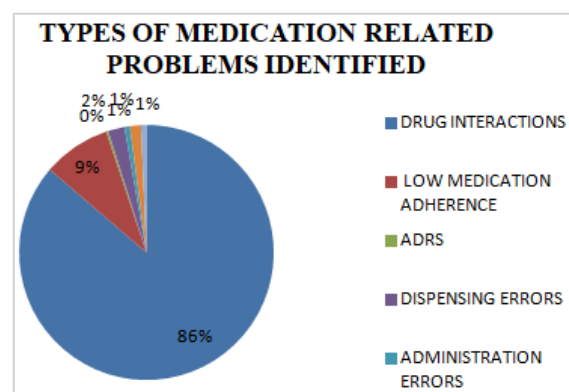


Figure 7 Types Of Medication Related Problems

Among 125 patients 2 (0.277) adverse drug reactions were observed and according to naranjo 's probability assessment scale these adverse drug reactions were possible ADRs and 15(2.08%) dispensing errors,10(1.386%) prescription errors where majority of prescription errors are due to missed written frequencies in the prescriptions.5(0.693%) administration errors,5 (0.693%)untreated indications were observed .

DISCUSSION

Ageing along with its associated physiological and pathological changes—places individuals at a higher risk of multi morbidity and treatment-related complications. Today, poly pharmacy, a common and important problem related to drug use, occurs subsequent to this multi morbidity in the elderly in all populations. Due to these chronic co-morbidities, elderly patients are generally exposed to poly pharmacy. Poly pharmacy is the most common reason for medication related problems. In this study a total of 1158 drugs were prescribed for 125 geriatric patients, due to poly pharmacy nearly 622 drug interactions were observed. This study showed that 48% of the patients were not taking their medications according to the prescription or having poor medication adherence. The low economic status of the patients, complex dosing regimen, confusion, forgetfulness, visual impairment, impaired dexterity, illiteracy, lack of information, etc. might be the reason for noncompliance in these patients. The majority of geriatrics had MRP because of more medication requirements and long hospital stay due to their multiple co morbidities. The most commonly observed are need of counseling, drug interaction, medical chart error monitoring, etc. This might be the reason for geriatric inpatients having more MRP than outpatients.

CONCLUSION

Ageing is inevitable .Ageing is progressive and irreversible. Ageing can't be stopped but for death. As man is science we can only decrease the wear and tear but can't stop age .As age progress so do the number of diseases and disorders. In today's world with fine tuned armament of medicines the choices are limitless. To take all diseases and disorders equally a multitude of drugs, Neff to be used. Current prescribing pattern of geriatrics patients showed an unfitting medication, increased potential drug interactions, the complicated medication and an inability to recall the drug regimen, lack of patient education about their medication, poverty, etc. were the risk factors for DRP/noncompliance. This study strongly suggested that there are more existences of DRP due to polypharmacy. Therefore we under the watchful eye of a clack pharmacist a disastrous event can be prevented and will enable us to deliver excellent modern medical care to all people.

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TESTING THE KNOWLEDGE AND ATTITUDE REGARDING PAP SMEAR TEST AMONG CERVICAL OMEN IN TERTIARY CARE HOSPITAL

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ABSTRACT

Cervical cancer is the leading cause of cancer related death among women next to breast cancer. It is usually a slow-growing cancer that may not have symptoms but can be found with regular Pap tests. A prospective, cross-sectional study was carried out among cancer women in bommidala cancer hospital. A total of 150 respondents are selected and Interview schedule with a structured questionnaire was used to assess the knowledge of cervical cancer, knowledge and attitude regarding Pap smear screening test. A self-designed structured questionnaire with mainly closed-ended questions and a few open- ended questions are used. Most of the women who had never screened for CC due to lack of information. The results of this study showed that 8.6% of the respondents are informed by health care professionals on cervical cancer screening that's why they attend for screening. The government should play its part by increasing health care budgets and put priority on cervical cancer prevention by establishing a national awareness campaign, spreading screening services all over the country especially in rural areas using screening procedures that have shown to have reasonable sensitivity and specificity.

KEY WORDS: *Awareness, Attitude Papanicolau test, Cervical carcinoma*

INTRODUCTION

Cervical cancer is the leading cause of cancer related death among women next to breast cancer. It is usually a slow-growing cancer that may not have symptoms but can be found with regular Pap tests (a procedure in which cells are scraped from the cervix and looked at under a microscope). Cervical cancer is almost always caused by Human papilloma virus (HPV) infection.¹ Most of the CCs develop slowly in the lining of the cervix as precancerous lesions if not detected earlier or treated properly, leads to CC. The precancerous lesions can be detected through screening, so this is the easiest cancer to prevent. CC screening is essential because it is asymptomatic and presents itself when a woman has late symptoms such as vaginal bleeding and abnormal discharge.²

Cervical Cancer Screening

Several tests such as Visual inspection with acetic acid (VIA), Visual inspection with Lugol's iodine (VILI), Human Papilloma virus deoxyribonucleic (HPV DNA) test and Pap smear test can be used in screening for cervical cancer, According to IARC estimate, among well-screened women aged 30-64 years, Pap smear screening prevented 80% of cervical cancers.⁵ Though specificity of Pap smear is generally agreed over 90% its limited sensitivity has been acknowledged openly and it has been estimated to be range from 50-80%. But this lower sensitivity is masked by regular screening, which has cumulative Sensitivity of over 90% for three successive smears.⁶ The False negative error rate of Pap smear test is around 10% to 20%. most of the false negative tests from sampling errors. When the Pap smear test was abnormal, a coloscopic examination of cervix and vagina is preformed to delineate the extent of the lesion and to target The area to be biopsied.⁴

HPV vaccination

In 2006 the FDA licensed a quadrivalent, prophylactic HPV vaccine for HPV types 6, 11, 16, and 18. this vaccine is designed to reduce the incidence of cervical cancer caused by HPV 16 and HPV 18. since the HPV vaccine does not eliminate the risk of cervical cancer completely due to other oncogenic HPV types, cervical screening should still continue according to past Guidelines to minimize cancer incidence⁵.

AIM: To determine knowledge of cervical cancer, knowledge and attitude of cervical cancer screening among cervical cancerous women.

OBJECTIVE OF STUDY

1. Testing the knowledge regarding cervical cancer.
2. Testing the knowledge regarding Pap smear screening.
3. Testing the attitude regarding Pap smear screening.

METHODOLOGY

STUDY DESIGN: A prospective, cross-sectional study.

STUDY DURATION: 6 Months (October 2017 to march 2018).

STUDY APPROVAL: This study was approved by the institutional human ethical committee of the priyadarshini institute of pharmaceutical education and research, Guntur.

STUDY METHOD: A prospective, cross-sectional study was carried out among cancer women in bommidala cancer hospital. A total of 150 respondents are selected and Interview schedule with a structured questionnaire was used to assess the knowledge of cervical cancer, knowledge and attitude regarding Pap smear screening test. A self-designed structured questionnaire with mainly closed-ended questions and a few open- ended questions are used. Data is collected from all the women by interviewing based on designed structured questions. Inform consent form is also obtained from study subjects. Collected data is analyzed and percentage of their knowledge of cervical cancer, knowledge and attitude of Pap smear screening are calculated.

Questionnaire includes

- Demographic data of the respondents.
- Knowledge regarding CC.
- Knowledge regarding Pap smear test.
- Attitude towards Pap smear test.

INCLUSION CRITERIA

- Women who are willing to participate.
- Women who are suffering with cervical cancer.
- Women who are married.

EXCLUSIVE CRITERIA

- Women who are unmarried.
- Women who are physically challenged.
- Women who has psychological illness.
- Women who underwent total hysterectomy.
- Women who are not willing to participate.

RESULTS AND DISCUSSION

Table.1: The socio-demographic information and frequencies of the respondents

Variables	Frequency	Percentage
Age:		
<35	19	12.66%
≥35	131	87.33%
Married at age of		
<18	108	72%
≥18	42	28%
No. of child		
≤2	85	56.66%
≥3	65	43.33%
Educational status None	84	56%

Primary	42	28%
Secondary	24	16%
Higher education	0	0%
Economical status		
Lower class	133	88.66%
Middle class Upper	17	11.34%
Middle class	0	0%
Menstrual status		
Regular	62	41.3%
Irregular	37	24.6%
Menopause	51	34%

Total sample size is 150 with overall mean age of the study women is 47.9 years ranging from 22 to 82 years. The data shows the socio economical status, that most of the respondents are lower class 133 (88.66%), few only middle class 17(11.34%) and none of are upper middle class. 84 (56%) women has never went to school or 42(28%) has primary education only. 24(16%) are only has education up to secondary. Study includes only married women mostly married at early age with mean duration of 15.8 years. Most of the respondents 85(56.66%) have 1 or 2 children's and 65(43.33%) have 3 or more children's. The date also shows that 62(41.3%) are having regular menstrual cycle, 37(24.6%) has irregular cycle and 51(34%) has menopause. Out of 150 respondents 110(73.3%) are aware of cervical cancer and remaining 40(26.6%) cases are not aware of cervical cancer. we found that 127(84.6%) are aware of that cervical cancer is preventable and remaining 23(15.4%) are known as cervical cancer has no prevention and also analyzed that none of respondents has a family history of cervical cancer. Among 150, 95(63.4%) respondents are heard about cervical cancer symptoms and remaining 55(36.6%) are unaware of cervical cancer symptoms and 120(80%) respondents agree with family history increases the risk of cervical cancer and remaining 30(20%) are seem to be unknown of this. Among 150 cases 137(91.33%) undergone biopsy test only and remaining 13(8.67%) undergone both biopsy and Pap smear screening test. no one is aware of cervical cancer vaccination among 150 cases. Based on the above table, frequency and percentages are calculated, among 150 cervical cancer patients only 13(8.66%) are heard about pap smear and only 13(8.66%) had pap smear and 13(8.66%) agree with the purpose of pap smear test is for early detection of cervical cancer. 13(8.66%) agree with the physician opinion that women above or equal to 35yrs should be screened for cervical cancer through pap smear test and rest of the patients don't know the necessity of pap smear and its importance in detection of cervical cancer. 13(8.66%) respondents are aware of these screening and had screening one time in past on advise of physician.

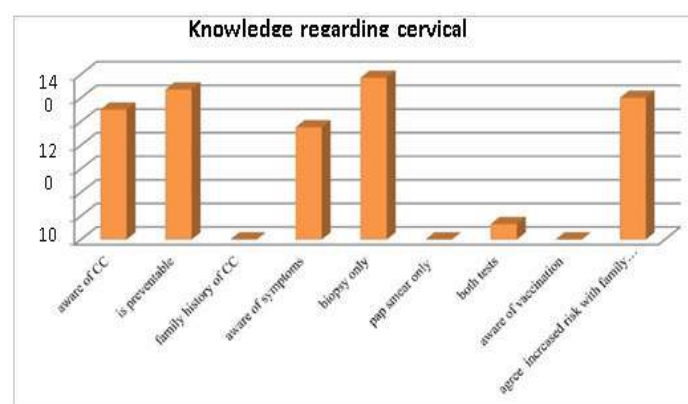


Figure 1: The distribution of respondents in knowledge regarding pap smear

PATIENTS WITH AWARE OF CERVICAL CANCER AND PAP SMEAR

Out of 150 women studied, 110 are aware of cervical cancer and 13 are aware of Pap smear

Awareness	Cervical cancer	Papsmear
Yes	110	13
No	40	137

The chi-square value for awareness of cervical cancer and pap smear was found to be 9.654. Degree of freedom =1 at the level of a significance 0.0432

Among 150 cervical cancer patients we observed that 13(8.66%) patients agreed Pap smear is good for early detection of cervical cancer and 137(91.33%) ever think of undergoing pap smear. Only 13(8.66%) think that pap smear test can prevent from cervical cancer, 6(4%) respondents not think so. Among 150 CC Patients 8(5.33%) feel comfortable when they are going through pap smear test and 5(3.33%) patients don't feel comfortable with pap smear test. out of 150 CC women 13 (8.66%) are satisfied with pap smear test and remaining don't know because they doesn't underwent pap smear screening and don't show attitude regarding pap smear test.

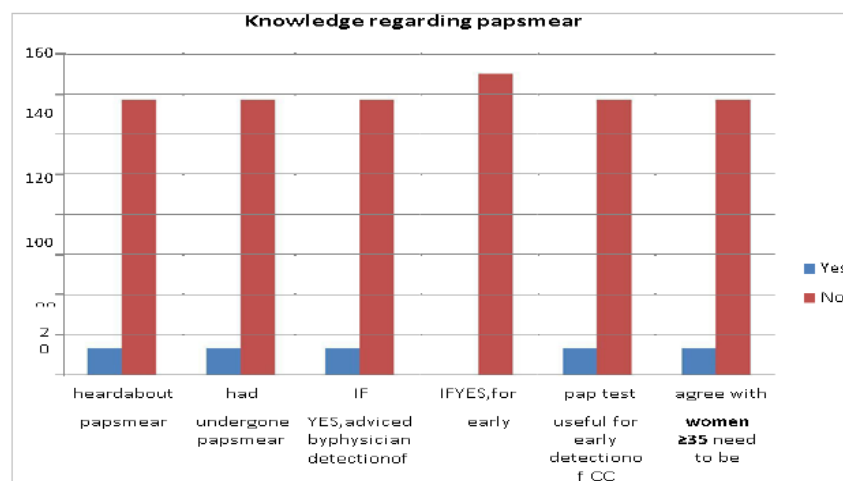


Figure 2: The distribution of respondents showing attitude regarding pap smear test.

CONCLUSION

The knowledge of CC, knowledge and attitude regarding Pap smear test, which are the major factors that determine the success of any screening program. Knowledge of women on cervical cancer and screening and socio-demographic characteristics influencing screening are also determined and characterized. The sample size of study is n=150 from age of 22 years and above with mean average age of 46.9 years. All the respondents are cancer patients from rural areas and none of women has family history of CC. Most of the respondents 88.6% (133) are from low economical status, 11.34% women (n=17) are from middle class and none of women from higher economical status. This much of low economical status does not influence the women as barrier to attend for CC screening because free screening programs are available at government hospitals. Most of the women who had never screened for CC due to lack of information. The results of this study showed that 8.6% of the respondents are informed by health care professionals on cervical cancer screening that's why they attend for screening. Lack of knowledge is main barrier for screening for early detection of CC. This study showed a direct relationship between higher educational level and adequate knowledge and attitude of Pap smear test. Health care workers also should play a vital role in educating communities on cervical cancer. Health personnel as the source of information showed a significant relation with adequate practice of Pap smear test which also emphasizes the important role of health personnel in increasing the awareness regarding CC and Pap smear test and finally decreasing the CC burden.

FUTURE SCOPE

- Efforts to promote cervical cancer screening among women should focus on informing women of their susceptibility to cervical cancer and encouraging a belief that active and regular screening can detect the pre-cancerous stage, hence enabling early treatment and prevention of cancer development.
- Emphasis on more media coverage through TV and Radio regarding the problems of female cancers especially, on cervical cancer to create community awareness.
- The government should play its part by increasing health care budgets and put priority on cervical

cancer prevention by establishing a national awareness campaign, spreading screening services all over the country especially in rural areas using screening procedures that have shown to have reasonable sensitivity and specificity.

- There is greater need of health awareness camps in rural areas. Healthcare professionals as source of information of CC and CC screening has significant role on adequate practice of pap smear screening.

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EVALUATION OF ANTI-CONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF PLUMBAGO ZEYLANICA

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ABSTRACT

The purpose of this study is to explore the anticonvulsant effect of the leaf gall extract of *Plumbago zeylanica* using pentylene tetrazole induced convulsion (PTZ) in rats. *Plumbago zeylanica* is an indigenous plant belonging to the family Fabaceae (Papilionaceae) commonly known as Karanj. Freshly powdered flowers were extracted with 70% ethanol. The convulsion is induced by administration of pentylene tetrazole (80 mg/kg, i.p.) to wistar albino rats and those showing response were divided into three groups of six animals each. The group I treated with 1% normal saline (1ml/100gm, orally), Groups II treated with phenytoin sodium (25 mg/kg, i.p.) and Groups III treated with ethanolic extract at a dose of (250 mg/kg, i.p.). The ethanolic extract showed significant anticonvulsant activity by lowering the duration of extension phase (3.72 ± 0.65) when compared to control group (8.94 ± 0.42). From the experiment we can say *Plumbago zeylanica* had significant anticonvulsant activity.

KEYWORDS: *Plumbago zeylanica* Phenytoin sodium, Pentylene tetrazole induced convulsion, anticonvulsant activity

INTRODUCTION

Plumbago zeylanica is a herbaceous plant with glabrous stems that are climbing, prostrate, or erect. The leaves are petiolate or sessile and have ovate, lance-elliptic, or spatulate to oblanceolate blades that measure $5-9 \times 2.5-4$ cm in length. Bases are attenuate while apexes are acute, acuminate, or obtuse. Inflorescences are 3–15 cm in length and have glandular, viscid rachises. Bracts are lanceolate and $3-7 \times 1-2$ mm long. The heterostylous flowers have white corollas 17–33 mm in diameter and tubes 12.5–28 mm in length. Capsules are 7.5–8 mm long and contain reddish brown to dark brown seeds.¹ It has been recognized in different system of traditional medicines for the treatment of different diseases and ailments of human beings^{1, 2}. It contains several phytoconstituents belonging to category flavonoids and fixed oils. The flowers and sprouts of *plumbago zeylanica* were used in folk remedies for tumors³. *Plumbago zeylanica*, commonly known as Ceylon leadwort, doctorbush or wild leadwort is a species of *plumbago* with a pantropical distribution. Seed extract of this plant has hypotensive effects and produce uterine contractions. Powdered seed is used in bronchitis, chronic fever, whooping cough and chronic skin diseases and painful rheumatic joints⁴. Leaves are active against *Micrococcus*; their juice is used for cold, cough, diarrhoea, dyspepsia, flatulence, gonorrhoea⁵ and leprosy. Roots are used for cleaning gums, teeth and ulcer. *Plumbago zeylanica* plant is used for anti-inflammatory⁵, anti-plasmodial, anti-nociceptive, anti-hyperglycaemics, anti-lipidoxidative, antidiarrhoeal, anti-ulcer, anti-hyperammonic, CNS depressant activity⁶ and antioxidant.

2. MATERIALS AND METHODS

2.1 Collection of Plant material

Collection and authentication of plant material The leaf galls of *Plumbago zeylanica* was collected from Maruthi nagar, Guntur, Andhra Pradesh, India and material was identified and authenticated by Dr. B.R.C Murthy, reader in Botany, Hindu college, Guntur, India.

2.2. Chemicals

Phenytoin sodium, pentylene tetrazole, ethanol, hexane and all other solvents and chemicals used were of laboratory grade, purchased from local market.

Preparation of *Plumbago zeylanica*

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After collection, the flowers were shade dried, pulverized using an electrical grinder and passed through the sieve (40 mesh size). The extract was prepared cold maceration method by taking 100g of powder in ethanol and soaked about 7 days. The extract was filtered using muslin cloth, followed by whatsmann filter paper (No.1) and concentrated at reduced temperature on rotary evaporator. The extract was evaporated to dryness to achieve the yield of 8% (w/w) of the extract and kept in a dessicator. We made experiments to collect the fraction of extract using column chromatography with different solvents of varying polarity and finally the ethalonic fraction was selected for the study as it has given number of colored bands. The 39th fraction (C-39) of entanolic extract was collected and used for the evaluation of anti-convulsant activity.⁶

2.3. Experimental Animals

Animal's Five male white albino wistar rats weighing in between 150-200g were selected and procured from the animal house of Hindu college of pharmacy, Guntur. The animals were maintained in well- cages. They were fed with rodent diet and water adlibitum. Animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) guided by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration number 1263/PO/ac/09/CPCSEA, dated 05/05/2014. All efforts were made to minimize animal suffering and to reduce the number of animals used. Institutional animal ethical committee has approved the protocol of the study.

2.4. Experimental design

The anticonvulsant activity of *Plumbago zeylanica* flower extract was evaluated for pentylenetetrazole induced convulsion (PTZ) in rats⁸. The convulsion is induced by administration of pentylenetetrazole (80 mg/kg, i.p.) to wistar albino rats. Rats those showing response were divided into three groups of six animals each. The first group of animals were administered 1% normal saline (1ml/100gm) orally which served as negative control. Group of II animals were treated with phenytoin sodium (were obtained from Merck, India) (25 mg/kg, i.p.) which served as positive control⁹. Group of III animal (Ps were treated with ethanolic extract of PPLE at a dose of (250 mg/kg i.p.). Drug pretreatment was given 1 hr prior to the administration of pentylenetetrazole and each animal were placed in individual plastic cage and observed initially 30 min. and later up to 24 hrs for the duration of tonic, flexion, tonic extension, clonus and death/recovery. Statistical analysis Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Student's t-test. Results are expressed as means $\pm$ SEM from six rats in each group.

RESULTS

Plumbago zeylanica flower extract was subjected for anticonvulsant effect using PTZ induced convulsion model in rats. PPLE exhibit significant anticonvulsant activity by lowering the duration of extension phase when compared to control group. The duration of tonic and hind limb extension in rats with 70% ethanolic extract was 3.72 ± 0.65 at a dose 250 mg/kg. The activity of ethanolic extract was comparable ($P > 0.001$) to that produced by standard drug phenytoin sodium (Table 1).

Table 1. Anticonvulsant activity *Plumbago zeylanica* flower extract on Pentylenetetrazole induced convulsion in rats

Treatment	Dose	Time in seconds of various phase of convulsion Flexion				
		Flexion Recovery/Death	Extension	Convulsion	Stupor	
Control(nor mal saline)	1ml/100gm orally	3.83 ± 0.36	$3.83 \pm 0.368.94 \pm 0.42$	4.65 ± 0.71	104.10 ± 8.74	Recovery
Phenytoin sodium	25 mg/kg ip 2.15 ± 0.21	2.15 ± 0.21	-	7.86 ± 0.35	91.55 ± 5.85	Recovery
Ethanolic extract (c-39)	250mg	1.90 ± 0.25	$3.72 \pm 0.6^*$	8.96 ± 2.00	102.00 ± 8.75	Recovery

Data are expressed in mean $\pm$ SEM, n = 6 in each gro ups *P < 0.001 vs Phenytoin sodium – treated rats by student's t-test

DISCUSSION

Pentylentetrazole is a selective blocker of the chloride ionophore complex to the GABA-A receptor, and after repeated or single dose administration leads to a decrease in GABAergic function and to the stimulation and modification of density or sensitivity of different glutamate receptor subtype in many brain regions. Pentylentetrazole may also trigger a variety of biochemical processes including the activation of the membrane phospholipase, proteases and nucleases. Alteration in membrane phospholipids metabolism cause liberation of free fatty acids, diacylglycerols, eicosanoids, lipid peroxidase and free radicals. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure¹¹. Phytochemicals such as kaempferol and fixed oils are the active principles responsible for the anticonvulsant activity of *Plumbago zeylanica*; it is likely that flavonoidal compounds, present in this plant may be involved in this action. Hence this drug may able to modulate the function of GABA or glutamate receptors.¹²

CONCLUSION

The present study indicates that the plant *Pongamia glabra* (L.) has potential anticonvulsant activity against chemically induced convulsion in experimental animals. This activity of plant probably due to the compounds present such as quercetin, kaempferol, flavonoidal compounds and fixed oils¹³. So the plant *Pongamia glabra* (L.) uses for both ayurvedic and modern drug development areas because of its phytomedicinal uses.

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PREPARATION AND PRELIMINARY EVALUATION OF READY TO SERVE HERBAL BEVERAGE

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ABSTRACT

The study of the ready to serve herbal drink based on *Hemidesmus indicus* (sugandhipala), *Madhuca longifolia* (iluppai or vippapuvvu), *Phoenix dactylifera* L. (date palm), *Vitis vinifera* grapes, *Punica granatum* pomegranate, lime and herbal extracts revealed that this drink is palatable and acceptable throughout its storage period. There were insignificant changes in acidity of the drink and the final product was accepted by the panelists. No preservative was added to increase the shelf life of the drink and the natural herb extracts present in the developed drink have contributed in keeping the bacterial count at bare minimum level. This drink is a blend of various essential vitamins and minerals. So instead of consuming various products, this one drink would be sufficient to replenish the needs of the body. The ready to serve herbal drink provides minimal calories for diabetic patients. The test was conducted once in the beginning of the storage and once at the end of the 30 day period. The microbial test revealed that no growth of microbes was observed on day 0, in nutrient agar condition. Whereas there was insignificant growth of microbes, even after day 30. The drink was acidic in nature and this might be a cause for the endurance of inhibition of microbial growth. The results were observed and the feedback was obtained by using hedonic scale.

KEY WORDS: *Herbal Beverage, Anti Oxidant, Energetic Drink.*

INTRODUCTION

Herbs and spices play a significant part in the production of beverages and have been used for many centuries. Some of them were already in use in pre-history. Various ingredients have their powerful effect on the well being of the drink. These herbal preparations are more readily absorbed into the system and known to cure various ailments. The herbal mix is expected to provide a refreshing drink with sweet taste and minimal calories for those who have to restrict sugar in their diet. *Hemidesmus indica* is used in blood diseases, dysentery, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, epileptic, kidney and urinary disorders, loss of appetite, burning sensation, dyspepsia, nutritional disorders, ulcer and rheumatism.¹ *Madhuca longifolia* flowers have been traditionally used as Cooling agent, tonic. *Phoenix dactylifera* a high tannin content and are used medicinally as a detergent (having cleansing power) and astringent in intestinal troubles²⁻⁵. *Zingiber officinale* used to treat common ailments such as Headaches, Nausea, Vomiting, Indigestion. Honey is used to treat and protect against gastrointestinal infection such as gastritis, duodenitis and gastric ulceration caused by bacteria and rotavirus. *Mentha piperita* used in combination with other drugs for the digestive function or to release the mucus in the respiratory tract. *Citrus aurantium* traditionally used in asthma, to reduce vomiting, to purify the blood, for coughs, fevers, thirst, hiccoughs and for indigestion. *Vitis vinifera* fruit as demulcent, cooling, sweet, laxative, stomachic, used in thirst, heat of body, coughs, hoarseness. Pomegranate fruit juice is also known to be very helpful in treating issues of erectile dysfunctions. Lemon juice, rind, and peel are used in a wide variety of foods and drinks.

MATERIALS AND METHODS

A. Extractive from Mint & Ginger

The fresh Mint and Ginger were bought from the market and were properly washed. Then a paste was made of all the 2 ingredients separately and was dried and powdered and sieved through 250 meshes and stored in

polyethylene packs. Later each ingredient was mixed in distilled water in a ratio of 10:1 (water: powdered ingredient) and kept in shaker for 1 hour and then filtered using vacuum funnel.

B. Extraction of grapes, pomegranate, orange and Lime Juice

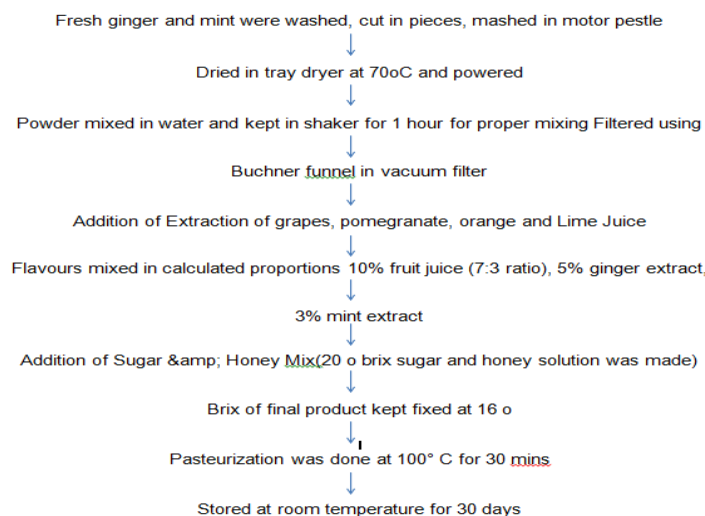
All fruits were separately washed and the juice was extracted and strained to remove seeds and fibres using cheese cloth. A trial was done on grapes, pomegranate, orange and Lime Juice was used in the various ratios.

C. Preparation of Sugar & Honey Mix

Sugar was powdered for easier mixing and honey was diluted in distilled water. The final brix was maintained at 16 ° brix (Degrees Brix (symbol °Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass.).

Powdered drug extract:

Hemidesmus indicus (Sugandhipala), Madhucal longifolia (Iluppai or vippapuvvu), Phoenix dactylifera L. (date palm) were shade dried and powdered. Equal quantities of herbal powder added to water and concentrated to one fourth of its volume. This concentrate gives about 1 liter of syrup. The coolant herbal beverage was served to judges to evaluate appearance, colour, taste, flavour, acceptability by using 9-point hedonic scale. Finally five formulations i.e. F1, F2, F3, F4 and F5 were tried. The final formulation was made after going through rigorous mix and matches of flavors. The sensory analysis showed that flavor retention was desirable.



Analysis

A. Microbial Test

The test of Total Plate Count (TPC) was done using peptone, beef extract and agar, keeping the incubation temperature at 37°C for 48 hours.

B. Sensory Evaluation

The prepared herbal drink was subjected for sensory evaluation by a panel of nearly 50 members involving staff and students of the institution. The beverage samples were tested for colour, appearance, flavour, taste and overall acceptability. The results observed that, colour, flavour, taste and overall acceptability were found to be good and widely accepted. The prepared drink is analysed for sensory evaluation on a 9 point hedonic scale depending on feedback obtained.

C. storage studies

Ethno herbal based RTS beverages were subjected to storage studies at room temperature for a period of 30 days by drawing samples at five days interval to evaluate changes in biochemical, microbiological and organoleptic parameters.

RESULTS AND DISCUSSION

In the primary sensory evaluation test, the panelists judged the formulation containing *Hemidesmus indicus* (Sugandhipala), *Madhuca longifolia* (vippapuvvu), *Phoenix dactylifera* L. (date palm) acceptable in terms of colour, flavour and sweetness.

CHARACTERISATION OF PRODUCT

Table-1: Characteristics Of Formulations

S.No	Particulars	F1	F2	F3	F4
1	Colour	Peach	Peach	Peach	Peach
2	Brix	14	14	15	16
3	Acidity	----	0.2	0.2	0.4
4	p ^H	2.9	2.9	3.7	4.8
5	Taste	Average	acceptable	Good	excellent

Table-2 :Composition Of The Formulation

S. No	Ingredients	F1	F2	F3	F4	F5
1	<i>Hemidesmus indicus</i> (Sugandhipala)	—	+	+	+	+
2	<i>Madhuca longifolia</i> (Iluppai or vippapuvvu)	+	—	+	+	+
3	<i>Phoenix dactylifera</i> L. (date palm)	+	+	—	+	+
4	<i>Vitis vinifera</i> (grapes)	+	+	+	+	—
5	<i>Punica granatum</i> (pomegranate)	+	+	+	+	+
6	<i>Citrus aurantium</i> (orange)	+	+	+	+	+
7	<i>Mentha piperita</i> (saharipudina)	+	+	+	+	+
8	<i>Citrus limon</i> (lemon)	+	+	+	+	+
9	<i>Zingiber officinale</i> (ginger)	+	+	+	+	+
10	<i>Apis mellifera</i> (honey)	+	+	+	+	+

Microbial testing

This test was conducted once in the beginning of the storage and once at the end of the 30 day period. The microbial test revealed that no growth of microbes was observed on Day 0, in nutrient agar condition. Whereas there was insignificant growth of microbes, even after Day 30. This study shows the proper method of sterilisation was taken care of. The drink was acidic in nature and this might be a cause for the hindrance of microbial growth. The following observation was recorded (Table. 3).

Table-3: Microbial Growth Test

S NO	DAYS	Bacterial count (upon serial dilution)
1	0	Below Predictable Limit (BPL)
2	30	Below Predictable Limit (BPL)

Sensory Evaluation

The panel members observed that the dried ginger shreds and fruits concentrate are suitable for texture and colour of herbal preparation. The optimum levels of all herbs were finalized by further sensory analysis of beverage (Table 4). The formulation F4 was the most optimum.

Table-4 :Sensory evaluation by hedonic scale

Nine point hedonic scale	
9	Excellent
8	Extremely good
7	Very good
6	Moderately good
5	Good
4	Very fair

3	Fair
2	Poor
1	Very poor

FEEDBACK							
S.NO	APPEARANCE	COLOUR	TEXTURE	FLAVOUR	TASTE	ACCEPTABILITY	SIGNATURE
1	8	7	7	9	9	8	S. K. S.
2	8	8	8	9	9	9	S. K. S.
3	9	9	8	9	9	8	S. K. S.
4	7	7	8	9	9	9	S. K. S.
5	5	7	7	9	8	9	S. K. S.
6	8	8	8	9	9	9	S. K. S.
7	8	8	8	9	9	8	S. K. S.
8	9	9	8	9	9	9	S. K. S.
9	9	8	7	9	9	9	S. K. S.
10	9	8	8	9	8	9	S. K. S.
11	9	8	9	9	9	9	S. K. S.
12	9	8	9	9	8	9	S. K. S.
13	8	7	7	9	9	9	S. K. S.
14	9	8	8	9	9	9	S. K. S.
15	9	8	8	9	9	9	S. K. S.
16	8	8	8	9	9	9	S. K. S.
17	8	8	8	9	9	9	S. K. S.
18	9	9	9	9	9	9	S. K. S.
19	9	8	8	9	9	9	S. K. S.
20	8	8	8	9	9	9	S. K. S.

Feed back

Storage Study

The RTS Herbal Drink was stored in a pre-sterilised glass bottle and was corked. The bottles were labelled and kept at room temperature for 30 days. At every 05 days interval, bottles were open and the sample was used for determination of acidity, microbial testing and the organoleptic evaluations.

CONCLUSION

The study of the Ready to Serve Herbal Drink based on *Hemidesmus indicus* (Sugandhipala), *Madhucal longifolia* (Iluppai or vippapuvvu), *Phoenix dactylifera* L. (date palm), *Vitis vinifera* (grapes), *Punica Granatum* (pomegranate), lime and herbal extracts revealed that this drink is palatable and acceptable throughout its storage period. There were insignificant changes in acidity of the drink and the final product was accepted by the panellists. No preservative was added to increase the shelf life of the drink and the natural herb extracts present in the developed drink have contributed in keeping the bacterial count at bare minimum level. The added herbs increased the nutritional and medicinal properties of the drink and made the drink more acceptable to the consumers. This drink is a blend of various essential vitamins and minerals. So instead of consuming various products, this one drink would be sufficient to replenish the needs of the body. The herbal mix is expected to provide nutrients with sweet taste.

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SP-137

FORMULATION AND EVALUATION OF MUCOADHESIVE LOSARTAN POTASSIUM BUCCAL FILMS

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ABSTRACT

The aim of this work was to develop buccal film and enhance the bioavailability of losartan potassium by using solvent casting method. In the present study losartan potassium buccal films were prepared by solvent casting method using different film forming polymers like HPMC, PVP K30 and Propylene glycol as plasticizer. Buccal films of losartan potassium formulated from F1 to F10 are smooth, translucent with good flexibility were evaluated and characterized. Among all formulations of buccalfilms, F7 formulation exhibited good physical appearance, uniformity in weight, thickness, folding endurance, and surface pH. It showed better drug release of 86.24% for losartan potassium in 60 minutes. The drug content is 97.72% for losartan potassium.

KEYWORDS: *Bioavailability, Losartan potassium, solvent casting method, HPMC, PVP K30, Propylene glycol.*

INTRODUCTION

Among the different routes of drug delivery transmucosal drug delivery consists of various advantages over peroral administration for systemic effect on comparing to other transmucosal routes buccal mucosa is most suited for local, as well as systemic delivery of drugs.¹ Buccal films have more importance as efficacious and novel drug delivery systems and are cost effective with a good patient compliance. Buccal films have direct access to the systemic circulation through the internal jugular vein, which bypasses the drug from the hepatic first pass metabolism causes high bioavailability. Losartan potassium (LP) is a potent, highly specific angiotensin II type 1 receptor antagonist with anti-hypertensive activity. The drug is orally administered as 25 mg tablets once or twice daily with total daily doses ranging from 25 to 100 mg. Following oral administration, it is readily absorbed from the GI tract with an oral bioavailability of about 33% and a plasma elimination half-life ranging from 1.5 to 2.5 h.

MATERIALS

Losartan potassium was procured from TCI Chemicals, Chennai. HPMC Polymer, PVP K30, Propylene Glycol, Sodium chloride was procured from Bross Scientifics, Tirupati. Aspartame, citric acid, Disodium hydrogen phosphate, was procured from S.D. Fine Chem Ltd., Mumbai.

METHODOLOGY

Preparation of Calibration Curve

Losartan potassium standard stock solution (100 µg/ml) was prepared by dissolving 10 mg of drug in salivary simulated fluid pH 6.8 and final volume was made up to 100 ml with the same fluid. From this solution, aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 were prepared and transferred to a series of 10 ml volumetric flasks and the final volume was made with salivary simulated fluid pH 6.8 to get drug concentrations of 2 to 10 µg/ml and observed at 234 nm.² A graph was plotted with concentration on X-axis against absorbance on Y-axis that is shown in fig no.1.

FT–IR Spectroscopy

Fourier transform infrared (FT– IR) spectral measurements were performed using Thermo-IR 200 FT–IR spectrophotometer. Potassium bromide pellet method was employed. The pure drug and the pure drug along with the polymer mixture used for the preparation of films was finely grounded with KBr to prepare pellets under a hydraulic pressure of 600psi and a background spectrum was collected under identical conditions. Each spectrum was derived from 16 single average scans collected in the range of 4000- 400 cm⁻¹.² The FTIR Spectroscopy of losartan potassium was shown in fig no.2 and the FTIR Spectroscopy of Mixture (losartan potassium +HPMC +PVPK30) was shown in fig no.3.

Preparation Of Buccal Films

From the several trails, optimization was further performed for the polymer and the plasticizer compositions which showed good film properties. The losartan potassium buccal films were prepared by solvent casting method.

Solvent casting method

Weigh sufficient amount of polymer. Add sufficient water to dissolve, slightly heat it with continuous stirring and plasticizer was added. Drug, saliva stimulating agent and sweetening agent was dissolved in sufficient amount of water and added to polymeric solution. Add flavouring agent and kept aside. The final solution was cast into the mould and kept aside undisturbed at temperature 40-45⁰c for time period of 24 hours. After complete drying the film was carefully removed from the mould and sized to 2×2 cm and packed in a butter paper and stored in a dessicator for further evaluation.³

Table 1. Composition of the buccal films of the Losartan potassium buccal films

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Drug (mg)	25	25	25	25	25	25	25	25	25	25
HPMC (mg)	0.4	0.5	0.6	0.7	0.8	0.9	0.9	0.9	0.9	0.9
PVP K30 (mg)	0.01	0.01	0.01	0.01	0.01	-	0.01	0.02	0.03	0.04
PG (%v/v)	1	1	1	1	1	1	1	1	1	1
Dis.water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Aspartame (mg)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Citric acid (mg)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Flavouring agent	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

EVALUATION OF BUCCAL FILMS

Film Weight uniformity

The weight of the film was measured using a digital balance among the three films of every formulation and the results were observed. The thickness of each film was measured using a digital vernier calipers at different locations i.e., at different corners and at the center of the film and the results were observed.

Folding Endurance

Folding endurance of the films was checked by repeatedly folding the same film at the same place till it broke or folded up to the appearance of the crack manually. The number of times the film can be folded at the same place without breaking or cracking gives the value of folding endurance was observed.

Surface pH

Film of 2×2 cm size was dissolved in 1 ml of distilled water and the pH was determined using pH meter.

Swelling Index

The prepared films were taken and weighed individually and placed in 1-2 ml of distilled water and kept in an incubator maintained at 37±0.2⁰c and the samples were allowed to swell. An increase in the weight of the film was noted at regular intervals of time and the weight was calculated. The percentage of swelling, %S was calculated using the following equation:

$$\text{Percent Swelling (\%S)} = (X_t - X_o / X_o) \times 100$$

Where X_t = the weight of the swollen film after time t, X_o = the initial film weight at zero time.

Moisture Content

The formulated films were weighed separately and kept in a desiccator containing anhydrous calcium chloride at room temperature for 24h. After a specified interval, the films are to be weighed again until they show an unvarying weight. The percentage of moisture content was calculated by using the following formula.

$$\% \text{ moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

Drug content uniformity

The drug content was performed to make sure of uniform distribution of drug. 2×2 cm of the film was cut into pieces, weighed and dissolved in 25 ml of simulated salivary fluid. From the above filtered solution 1 ml was withdrawn and then transferred to the 10 ml volumetric flask and make up the solution with the simulated salivary fluid. Secondary dilution was done and the serial aliquots upto 10 µg/ml. The resulted solutions has to be filtered, diluted with suitable solvent and analyzed at a particular nm in UV-visible spectrophotometer at 234nm.

$$\% \text{ Drug content} = \frac{\text{Observed value}}{\text{Actual value}} \times 100$$

Dissolution

Dissolution studies of the buccal films of losartan potassium was carried out by using USP II (paddle apparatus) with 900 ml of simulated salivary fluid of pH 6.8 as a dissolution medium maintained at $37 \pm 0.5^\circ\text{C}$ at 50 rpm. The particular amount of the sample had been withdrawn at regular intervals of time. After the dilution of the samples was analysed spectroscopically at the wave length of 234 nm.⁴

In-vitro permeation study

The in-vitro drug release studies were carried out using open ended cylinder method with the use of a membrane that is semi-permeable. There are normally two compartments in the open ended cylinder that is donor and receptor compartments. The top portion of the donor compartment was opened which was exposed to the environment and egg membrane was used as a semi- permeable membrane to separate the receptor compartment. Simulated salivary fluid of pH 6.8 was used as the diffusion medium. Films were then placed on the membrane which was initially soaked in the mixture of water. Temperature was maintained at 37°C at 50 rpm. 5ml of the sample was withdrawn after every half an hour for 5 consecutive hours and simultaneously the receptor compartment was replaced with the fresh buffer⁶⁸. The absorbance was determined using UV spectrophotometer at 234nm.

Drug release kinetics

As a model-independent approach, comparison of the time taken for the given proportion of the active drug to be dissolved in the dissolution medium and figures such as T50 and T90 calculated by taking the time points of 50% and 90% of the drug dissolved and another parameter dissolution efficiency (DE) suggested by Khan were employed. DE is defined as the area under the dissolution curve up to the time 't' expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

Dissolution Efficiency (DE)

The dissolution efficiency can have a range of values depending on the time interval chosen. In any case constant time intervals should be chosen for comparison. For example, the index DE30 would relate to the dissolution of the drug from a particular formulation after 30 minutes could only be compared with DE30 of other formulations.

Characterization of buccal films

The prepared buccal films of losartan potassium were characterizes for following studies.

Scanning electron microscopy (SEM)

The surface morphology of the pure drug and the formulated films were assessed using a scanning electron microscope JSM – 6610. Samples were mounted on the round brass stubs (12mm diameter) using double – backed adhesive tape and then sputter coated for 8 minutes under argon atmosphere with gold before examination under the scanning electron microscope. Pictures were taken at a excitation voltage of 15Kv. ^[5]

RESULTS AND DISCUSSIONS

Calibration curve of Losartan potassium

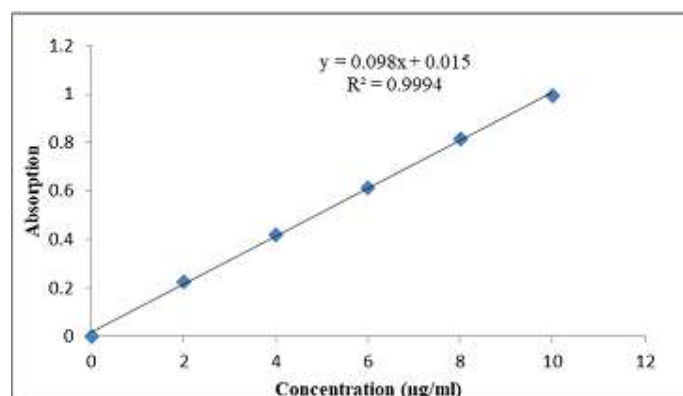


Figure 1. Standard calibration curve of Losartan potassium in salivary simulated fluid pH

FTIR SPECTROSCOPY

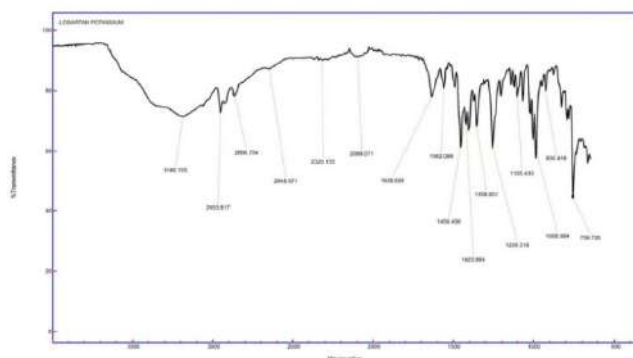


Figure 2. FTIR Spectroscopy of Losartan potassium

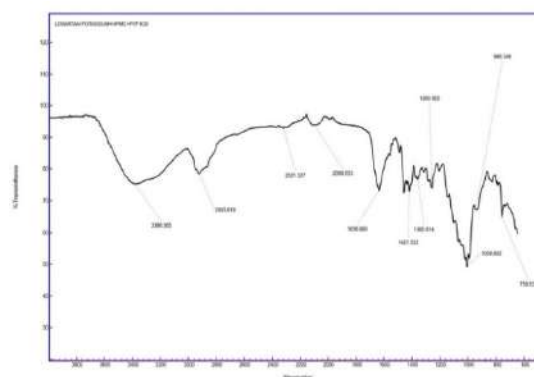


Figure 3. FTIR Spectroscopy of Mixture (losartan potassium +HPMC +PVPK30)

In the FTIR spectroscopy of losartan potassium- Film weight uniformity, the weight of the film varies from 71.08 mg to 96.43 mg ,The results obtained shown that, the selection and the proportion of the carriers used for the preparation of the films have reduced the weight variation and improves the uniformity of drug distribution in casted films. The average film weight was found to be 87.36 mg. The thickness of the films was found to be in the range of 0.13 to 0.21 mm .The average thickness of the films was found to be 0.16 mm. The folding endurance of the films was found to be in the range of 268 to 330 times F1 shows low folding endurance because it consists of low concentration of HPMC and PVP K30. It shows high folding endurance in F10 as it contains high concentration of HPMC. The average folding endurance of the films was found to be 305 times.The surface pH was found to be in the range of 6.68 to 6.92. From the results it is clear that all films have the pH value closer to the neutral pH, which indicates films do not cause irritation to the buccal mucosa. Swelling index of the films was found in the range of 10-18%. It was high in F9 as it contains increased concentration of PVP K30 as it increases swelling index also increases. It was found decreased where HPMC was in high concentrationThe percentage drug content of the films was found between 81.42- 97.72%. It was observed that there was no major difference in the uniformity of drug

content Moisture content was calculated for the developed films and it was in the range of 5.82-10.32. And the percentage drug content of the films was found between 81.42- 97.72%. It was observed that there was no major difference in the uniformity of drug content.

8. In-vitro Drug Release studies ⁶

In-vitro drug release study was carried out for 60 min with sampling at specific intervals. The release of the drug was based on the concentration of the polymers used in the formulation. Based on this, the formulation F7 with 0.9mg HPMC, 0.01mg PVP K30, 1% PG exhibited drug release of 86.24% in 60 min and F7 was selected as optimized formulation.⁷⁻⁸ The results obtained were represented graphically in Figure 4.

In-vitro Permeation studies

In-vitro permeation studies were carried out using franz diffusion cell for 5 hour duration by withdrawing samples at specific time intervals. The results obtained were shown graphically in figure no. 8. Based on the drug permeated, formulation F7 which consists of 0.9mg HPMC, 0.01 PVP K30, 1% PG exhibited a drug release of 82.90% in 5 hours. F7 was selected as an optimized formulation. The results are represented graphically in fig 5.

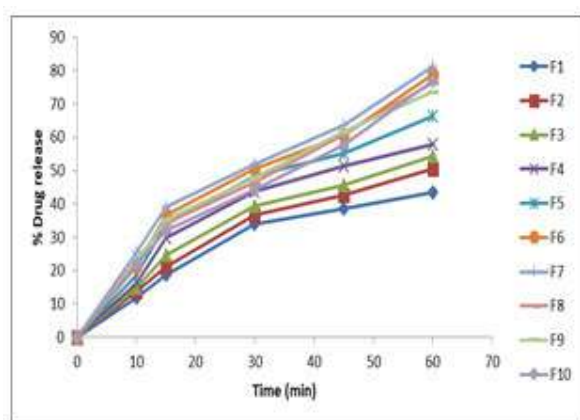


Figure 4. Graphical representation of in vitro drug release studies

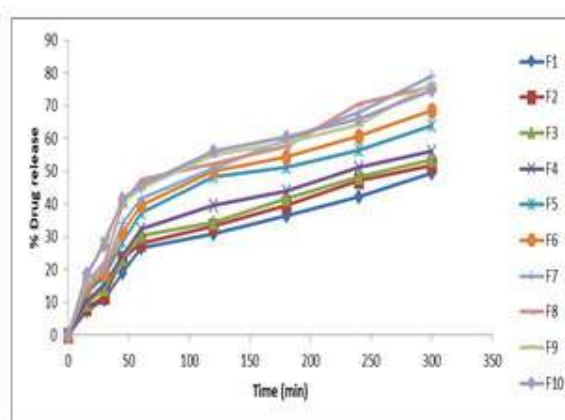


Figure 5. Graphical representation of In-vitro permeation studies of Losartan potassium buccal films

10. Drug release kinetics

Table 2. R2 values of F7 formulation of Losartan potassium buccal films

Formulation	Zero order plot	First order plot	Higuchi plot	Peppas plot	Hixson-Crowell
F7	0.892	0.972	0.983	0.872	0.956

The drug release profiles of the losartan potassium buccal films were applied to various kinetic models such as zero order, first order, higuchi plot, korsmeyer-peppas and Hixson-crowell models. The drug release kinetic results showed in table 2

F. Characterization studies

1. Scanning Electron Microscopy

From the below SEM images it is clearly evident that the films made up of drug and HPMC, PVP K30 combination has uniform distribution of the drug and the film appears clear. This is due to the less viscous nature of PVP K30 and HPMC and its freely soluble nature made them uniform distribution of drug. The SEM images of the losartan potassium buccal films of F7 formulation made up of polymers like HPMC and PVP K30 were shown in the figure no.6 respectively

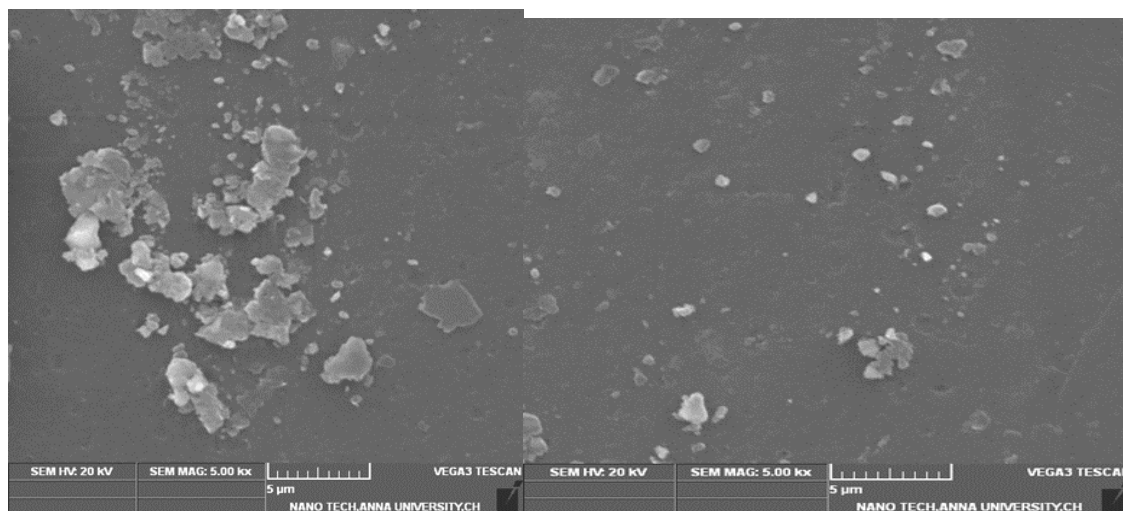


Figure No.6 Scanning electron microscopy of Losartan potassium buccal film (F7)

CONCLUSION

Losartan potassium was successfully formulated as buccal films by using film forming agents in combination and plasticizer by solvent casting method. All the films are in smooth textured. Amongst all the prepared formulations from F1 to F10, the F7 formulation possessing HPMC and PVP K30 as film forming agents and PG as plasticizer considered as optimized formulation on the basis of % drug release, % drug diffused and surface morphology. The optimized formulation F7 of losartan potassium releases 86.24 % of its drug release in 60 min. 82.90 % losartan potassium drug was diffused at the end of the 5 hour, the drug release kinetics follows Higuchi model for both the moieties.

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MOUTH DISSOLVING FILMS: A FAST ABSORBING DRUG DELIVERY SYSTEM: A REVIEW

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ABSTRACT

As the discovery of new molecules is a time consuming and expensive process, pharmaceutical companies have focussed on developing innovative drug delivery system for the existing drugs. Mouth dissolving films (MDF's) is one of the best examples of such drug delivery system. It was mainly designed for special category patients like pediatric, geriatric, bedridden and nauseous patients who have the fear of swallowing by the use of hydrophilic polymers and other excipients like sweeteners, saliva stimulating agents drug gets rapidly dissolved in the saliva with a good mouth feel. This article focuses on evolution of MDF's, formulation consideration and methods to prepare MDF's.

KEY WORDS: *Mouth Dissolving Films (MDF's), Buccal drug delivery system (BDDS), patient compliance, disintegrates, polymers, low dose.*

1. INTRODUCTION

The sound for the design of an effective dosage form has been there since long ago, even though many formulation scientists had designed different dosage forms but the people are not happy enough with these type of dosage forms because of the drawbacks that are associated with them. The dosage forms like injections are patient in compliance as their administration are associated with pain¹, transdermal patches need special knowledge whereas administration of dosage forms through the oral route doesn't require any skill and prior knowledge and self-medication is possible. Due to the advantages associated with oral dosage forms such as portability, cost-effectiveness and ease to administer accurate dose and avoiding first pass metabolism, oral formulations got popular and most of the marketed formulations are headed by the dosage forms that can be administered through oral route². Conventional tablets and capsules are the most widely used dosage forms but these undergo hepatic first pass metabolism and to overcome this problem new dosage forms were developed which is known as Buccal Drug delivery system (BDDS)³. However, these BDDS offer patient in compliance because of the size of tablet especially for the age specific patients like pediatrics and geriatrics and in this context MDF's were developed. MDF's are the solid oral dosage forms which appear as thin polymeric strip and dissolve immediately after placing on the tongue with the help of small amount of saliva and show bioavailability similar to liquids. MDF's can be administered easily without any fear of choking it is a suitable dosage form for these special category patients and faster therapeutic activity was attained as it avoids first pass metabolism and oro-mucosal absorption of drug occurs directly from site of administration to the systemic circulation⁴.

2. IDEAL CHARACTERISTICS OF DRUG TO BE SELECTED⁵

To formulate the drug as MDF it should have low dose, should be capable to permeate through the oral cavity and drug should be partially unionized at the pH of oral cavity.

3. FORMULATION CONSIDERATIONS

MDF's are formulated by using low dose of drug and suitable excipients. The standard composition of the film is shown in Table 2.

4.METHODS OF MANUFACTURING

MDF'S are mainly manufactured by the following methods ^{7,8}.

4.1Solvent casting method

In this method accurate weighed amount of drug was added to the solvent and to this solution plasticizer and remaining excipients were added one after other. On the other hand, polymer was dissolved in water. Both the solutions were mixed together and sonicated to remove the entrapped air bubbles if present any. The prepared solution was casted on the petri plate and is dried [5]. The films can be casted either in the petri plate or by using the specific types of equipment like rollers. The solution was poured at one end of an inert base and film was spreaded uniformly by the roller. The thickness of the film was based on the distance between the roller and substrate ⁹.

Table2: standard composition of Mouth dissolving films⁶

Ingredients	Amount	Examples
Drug	5-30% w/w	Antiallergic, antiemetic, antiepileptic, antimigrant.
Hydrophilic polymer	45%w/w	HPMC E3, E5, E15, K-3, Methyl Cellulose A-3, A-6, A-15, Pullulan, carboxy methyl cellulose cekol 30, polyvinyl pyrrolidone PVP K-90, Pectin, gelatin, sodium alginate, hydroxy propyl cellulose, polyvinyl alcohol, maltodextrins.
Plasticizers	0-20% w/w	Glycerol, dibutyl phthalate, poly ethylene glycol etc.
Surfactants	q.s	Sodium lauryl sulfate, benzalkonium chloride, tween etc
Sweetening agents	3-6% w/w	Saccharin, aspartame and cyclamate.
Saliva stimulating agents	2-6% w/w	Citric acid, malic acid, lactic acid and ascorbic acid
Fillers, colours, flavour's	q.s	FD&C C colours, USFDA approved flavour's

4.1Semi solid casting

In semi solid casting method, acid insoluble polymer solution was prepared in sodium hydroxide or ammonium. To this hydrophilic polymer solution was added. Suitable amount of plasticizer is added to obtain as a gel and the formed gel mass was casted in to films using a controlled heat source⁹.

4.2Solid dispersion

In this method weighed amount of drug was mixed with suitable solvent and this mixture was added to melted polymer along with immiscible components. Cutting of solid dispersion in to film⁷.

4.3Rolling method

In this method, drug solution was mixed uniformly along with the polymer solution and the resultant solution was poured on the roller. The film on rollers was dried and cutted in to desired shapes and sizes¹⁰.

4.4 Hot -MeltExtrusion

This process involves uniform mixing of API and excipients in a dry state with heating. The granular material is introduced in to the extruder for the processing up to 3-4 mins. The extrudate was pressed in to cylindrical calendar for films^{5,11}.

EVALUATION TESTS

Visual inspection and surface morphology ⁷

Visual inspection provides information about colour, homogeneity & transparency. In case of surface morphology scanning electron microscopy is performed. Absence of pores shows that films are of good quality.

Thickness Test

Thickness of the MDF's should be measured as it shows its impact directly on the drug content within the MDF's and the thickness of MDF's was measured using thickness gauge.¹²⁻¹³. The measurements were carried out in triplicate and the mean values were noted.

Weight variation

Weight variation test was performed to ensure that each individual film contains uniform amount of drug without any significant change and the weight of the film mainly depends upon the thickness of the MDF's. Weight variation was calculated in triplicate by checking the weight of individual film and the average values were noted.¹³

Dryness test/Tack test

The ability of a film to adhere to a piece of paper when pressed between strips is tested by the dryness test. Obstinacy to which film adheres with the piece of paper or any other accessory prepared in between the film is known as tack.⁷

Tensile strength

It is the maximum stress applied to the point at which the oral film breaks and this test was useful to determine the mechanical strength of the MDF¹⁴. Tensile strength for the MDF's was calculated using the following formula.

$$\text{Tensile strength} = (\text{load at failure} / \text{strip thickness} \times \text{strip width}) \times 100.$$

Percentage elongation

By applying stress to the film, it stretches and this is referred as strain and the distance travelled by the pointer before breaking the film on the graph was noted as Percentage elongation. It is determined by the following formula and percent elongation depends upon the plasticizers used in the film.¹⁵

$$\% \text{ elongation} = (\text{change in length} / \text{initial length}) \times 100$$

Tear resistance

Maximum force that is required to tear the film is measured as tear resistance value & it is the ultimate point at which the film ruptures.¹⁶

Young's modulus

Young's modulus is used to determine the rigidity of the film. It is represented as ratio of applied stress to the strain and is a measure of film stiffness¹⁴. The formula is as follows.

$$\text{Young's modulus} = (\text{slope} / \text{strip thickness} \times \text{cross head speed}) \times 100$$

Folding endurance

Folding endurance was determined by repeatedly folding the film at the same place till it breaks. The number of times the film was folded without breaking was noted as folding endurance value².

Swelling property¹

Swelling property of the MDF was determined by using simulated saliva solution. Each film specimen was weighed and was placed in a preweighed steel mesh and this was introduced in to a beaker containing 15ml of saliva solution and the degree of swelling was calculated using the following formula:¹⁴

$$\text{Degree of swelling} = (\text{final weight} - \text{initial weight}) / \text{initial weight}$$

Content uniformity

Content uniformity is determined to know the amount of API present in individual strip and was determined according to the standard assay method for the particular API. Limit of content uniformity is 85-115 percent.¹⁷

Disintegration time

Disintegration and dissolution characteristics of the MDF's can be estimated through the disintegration time. In case of MDF's, both the disintegration and dissolution procedures are difficult to distinguish due to the characteristic of the MDF to dissolve immediately after disintegration within a small amount of saliva and it becomes difficult to mimic these natural conditions and measure with an adequate method¹⁸.

Disintegration of MDF's was mainly determined by 2 methods:

a) Drop method

In this method one drop of distilled water was dropped through the pipette onto the film which was placed on a glass slide planar on a petri dish and the time until the film dissolves and causes a hole within the film was measured as disintegration time.

b) *Petri dish method*

In this method 2 ml of distilled water was placed in a petri-dish and a film of 1x1 cm² was placed on the surface of the water and the time required to dissolve the film completely was measured. The estimations were carried out in triplicate.

In-vitro dissolution test

In-vitro drug release studies were carried out to determine the Cumulative percent of drug released and the percent retained and the process was carried out using USP Type 5 apparatus and the selection of dissolution medium and volume depends upon the drug used in the formulation. Temperature was maintained at 37±0.5°C. Samples were collected at predetermined time intervals and replaced with same amount of buffer to maintain the sink conditions and the collected samples were analysed at appropriate time intervals using double beam UV-Visible spectrophotometer.¹⁹

5. CONCLUSION

MDF's are novel drug delivery systems which are mainly suitable for pediatric, geriatric patients who have the fear of choking & difficulty in swallowing. MDF's show rapid onset of action as drug is directly reached in to the systemic circulation without first pass metabolism. The major advantage of these dosage form is it can be administered at any time without the need of water and suitable in conditions such as asthma, hypertension, vomiting's, allergy, acidity where the drug should be released immediately.

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EVALUATION OF WOUND HEALING ACTIVITY OF HESPERIDIN ISOLATED FROM CITRUS FRUITS PEEL

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1.ABSTRACT

Hesperidin is a flavanone-on glycoside found in citrus fruits used traditionally for treatment of wounds. However, there are no scientific reports documented so far on the wound healing activities of this isolated compound. Thus, the present study provides a scientific evaluation for the wound healing potential of hesperidin. The compound was prepared in 5%(w/w) and 10% (w/w) ointment and evaluated for wound healing activity using excision, infected, and incision wound models in Swiss albino mice. Both 5% and 10% (w/w) ointments significantly reduced period of epithelialization and increased wound contraction rate and tensile strength compared to the negative control group ($P < 0.05$). The wound healing activity of 10% (w/w) ointment treated groups was greater than 5% (w/w) and nitrofurazone ointment treated groups in *S.aureus* infected wound model. These results demonstrate that hesperidin possesses wound healing activities. This justifies the traditional claimed use of the hesperidin for treating uninfected and infected wounds caused by *S.aureus*.

KEYWORDS: *Hesperidin, nitrofurazone ointment, S.aureus.*

2. INTRODUCTION

Wound defined as the cellular and anatomic disruption of a tissue may be caused by chemical, physical, microbial, thermal, or immunological damage to the tissue.¹ Wound healing is restoration of structure and function of an injured tissue in order to approximate prewound characteristics. The genus citrus includes sweet oranges (*Citrus sinensis*), grapefruits (*Citrus paradisi*), limes (*Citrus aurantifolia*), lemons (*Citrus aurantium*).⁸ These fruits have been cultivated since ancient times. They have probably originated from Australia, New Caledonia and New Guinea, although some research points to them originating in southeast Asia. Many of the species are hybrids and there is speculation that even the wild true-breeding species are actually hybrids originally. Rutaceae, the rue family of flowering plants (order Sapindales), composed of 160 genera and about 2,070 species.¹¹ Rutaceae includes woody shrubs and trees (and a few herbaceous perennials) and is distributed through the world, especially in warm temperature and tropical regions.¹² Citrus fruit peel contains carbohydrates, pectin and fiber, that giving it to better functional properties. Thus, it can be used to increase viscosity, water and oil absorption capacity in food products to be utilized later, they need to be preserved. Citrus peels are packed with immune-boosting vitamin C, bone-building calcium and anti-inflammatory, antioxidants, bioflavonoids.^{13,14} They also provide potassium, which helps to keep blood pressure in check, and limonene, a phytochemical that may have anti-cancer effects and help with heart burn.¹³ Hesperidin is also used for prevention and management of capillary fragility so used in hypertension, CVS disorders, cerebrovascular diseases. Hesperidin is thought to have beneficial effects on the blood vessels. It is used as a natural remedy for a number of health problems, including: *Allergies, *Hemorrhoids and Varicose Veins⁷, *Chronic Venous Insufficiency, *High Blood Pressure, *High Cholesterol, *Cancer

3. MATERIAL AND METHODS

3.1 Wound Healing Activity Testing:

3.1.1 Grouping and Dosing of Animals.

Healthy, adult white albinomice of either sex (25–35 g, and 6–8 weeks of age) were used. Four groups of mice, each containing six mice, were used for excision model. Animals in group I were treated

Based Complementary and Alternative Medicine 3 with simple ointment (as negative control), whereas group II was treated with nitrofurazone (0.2 %) ointment (as a standard drug). Group III and group IV were treated with 5% (w/w) and 10% (w/w) extract ointments, respectively. Four groups of mice, each containing six mice, were used for incision wound model. The animals of groups I-IV were treated in a similar fashion with excision wound model and infected wound model. All the experiments were conducted in accordance with the internationally accepted guideline for laboratory animal use and care.¹

3.1.2. Excision Wound Model

On wounding day, animals were anesthetized using subcutaneous injection of ketamine (1 ml/kg) and diazepam (1 ml/kg). After wound area preparation with 70% alcohol, the dorsal fur of the animals was shaved with shaving machine and the anticipated area of the wound to be created was outlined on the back of the animals on the dorsal thoracic region 1 cm away from vertebral column on the anesthetized mouse. Full thickness circular excision wounds sized about 300 mm² were created along the markings using toothed forceps, scalpel, and scissors. Hemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The entire wound was left open. The mice were divided into four groups (6 mice per group) randomly and each mouse was placed in a separate cage. The treatment was done once daily topically in all the cases. The wounding day was considered as day 0. The standard drug, extract, and simple ointment were applied topically to the respective groups till the wound was completely healed.¹⁸⁻¹⁹

3.1.3 Infected Wound Model

Full thickness circular excision wounds sized about 300 mm² were created along the markings using toothed forceps, scalpel, and scissors using similar procedures to those in the excision wound model. After achieving complete haemostasis by blotting the wound with cotton swab soaked in normal saline, the wound was inoculated with standard strain of *S. aureus* culture (24 hrs) obtained from Department of Microbiology, Nirmala college of pharmacy, Atmakur, Mangalagiri. After 24 hrs of contamination with *S. aureus* suspensions (1×10^6 CFU/mL), the experimental animals were randomized into four groups.¹⁻⁴ The animals were placed singly in separate cages and treated in similar procedures to those followed in the case of excision wound model. Treatments of infected wounds commenced on the 2nd day to allow establishment of infection on the wound. The wound area was measured with a translucent paper and a 1 mm² graph sheet on day 0, day 2, day 4, and thereafter every other day until wound closure is completed was recorded. Wound contraction was calculated as a percentage of the original wound size. Throughout the experiment, presence or absence of phlogistic characteristics (infiltration, edema/localized swelling, abscess, or lesion and exudates) were monitored every 24 hours.⁶

3.1.4 Measurement of Wound Contraction

The wound closure rate was assessed by tracing the wound on days 2, 4, 6, 8, 10, 12, 14, and 16 using transparent paper and a permanent marker. In case of infected wound model, measurement of wound contraction was prolonged till 18 days. The wound areas recorded were measured using 1 mm² scale of graph paper. Changes in wound area were evaluated, giving an indication of the rate of wound contraction and epithelialisation period. The evaluated surface area was used to calculate the percentage of wound contraction, taking initial size of the wound as 100%.¹⁸

3.1.5 Incision Wound Model

Animals were anesthetized in the same manner described for excision wound model. The dorsal fur of each mouse was then shaved and a 3 cm long longitudinal paravertebral incision 1 cm away from vertebral column was made through the skin and subcutaneous tissue. The parted skin was then sutured 1 cm apart using a surgical thread (silk no. 00 round) as described by Ehrlich and Hunt with slight modification.¹⁶ After 24 hrs of wound creation (on 1st day), animals were treated as described under grouping section, with topical formulation of nonmedicated simple ointment, HO, and standard drug once daily for nine days. The suture was removed. Animals were anesthetized in the same manner described for excision wound model. The removed on day 8 after incision and tensile strength was measured on the 10th day after wounding using continuous water flow technique.¹⁹

4. RESULTS

Table 1: Effect of topical application of the 80% isolated hesperidin ointment from citrus fruits peel on wound contraction of excision wound model in mice.

	Days	SO	NF	5% HO	10% HO
Wound area (mm ²) after wounding days	0	298.11±5.14	298.11±5.14	290.60±5.84	298.11±5.14
	2	275.71±8.13	254.51±12.18	270.5±5.59	260.20±6.08
	4	235.51± 6.03	205.21±11.45	220.51± 8.09	205.55±5.26
	6	211.12 ±8.24	150.86±15.22 ^{a2}	162.21±7.55 ^{a1}	119.53±9.37 ^{a3c1}
	8	159.46 ±9.27	88.22 ±10.98 ^{a3}	90.68± 8.15 ^{a3}	60.79± 7.58 ^{a3}
	10	125.51±11.52	45.60 ±4.35 ^{a3}	50.89 ±5.03 ^{a3}	23.49 ±5.19 ^{a3c1}
	12	92.05 ±12.13	22.61 ±4.56 ^{a3}	21.58±43.12 ^{a3}	3.04 ±1.19 ^{a3}
	14	48.58 ±9.95	4.59 ±1.99 ^{a3}	7.02 ±3.08 ^{a3}	0.00 ±0.00 ^{a3}
	16	29.12 ±5.13	0.00 0.00 ^{a3}	1.52 ±1.16 ^{a3}	0.00 ±0.00 ^{a3}

SO, simple ointment base; HO, hesperidin ointment; NF, nitrofurazone; n = 6 animals in each group. Values are expressed as mean ± SEM (n = 6), one-way ANOVA. ^aAgainst control and ^cagainst 5%(w/w) HO ; ¹P< 0.05, ²P<0.01, and ³P< 0.001.



Figure 1: Photograph of incision wound on day 0 and measurement of tensile strength on 10th day using water flow technique.

Table 2: Effect of topical application of the 80% isolated hesperidin ointment from citrus fruits peel on period of epithelialization (number of days).

Treatment groups	Period of epithelialization(days) Mean ±SEM	%decrease in epithelialization periods
Simple ointment base	19.9±1.01	-
Nitrofurazone(0.2%w/w)	14.2±0.41 ^{a3}	25.4
5%Hesperidin ointment(w/w)	14.8±0.55 ^{a3}	23
10%Hesperidin ointment(w/w)	12.17±0.30 ^{a3c1}	35.8

Note: values are expressed as mean ± SEM (n = 6), one-way ANOVA. ^aCompared to negative control (simple ointment); ^c compared to 5% ointment; ¹P<0.05, ²P<0.01, and ³P< 0.001, when compared to control group, one-way ANOVA.

Table 3: Effect of topical application of 80% isolated hesperidin ointment from citrus fruits peel on wound contraction of infected wound model in mice.

	Days	SO	NF	5% HO	10% HO
Wound area (mm ²) after wounding days	0	295.10±5.84	299.11 ±5.55	295.49 ±5.84	299.11± 5.59
	2	275.71± 8.13	250.35 ±7.73	262.77 ±5.50	250.19±4.14
	4	250.67±10.12	201.61±10.01 ^{a2}	201.5±5.39 ^{a1}	200.12±6.19 ^{a2}
	6	210.34±10.59	140.11±10.75 ^{a2}	145.66±8.51 ^{a2}	123.71±9.51 ^{a3}
	8	182.47±16.40	83.04±8.52 ^{a3}	95.01 ±9.03 ^{a3}	65.18 ±8.07 ^{a3}
	10	125.51±11.52	45.60 ±4.35 ^{a3}	50.89 ±5.03 ^{a3}	23.49±5.19 ^{a3c1}
	12	116.24±18.51	19.43 ± 5.63 ^{a3}	26.05±8.00 ^{a3}	7.01 ±3.12 ^{a3}

	14	105.02±18.27	5.33 ± 3.04 ^{a3}	11.02±5.32 ^{a3}	1.60 ± 1.15 ^{a3}
	16	83.21 ±18.39	0.63 ± 0.50 ^{a3}	2.45 ± 3.50 ^{a3}	0.00 ± 0.00 ^{a3}
	18	65.04 ± 16.89	0.00 ± 0.00 ^{a3}	0.00 ± 0.00 ^{a3}	0.00 ± 0.00 ^{a3}

Note: SO, simple ointment base; HO, hesperidin ointment; NF, nitrofurazone; n = 6 animals in each group. Values are expressed as mean ± SEM (n = 6), one-way ANOVA. ^aAgainst negative control, ¹P< 0.05, ²P< 0.01, and ³P< 0.001.

Table 4: Effect of topical application of the 80%isolated hesperidin ointment from citrus fruits peel on the period of epithelialization of infected wound model in mice.

Treatment groups	Period of epithelialization(days)
Simple ointment	FR
Nitrofurazone(0.2%w/w)	15.17 ± 1.73 ^{a3}
5%HO(w/w)	17.00 ± 0.85 ^{a3}
10%HO(w/w)	14.07 ± 1.14 ^{a3c1}

Note: FR: failed to reepithelize. Values are expressed as mean ± SEM (n = 6), one-way ANOVA. ^aCompared to negative control (simple ointment); ^ccompared to 5% ointment; ¹P< 0.05 and ³P< 0.001.

Table 5: Effect of topical application of the 80%isolated hesperidin ointment from citrus fruits peel on tensile strength of incision wound model in mice.

Treatment group	Tensile strength(g) (mean±SEM)	%tensile strength
Simple ointment	195.00 ± 5.15	-
Nitrofurazone(0.2%)	259.23 ± 5.69 ^{a3}	30.12
5%HO	255.49 ± 7.11 ^{a3}	31.65
10%HO	265.50 ± 7.51 ^{a3}	34.69

Note: values are expressed as mean ± SEM (n = 6), one-way ANOVA. ^aCompared to negative (simple ointment) control; ³P <0.001, when compared to control group, one-way ANOVA.

5. DISCUSSION

Traditionally, the isolated hesperidin used for woundhealing activity mixed with butter, which is hydrophobic. Applying the extract directly on the affected wound cannot bring the desired effect as it does not stay longer on the wounded skin of the experimental animals. Ointment is necessary to achieve a sustained drug release at the application sites. Hence, a hydrophobic base was selected based on traditional claim and active metabolites of hesperidin is predominate polar components, which would be released better from the nonpolar base and vice versa.¹⁵ The ointment base has additional roles like formation of occlusive barrier for moisture by hard and white soft paraffin. Wool fat and cetostearyl alcohol are thickeners and they are used for stabilization of ointment. The results of this study on wound healing activity revealed that the crude extract significantly increases wound healing effects with both 10% (w/w) and 5% (w/w) hesperidin ointment treated groups in the excision, infected, and incision wound models. This can be supported by the fact that the greater the reduction in the rate of wound contraction is, the better the efficacy of medication is and the wound will close at faster rate if the medication is more efficient. In excision wound healing model, the 80% isolated hesperidin showed statistically significant wound area contraction compared to the negative control. The 10% (w/w) hesperidin ointment treated group revealed faster wound area contraction from day 6 to day 14, whereas the 5% (w/w) hesperidin ointment treated group showed statistically significant wound area contraction starting from the 8th day onwards. The higher wound contraction rate of the extract ointment may be due to either its dose-dependent antibacterial effect or induction of macrophage cell proliferation.¹⁷ Furthermore, the period of epithelialization was significantly reduced from 20 days (negative control) to 15, 15, and 13 days for 5% HO, nitrofurazone, and 10% HO treated groups, respectively. The shorter period of epithelialization and faster wound area contraction could be due to the ability of isolated hesperidin from citrus fruits peel enhance collagen synthesis, induction of cell proliferation, and antimicrobial activities of bioactive constituents.⁹ In the case of infected wound model, the ointments of crude extract revealed statistically significant wound healing effect in mice infected with S.aureus. The infiltration, blister formation, edema, and exudates exhibited on the wounds of mice before treatment vanished in all treated groups except the negative control. Groups treated with 10% hesperidin ointment

showed faster rate of wound contraction than nitrofurazone and 5% hesperidin ointment treated groups. Additionally, the period of epithelialization was shorter in 10% HO followed by nitrofurazone and 5% HO. This finding indicated that the wound healing activity of the hesperidin in infected wound model was presumed to be dose-dependent. In this study, the antibacterial activity of the hesperidin was conformed against common wound infecting pathogens, which might contribute remarkably to the faster wound healing rate. Supporting evidence explained that the eradication of the colonizing organisms from infected wounds creates a suitable environment for wound healing to take place.

6. CONCLUSION

In this study, in all the three models, the different phases of wound repair, wound contraction, epithelialization, and tensile strength, were enhanced by the 80% hesperidin ointment from the isolated citrus fruits peel as compared to the negative control group. These results collectively demonstrate that the 80% HO possesses wound healing activity and this justifies the use of the hesperidin for treatment of wounds as claimed in the folklore literature. This study also showed that the isolated hesperidin was endowed with significant antibacterial activities that explain at least in part its wound healing activity.

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A REVIEW OF THE ETHNOPHARMACOLOGY, PHYTOCHEMISTRY AND PHARMACOLOGY OF FOOD MEDICINE *TALINUM PORTULACIFOLIUM*

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ABSTRACT

Medicinal herbs are used since ancient times for their therapeutic benefits. Now-a-days chronic diseases have become a global threat. In order to safeguard human health one should adopt some eating habits. Supplementing the daily eaten food with some herbs increases body's immunity towards stress and other unfavourable pathologies of human body. With this regard the plant selected was *Talinum portulacifolium*. It is often used as a vegetable, ornamental and medicinal plant in some traditional cultures. The plant has rich mineral nutritional value and used to treat certain ailments. The current review discusses its traditional, phytochemical, and pharmacological aspects. The literature review suggests that the plant needs to be explored phytochemically and pharmacologically.

KEYWORDS: *Medicinal herb, mineral content, food, therapy*

INTRODUCTION

Medicinal plants have been the source of drugs since decades. Chronic diseases have become global health burden. The treatment options have become costly, and unsafe. Inclusion of certain medicinal plants to our daily diet keeps away health hazards and gives us long, healthy life. Keeping the health prospective in view the present article aims to describe the traditional uses, phytochemistry, and phytopharmacological preview of the plant *Talinum portulacifolium* (Forsk.) Asch. & Schwe commonly called as flame flower. It widely grows in tropical regions as a leaf vegetable. It belongs to the family Talinaceae or Portulacaceae. The number of species present all over the world account for 500 from the genus *Talinum*. The family is cosmopolitan. It has 19 genera and more centered in South Africa (7 species) and Southern America (40 species).¹ The plant is common in a wide range of habitats, from rocky locations to grass land, in open bushland and thickets, along water courses and as a weed in fields, from sea-level up to 2200m altitude. It is distributed from Nigeria east to Ethiopia, Eritrea, south to northern South Africa, Eastern Cape South Africa, Arabia, China, India and Nepal. Most of the species are of warm American, African and Asian origin. In India it is distributed from Rajasthan to southwards to the peninsular region.² It consists of about 50 species, widely distributed in Talakona forest in Chittoor district of Andhra Pradesh. In the state of Tamilnadu, it is available from wild sources found in Madurai, Thiruchirappalli, and Tirunelveli districts. It is used as a green leafy vegetable due to its rich vitamin A and mineral content.³ The plant *T. portulacifolium* is confused with *T. triangulare*, which is also used as a vegetable. The latter species differs in its pink flowers arranged in cymes on triangular stalks, sepals prominently 3-veined and tuberculate seeds.⁴ In Africa it is occasionally cultivated like spinach. It is mostly collected from the wild and occasionally from cultivated source. Propagation is done by seeds and cuttings. The nutritional composition of leaves is comparable with *T. triangulare* (acq.) wild. The plant species is a perennial herb with succulent greyish stems, thickened, creeping roots helpful in sprouting. The stems, which can sometimes become soft woody, usually grow up to 1 metre long, occasionally to 3 metres.⁵

TRADITIONAL USES

For a long time, the tribal people of the Rayalaseema region in Andhra Pradesh, India, have used the leaves of the plant to keep away from diabetes, ulcer, and fevers. The leaf powder of this plant is mixed with boiled milk to treat diabetes.^{6,7} The leaf powder of the plant is mixed with leaf powder of *Gymnema sylvestre* and taken one spoon full in milk twice a day for 30 days in the treatment of diabetes.⁸ Since ages, the plant has been used as an aphrodisiac. The leaves are used against stomach ache as an ancient medicine.⁹ In Uganda, people use it against demonic effects of malaria and mouth ulcers.¹⁰

PHYTOCHEMISTRY

Leaves contain scopoletin, phytoconstituents like tannins, phosphates, urea and various minerals with a larger amount of magnesium.¹¹ Highest amount of total phenolic content was observed in chloroform extract 39 µg gallic acid/100gm dry weight of plant material.^{12,13} The leaves were found to contain minerals, calcium 10578 mg/Kg, magnesium 22101 mg/Kg, sodium 365 mg/Kg, potassium 51424 mg.Kg⁻¹ and phosphorus 367.12 mg.Kg⁻¹. The stems of the plant contain magnesium 547 mg.Kg⁻¹, sodium 156 mg.Kg⁻¹, potassium 41511 mg.Kg⁻¹ and phosphorus 262.33 mg.Kg⁻¹ respectively. The plant extracts were found to contain flavonoid quercetin by HPLC. The study also includes determination of total phenol, 9000 and 2880 mg gallic acid equivalents/100gm, total flavonoid 8800 and 960 mg quercetin equivalents/100gm and total flavonol content 1840 and 360 mg quercetin equivalents/100gm respectively for the leaf and stem extracts. The results indicate that leaf extract possesses more mineral content, total phenol, total flavonoid, total flavonol than the stem extract. Two flavonoids, luteolin and kaempferol were isolated and characterized from the methanolic extract of plant leaves.¹⁰ Acetone extract of chloroform fraction of leaves of the plant subjected to GC-MS analysis. The phytochemical marker kaempferol was quantified in the hydroalcoholic extracts of the plant leaves by HPLC.¹⁵

PHARMACOLOGICAL STUDIES

Antioxidant activity

Petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of leaves of the plant were studied by 2,2-diphenyl-1-picryl hydrazyl radical (DPPH), nitric oxide radical (NO), superoxide radical, hydrogen peroxide radical (H₂O₂) scavenging assays. The results indicate that chloroform extract of leaves showed highest antioxidant activity with IC₅₀ values of 133.26, 165.5, 156.34 and 135.29 µg.ml⁻¹ and highest amounts of phenolics with a direct relationship between activity and concentration of extracts (15-240 µg.ml⁻¹). The extracts showed significant activity but lower than the standard ascorbic acid¹³. The methanol extracts of stem and leaf of the plant were studied for *in vitro* antioxidant activity reducing power, chelating capacity (ICC) and ferric reducing antioxidant power (FRAP). The results indicate that leaf extract possesses more mineral content, total phenol, total flavonoid, total flavonol and antioxidant activity than the stem extract. The antioxidant activity was attributed to the presence of high amount of polyphenolic content.¹⁶

Antidepressant activity

The ethanolic extract of the whole plant was studied for antianxiety and antidepressant activities by the open field test, the light/dark box, the elevated plus maze test (EPM), the forced swimming test (FST), and tail suspension test (TST). The extract showed antidepressant activity (400mg.Kg⁻¹) in dose dependant manner.¹⁷

Analgesic activity

The methanolic extract was studied *in-vivo* for analgesic activity by acetic acid induced writhing response in albino mice. The extract showed significant (p<0.05) dose dependent analgesic properties at 2000 mg.¹⁸

Antibacterial and antifungal activity

Antibacterial and antifungal properties of methanolic extract of leaves of the nutraceutical plant were found to be inactive against *S. aureus*, *E. coli*, *P. aeruginosa* and *C.albicans*.¹⁹

Antiulcer activity

Ethanol extract of the whole plant, acted against histamine and ethanol induced ulcerations compared to standard drug Ranitidine.²⁰

Inhibition of carbohydrate digestive enzymes

The methanolic leaf extract was studied for inhibition of carbohydrate digestive enzymes and its activity on glucose uptake in isolated rat hemidiaphragm by alpha- glucosidase inhibitory assay using mouse small intestine. The methanolic extract effectively inhibited alpha-glucosidase (86.00%) and alpha-amylase (90.66%) at 500 µg.ml⁻¹. The results showed that the extract may act as a potent antihyperglycemic agent.¹³

Antihyperglycemic and antioxidant effects

The hexane extract at a dosage of 0.5g.Kg⁻¹ b.w has shown maximal (64.7%) blood glucose lowering effect. The activity was higher than that of glibenclamide. Hexane extract showed both antihyperglycemic and antioxidant activities where as ethanol and aqueous extracts showed only antioxidant activity. The highest total phenolic content of aqueous extract was found to be 114 mg.gm⁻¹.²¹

Antidiabetic activity by alloxan induced method

The methanolic extract of the plant leaves was studied for antihyperglycemic activity against alloxan induced diabetes. The effect on blood glucose, serum lipid profiles (triglycerides and total cholesterol), liver glycogen, malondialdehyde and reduced glutathione (GSH) levels. The blood glucose, lipid profile and malondialdehyde levels were decreased while liver glycogen and reduced glutathione were increased significantly. Histopathological studies of pancreas showed regeneration.²

Antidiabetic and antioxidant activity by streptozocin induced method

Antidiabetic and antioxidant activity of methanolic extract of edible plant leaves was studied by streptozocin induced method. The antioxidant activity was studied in liver and kidney tissues by measuring malondialdehyde (MDA), reduced glutathione (GSH) and catalase levels. The plant extract improved HDL/LDL (High density lipids and low density lipids) ratios, decreased cholesterol and triglyceride levels. Histopathological studies of pancreas showed regeneration compared to glibenclamide.²²

Antidiabetic evaluation of ethanolic extract loaded solid lipid nano particles

The ethanolic extract of the plant was loaded in to suitable lipids to prepare solid lipid nano particles using ultrasonication homogenization method to improve the solubility and bioavailability of extract. The extract was also investigated for antidiabetic activity in streptozocin and high fat diet induced diabetic rats. The parameters like blood glucose level, serum insulin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lipid peroxidase, catalase, and glutathione were evaluated. It was shown that the extract reduced all the elevated parameters at 250mg/Kg. Histopathological studies showed regeneration of damaged pancreatic cells.²³

Antiasthmatic activity

The plant leaf acetone extract showed antihistaminic bronchospasmolytic activity better than standard drug chlorpheniramine¹⁴.

CONCLUSION

The review of literature indicates that the plant is used traditionally and was not explored phytopharmacologically totally. The reported pharmacological activities suggest the potential use of the plant in treating diabetes, liver problems and other chronic inflammatory disorders. It may be studied further to isolate and characterise the phytoconstituents which may responsible to treat the disorders safely.

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AUTHORS CONTRIBUTION STATEMENT

Mamillapalli Vani., Shaik Abdul Rahaman, and Avula Prameela Rani have equally contributed to the design, collection of data and to the writing of the manuscript.

CONFLICTS OF INTEREST

None

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A REVIEW ON PHYTOCHEMICALS AS IMMUNO STIMULANTS IN FISHES

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ABSTRACT

Natural products, like plant extracts are most effective in controlling diseases and replace chemotherapeutic agents. Immunostimulants are natural or synthetic products with different chemical characteristic and mechanism of action. Immunostimulants activate the immune system of humans and animals. They reinforce a body's natural resistance to cope with various viral and bacterial infections. Immunostimulants are used in the clinical practice of the non-specific immunotherapy by stimulating the main factors of the immune system like the phagocytosis, properdin and complement system, T- and B-lymphocytes, synthesis of specific antibodies and cytokines and synthesis of pulmonary surfactant. Several studies indicated that immunostimulants can enhance resistance of fish to several bacterial pathogens including *Aeromonas hydrophila*. An all India ethnobiological survey carried out by the Ministry of Environment and Forests, Government of India reported that more than 8000 species of medicinal plants are being used by the Indians. The herbal extracts are used to control numerous diseases of bacterial, viral, fungal and parasitic etiologies like skin diseases, cough, cold, fever, headache, migrane, eye infections, respiratory tract infections, diarrhoea, hepatotoxicity and anticancer, antistimulant, neurostimulant, antiageing, antirheumatic, adaptogenic and antistress etc. Herbal medicines are effective, inexpensive, eco and farmer friendly, easily biodegradable, and without much side effects. Phytogenic feed additives are available with low cost or no cost as they are available in local areas and showed improvement in feed conversion ratio and in gaining weight. They can be mixed with feed or they can also be mixed with drugs in aquaculture disease management.

KEYWORDS: Phytochemicals, immunostimulants, Aquaculture, *Aeromonas hydrophila*

INTRODUCTION

Aquaculture is the fastest growing industry in the world with around 80 million tones produced annually. It is an important sub-sector in agriculture industry in India which produces protein rich food for human consumption. In recent years there has been an increasing number of fish and fish product consumers due to an increased awareness of nutritional values and health benefits of fish and its products. At the same time consumers wants an assurance that the food they eat is produced without any harm to environment and is safe to eat. This is due to the incidence of many food scares like madcow disease, antibiotic and chemical residues in sea food. As a result, there is a considerable shift in aquaculture disease management practices mainly from chemotherapy to phytotherapy. Many investigators reported that bacterial, algal, animal and plant products can be used as immunostimulants to stimulate and/or enhance non-specific immune response in cultured organisms. Immunostimulants are dietary additives/chemical substances which activate leukocytes. Adjuvants and glucans which are polymers of glucose, found in the cell walls of plants are used in fish and shrimp culture ponds orally. A polysaccharide which constitutes the principal components of exoskeletons of crustaceans and insects and cell walls of few fungi can stimulate macrophage activity and produce resistance from certain bacteria. Enhancement of disease resistance by using herbal immunostimulants in Aquaculture is an ecofriendly practice. (Arun Sharma *et al.*, 2010)⁴ studied the efficacy of dietary doses of *Withania somnifera* (Ashwaganda) root powder on immunological parameters and disease resistance against the bacterial pathogen *A. hydrophila* infections in *L. rohita* fingerlings. The results demonstrated that fishes fed with *W. somnifera* root showed enhanced respiratory burst activity of leucocytes, phagocytic activity, total immunoglobulin level and lysozyme activity compared with the control group. Also, it is reported that the root powder of *W. somnifera* induced immunostimulation thereby

increasing the disease resistance against aeromoniasis. (Hanifa and Kavitha 2012)¹⁴ employed 15 selected medicinal herbs to evaluate antibacterial activity against *A. hydrophila* and found to be effective.

Major Constraints In Aquaculture

Several factors like poor managerial practices, environmental conditions and disease outbreaks are the major constraints in the development and sustainability of aquaculture. Bacterial diseases are causing major loss to the fish farmers (Almeida *et al.*, 2009)². Stress factors such as overcrowding, transport, grading, poor handling and low water quality adversely affect the health of culture fish resulting in immunosuppression and disease outbreaks (Smith *et al.*, 2003)²⁹; (Li *et al.*, 2004)¹⁸; (Quesada *et al.*, 2013)²². Antibiotics and chemotherapeutics are commonly used in aquaculture to prevent disease outbreaks (Rico *et al.*, 2013)²⁴. Consumption of muscles containing antibiotic residues causing various health hazards in humans (Seyfried *et al.*, 2010)²⁸; (Romero Ormazahal *et al.*, 2012)²⁵; (Forwood *et al.*, 2013)¹². Antibiotics are proved non-effective against bacterial pathogens; the other alternative to control diseases is to strengthen/stimulate non-specific immunity (Anderson, 1992)³; (Ashley, 2007)⁵.

ROLE OF HERBAL IMMUNOSTIMULANTS

Fish immune system can be modulated by the use of herbal extracts (as feed supplement) as alternative chemotherapeutic agents (Raman, 2007)²³; (Kumar *et al.*, 2013)¹⁷. (Vaseeharan and Thaya 2013)³¹ found growth promotion, stimulation of appetite and immunity and increase of antimicrobial properties in fish due to the use of plant extracts. Immuno stimulants are substances, which enhance the non-specific defense mechanism and provide resistance against pathogenic organisms (Citarasu *et al.*, 2006)¹¹. There are many scientific publications looking at different mechanisms and ways to enhance the specific and non-specific immune systems in fish. Many plant derived compounds have been found to have non-specific immunostimulating effects in animals, of which more than a dozen have been evaluated in fish and shrimp (Citarasu *et al.* 2002)¹⁰; (Sakai, 1999)²⁶. Many herbs and plants have been used for millennia as home remedies in many cultures around the world for both human and animals. Some of these remedies have potent anti-viral as well as anti-bacterial and anti-fungal properties. Moreover, these substances also possess other valuable properties; they are nontoxic, biodegradable and biocompatible. No herbal-resistance immunity has been found by any pathogen to date. Although the properties of herbs and plants are well known, documented, and in use in human herbal medicine around the world. Immunostimulants are widely used in aquaculture for health management. Fish fed with immunostimulants along diet showed resistance against various pathogens. (Yano *et al.*, 1990)³³ found enhancement of disease resistance due to yeast β -1, 3 glucan in *C. carpio* against several bacterial pathogens, including *A. hydrophila*. (Baulny *et al.*, 1996)⁸ suggested that disease prevention is possible by immunostimulation (through alteration of diet and feeding practices) in turbot, *Scophthalmus maximus*. (Selvarj *et al.*, 2005)²⁷ investigated the protective effect of yeast glucan in *C. carpio* infected with *A. hydrophila*; intraperitoneal injection of glucan enhanced the relative percent survival and some non-specific and specific immune parameters compared to bathing and oral route of glucan application. Also, the higher concentration of glucan treatment resulted in the increase of leucocytes, neutrophils and monocytes, and superoxide anion production that may be involved in the destruction of *A. hydrophila*. (Sakai, 1999)²⁶ reported increased resistance against infectious diseases in fish; protection was by enhancing both non-specific and specific (cellular and humoral factors) immune responses. (Pratheepa *et al.*, 2010)²¹ reported enhanced resistance and non-specific and specific immune response in *C. carpio* treated with various concentrations of *Aegle marmelos* (leaf extract) and ten plant extracts supplemented diets. (Baba *et al.*, 1993)⁷ and (Gopalakannan and Venkatesan 2006)¹³ reported the enhancement of non-specific immune response and resistance to experimental challenge with *A. hydrophila* in *C. carpio* treated with the immunostimulant, levamisole. (Wang *et al.*, 2015)³² and (Baba *et al.*, 2016)⁶ observed enhancement of growth and positive effects of dietary supplements in *C. carpio*. (Harikrishnan *et al.*, 2003)¹⁵ and (Jagruhi *et al.*, 2015)¹⁶ recorded lower mortality on *A. hydrophila* challenge in *C. carpio* fed with immunostimulants. (Thiyagarajan *et al.*, 2014)³⁰ reported that use of medicinal plant extracts (*A. paniculata* and *A. indica*) can enhance the resistance of fish and control *A. hydrophila* infection. Dietary blackberry syrup was studied for growth, antioxidation, immune response, and resistance against *Pleiomonas shigelloides* in *O. niloticus* (Yilmaz, 2019)³⁴. Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (*Cyprinus carpio*) fingerlings (Yousefi *et al.*, 2019)³⁵. Dietary ginger (*Zingiber officinale*) alter biochemical and immunological parameters and gene

expression related to growth, immunity and antioxidant system in zebrafish (*Danio rerio*) (Ahmadifar et al., 2019)¹

METHOD OF ADMINISTRATION

Method of application of phytochemicals as immunostimulants in Aquaculture is a challenging and laborious process. Even though different practices are used in different culture systems immersion method is used for juveniles during early stages because the fish is very sensitive and delicate at this stage. Second method which is in common practice as well as induces strong immune response in fish is injection. But it can be applied only to large sized animals and needs technical expert and is costly procedure. Third method is the oral ingestion method in which phytochemicals are mixed with feed by using fish oils like cod-liver oil as coating. This method of application is farmer friendly and most effective method in large scale application.

Mode of action

The most proven effect of immunostimulants is to facilitate the function of phagocytic cells and increase their bactericidal and fungicidal activities. Immunostimulants can promote recovery from immunosuppressive states caused by any form of stress. Various studies proved that phytochemicals when injected reduces the mortality due to infections and improves the general health of fish by enhancing non-specific immune response.

CONCLUSION

Herbal extracts in fish health management and curing of diseases proves to be prompt alternate. However, the lack of sufficient knowledge on herbal extracts limits the cost-effective use of herbal extracts in aquaculture. Therefore, more research is required to further validate herbal extracts with their allied growth-promoting effects and immune-stimulating effects for sustainable aquaculture.

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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DAUNORUBICIN AND CYTARABINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

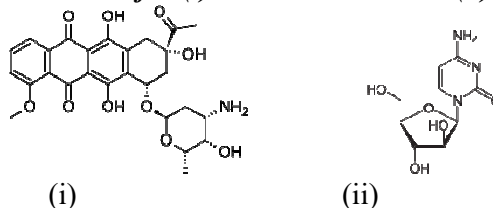
An economical UPLC method was developed for the simultaneous estimation of the Daunorubicin and Cytarabine in vial dosage form. In present study, the Chromatogram was run through X-Bridge C18 (100 x 2.1 mm, 1.6µm) column with mobile phase containing Buffer 0.01N KH₂PO₄: Acetonitrile taken in the ratio 50:50, was pumped at a flow rate of 0.3 ml/min. Buffer used in this method was 0.01N KH₂PO₄ (3.5 pH) and buffer pH was adjusted with 0.1% OPA. Optimized wavelength selected was 240.0 nm. Retention time of Daunorubicin and Cytarabine were found to be 0.958 min and 1.510 min. % RSD of the Daunorubicin and Cytarabine were found to be 0.8 and 0.6 respectively. % Recovery was obtained as 99.37 % and 100.10 % for Daunorubicin and Cytarabine respectively. LOD, LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.14, 0.41 and 0.33, 1.00 respectively. Linearity equations were obtained for Daunorubicin was $y = 2177.2x + 718.38$ and Cytarabine was $y = 4896.8x + 2531.1$ respectively. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: *Daunorubicin, Cytarabine, UPLC, Validation.*

1. INTRODUCTION

Cytarabine IUPAC name is 4-amino-1-[(2R, 3S, 4S, 5R)-3, 4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl] pyrimidin-2-one (Figure 1). It is a chemotherapy drug used to treat acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and non-Hodgkin's lymphoma. Daunorubicin IUPAC name is (1S, 3S)-3-Acetyl-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1, 2, 3, 4, 6, 11-hexahydro-1-tetracyclic 3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoside (Figure 1). It is used to treat acute leukemia, lymphocytic leukemia, chronic myelogenous leukemia and Kaposi's sarcoma.¹⁻⁵

Figure 1: Structures for (i) Daunorubicin and (ii) Cytarabine



A detailed Literature review⁶⁻¹⁰ reveals that different methods RP-HPLC, UV, LCMS for its analysis in formulations, but there is no UPLC method was reported. Hence our present plan is to develop a new, sensitive, robust & accurate method for its analysis in formulation, after a detailed study, a new UPLC method was decided to be developed and validated as per ICH norms.¹¹⁻¹²

2. MATERIALS AND METHODS

2.1. Instruments and Reagents Used

Electronics Balance-Denver, pH meter -BVK enterprises, India, Ultra sonicator-BVK enterprises, WATERS

UPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer integrated with UV win 6 Software was used for measuring absorbance. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Ortho-phosphoric acid [All are HPLC grade], potassium dihydrogen phosphate buffer [AR].

2.2. Analytical methodology

2.2.1. Preparation of Standard and Sample stock solutions

Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25ml volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (440 µg/ml of Daunorubicin and 1000 µg/ml Cytarabine)

2.2.2. Preparation of Standard and Sample working solutions (100 % solution)

1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (44 µg/ml of Daunorubicin and 100 µg/ml of Cytarabine)

3. METHOD DEVELOPMENT

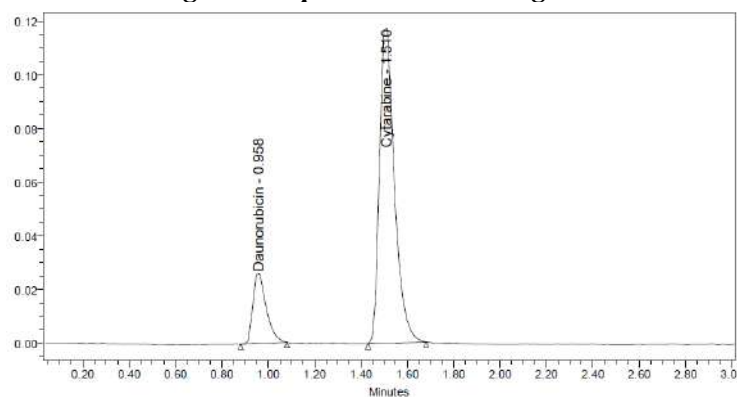
3.1. Optimized method

Trials were performed for the method development and the best peak with least fronting factor was found to be with RT = 0.958 min., for Daunorubicin and 1.510 min., for Cytarabine respectively (Figure 2; Table 1).

Table 1: Optimized Chromatographic conditions

Parameter	Content
Column	X-Bridge C18 (100 x 2.1 mm, 1.6 µm)
Mobile Phase	50% 0.01N KH ₂ PO ₄ : 50% Acetonitrile
Flow Rate	0.3 ml/min.
Temperature	30 ⁰ C
Injection Volume	0.30 µl
Wavelength	240 nm

Figure 2: Optimized chromatogram



4. METHOD VALIDATION

4.1. Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.37% and 100.31 % for Daunorubicin and Cytarabine respectively (Table 2).

Table 2: Recovery studies for Daunorubicin and Cytarabine

% Concentration	Daunorubicin			Cytarabine		
	50 %	100 %	150 %	50 %	100 %	150 %
Trail-I	98.55	99.92	99.60	100.71	99.08	100.50
Trail-II	99.12	99.04	100.21	99.95	100.01	100.31
Trail-III	98.90	99.80	99.18	100.53	99.95	99.85
AVG (% Recovery)	98.9	99.59	99.66	100.39	99.68	100.22
SD	0.29	0.48	0.52	0.396	0.5178	0.3363
% RSD	0.29	0.48	0.52	0.39	0.52	0.34
Mean % Recovery	99.37±0.54			100.31±0.49		

4.2. Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned in table 3. Precision % RSD values obtained as 0.3 % and 0.9 % and Intermediate precision values obtained as 0.6 % and 0.6 % respectively for Daunorubicin and Cytarabine. As the limit of Precision was less than "2" the system precision was passed in this method (Table 3).

Table 3: System precision table of Daunorubicin and Cytarabine

S. No	Peak area of Daunorubicin		Peak area of Cytarabine	
	Precision	Day_Day Precision	Precision	Day_Day Precision
1.	103838	103398	492723	451949
2.	103225	105155	500146	459018
3.	103409	102473	496550	457349
4.	104038	103028	495758	456419
5.	103249	103268	495001	453657
6.	103294	102673	499675	453511
Mean	103509	103333	496642	455317
S.D	344.3	958.8	2839.7	2698.2
% RSD	0.3	0.9	0.6	0.6

4.3. Linearity

Six linear concentrations of Daunorubicin (11-66 µg/ml) and Cytarabine (25-150 µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Daunorubicin was $y = 2177.2x + 718.38$ and of Cytarabine was $y = 4896.8x + 2531.1$. Correlation coefficient obtained was 0.999 for the two drugs (Figure 3&4; Table 4).

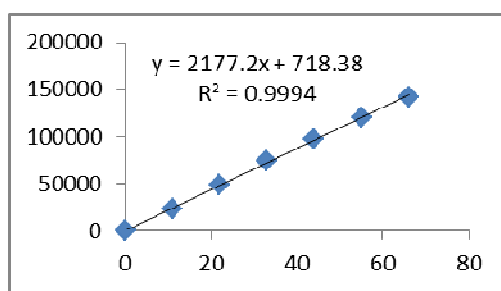
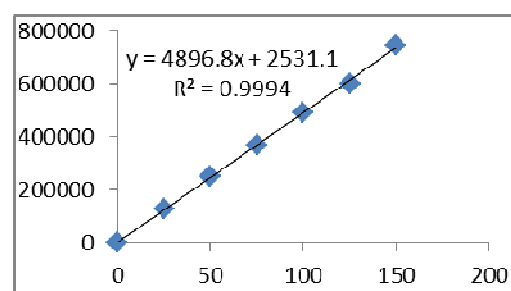
Figure 3: CalibrationcurveofDaunorubicin**Figure 4: CalibrationcurveofCytarabine**

Table 4: Linearity table for Daunorubicin and Cytarabine

Daunorubicin		Cytarabine	
Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
0	0	0	0
11	23739	25	126910
22	48836	50	251164
33	74414	75	371185
44	97759	100	491169
55	120501	125	602795
66	142708	150	745339

4.4. Robustness

Robustness conditions like Flow minus (0.20 ml/min), Flow plus (0.40 ml/min), mobile phase minus (45B:55A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit (Table 5).

Table 5: Robustness data for Daunorubicin and Cytarabine

S. No	Condition	% RSD of Daunorubicin	% RSD of Cytarabine
1	Flow rate (-) 0.2 ml/min	0.9	0.9
2	Flow rate (+) 0.4 ml/min	0.4	0.8
3	Mobile phase (-) 45B:55A	0.3	0.3
4	Mobile phase (+) 55B:45A	0.4	0.5
5	Temperature (-) 25°C	0.6	0.4
6	Temperature (+) 35°C	0.7	0.8

4.5. DEGRADATION STUDIES

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 6).

Table 6: Degradation Data of Daunorubicin and Cytarabine

S. No	Degradation Condition	% Drug Degraded	
		Daunorubicin	Cytarabine
1	Acid	9.36	3.41
2	Alkali	8.78	3.07
3	Oxidation	8.38	2.52
4	Thermal	2.08	2.43
5	UV	1.23	1.81
6	Water	1.23	0.44

5. CONCLUSION

A simple, precise method was developed for the simultaneous estimation of the Daunorubicin and Cytarabine in injection form. Retention time of Daunorubicin and Cytarabine were found to be 0.958 min., and 1.510min. % RSD of the Daunorubicin and Cytarabine were found to be 0.8 and 0.6 and % Recovery was obtained as 99.33 % and 99.70 % respectively. LOD, LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.14, 0.44 and 0.33, 1.00 respectively. Regression equation of Daunorubicin is $y = 2177.x + 718.3$, and $y = 4896.x + 2531.1$, of Cytarabine. Retention times were decreased

and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

6. ACKNOWLEDGEMENT

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DEVELOPMENT, CHARACTERIZATION AND PRE CLINICAL EVALUATION OF POLYHERBAL SYRUP FOR ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

The liver problems are on rise which necessitates development of better remedies to contain them. Hence the present study was intended to develop, characterize and evaluate polyherbal syrup containing *Cicer arietinum*, *Tabebuia argentea*, *Acacia leucophloea*, *Biophytum sensitivum* for its hepatoprotective and antioxidant activity. Polyherbal syrup was prepared by taking equal proportions of methanolic extracts of selected plants and simple syrup in 1:5 proportions. The formulation was then characterized for its organoleptic parameters, physicochemical parameters, stability testing and refractive index. It was later evaluated for hepatoprotective and antioxidant activity in CCl₄ induced hepatotoxicity model. The formulation showed significant hepatoprotective and antioxidant activity by restoring altered biochemical and antioxidant enzyme levels in both the models which proved its efficacy in alleviating liver disorders. The study provides a better remedy for liver disorders which needs further substantiation in clinical studies.

KEY WORDS: *Hepatotoxicity, Polyherbal formulation, Oxidative stress.*

1. INTRODUCTION

Liver diseases have turned into a global concern worldwide¹. The exposure to various organic compounds (drugs, chemicals, etc.) and environmental pollutants, to form highly reactive substances like reactive oxygen species (ROS) directly or through metabolic activation, which results change in anatomy or functions of liver². The management of liver disorders is a big challenge to the modern medicine. The modern allopathic drugs are unsatisfactory in alleviation of hepatic ailments and some of these drugs adversely affect the liver function. The traditional system of medicine like ayurveda and siddha system of medicine have a crucial role in curing of liver ailments³. Owing to good safety profile, use of herbal medicine for various diseases have received much attention in worldwide and in India⁴. The herbal formulations that have attained widespread acceptability as therapeutic agents include antidiabetics, hepatoprotective agents, and lipid-reducing agents⁵. However, there are many limitations such as collection, storage, doses, and duration regarding the safety and efficacy of these preparations. A research has been carried out to evaluate hepatoprotective and antioxidant activity of herbal agents as formulation⁶. The formulation contains methanolic extracts of aerial parts except seeds and seed coat of *Cicer arietinum* (Fabaceae), leaves of *Tabebuia argentea* (Bignoniaceae), *Biophytum sensitivum* (Oxalidaceae) and bark of *Acacia leucophloea* (Mimosaceae).

2. MATERIALS AND METHODS

2.1. Preparation of formulation

All the plant materials were collected and authentication was done by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh. Methanolic extracts of each plant prepared by using continuous hot percolation method. These extracts of each plant in equal proportions were mixed with simple syrup in 1:5 v/v ratio. The final liquid dosage form was then subjected to evaluation of quality and Pharmacological activity of the formulation as per official standards.

2.2. Chemicals

Carboxy methyl cellulose, Carbon tetrachloride, Silymarin was purchased from Sri Sai Krishna Enterprises, Hyderabad. Chem. Kit for SGOT, SGPT, SALP, and Total Protein and formalin were purchased from SD Fine chemicals, Chennai.

2.3. Evaluation of polyherbal syrup

The poly herbal formulation was evaluated for organoleptic parameters (color, odour, taste, appearance), physicochemical parameters (pH, specific gravity and viscosity, turbidity, homogeneity).⁷ The syrup was subjected for stability testing at temperatures 4°C and 47°C. All the physicochemical parameters were evaluated at intervals of 24 hours, 48 hours and 72 hours to observe any change⁷. Refractive index was measured by Abbe's refractometer.⁸

2.3. Experimental animals

Wistaralbino rats weighing 150-200g were acclimatized for one week in experimental room. The animals were selected for final allotment of the study. Feed and water were given *ad libitum* throughout the study. All the animal experiments were conducted according to the ethical norms approved by the Institutional ethical committee of CPCSEA, New Delhi (Reg.No: 1722/Ro/Ere/S/13CPCSEA).

2.4. Carbon tetrachloride induced hepatotoxic model

Hepatoprotective activity was evaluated using CCl₄ induced hepatotoxicity. In each models 5 groups (n=6) i.e group-I (normal control), group-II (disease control), group-III (standard control), group-IV & group-V received PHF 200 and 400mg/kg b.w/day. Treatment duration in CCl₄ model was 21 days. Normal control received 1ml of CMC; standard control received silymarin 50mg/kg/day; disease control groups received CCl₄ – 1ml/kg/day. Blood was collected by retro orbital plexus and transferred to sterilized non-heparinized syringes to separate serum for biochemical analysis and animals were scarified and liver was isolated and preserved for histopathological examinations. The serum was stored at -10 °C until biochemical analysis which was carried out within 24 hrs.

2.5. Assessment of hepatoprotective activity

The biochemical parameters includes serum enzymes Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT) by (uv kinetic method)⁹, Alkaline phosphatase (SALP) by p-NPP method¹⁰ by Schlebush *et al.*, 1974., Total Bilirubin by Taylor RLS *et al*, 1996¹¹ and Total Protein by Lowry's method by Dunn *et al*, 1992¹² were estimated.

2.6. Determination of anti oxidant parameters from liver homogenate

Anti oxidant parameters includes superoxide dismutase (SOD) by method of pyrogallol¹³, catalase (CAT) by the method of Aebi (1974)¹⁴, reduced glutathione (GSH) by the method of Ellman, (1959)¹⁵ and malondialdehyde (MDA) by Ohkawa *et al*, 1979 by using liver homogenate.

3. STATISTICAL ANALYSIS

The statistical significance was analyzed by using one way analysis of variance (ANOVA) followed by Dunnett multiple comparisons test using Graph Pad Instat 3.

4. RESULTS AND DISCUSSION

4.1. Evaluation of Physicochemical properties and Stability studies:

4.1.1. Evaluation of Physicochemical properties

The density of the polyherbal syrup was found to be 1.103 g/cm³, specific gravity - 1.106 g/mL, Viscosity - 4.53 cps, pH- 6.7, Refractive index - 1.341. All the physicochemical parameters were found to be in permissible range

4.1.2. Accelerated stability studies

The stability of polyherbal syrup was studied at 4°C, room temperature, and 47°C. There was no change in colour, odour and turbidity. It proved stability of polyherbal syrup at various temperatures.

4.2. Carbon tetrachloride induced Toxicity

Table: 1 Effect of Polyherbal formulation on various biochemical parameters in CCl₄ induced hepatotoxicity model in rats.

Parameters	Treatment groups				
	Group-I	Group-II	Group-III	Group-IV	Group-V
AST (U/L)	52.625±0.78	203.49±5.82 ^{##}	72.95±1.92 ^{**}	81.77±1.61 ^{**}	77.47±0.92 ^{**}
ALT (U/L)	53.26±4.23	233.38±12.1 ^{##}	101.52±0.92 ^{**}	87.85±2.91 ^{**}	77.92±2.44 ^{**}
ALP U/L	114.18±2.88	316.08±4.19 ^{##}	128.58±1.57 ^{**}	137.36±1.77 ^{**}	123.69±1.70 ^{**}
TBL (mg/dl)	0.57±0.04	6.25±0.12 ^{##}	1.15±0.06 ^{**}	1.44±0.10 ^{**}	1.12±0.12 ^{**}
TP U/L	9.255±0.59	2.98±0.14 ^{##}	7.23±0.09 ^{**}	6.44±0.10 ^{**}	7.01±0.16 ^{**}
Liv.wt (g/100g)	3.56±0.10	6.21±0.16 ^{##}	4.13±0.04 ^{**}	4.57±0.05 ^{**}	4.28±0.08 ^{**}
liv.vol (ml/100g)	4.34±0.12	6.82±0.11 ^{##}	5.03±0.15 ^{**}	5.49±0.09 ^{**}	5.23±0.11 ^{**}

Values were expressed as Mean±SEM (n=6), the statistical significance was analyzed by using one way analysis of variance (ANOVA) followed by Dunnett multiple comparisons test using GraphPadInstat3. P values: [#]P< 0.05 or ^{*}P< 0.05 (Significant), ^{##}P< 0.01 or ^{**}P< 0.01 (Highly significant), ^{###}P< 0.001 (Very highly significant) and compared to normal control group. ^{**}Values were highly significant at P< 0.01 when compared to control group. ^{ns}Values were Not significant at P> 0.05 when compared to toxic control group. (#-Vs Normal control; * - Vs disease control)

Table: 2 Effect of Polyherbal formulation on antioxidant parameters in CCl₄ induced hepatotoxicity model in rats.

Treatment	Treatment Groups				
	Group-I	Group-II	Group-III	Group-IV	Group-V
SOD(u /mg of protein)	182.32±1.11	71.12±0.12 ^{##}	161.2±1.13 ^{**}	143.21±1.21 ^{**}	156.21±1.14^{**}
CAT (u M/min/ mg of protein)	28.92±0.31	7.32±2.12 ^{##}	21.10±0.98 ^{**}	19.23±0.78 ^{**}	22.12±0.98^{**}
GSH (μ mol / 100 mg of protein)	88.12±1.49	30.24±2.1 ^{##}	71.32±1.12 ^{**}	65.12±1.14 ^{**}	74.31±0.98^{**}
MDA (n moles /100 mg of tissue)	0.39±0.12	5.42±1.12 ^{##}	1.1±0.43 ^{**}	1.56±1.14 ^{**}	1.21±0.09^{**}

Values were expressed as Mean±SEM (n=6), the statistical significance was analyzed by using one way analysis of variance (ANOVA) followed by Dunnett multiple comparisons test using GraphPadInstat3. P values: [#]P< 0.05 or ^{*}P< 0.05 (Significant), ^{##}P< 0.01 or ^{**}P< 0.01 (Highly significant), ^{###}P< 0.001 (Very highly significant) and compared to normal control group. ^{**}Values were highly significant at P< 0.01 when compared to control group. ^{ns}Values were Not significant at P> 0.05 when compared to toxic control group. (#-Vs Normal control; * - Vs disease control).

A strong exposure to CCl₄ leads to severe liver damage which is stimulated by cytochrome P-450 dependent mixed oxidases and produce free radical trichloromethyl (CCl₃ and Cl₃COO), which acts on the membrane of the endoplasmic reticulum and other cellular membranes resulting in an increase in calcium ion permeability through the plasma membrane causing severe instabilities of calcium homeostasis leading to necrotic cell death. These changes cause marked disruption of cell integrity, followed by excessive leakage of transaminases into the blood, leading to substantial rise of its enzymes (AST, ALT, and ALP) and total bilirubin. These elevations were observed in disease control group which indicated that the hepatotoxicity was developed by CCl₄. Higher and lower doses of polyherbal formulation 400 mg/kg, 200 mg/kg showed significant decrease in liver enzymes (AST, ALT and ALP) and total bilirubin and increase in total protein levels (Table: 1). Chronic exposure of CCl₄ cause excess production of free radicals leading to depletion of natural antioxidant enzymes like superoxide dismutase, catalase and reduced glutathione.¹⁶ As a result of reduction of these antioxidant enzymes the free radicals cause the oxidation of lipids or thiol moiety of biomembranes of hepatocytes leading to lipid peroxidation and release of thiobarbituric acid reactive substances (TBARS).¹⁷ Significant reduction in the levels of antioxidants like SOD, CAT, GSH and increase

in lipid peroxidation in CCl₄ treated group indicates the induction of hepatotoxicity. This herbal syrup exhibited dose dependant protective effect on hepatic parameters. High doses of the formulation (400mg/kg) significantly normalized the altered the biochemical and antioxidant parameters (Table: 2).

5. CONCLUSION

Our investigations confirmed the hepatoprotective effect of the herbal formulation and revealed its effect in alleviating oxidative stress induced by CCl₄ in a dose-dependent manner. Herbal formulation significantly reduced the levels of AST, ALT, ALP, TB, and increase the level of TP, SOD, CAT and GSH. These findings are also confirmed by histopathological observation. Traditionally these plants were reported with hepatoprotective activity. The hepatoprotective and antioxidant activity of polyherbal formulation could be attributed to synergistic interaction of phytochemicals present in it. Further studies are warranted to confirm its effect clinically.

6. ACKNOWLEDGEMENTS

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A CASE REPORT ON CAPTOPRIL INDUCED PEMPHIGUS VULGARIS

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ABSTRACT

Pemphigus is an autoimmune bullous disease that may be influenced by genetic and exogenous factors. The characteristic presentation of Pemphigus is the presence of widespread flaccid blisters and erosions erupting on the skin and oral mucosa. Drugs are a top cause of pemphigus. There is an essential requirement during taking the systematic history to achieve the medication causing this same considering the challenges required in the diagnosis of drug-induced pemphigus. Patients frequently use various drugs, for different comorbid diseases. Some of the drugs used by the patient may have an extended latency period from the time of exposure to the onset of the disease. Discontinuing the causative medication in question should decrease the clinical manifestations and decrease the overall expense of management.

KEYWORDS: Autoimmune Diseases, Skin, Drug, Captopril.

INTRODUCTION

Pemphigus consists of a unique combination of bullous autoimmune diseases affecting the skin and mucosal membranes. The presence of flaccid blisters and erosions (see Figure 1) while the skin and oral mucosa is a special feature of Pemphigus¹. Drug-induced Pemphigus is precipitated with a series of biochemical interactions and in-advertent stimulation of host B cells resulting in the generation of intracellular IgG antibodies. Those autoantibodies target and attack desmogleins, resulting in Acantholysis (see Figure 2). Between all other factors associated to trigger the disease, drugs resume being the cause behind Pemphigus. Antihypertensive drugs are suspected to trigger Pemphigus, Captopril, an angiotensin-converting enzyme (ACE) inhibitor used to treat hypertension and congestive heart failure, has been reported to induce pemphigus, although less frequently than does penicillamine². Captopril was the first ACE inhibitor available for clinical use. The six other ACE inhibitors in use are enalapril, lisinopril, benazepril hydrochloride, fosinopril sodium, quinapril hydrochloride, and ramipril. Enalapril is a newer ACE inhibitor that lacks a sulfhydryl group but shares a carboxyl-containing C-terminal proline with captopril. Adverse cutaneous reactions are reported in 4 to 7% of patients taking captopril; this rate is more frequent than that with the other ACE inhibitors. The reactions include pruritus, urticaria, maculopapular eruptions, lichen planus pemphigoides with pemphigus-like features, aphthous ulcers, and pemphigus.³

CASE REPORT

A 60 years old male patient got admitted to Male dermatology ward of government general hospital Ongole with chief complaints of fluid filled blisters on upper body, lower limbs scalp and under arms. Blisters are associated with pain. He had a history of hypertension in the last 5 years he had claimed to take Tab.Captopril dose 10 mg once daily for the treatment of hypertension since 5 years. He also claimed to be suffering with the above-mentioned complaints since one and half year and was on medication with (Cyclophosphamide) of dose 100 mg once daily.His family history and social history have nothing in significant. His general examinations included general condition: fair; BP: 130/90 mmHg;

DISCUSSION

The frequency of Pemphigus is rare and has been predictable to be 1 in 100,000 people. Many factors trigger Pemphigus, Whatever what it may be Pemphigus Vulgaris is mostly induced by the using Medications. This

disease influence both Genders are equally. D-Penicillamine, captopril, and penicillin drugs are the most common offending drugs to affect people from the Pemphigus. Drug-induced pemphigus is diagnosed when three criteria are met: circumstantial or definitive sign that the onset of lesions is associated to the intake of a certain drug, Acantholysis of epidermal cells noticed on histopathologic examination, and positive direct immunofluorescence staining of intercellular substance with either IgG or C3 or both as shown in Figure 1⁴. Patients with pemphigus vulgaris have circulating autoantibodies directed toward a 130-kd cell surface keratinocyte protein that is a member of the cadherin family. Pemphigus vulgaris antigen and desmoglein-I share sequence homology and bind to the 85-kd plakoglobin by disulfide bonds. Pemphigus vulgaris antigen is limited to stratified squamous epithelium, whereas desmoglein-I is present in all desmosome-containing tissues. The sole peptide structure of pemphigus vulgaris antigen and desmoglein-I have important inferences in the pathogenesis of drug-induced pemphigus. Patients with drug-induced pemphigus may or may not have circulating intercellular substance autoantibodies. Many drugs have been reported to cause pemphigus; 80% of them are sulfhydryl-containing compounds. The first drug reported to cause pemphigus was penicillamine. Captopril, another sulfhydryl-containing compound, is the next most common drug reported to induce pemphigus. Two theories account for the acantholysis noted in drug induced pemphigus. Acantholysis may be mediated by a direct effect of the drug on the epidermis or indirectly by inducing the immune system to manufacture anti-intercellular substance antibodies. A combination of the two mechanisms is probably involved in most cases of drug-induced pemphigus.

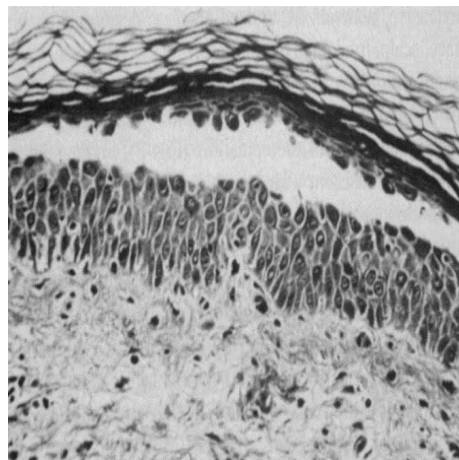


Figure 1. Wide-spread skin blisters and crusted erosions⁴.



Figure 2. Superficial acantholysis of Epidermal cells consistent with pemphigus⁵

CONCLUSION

Finally in this case study we Conclude that previous studies and Literature is strongly support when a patient taking Captopril, we observe pemphigus symptoms on the patient after taking the medication, so be aware from adverse drug reactions as a Trainee Clinical Pharmacist we are educate the patients as well as health care professionals to avoid any Adverse drug reactions after taking the medications.

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SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND ANTIOXIDANT EVALUATION OF SOME COUMARIN-CHALCONE DERIVATIVES

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ABSTRACT

Coumarin and Chalcones derivatives are two different chemical moieties occur from natural as well as synthetic origin, which possesses a wide range of pharmacological activities. Molecular hybridization is the novel drug design technique that combines two or more pharmacophores for the development of new chemical hybrids which can boost up the pharmacological activities. This paper explains the synthesis, spectral characterization, and screening for antimicrobial and antioxidant activity of some new coumarin-chalcone derivatives (DPCU 1-13 and DPCT 1-13). Results indicated that the coumarin-chalcone derivatives synthesized with good yield, and were confirmed by spectral data. Among all synthesized derivatives, DPCU 9 showed potent antibacterial activity, DPCU 13 showed antifungal activity, and DPCT 7 showed potent antioxidant activity.

KEYWORDS: *Coumarin; Chalcone; Coumarin-chalcone; Antimicrobial; Anti-oxidant.*

1. INTRODUCTION

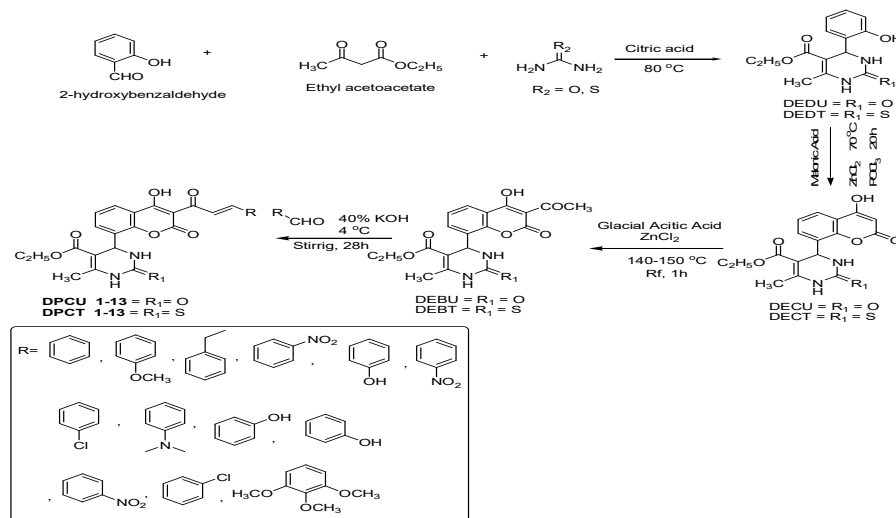
Modern drug design and development utilize molecular hybridization for the production of hybrid molecules which can target multiple sites in biological proteins through chemical bonds. Chemical hybridization includes combining the two or more chemical entities through breakable or unbreakable bonds, to potentiate the combined activity, improve the pharmacological parameters and minimize the individual side effects¹. Coumarins are pharmacologically important compounds obtained from natural sources. The coumarins are the largest class of 1-benzopyran derivatives. They are found mainly in higher plants. Most natural coumarins are oxygenated at C-7; Umbelliferone (7-hydroxycoumarin) being regarded as the structural and biogenetic parent of the more highly oxygenated coumarins². Coumarin and its derivatives can be synthesized by different methods like Pechmann, Knoevenagel, Perkin, Friedel-Crafts, Reformatsky and Wittig reactions.^{3,4} Chalcones are secondary metabolites obtained from the main precursors of flavonoids and isoflavonoids biosynthesis occurred in from terrestrial plants. Chalcones (1,3-diaryl-2-propane-1-ones) one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soya-based foodstuff⁵. Chemically chalcones contain open-chain flavonoid moiety whose basic structure includes two aromatic rings which are bound by three carbons α , β -unsaturated carbonyl system⁶. Chalcones and its derivatives can be synthesized by different methods but, Claisen-Schmidt condensation still holds a high position.⁷ The existing scientific literature describes about the different types of coumarinyl chalcones are designed, screened for antimicrobial activities⁸, anti-oxidant^{9, 10} along with other pharmacological activities. This paper explains the synthesis, characterization, and biological evaluation of some new coumarin-chalcone derivatives for their antimicrobial and antioxidant activity.

2. MATERIALS AND METHODS

The chemicals used for synthesis were purchased from Loba Chemie Pvt. Ltd., Thane, SDFCL, Mumbai, Fisher Scientific, Mumbai, Merck, Mumbai, Avra Synthesis Pvt. Ltd., Hyd. Melting points of synthesized compounds were determined by Dalal Melting point Apparatus using open capillary tubes. Infrared spectra were recorded using the KBr Pellet method. ¹H-NMR and ¹³C-NMR spectra were determined by using a

Bruker-NMR Spectrometer 400 MHz at Laila Impex, Vijayawada. Mass spectra were measured with Shimadzu GCMS QP 5000 mass spectrometer at Laila Impex, Vijayawada. Other chemicals were of commercial grade and used without further purification. The homogeneity of the compounds was checked by thin-layer chromatography (TLC) on TLC silica gel F₂₅₄ aluminum plates (E. Merck, Germany) using chloroform-methanol (3:1) mixture as mobile phase and visualized using iodine vapors. Completion of synthetic reactions and purity of end products of reactions were confirmed by Thin Layer Chromatography on TLC silica gel F₂₅₄ aluminum plates (E. Merck, Germany), using acetone and ethyl acetate as mobile spots are visualized with UV chamber and iodine vapors.

2.1. Scheme



2.2. Procedure for synthesis of Coumarin-Chalcone derivatives (DPCU 1-13 & DPCT 1-13)

Two series of coumarinyl chalcone derivatives (DPCU 1-13, and DPCT 1-13) were designed and synthesised Biginelli synthesis¹¹, Pechmann condensation¹², Acetylation¹³ and Claisen- Schmidt reactions¹⁴ with good yield (54-78%) as described in experimental procedures. Intermediate products DEDU, and DEDT were synthesised by reacting Salicylaldehyde, Ethylacetoacetate, Urea (DEDU) or Thiourea (DEDT) and Citric acid, products DECU, and DECT were synthesised by adding corresponding first step products with malonic acid to preheated mixture of phosphorus oxychloride and anhydrous zinc chloride at 70 °C for 20 h. Final derivatives (DPCU 1-13 and DPCT 1-13) were synthesised by reacting DECU and DECT with different aromatic aldehydes in the presence of Sodium hydroxide. Completion of synthetic reactions and purity of end products of reactions were confirmed by Thin Layer Chromatography on TLC silica gel F254 aluminium plates (E. Merck, Germany), using acetone and ethyl acetate as mobile spots are visualized with UV chamber and iodine vapours.

3. BIOLOGICAL ACTIVITY

3.1. In-vitro Anti-microbial activity

The *in-vitro* antimicrobial assay was performed by Agar well diffusion method. The results were expressed as minimum inhibition concentration (MIC). The MIC in µg/ml of the synthesized compounds was compared MIC of standard drugs Ciprofloxacin and Fluconazole for antibacterial and antifungal activity respectively. The *in-vitro* antimicrobial assay was performed as per procedure described by Bauer, et.al¹⁵ The MIC values were determined for each test compound along with standard. The results of antimicrobial activity were tabulated in Table 1.

3.2. In-vitro Anti-Oxidant activity

The percentage of antioxidant activity (AA%) of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity¹⁶ of samples by reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of 0.5 mM DPPH radical solution in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read Absorbance at 517 nm after 30 min of reaction using a UV-VIS spectrophotometer. The mixture

of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined according to following formula.

$$AA\% = 100 - \left[\frac{(\text{Absorbance sample} - \text{Absorbance blank})}{\text{Absorbance control}} \right] \times 100$$

4. RESULTS AND DISCUSSION

4.1. Chemistry

The title derivatives were synthesized by hybridization of dihydropyrimidinone substituted coumarin with chalcone as shown in Synthetic scheme through aromatic addition, condensation, Acetylation, and Claisen-Schmidt reactions. The final derivatives were recrystallized from suitable solvents and confirmed by IR, NMR, and Mass spectral data given in Table 1.

4.2. Antimicrobial activity

The antibacterial screening data of the two series of compounds (DPCU 1-13, DPCT 1-13) disclosed that the compound DPCU 9, DPUC 4, DPCU 3 had 2-OH, 2-NO₂, 1-C₂H₅, substitutions respectively on B ring of chalcone part showed good and DPCU 1, DPCU 2 had no, 4-OCH₃ substitution respectively on B ring of chalcone part showed moderate antibacterial activity against gram-positive and gram-negative bacteria when compared to Ciprofloxacin as standard. The MIC values of the compounds showed good and moderate antibacterial activity is in the range of 25 - 30 µg/ml and 30-60 µg/ml respectively. The antifungal evolution data reveals that the compound DPCU 13, DPCU 10 had 3, 4, 5 trimethoxy, 3-OH substitutions respectively.

Table 1: Spectral Data of Coumarin-Chalcone derivatives

S. No.	Compound	IR	NMR	Mass
1	DPCU 1	3482(O-H), 3270(N-H), 3069 (C-H Ar), 2952(C-H), 1724(C=O), 1671(C=O), 1600, 1474 (C=C), 1281 (C-O), 760;	¹ H-NMR (400 MHz, DMSO-d ₆ , δ/ppm): 1.41(t, 3H, CH), 1.95(s, 3H, CH), 4.73(q, 2H, CH), 5.21(s, H, CH-Ar), 6.19(s, 2H, NH), 6.93-7.34 (m, 8H, Ar), 7.78(d, 2H, HC=CH), 11.23 (s, H, OH).	475 (M + 1, 9.6), 402 (M+, 36), 273 (M+2, 100), 162 (M+, 49), 132 (27), 74(M+, 16)
2	DPCU 2	3399(O-H), 3249(N-H), 2920 (C-H Ar), 2851 (C-H), 1735(C=O), 1669(C=O), 1601, 1474 (C=C), 1251 (C-O), 831 (P-sub)	1.27(t, 3H, CH), 1.64(s, 3H, CH), 3.54(s, 3H, OH) 4.48(q, 2H, CH), 5.11(s, H, CH-Ar), 6.07(s, 2H, NH), 6.73-7.23 (m, 7H, Ar), 7.56(d, 2H, C=CH), 11.46 (s, H, OH)	505 (M + 1, 10.5), 432 (100), 322 (31), 184 (17), 74 (44)
3	DPCU 3	3473(O-H), 3236(N-H), 2921 (C-H Ar), 2851 (C-H), 1749(C=O), 1675(C=O), 1601, 1474 (C=C), 1282 (C-O), 757	1.62 (t, 3H, CH), 2.57(s, 3H, CH), 4.25(q, 2H, CH), 5.14 (s, H, CH-Ar), 6.12 (s, 2H, NH), 6.65-7.57 (m, 12H, Ar), 9.45 (s, H, OH).	501 (M + 1, 9.2), 428 (100), 318 (27), 184 (38), 74(32).
4	DPCU 4	3348(O-H), 3257(N-H), 2922 (C-H Ar), 2851 (C-H), 1732(C=O), 1668(C=O), 1610, 1474 (C=C), 1257 (C-O), 868 (o-sub)	1.53(t, 3H, CH), 2.11(s, 3H, CH), 4.17 (q, 2H, CH), 5.09 (s, H, CH-Ar), 6.03 (s, 2H, NH), 6.85-8.24 (m, 7H, Ar), 8.43 (d, 2H, CH=CH), 9.83 (s, H, OH).	520 (M + 1, 8), 447 (100), 337 (45), 184 (19), 74 (32)
5	DPCU 5	3368(O-H), 3220(N-H), 2921 (C-H Ar), 2851 (C-H), 1745(C=O), 1674(C=O), 1601, 1474 (C=C), 1283 (C-O), 759 (p-sub)	1.62 (t, 3H, CH), 1.98(s, 3H, CH), 4.02(q, 2H, CH), 4.95 (s, H, CH-Ar), 5.58 (s, H, OH), 6.09 (s, 2H, NH), 6.62-7.24 (m, 7H, Ar), 7.94 (d, 2H, CH=CH), 9.44 (s, H, OH).	491 (M + 1, 9), 398 (28), 308 (100), 184 (53), 94 (19)
6	DPCU 6	3471(O-H), 3156(N-H), 2922 (C-H Ar), 2852 (C-H), 1731(C=O), 1667(C=O), 1611, 1474 (C=C), 1255 (C-O), 758 (p-sub)	1.41 (t, 3H, CH), 2.04 (s, 3H, CH), 4.12 (q, 2H, CH), 4.93 (s, H, CH-Ar), 6.13 (s, 2H, NH), 6.95-8.24 (m, 9H, Ar), 9.38 (s, H, OH).	520 (M + 1, 7), 398 (47), 334 (100), 184 (28), 123 (31).

7	DPCU 7	3341(O-H), 3009(N-H), 2924 (C-H Ar), 2852 (C-H), 1737(C=O), 1671(C=O), 1601, 1475 (C=C), 1284 (C-O), 763 (p-sub)	1.27 (t, 3H, CH), 1.93 (s, 3H, CH), 4.06 (q, 2H, CH), 4.92 (s, H, CH, Ar), 6.10 (s, 2H, NH), 6.90-7.29 (m, 7H, Ar), 7.79 (d, 2H, CH=CH), 9.31 (s, H, OH).	509 (M + 1, 10), 398 (42), 326 (100), 184 (34), 112 (41).
8	DPCU 8	3403(O-H), 3165(N-H), 2923 (C-H Ar), 2854 (C-H), 1727(C=O), 1662(C=O), 1611, 1474 (C=C), 1267 (C-O), 767 (p-sub)	1.42 (t, 3H, CH), 2.21 (s, 3H, CH), 2.94 (s, 6H, CH), 4.14 (q, 2H, CH), 5.02 (s, H, CH), 6.15 (s, 2H, NH), 6.52-7.20 (m, 7H, Ar), 7.75 (d, 2H, CH=CH), 9.26 (s, H, OH).	518 (M + 1, 8), 398 (56), 335 (100), 174 (26), 121 (39).
9	DPCU 9	3428(O-H), 3171(N-H), 2925 (C-H Ar), 2850 (C-H), 1733(C=O), 1670(C=O), 1611, 1474 (C=C), 1281 (C-O), 757 (o-sub)	1.32 (t, 3H, CH), 2.14 (s, 3H, CH), 4.11 (q, 2H, CH), 4.94 (s, H, CH, Ar), 5.14 (s, H, OH), 6.06 (s, 2H, NH), 6.70-7.20 (m, 7H, Ar), 8.12 (d, 2H, CH=CH), 10.21 (s, H, OH).	491 (M + 1, 11), 398 (45), 308 (100), 184 (28), 94 (22)
10	DPCU 10	3420(O-H), 3239(N-H), 2924 (C-H Ar), 2853 (C-H), 1733(C=O), 1672(C=O), 1611, 1474 (C=C), 1282 (C-O), 758 (m-sub)	1.46 (t, 3H, CH), 2.15 (s, 3H, CH), 4.09 (q, 2H, CH), 5.01 (s, H, OH), 5.21 (s, H, CH, Ar), 6.15 (s, 2H, NH), 6.60-7.19 (m, 7H, Ar), 7.70 (d, 2H, CH=CH), 9.52 (s, H, OH).	491 (M + 1, 9), 418 (30), 326 (100), 215 (43), 94 (25)
11	DPCU 11	3344(O-H), 3011(N-H), 2926 (C-H Ar), 2853(C-H), 1736(C=O), 1670(C=O), 1609, 1475 (C=C), 1284 (C-O), 764 (m-sub)	1.52 (t, 3H, CH), 2.14 (s, 3H, CH), 4.13 (q, 2H, CH), 5.11 (s, H, CH, Ar), 6.07 (s, 2H, NH), 6.90-8.32 (m, 9H, Ar), 9.57 (s, H, OH).	520 (M + 1, 8), 398 (31), 337 (100), 184 (57), 123 (38)
12	DPCU 12	3342(O-H), 3011(N-H), 2926 (C-H Ar), 2854 (C-H), 1738(C=O), 1666(C=O), 1602, 1475 (C=C), 1284 (C-O), 765 (o-sub)	1.37 (t, 3H, CH), 1.92 (s, 3H, CH), 4.03 (q, 2H, CH), 5.22 (s, H, CH, Ar), 6.04 (s, 2H, NH), 6.90-7.30 (m, 7H, Ar), 7.98 (d, 2H, CH=CH), 9.62 (s, H, OH).	510 (M + 2, 10), 398 (47), 326 (100), 184 (26), 112 (17)
13	DPCU 13	3400(O-H), 3120(N-H), 2936 (C-H Ar), 2850 (C-H), 1734(C=O), 1675(C=O), 1611, 1474 (C=C), 1284 (C-O), 759 (3,4,5-sub)	1.27 (t, 3H, CH), 2.03 (s, 3H, CH), 3.79 (s, 9H, CH), 4.31 (s, 2H, CH), 6.05 (s, 2H, NH), 6.20-7.15 (m, 5H, aromatic), 7.84 (d, 2H, CH=CH), 9.52 (s, H, OH).	565 (M + 1, 7), 398 (59), 382 (38), 216 (100), 184 (23), 168 (15).
14	DPCT 1	3458(O-H), 3128(N-H), 2929 (C-H Ar), 2863(C-H), 1746(C=O), 1602, 1475(C=C); 1257 (C-O), 765	1.25 (t, 3H, CH), 1.84 (s, 3H, CH), 2.35 (s, 2H, NH), 4.31 (q, 2H, CH), 5.21(s, H, CH-Ar), 6.90-7.38 (m, 8H, Ar), 7.63 (d, 2H, HC=CH), 9.74 (s, H, OH).	491 (M+1, 8), 414 (47), 292 (100), 200 (34), 132 (27), 78(19)
15	DPCT 2	3379(O-H), 3238(N-H), 2932 (C-H Ar), 2875(C-H), 1730(C=O), 1612, 1474 (C=C); 1281 (C-O), 755(p-sub)	1.31 (t, 3H, CH), 1.79 (s, 3H, CH), 2.16 (s, 2H, NH), 3.67 (s, 3H, CH), 4.28 (q, 2H, CH), 5.07 (s, H, CH-Ar), 6.70-7.20 (m, 7H, Ar), 7.70 (d, 2H, HC=CH), 9.54 (s, H, OH).	521 (M+1, 11), 414 (62), 322 (23), 200 (14), 108 (35)
16	DPCT 3	3379(O-H), 3265(N-H), 2935 (C-H Ar), 2881(C-H), 1732(C=O), 1600, 1474 (C=C); 1281 (C-O), 751	1.51 (t, 3H, CH), 1.97(s, 3H, CH), 2.31 (s, 2H, NH), 4.52 (q, 2H, CH), 5.27 (s, H, CH-Ar), 6.85-7.38 (m, 10H, Ar), 7.85 (d, 2H, CH=CH), 9.22 (s, H, OH).	517 (M+1, 10), 440 (61), 318 (52), 200 (47), 78(29)
17	DPCT 4	3347(O-H), 3094(N-H), 2930 (C-H Ar), 2867(C-H), 1735(C=O), 1602, 1475 (C=C); 1257 (C-O), 753(o-sub)	1.27 (t, 3H, CH), 1.93 (s, 3H, CH), 2.48 (s, 2H, NH), 4.26 (q, 2H, CH), 5.12 (s, H, CH-Ar), 6.82-8.18 (m, 7H, Ar), 8.31 (d, 2H, CH=CH), 9.62 (s, H, OH).	536 (M+1, 9), 414 (49), 337 (100), 200 (24), 123 (62)
18	DPCT 5	3342(O-H), 3217(N-H), 2935 (C-H Ar), 2859(C-H), 1739(C=O), 1610, 1473 (C=C); 1282 (C-O), 754(p-sub)	1.27 (t, 3H, CH), 1.78 (s, 3H, CH), 2.06 (s, 2H, NH), 4.16 (q, 2H, CH), 4.82 (s, H, CH-Ar), 5.11 (s, H, OH), 6.55-7.20 (m, 7H, Ar), 7.79 (d, 2H, CH=CH), 9.57 (s, H, OH).	507 (M+1, 12), 414 (56), 308 (100), 200 (39), 94 (23)
19	DPCT 6	3370(O-H), 3281(N-H),	1.39 (t,3H, CH), 1.88 (s, 3H, CH), 2.26	536 (M+1, 10), 414

		2943(C-H Ar), 2870(C-H), 1735(C=O), 1613, 1474 (C=C); 1257 (C-O), 751(p-sub)	(s, 2H, NH), 4.02 (q, 2H, CH), 5.20 (s, H CH-Ar), 6.87-8.20 (m, 9H, Ar), 9.48 (s, H, OH).	(52), 337 (100), 200 (37), 123 (46)
20	DPCT 7	3368(O-H), 3010(N-H), 2938 (C-H Ar), 2841(C-H), 1738(C=O), 1601, 1474 (C=C); 1286 (C-O), 755 (p-sub)	1.06 (t, 3H, CH), 1.72 (s, 3H, CH), 2.39 (s, 2H, NH), 4.37 (q, 2H, CH), 5.41 (s, H, CH, Ar), 6.83-7.37 (m, 7H, Ar), 7.85 (d, 2H, CH=CH), 9.44 (s, H, OH).	525 (M+1, 6), 414 (33), 326 (100), 200 (58), 112 (27)
21	DPCT 8	3400(O-H), 3281(N-H), 3003 (C-H Ar), 2857(C-H), 1739(C=O), 1615, 1475 (C=C); 1281 (C-O), 764 (p-sub)	1.22 (t, 3H, CH), 1.90 (s, 3H, CH), 2.21 (s, 2H, NH), 2.88 (s, 6H, CH), 4.21 (q, 2H, CH), 5.17 (s, H, CH), 6.50-7.28 (m, 7H, Ar), 7.88 (d, 2H, CH=CH), 9.51 (s, H, OH).	534 (M+1, 11), 414 (54), 335 (100), 200 (19), 121 (61)
22	DPCT 9	3426(O-H), 3104(N-H), 2934 (C-H Ar), 2867(C-H), 1737(C=O), 1611, 1474 (C=C); 1287 (C-O), 751(o-sub)	1.24 (t, 3H, CH), 1.81 (s, 3H, CH), 2.18 (s, 2H, NH), 4.22 (q, 2H, CH), 5.08 (s, H, CH, Ar), 5.52 (s, H, OH), 6.55-7.31 (m, 7H, Ar), 8.02 (d, 2H, CH=CH), 10.13 (s, H, OH).	507 (M+1, 7), 414 (59), 308 (100), 200 (20), 94 (35)
23	DPCT 10	3346(O-H), 3219(N-H), 2939 (C-H Ar), 2885(C-H), 1731(C=O), 1611, 1474 (C=C); 1251 (C-O), 757(o-sub)	1.28 (t, 3H, CH), 1.88 (s, 3H, CH), 2.20 (s, 2H, NH), 4.27 (q, 2H, CH), 4.69 (s, H, OH), 5.37 (s, H, CH, Ar), 6.51-7.10 (m, 7H, Ar), 7.86 (d, 2H, CH=CH), 9.69 (s, H, OH).	507 (M+1, 6), 414 (63), 308 (100), 200 (26), 94 (44)
24	DPCT 11	3458(O-H), 3128(N-H), 2929 (C-H Ar), 2863(C-H), 1746(C=O), 1602, 1475 (C=C); 1257 (C-O), 765 (m-sub)	1.31 (t, 3H, CH), 1.69 (s, 3H, CH), 2.10 (s, 2H, NH), 4.22 (q, 2H, CH), 5.25 (s, H, CH, Ar), 6.79-8.30 (m, 9H, Ar), 9.45 (s, H, OH).	536 (M+1, 12), 414 (55), 337 (100), 184 (67), 123 (28)
25	DPCT 12	3369(O-H), 3249(N-H), 2939(C-H Ar), 2865(C-H), 1732(C=O), 1600, 1474 (C=C); 1281 (C-O), 765(m-sub)	1.42 (t, 3H, CH), 1.85 (s, 3H, CH), 2.17 (s, 2H, NH), 4.33 (q, 2H, CH), 5.03 (s, H, CH, Ar), 6.88-7.39 (m, 7H, Ar), 7.63 (d, 2H, CH=CH), 9.41 (s, H, OH)	526 (M+2, 13), 414 (38), 326 (100), 200 (69), 112 (48)
26	DPCT 13	3356(O-H), 3095(N-H), 2935 (C-H Ar), 2861(C-H), 1735(C=O), 1610, 1474 (C=C); 1281 (C-O), 755 (3,4,5-sub)	1.36 (t, 3H, CH), 1.81 (s, 3H, CH), 2.17 (s, 2H, NH), 3.88 (s, 9H, CH), 4.14 (s, 2H, CH), 6.15-7.10 (m, 5H, Ar), 7.68 (d, 2H, CH=CH), 9.72 (s, H, OH).	581 (M+1, 9), 414 (48), 382 (100), 200 (23), 168 (15)

Table 2: MIC values of the synthesized compounds against bacterial and fungal strains

S. No.	Compound	MIC (µg/ml)			S. No.	Compound	MIC (µg/ml)		
		<i>S.aureus</i>	<i>E.coli</i>	<i>A.nigir</i>			<i>S.aureus</i>	<i>E.coli</i>	<i>A.nigir</i>
1	DPCU 1	25	60	32	29	DPCT 1	60	100	50
2	DPCU 2	30	57	30	30	DPCT 2	R	75	75
3	DPCU 3	32	20	25	31	DPCT 3	R	85	R
4	DPCU 4	28	25	25	32	DPCT 4	R	75	R
5	DPCU 5	R	R	R	33	DPCT 5	75	75	R
6	DPCU 6	50	75	28	34	DPCT 6	R	85	R
7	DPCU 7	87	75	30	35	DPCT 7	75	R	R
8	DPCU 8	50	100	25	36	DPCT 8	R	R	R
9	DPCU 9	25	30	25	37	DPCT 9	80	60	R
10	DPCU 10	50	R	20	38	DPCT 10	75	R	R
11	DPCU 11	R	R	R	39	DPCT 11	60	100	100
12	DPCU 12	R	R	R	40	DPCT 12	R	R	R
13	DPCU 13	50	50	20	41	DPCT 13	R	R	100

14	Ciprofloxacin	0.75	0.20	-	28	Flucanazole	-	-	0.50

on B ring of chalcone part showed good antifungal activity with MIC value as 20 µg/ml and compounds DPCU 9, DPCU 8, DPCU 4, and DPCU 3 had 2-OH, 4-dimethyl amino, 2-NO₂, and 1-C₂H₅substitutions respectively on B ring of chalcone part showed moderate antifungal activity with MIC value as 25 µg/ml. the MIC values of the synthesized compounds against bacterial and fungal strains were given in Table 2.

4.3. Anti-oxidant activity

The anti-oxidant activity indicated by the % Scavenging Activity of synthesized compounds (DPCU 1-13, DPCT 1-13) was determined by the DPPH assay method given in Table 3. The compounds DPCT 8, DPCU 2, DPCU 5, DPCT 13 and DPCT 7 showed significant anti-oxidant activity with % scavenging activity in the range of 63-70 %. Especially the compound DPCT 8 which bearing the para dimethyl amino substitution on 'B' ring of chalcone and Sulphur in dihydropyrimidinone moiety showed better ant-oxidant activity among all the compounds.

Table 3: The % Scavenging Activity of synthesized compounds

S. No	Compound Code	Concentration (µg/ml)	% Scavenging Activity*	S. No	Compound Code	Concentration (µg/ml)	% Scavenging Activity*
1	DPCU 1	100	52.70±0.94	14	DPCT 1	100	38.12±0.79
2	DPCU 2	100	69.51±1.03	15	DPCT 2	100	24.11±0.48
3	DPCU 3	100	58.55±0.74	16	DPCT 3	100	12.37±0.75
4	DPCU 4	100	35.88±0.29	17	DPCT 4	100	27.82±0.44
5	DPCU 5	100	26.72±0.59	18	DPCT 5	100	37.59±0.29
6	DPCU 6	100	65.16±1.23	19	DPCT 6	100	30.30±0.36
7	DPCU 7	100	42.94±0.68	20	DPCT 7	100	63.36±0.97
8	DPCU 8	100	10.11±0.55	21	DPCT 8	100	70.12±1.43
9	DPCU 9	100	74.32±0.28	22	DPCT 9	100	25.93±1.05
10	DPCU 10	100	60.66±0.10	23	DPCT 10	100	35.29±0.89
11	DPCU 11	100	17.22±0.82	24	DPCT 11	100	40.33±0.56
12	DPCU 12	100	22.15±1.27	25	DPCT 12	100	20.18±0.74
13	DPCU 13	100	36.63±0.85	26	DPCT 13	100	63.96±1.18

* Mean of three replications

5. CONCLUSION

Two series (DPCU 1-13, DPCT 1-13) of a total of 26 new coumarin-chalcones were synthesized efficiently with good yield; the proposed structures were confirmed by spectral data analysis. The antimicrobial screening of all the synthesized compounds was done by *in-vitro* well diffusion method against gram +ve, gram -ve, and fungal strains, the results concluded that the compound DPCU 9 bearing 2-OH substitution showed potent antibacterial activity with MIC value as 25 and 35 against gram +ve (*Staphylococcus aureus*) and gram -ve (*Escherichia coli*) organisms respectively, and compound DPCU 13 bearing 3,4,5-trimethoxy substitution showed potent antifungal activity with MIC value as 20 µg/ml against *Aspergillus niger*. The anti-oxidant activity screening was carried out by *in-vitro* DPPH assay; results indicated that the compound DPCT 8 showed potent anti-oxidant activity with % scavenging activity as 70.12%. All the conclusions drawn regarding the antimicrobial and antioxidant activity of new synthesized coumarin-chalcone hybrids are helpful for further research in the development of new drugs that exhibit better efficacy and potency with fewer complications.

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AN OVERVIEW ON APPLICATION OF NANO TECHNOLOGY IN ENVIRONMENT AND MEDICAL

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ABSTRACT

“Now a day’s nano technology is most widely used in different fields to make the task of living better. The different outcomes of the nanotechnology like Nano fibers, Nano particles and Nano robots are commonly used. Nano fibers are useful in the purification of the polluted water. Nano fibres are helpful in removal of acids, Heavy metals and minute nano particle wastes from the water and thus it makes the impure water to the safe drinking water. Whereas nano particles is useful in the medical fields to correct disposition of the drug at respective sites and to decrease the systemic side effects of drugs, Nano particles are also used in the treatment of cancer to avoid necrosis of normal healthy cells. Nanorobots are used in diagnostic tests and also to deliver the drug to the appropriate site and identification of the foreign organisms present within the body. So, here the Nanotechnology plays a vital role in further medical and other fields.”

KEYWORDS: *Applications of Nanotechnology, Nanofibers, Nanorobots, nano particles, Nanoinformatics.*

INTRODUCTION

“It is defined as science that involves in research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1 to 100 nm range¹”. Thenanoscience and nanotechnology community has moved toward a more comprehensive integration of nanosciences and nanotechnologies with other emergent technologies and topics of interests such as biotechnology, biomedical engineering, environmental remediation, molecular communication networks, quantum computing, and data-driven design of new materials (materials informatics). Nanoinformatics along with materials science modeling has caught the attention of materials scientists because of several reasons¹: it is aimed at creating comprehensive databases of physical and chemical properties of materials based on which quantitative structure-activity (QSAR) and structure properties (QSPR) relationships might be advanced²; by incorporating optimization methods (Monte Carlo, genetic algorithms, neural networks) and data analytics techniques (data mining, network analysis, k-clustering, machine learning, and deep learning), it helps to optimize quantum mechanical calculations aimed at designing new molecules and materials, and it is permitting to reduce the time of design and translation into applications as well as the cost of new materials². Nanotechnology is used in different fields to improve the cost living better. Nanotechnology is used for the target release of drug, multi drug administration; sustain release of drug and to purify the polluted water. Nanorobots are widely used in diagnosis, Delivery of drug, cleaning of teeth, Treatment of diseases and detection of different organisms within the body. Especially Nanofibers are used in cleaning and in purification of polluted water. Nano particles are the basic structural component of nano medicine which is responsible for the specific target of drug deliver there by it increases the drug efficacy and decrease the side effects due to systemic distribution of drug while administered orally.

APPLICATIONS OF NANOTECHNOLOGY IN MEDICAL FIELD

Nanorobots, Nanoparticles are different types of the nano technological outcomes that are widely used in the medical field.

Nanoparticles

The history of Nanoparticles dates back to 9th century in Mesopotamia. Artisans used these to generate a glittering effect on the surface of pots^{2,3}. These particles are may be prepared by lipid layer or by with the metal ligands which are helpful for the target drug deliver.

APPLICATION OF NANO PARTICLES

- The major applications include Targeted drug delivery.
- Magnetic and gold nanoparticles are useful to detect cancer and bind to the certain tumour part and allows the penetration of the drugs of chemo therapy and radio therapy⁴.
- Nanoparticles are useful in gene probing, Protein detection, vaccines, Biodetection of pathogens and Killing of the bacteria, viruses and cancer cells.
- Nano particles are used in medical field namely MRI (Magnetic Resonance Imaging), Optical Imaging, gene delivery and Neuro degenerative diseases.
- Nano particles are used in the preparation of cosmetics especially sun screen lotions. As the particles are smaller they tend to absorb more UV light.
- Used in the preparation of chips, semiconductors³.

NANO ROBOTS

Nanorobots would constitute any smart structure capable of actuation, sensing, signaling, information, processing, intelligence, manipulation and swarmbehaviour at nanoscale(50-300nm)¹². Carbon is the primary component. These involve in repair or detect damages and infections. They destroy themselves and pass out from excretory system.

FUNCTIONAL REQUIREMENTS

- ✓ A navigational network.
- ✓ This will enable the physician to keep track of the various devices in the body.

APPLICATIONS OF NANOROBOTS

In Dentistry: The growing interest in the future of dental applications of nanotechnology is leading to the emergence of a new field called Nanodentistry. Nanorobots induce oral analgesia, Desensitize tooth; manipulate the tissue to re-align and straighten irregular set of teeth and to improve durability of teeth. Further it is explained that how nanorobots are used to do preventive, restorative, curative procedures. Nanodental techniques involve many tissue engineering procedures for major tooth repair. Mainly nanorobotics manufacture and installation of a biologically autologous whole replacement tooth that includes both mineral and cellular components which leads to complete dentition replacement therapy 16. Nanodentistry has given material that is nanostructured composite material, sapphire which increases tooth durability and appearance. Upper enamel layers are replaced by covalently bonded artificial material such as sapphire. This material has 100 to 200 times the hardness and failure strength than ceramic. Like enamel, sapphire is a somewhat susceptible to acid corrosion. Sapphire has best standard whitening sealant, cosmetic alternative. New restorative nano material to increase tooth durability is Nanocomposites. This is manufactured by nanoagglomerated discrete nanoparticles that are homogeneously distributed in resins or coatings to produce nanocomposites. The nanofiller include an aluminosilicate powder having a mean particle size of about 80nm and a 1:4ratio of alumina to silica. The nanofiller has a refractive index of 1.503, it has superior hardness, modulus of elasticity, translucency, esthetic appeal, excellent color density, high polish and 50% reduction in filling shrinkage. They are superior to conventional composites and blend with a natural tooth structure much better⁸.

Nano Impression

Impression material is available with nanotechnology application. Nanofiller are integrated in the vinylpolysiloxanes, producing a unique addition siloxane impression material. The main advantage of material is it has better flow, improved hydrophilic properties hence fewer voids at margin and better model pouring, enhanced detail precision⁹.

Nano medicine

They are used in the delivery of drugs rationally can be done by the Nanorobots. Nano robots are used in the

delivery of the drug in Asthma, HIV, TB, Diabetic in order to not miss the frequent administration of the drug and also to maintain the constant serum concentration of the drug.

In Cancer Detection and Treatment

Cancer can be successfully treated with current stages of medical technologies and therapy tools. However, a decisive factor to determine the chances for a patient with cancer to survive is: how earlier it was diagnosed; what means, if possible, a cancer should be detected at least before the metastasis has begun¹⁰. Another important aspect to achieve a successful treatment for patients is the development of efficient targeted drug delivery to decrease the side effects from chemotherapy. Considering the properties of nanorobots to navigate as bloodborne devices, they can help on such extremely important aspects of cancer therapy. Nanorobots with embedded chemical biosensors can be used to perform detection of tumour cells in early stages of development inside the patient's body¹¹. Integrated nanosensors can be utilized for such a task in order to find intensity of E-cadherin signals. Therefore a hardware architecture based on nanobioelectronics is described for the application of nanorobots for cancer therapy¹². Analyses and conclusions for the proposed model is obtained through real time 3D simulation¹¹.

In the diagnosis and treatment of Diabetes

Glucose carried through the blood stream is important to maintain the human metabolism working healthfully, and its correct level is a key issue in the diagnosis and treatment of diabetes. Intrinsically related to the glucose molecules, the protein hSGLT3 has an important influence in maintaining proper gastrointestinal cholinergic nerve and skeletal muscle function activities, regulating extracellular glucose concentration¹⁶. The hSGLT3 molecule can serve to define the glucose levels for diabetes patients. The most interesting aspect of this protein is the fact that it serves as a sensor to identify glucose. The simulated nanorobot prototype model has embedded Complementary Metal Oxide semiconductor (CMOS) nanobioelectronics¹⁵. It features a size of 2 micronmeter, which permits it to operate freely inside the body. Whether the nanorobot is invisible or visible for the immune reactions, it has no interference for detecting glucose levels in blood stream. Even with the immune system reaction inside the body, the nanorobot is not attacked by the white blood cells due to biocompatibility. For the glucose monitoring the nanorobot uses embedded chemosensor that involves the modulation of hSGLT3 protein glucosensor activity. Through its onboard chemical sensor, the nanorobot can thus effectively determine if the patient needs to inject insulin or take any further action, such as any medication clinically prescribed. The image of the NCD simulator workspace shows the inside view of a venule blood vessel with grid texture, red blood cells (RBCs) and nanorobots. They flow with the RBCs through the bloodstream detecting the glucose levels¹³. At a typical glucose concentration, the nanorobots try to keep the glucose levels ranging around 130 mg/dl as a target for the Blood Glucose Levels (BGLs). A variation of 30mg/dl can be adopted as a displacement range, though this can be changed based on medical prescriptions. In the medical nanorobot architecture, the significant measured data can be then transferred automatically through the RF signals to the mobile phone carried by the patient. At any time, if the glucose achieves critical levels, the nanorobot emits an alarm through the mobile phone¹⁴.

In Surgery

Surgical nanorobots could be introduced into the body through the vascular system or at the ends of catheters into various vessels and other cavities in the human body. A surgical nanorobot, programmed or guided by a human surgeon, could act as a semiautonomous on-site surgeon inside the human body¹¹. Such a device could perform various functions such as searching for pathology and then diagnosing and correcting lesions by nanomanipulation, coordinated by an on-board computer while maintaining contact with the supervising surgeon via coded ultrasound signals¹⁷. The earliest forms of cellular nanosurgery are already being explored today. For example, a rapidly vibrating (100 Hz) micropipette with a <1 micron tip diameter has been used to completely cut dendrites from single neurons without damaging cell viability. Axotomy of roundworm neurons was performed by femtosecond laser surgery, after which the axons functionally regenerated. A femtolaser acts like a pair of "nano-scissors" by vaporizing tissue locally while leaving adjacent tissue unharmed¹⁸.

Diagnosis and Testing

Nanorobots can perform a wide variety of diagnostic, testing and monitoring functions, in the body. These devices periodically record all vital signs from different regions of the body.

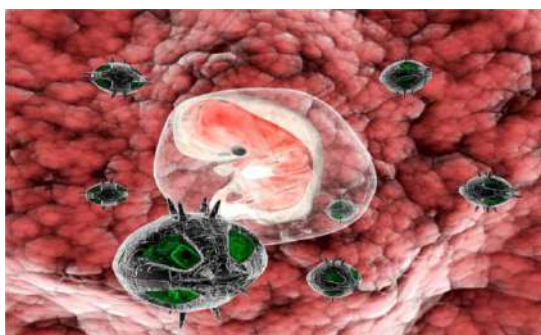


Figure 3:- nano robots in foetal surgery

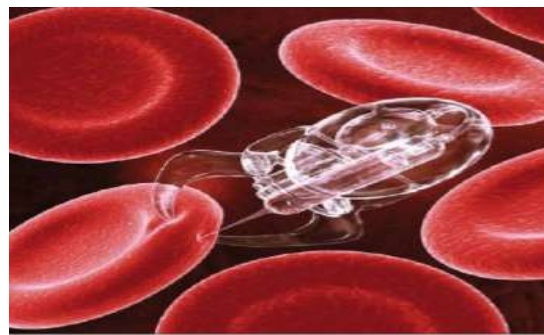


Figure 4:- Nano robots extracting sample

Nanorobots swallowed by a patient for diagnostic purposes approach the surface of the stomach lining to begin their search for signs of infection¹¹.

Atherosclerotic treatment

Devices working in blood stream could nibble away at athero-sclerotic deposits, widening the affected blood vessels. It prevents from most of the heart attacks.

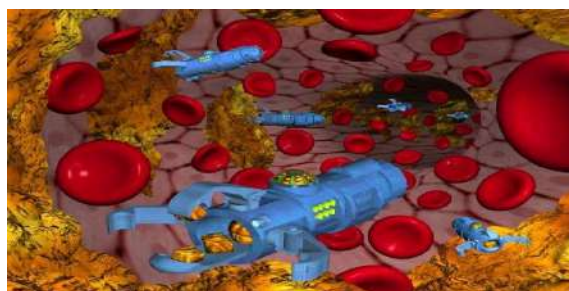


Figure 5:- Nano robot clearing the depositions in blood vessel.

In Gene Therapy

Medical nanorobots can readily treat genetic diseases by comparing the molecular structures of both DNA and proteins found in the cell to known or desired reference structures. Any irregularities can then be corrected, or desired modifications can be edited in place. In some cases, chromosomal replacement therapy is more efficient than in cytotrepair. Floating inside the nucleus of a human cell, an assembler-built repair vessel performs some genetic maintenance. Stretching a supercoil of DNA between its lower pair of robot arms, the nanomachine gently pulls the unwound strand through an opening in its prow for analysis. Upper arms, meanwhile, detach regulatory proteins from the chain and place them in an intake port¹⁹. The molecular structures of both DNA and proteins are compared to information stored in the database of a larger nanocomputer positioned outside the nucleus and connected to the cell-repair ship by a communications link²⁰. Irregularities found in either structure are corrected and the proteins reattached to the DNA chain, which re-coils into its original form. With a diameter of only 50 nanometers, the repair vessel would be smaller than most bacteria and viruses, yet capable of therapies and cures well beyond the reach of present-day physicians. With trillions of these machines coursing through a patient's bloodstream, "internal medicine" would take on new significance. Disease would be attacked at the molecular level, and such maladies as cancer, viral infections and arteriosclerosis could be wiped out²¹.

APPLICATION OF NANOTECHNOLOGY IN ENVIRONMENT

Water is the daily requirement of the life. Water is polluted by the effluents discharged from various industries and some of the pollutants are acids, dyes, heavy metals and nano particles. The polluted water bodies lead to the lack of safe drinking water²². Hence an efficient methods are practiced in removing these waste from the body of them usage of Nanofibers made the removal of different types of wastes easily. This

nanofibre diameter is within 100nm. Nanotechnology has great potential in filtration applications due to its capability to create precise structural controlled materials for such requirements. ENMs (Electrospun Nanofibrous membranes) are an innovative membrane technology that offer substantial high flux and high rejection rate compared to conventional membrane. The electrospinning process fabricates electrospun nanofibrous membranes with manageable whole dimension in the scale of micro and nano, which may substitute for the classical membrane through lesser schemes that function at minimum pressure. ENMs present an innovation in water and wastewater purification by offering a lightweight, cost effective, and less energy consuming process than conventional membranes.²⁸ ENMs possess high porosity and high surface area to volume ratio. Their porosity is generally around 80%, whereas conventional membranes have about 5–35%. The inter-connecting structure and maximum permeability of ENMs permit permeabilities much higher than their conventional counterparts. The important features of ENMs are pore size, porosity, and fiber diameter control flux, removal rate, and efficiency of ENMs.²⁷

CONCLUSION

The applications of nanotechnology in medical and environment helps to improve the comfort of living. Nanotechnology will play vital role in the future. Especially in medical field it is useful for the better efficacy in therapy, minimize the side effects, low trauma surgery can be also done and in cancer treatment nanotechnology provides less side effect therapy. So, in future we can expect the nano technological applications and appliances will become a routine and a part of our daily needs. This technology applications are now present in the developed countries like UK, America etc ... And we can hope of application of this technology in developing countries may also be happen in next two decades. And when comes to application of the Nanofibers make easier and effective purification of the water by removing the finest plastic particles from water and also useful for the removal of the microbes to some extent. These application of nano technology develop may increase in future.

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CURRENT ADVANCES IN BRAIN TARGETING MULTIPARTICULATE SYSTEM

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ABSTRACT

The brain is unique of the smallest accessible organs of the body, consequently creation the delivery of neurotherapeutics virtually a trial. Despite its reasonably high nutrient sustenance and interchange desires, the acceptance of any compound is austere regulated by the blood brain barrier (BBB). As a moment, BBB averts effective treatment of various austere and life menacing diseases like brain cancer, epilepsy, Alzheimer's disease, schizophrenia etc. Frequent drug delivery approaches have been advanced to circumvent this barrier. Unique such approach is the use of nanoparticles. Nanoparticles form solid, colloidal drug delivery system that entails of macromolecular constituents in which the active principle is dissolved, entrapped or encapsulated or onto which the active principle is adsorbed or involved. Brain targeted polymeric nanoparticles have been institute to upsurge the therapeutic efficacy and diminish the toxicity for a large number of drugs. By coating the nanoparticles with surfactants, advanced concentrations of drugs can be delivered to the brain. The manuscript presents innumerable approaches used in design and delivery of nanoparticles to brain. It also assessments various patents that designate the use of nanoparticles to deliver various neurotherapeutics and neurodiagnostics to brain.

KEYWORDS: *Nanoparticles, blood brain barrier, neuropharmaceuticals, targeting.*

1. INTRODUCTION

Drug targeting to specific organs and tissues has become one of the critical endeavors of the new century. The search for new drug delivery approaches and new modes of action represent one of the frontier areas which involves a multidisciplinary scientific approach to provide major advances in improving therapeutic index and bioavailability at site specific-delivery¹. The global drug development for brain diseases has to grow rapidly in the next 20 years as the populations of seniors and patients with CNS disorders are increasing. However, drug development for brain diseases has the poorest success rates compared to other therapeutic areas. The time for developing Central Nervous System (CNS) drugs is normally much longer than for non-CNS drugs². Only small molecules that are lipid soluble and also have a molecular weight <400 Da can cross the Blood Brain Barrier (BBB); most macromolecules cannot penetrate the brain endothelium.

1.1 Barriers to CNS drug delivery

The drug delivery to the brain for the successful treatment of the brain disease is very challenging because of its barriers present in it. The most limiting factor to successful treatment of brain disease is the BBB which prevents access of approx. 98% of current pharmaceutical agents to the brain when delivered intravenously³.

1.1.1 Blood Brain Barrier (BBB)

BBB shows close proximity of paracrine type of interactions between cerebral endothelial cells, pericytes, astrocytes, microglia and neurons. Anatomically, BBB is a continuous zipper-like tight junction of endothelial cellular layer. The microvascular endothelium shares a common basement membrane with astrocytes and pericytes. Astrocytes give out their foot processes to invest more than 90% of endothelial capillaries.

1.1.2 Blood cerebrospinal fluid barrier

The second barrier, located at the choroid plexus, is represented by the blood-cerebrospinal fluid barrier that separates the blood from the cerebrospinal fluid (CSF) which, in turn, runs in the subarachnoid space surrounding the brain⁴. The choroid plexus and the arachnoid membrane act together at the barriers between

the blood and CSF. The arachnoid membrane is generally impermeable to hydrophilic substances, and its role is formation of the Blood-CSF barrier, is largely passive.

1.2 General Transport Mechanisms across BBB

These include paracellular aqueous pathway, transcellular lipophilic pathway, transport protein pathway, receptor mediated transcytosis, and adsorptive transcytosis. Paracellular aqueous pathway is a rare pathway across BBB through which small water-soluble molecules diffuse into the brain. Lipophilic molecules such as alcohol, steroid hormones etc. In receptor-mediated transcytosis type of transport mechanism is for the selective uptake of macromolecules. These systems include receptors for transferrin, insulin, lipoprotein etc. and those are also explored for the ligand based formulations. In carrier-mediated transport, a protein transporter binds to glucose or amino acids which triggers a conformational change in the protein and helps in the transport of the molecule to the other side. Another kind of transport mechanism, called adsorptive-mediated transcytosis is triggered by an electrostatic interaction between a positively charged substance such as charged moiety of a peptide, and the negatively charged plasma membrane surface such as heparin sulphate proteoglycans⁵.

1.3 Factors that affect the drug transport across BBB

- ❖ Concentration gradient of drug/polymer
- ❖ Molecular weight of the drug
- ❖ Lipophilicity of the drug
- ❖ Pathological status
- ❖ Affinity for efflux proteins
- ❖ Molecular charge
- ❖ Cerebral blood flow
- ❖ Clearance rate of drug/polymer
- ❖ Cellular enzymatic stability⁴

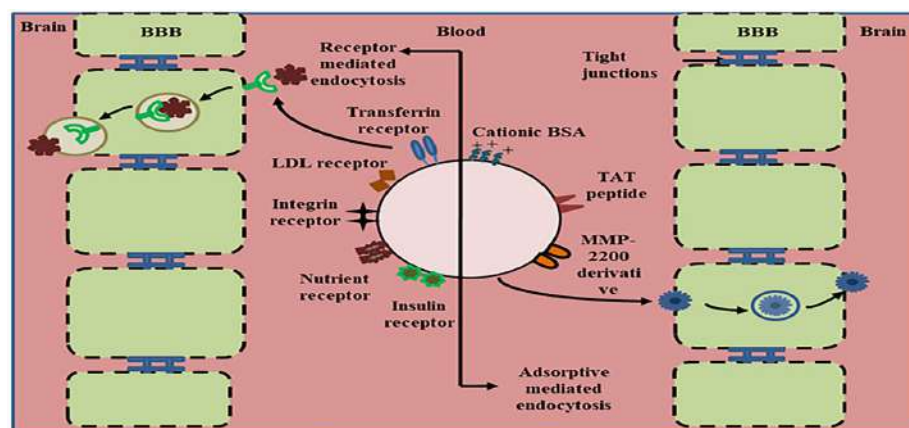


Figure 1: General transport mechanisms across blood-brain barrier

1.4 Drug delivery strategies for brain targeting

Various drug delivery strategies to disrupt or overcome the BBB and potentiate the transport of drug molecules across this barrier to the CNS have been studied. These strategies are divided into three main categories; invasive and non-invasive strategies and recent techniques for BBB disruption.

1.4.1 Invasive strategies

Temporary physiological disruption of endothelial integrity of the brain is one of the invasive strategies for drug delivery to the brain.

1.4.1.1 Chemical disruption of BBB

Osmotic disruption of BBB is one of the invasive techniques involving temporarily shrinkage of endothelial cells, opening of tight junctions and leakage of drug to the CNS. On injecting intracarotid hypotonic solution of mannitol, tight junctions were opened and subsequently promoted the delivery of chemotherapeutic agents to the brain. Osmotic disruption enhanced 2.5–7.6 fold transport of methotrexate, aminoisobutyric

acid and dextran 70 to CNS⁶⁻⁸. Vasoactive agents such as bradykinin and histamine disturb the BBB and improve the transportation of drugs to CNS. Purpose mechanisms involved in BBB opening effects of bradykinin are the activation of B2 receptors, leakage of endothelial cells based on modulation of caveolin-1 and caveolin-2 and permeability enhancement of brain tumor microvessels via (KATP) channels⁹. Osmotic disruption seems to be most successful in treating primary non-AIDS CNS lymphoma. The chemical induction of fenestrated capillaries is attributed to the production of the plasminogen activator urokinase, and is completely reversed 1-2 months after delivery of retinoic acid or PMA is stopped. Osmotic disruption also has been tested as a strategy for the delivery of macromolecular drugs such as monoclonal antibodies, nanoparticles and viruses.

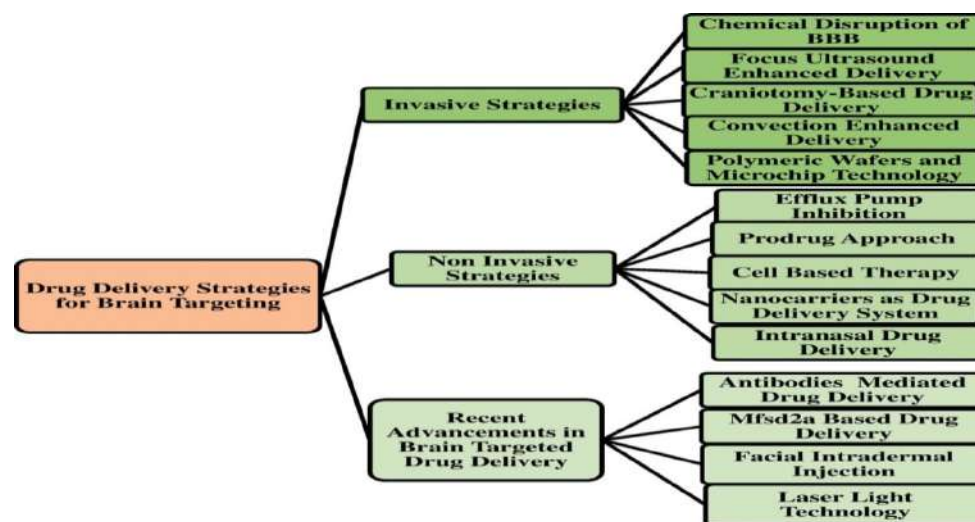


Figure 2: Schematic presentation of drug delivery strategies for brain targeting

1.4.1.2 Focus ultrasound enhanced delivery

Ultrasound based drug delivery utilized microbubbles (MBs) as a contrast agent¹⁰. These bubbles were administered systemically and worked on acoustic energy principle to exert pressure on endothelial cells and open the tight junctions, resulted in increased permeability of BBB and improved delivery of drug to the brain. MBs have diameter of 1–10 μm and are made of semi rigid lipid and albumin shells encapsulated with perfluorocarbon. These MBs operate in collaboration with low intensity Focus Ultrasound (FUS) and this combined system is called MB facilitated FUS. High-Intensity Focused Ultrasound (HIFU) is effectively applied for reversible disturbance of BBB and promotion of drug distribution to the brain in a précised and controlled way without toxicity to brain parenchyma tissues. This technique is valuable for tumor targeted delivery of drugs, genes and antibodies¹¹. Interstitial fluid pressure limited the distribution of NPs to the tumor. HFU increased the permeability of endothelial cells and NPs were moved from leaky endothelial cells to the tumor microenvironment resulting in improved distribution of anticancer agents to the tumor targeted area.

1.4.1.3 Craniotomy-based drug delivery

Craniotomy-based drug delivery is the direct way of targeting the specific part of the brain without exposure to peripheral organs via Intracerebral or intraventricular injection. Intraventricular system directly delivers the drug to the ventricles and subarachnoidal part of the brain and is suitable for therapy of meningioma and metastatic cells of CSF. Intracerebral system directly injects or infuses the drug into brain parenchyma through catheter and controlled devices maintain the delivery¹².

1.4.1.4 Convection-enhanced delivery (CED)

CED is a novel approach for the drugs which are used for the brain tumors. In this method the dug is infused via intra tumoral or intra parenchymal catheters continuously. A continuous infusion pressure gradient over hours to days results in distribution of therapeutic agents into the interstitial space. CED is a promising approach for the delivery of various agents including conjugates, monoclonal antibodies, antisense oligo-

nucleotides or viral vectors CED has also been applied in antibody-mediated therapies and immunotherapies with acceptable toxicities yet highly variable efficacy¹³.

1.4.1.5 Polymeric wafers and microchip technology

Wafers based on polyanhydride were implanted in tumor resection area, crossed the BBB, gradually released and distributed the drug into the brain and targeted site. Circumventing the BBB and providing the controlled release of drug at intracranial tumor using polymer devices, is a big achievement in the field of polymer nanotechnology¹⁴. Programmable microchips are intracranial devices implanted to control the release of drug at targeted site. Two types of chips are micro electro mechanical systems (MEMS) and the passive chip. Single and multiple doses could be delivered through chip technology¹⁵.

1.4.2 Non-invasive strategies

Noninvasive approaches exploit the endogenous mechanisms for transport of drug across the BBB. These strategies include prodrug approach, chemical modulation of BBB, efflux pump inhibition, alternative route of administration and nanocarriers based drug delivery to the brain.

1.4.2.1 Efflux pump inhibition

Efflux by the active P-glycoprotein (P-gp) presents on the apical membrane of the endothelial cells of BBB. P-gp has more association with lipophilic and cationic drugs. Within the brain capillary endothelium, it is assumed that Pgp is selectively localized at the luminal membrane, although the definitive immunogold electron-microscopic studies for this transporter have yet to be performed for brain¹⁶.

1.4.2.2 Prodrug approach

Prodrug approach provides an effective tool in solving drug formulation and delivery problems, such as poor aqueous solubility, chemical instability, insufficient oral absorption, rapid presystemic metabolism, inadequate brain penetration, toxicity and local irritation also be used to improve targeting of drug action¹⁷. Prodrug approach has been successfully utilized for delivery of neurotherapeutics to treat neurological disorders.

1.4.2.3 Cell based therapy

This therapy involves macrophages and many types of stem cells as carriers for delivery to brain. Macrophages are migrated to brain through paracellular and transcellular transport mechanisms. They have natural ability of phagocytosis, enables them to enter brain as Trojan horses¹⁸. Macrophages are suitable candidates for targeted delivery of Nano Particles (NPs) and diagnostic and imaging agents to the brain tumor and neurodegenerative diseases. Stem cells could be used as vector for delivery of cytokines, oncolytic viruses, and suicide genes to brain¹⁹.

1.4.2.4 Nanocarriers as drug delivery system

Nanotechnology offers the possibility to deliver small molecules/drugs against CNS disorders across BBB. Nanoscale entities comprises of a vast range of molecules among which nanomedicines includes a diverse range of polymers, peptides and liposomes, which are encapsulated or conjugated with therapeutic drugs. The strategic developments of more potent drug delivery systems to CNS via endocytic pathways appears to be the most promising approach. Endocytic pathways are essential for communication and transport of nutrients across BBB in endothelial cells²⁰. Liposomal delivery of cerebral ischemic agents, opioid peptides and antitumor agents has proved therapeutic importance of these nanocarriers for brain targeting delivery. Cationic liposomes are positively charged vesicles bind with negatively charged endothelium and delivered through adsorptive-mediated endocytosis or phagocytosis²¹. The specific targeting ligands, attached to the NPs, assists drug entry into the brain parenchyma via receptor mediated transcytosis. Essentially, targeting ligands employed for this purpose should possess their specific receptors, in abundance, on the brain capillary endothelium.

1.4.2.5 Intranasal drug delivery

Drug absorption through nasal respiratory epithelium follows transcellular and paracellular absorption, carrier-mediated transport, and absorption through transcytosis mechanism. Nasal drug delivery to the brain posed a big challenge of BBB-mediated restriction²². Administration of drug deep into the nasal cavity

approached nasal mucosa, led to direct transmission of drug into brain via olfactory pathway. Olfactory pathway consists of olfactory neurons that carry drugs from olfactory mucosa to the brain and is slow process of drug transmission. Olfactory epithelium pathway is faster way of drug transportation. Drug is passed through olfactory epithelium via paracellular mechanism into perineural space and transferred directly to the brain. Intranasal route of transportation directly delivers the drugs to brain without systemic absorption, thus avoiding the side effects and enhancing the efficacy of neurotherapeutics²³.

1.4.3 Recent advancements in brain targeted drug delivery

1.4.3.1 Antibodies mediated drug delivery

Monoclonal Antibodies (mAbs) are much large in size and unlike small molecules, unable to cross BBB and approach target sites in the brain. The immunogenicity of the mAbs was eliminated with genetic engineering and formation of a chimeric Human Insulin receptors (HIR) MAb, the chimeric HIRmAbs retained 100% affinity for the HIR following genetic engineering based on both western blot analysis and quantitative ELISA. Alzheimer's disease is accompanied by the abnormal folding and deposition of amyloid beta (A β) peptide and tau protein in the brain. Bispecific antibody (bsAb) is newly designed Ab with two different binding specificities. bsAbs are introduced for chemotherapy with one binding specificity targets the tumor cell and other targets the antigen on immune cells²⁴. Lipocalin-2 (LCN2) is a lipocalin protein secreted by the astrocytes and microglia during brain hemorrhage, ischemic stroke and brain injury. Astrocytes and microglia secreted LCN2 facilitates the inflammation during brain injuries²⁵.

1.4.3.2 Mfsd2a-based drug delivery strategy

Mfsd2a presents on the endothelial cells surface in the BBB, prevents the transmission of molecules through transcytosis and facilitates the transport of specific lysophosphatidyl-choline (LPC) derivatives. Mfsd2a works on principle inhibition of Mfsd2a promotes the transcytosis in endothelium and also by LPC mediated delivery resembling the glucose transporter (GluT1) and L-type amino acid transporter (LAT1) based transport²⁶.

1.4.3.3 Facial intradermal injection

Facial intradermal injection overcomes the BBB through trigeminal neural connection. Trigeminal pathway consists of vasculature, perineurium and epineurium interlinks facial skin with brain. This pathway is involved in facial intradermal targeting of brain. Facial dermal delivery explored epineurium, perineurium, neurons and Schwann cells for brain targeting²⁷.

1.4.3.4 Laser light based technology

Laser light causes defects in membrane of endothelial cells and endothelial cells become leaky, allow transport of therapeutic agents to parenchymal tissues. Photodynamic therapy (PDT) with using 5-aminolevulinic acid (5-ALA)-PDT is a valuable method for surgical navigation of glioblastoma. PDT is a form of therapy that combines a light source and nontoxic photosensitizing agents. The systematically or topically administered photosensitizer is specially accumulated in diseased tissue²⁸.

1.4.4 Miscellaneous

Overall, the idea of using an appropriate drug carrier to delivery across the BBB is reinforced. Nano-vectoring with tissue-specific targets is an ideal pathway since it delivers both hydro- and lipophilic drugs, as well as macromolecules such as peptides and genes through a controlled release profile over an extended period of time²⁹. The use of the specific peptides for targeting the receptor-mediated transcytosis across BBB can be a successful strategy for improving drug delivery to the brain. In this way, promising results have been achieved by directly delivering drugs to the brain interstitium through the design of polymer-based drug delivery systems. Another approach to Lipoprotein receptor-related proteins (LRP) receptor is by the use of a series of peptides called "angiopep" at the surface of nanoparticles which have shown specific targeting of the LRP³⁰. The most used is angiopep-2 as it shows enhanced transcytosis across the brain and it has been effective against glioblastoma. Other receptors that have been proposed to targeting are insulin, albumin, transferrin, lactoferrin and more recently, the glutathione receptor. In the last case, liposomes were coated with glutathione-conjugated PEG and they successfully delivered free drug doxorubicin or ribavirin. Scaffolds are implantable and can be used to treat a variety of conditions associated with brain injury and

diseases, for delivering drugs to treat neurological diseases such as Parkinson's disease and Alzheimer's disease. Delivery of therapeutic agents from scaffolds potentially helps to limit the damage to neurons while helping to preserve their function³¹. Viral vectors have a natural ability to infect cells with nucleic acids. Lentivirus, herpes simplex virus, Adenovirus and Adeno-Associated Virus (AAV) vectors have achieved gene transduction in the brain. AAV vector is a prominent vector used in current clinical trials of gene therapy for brain diseases³². Exosomes, a natural product of the body's own cells, can be tailored to cross the BBB, thus improving drug transport to the brain by decreasing mononuclear phagocyte system (MPS) drug clearance. These deliver small molecule drugs across the BBB to treat both brain cancers and neurological disorders.³³

2. CONCLUSION AND FUTURE PERSPECTIVES

This review has covered recent strategies to deliver drugs to the brain in the past years. To design effective drug delivery systems for brain diseases, detailed understanding of BBB disruption is necessary. Recent developments have done in drug delivery strategies which have been proven to be helpful to overcome barriers associated with brain drug delivery. Invasive strategies, non-invasive approaches were designed to treat life threatening brain disorders. P-gp efflux prohibits lipophilic and low molecular weight drugs to enter the brain. Prodrug approach is based on reducing the number of polar groups of hydrophobic drug or linking it with lipophilic moiety. Additionally, extent of impairment caused to the brain via nano-drugs should be another critical parameter while designing future drugs for crossing BBB.

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VALIDATED RP-HPLC METHOD DEVELOPMENT AND FORCED DEGRADATION STUDIES FOR THE QUANTITATION OF DOLUTEGRAVIR IN BULK AND TABLETS

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ABSTRACT

The present work was aimed on the development and validation of RP-HPLC method for the quantitation of Dolutegravir in bulk and tablets. Dolutegravir was eluted at 2.915 ± 0.1 mins with a run time of 6 mins. The developed analytical method was validated in accordance to ICH guidelines (ICH, Q2 (R1)). The linearity of the method was demonstrated in the concentration range of 2.5-12.5 µg/ml for Dolutegravir with a correlation coefficient (r^2) of 0.999, %recovery was found to be 99.2% to 99.57% and % RSD for intra-day and inter-day precision was less than 2. LOD and LOQ values were found to be 0.013 µg/ml and 0.039 µg/ml respectively, and assay of marketed formulation was found to be 99.28%. The developed RP-HPLC method was found to be simple, specific, sensitive, rapid, linear, accurate, precise and economical for the estimation of Dolutegravir in bulk and pharmaceutical formulation. So, it can be used for the routine quality control of Dolutegravir in bulk and pharmaceutical formulation.

KEY WORDS: *Dolutegravir, RP-HPLC, Waters symmetry C18 column.*

INTRODUCTION

Dolutegravir, a HIV-1 integrase inhibitor is used in the treatment of human-immunodeficiency virus. It is indicated in combination with other anti-retroviral agents for the treatment of HIV infection in adults and children with body weight of at least 30 kg. Dolutegravir increases the number of immune cells which helps fight infections in the body¹. Chemically it is 3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.0^{3,8}]tetradeca-10,13-diene-13-carboxamide with a molecular formula of $C_{20}H_{19}F_2N_3O_5$ and a molecular weight of 419.3 gm. The structure of Dolutegravir was shown in fig.1. Literature survey revealed that only one HPLC method was reported for the estimation of Dolutegravir in bulk and formulation²⁻⁷. So, an attempt was made to develop RP-HPLC method which is simple, rapid, accurate, precise, specific, economical and sensitive stability indicating for the estimation of Dolutegravir in bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals

Reference standard of Dolutegravir was obtained as gift sample from Hetero labs, Hyderabad, Telangana. Dolutegravir available as tablets under the trade name Doluvir (50mg) was procured from the local pharmacy. Ortho-phosphoric acid, HPLC grade Water, Acetonitrile and methanol were procured from Rankem chemicals limited.

Chromatographic conditions

WATERS HPLC system with auto-sampler and PDA-detector with empower-2 software was used. The optimized chromatographic conditions were shown in table: 1. Acetonitrile and water in the ratio of 1:1 was used as diluent.

Preparation of Standard stock solution

Dolutegravir standard (1000 μ g/ml) [stock-1] was prepared. Accurately 10mg was transferred to 10ml volumetric flask, 5ml of diluent was added, sonicated and made up to the mark with diluent, filtered through 0.45 μ m HV millipore membrane filter. Stock solution-2 was prepared by diluting suitable volume of stock-1 to 10ml using diluent. (100 μ g/ml)

Preparation of the sample stock solution

20 tablets were weighed and the average weight of tablet was calculated. The tablets were finely powdered and tablet powder equivalent to 10 mg of dolutegravir was weighed accurately. Stock solution-1 1000 μ g/ml and stock solution-2 100 μ g/ml were prepared similar to standard stock solution.

Preparation of standard and sample working solution

10 μ g/ml of standard and sample working solutions were prepared from the stock solution-II using the same diluent. 10 μ l of standard and sample working solutions were injected into the chromatographic system and chromatograms were recorded.

Method Validation

The developed method was validated in accordance with ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, Limit of Detection, Limit of Quantitation and robustness.⁶

Accuracy

Accuracy of the developed method was established in terms of recovery studies using three different levels 50%, 100% and 150% of the target sample concentration. Three injections of each level were injected and individual % recovery and mean % recovery values were calculated.

Precision

Precision of the developed method was established by performing intra-day and inter-day precision by injecting 10 μ g/ml concentration of sample solution at six different times on the same day and on three different days. The results of precision were expressed in terms of %RSD.

Linearity

Linearity of the developed method was established at a concentration range of 2.5-12.5 μ g/ml. Calibration curve of peak area versus concentration was plotted and the correlation coefficient was calculated.

Specificity

Specificity of the developed method was established by comparing the chromatograms of blank, placebo, standard and sample. It was found that there was no interference due to excipients and impurities at the retention time of the drug.

Limit of Detection and Limit of Quantitation

LOD and LOQ values were calculated using the formulae based on the standard deviation of the response and slope of the calibration curve. The LOD and LOQ for Dolutegravir were found to be 0.013 μ g/ml and 0.039 μ g/ml respectively.

Robustness

Robustness was demonstrated by making small intended changes in the optimized chromatographic conditions. Parameters like flow rate ($\pm$ 0.1ml/min), organic composition ($\pm$ 5ml) and temperature ($\pm$ 5 $^{\circ}$ C) were changed. Six replicate injections of each condition were injected and %RSD of peak area was calculated.

Stability of the solution

Stability of both the standard and sample solutions was checked up to 24 hours at room temperature.

Forced degradation studies

The concentration of stressors was selected on the basis of literature review. 1 ml of stock solution-2 of Dolutegravir was subjected to different stress conditions such as Acidhydrolysis, alkaline hydrolysis, Oxidative hydrolysis, dry heat degradation, photostability studies and neutral degradation studies. For HPLC study, the resulting solution was diluted to obtain 10 µg/ml solution. 10 µl solution of each stressed condition was injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

RP-HPLC method was developed for the estimation of Dolutegravir in bulk and tablet formulation. Detection wavelength was selected by scanning the standard concentration in the wavelength range of 200-400 nm, and λ_{max} was found to be 257 nm. Diluent was selected on the basis of solubility of the drug. Acetonitrile and water in the ratio of 1:1 was used as diluent. The method was developed employing Waters symmetry C18 column 150 x 4.6 mm, 5 µm particle size with 0.1% Ortho-phosphoric acid buffer and Acetonitrile in the ratio of 45:55 v/v as mobile phase at a column temperature of 30°C. Mobile phase was forced at a flow rate of 1.0 ml/min. The drug Dolutegravir was detected at a wavelength of 257 nm. 10 µl solution was injected into the chromatographic system. The total runtime was 5 mins with a retention time of 2.617 ± 0.1 mins. The chromatograms of the standard and sample were shown in fig.3 & 4. Suitability of the system was demonstrated by assessing various parameters from six replicate injections of the standard solution. Theoretical plates were found to be 5843, tailing factor of 1.19 and %RSD of peak area was 0.5. All the system suitability parameters were well within the limits, indicating the system was well suitable for performing the analysis. The developed method was validated. Accuracy was computed by recovery studies. The mean %recovery values for three levels were found to be 99.29, 99.5 and 99.57% were within the limits indicating the method developed was accurate. Calibration curve of peak area versus concentration for Dolutegravir was constructed [fig.2]. The linear regression equation obtained was $y = 62913x + 5260$ and a correlation coefficient of 0.999 indicating the good linear relationship between the concentration and peak area. The method was found to be linear in the concentration range of 2.5-12.5 µg/ml. The calculated LOD and LOQ values for the method were 0.013 µg/ml and 0.039 µg/ml (< 0.1 µg/ml) indicating the method was highly sensitive. The retention time of the standard and sample confirm the drug present in tablet formulation was Dolutegravir. Robustness of the method was designed by changing the optimized conditions adequately. On assessment of the results it can be deduced that the variation in the composition of mobile phase, flow rate and temperature does not affect the method significantly. %RSD of less than 2% specifies that the developed method was robust. The stability of the drug solutions was observed for 24 hours. %RSD of 0.91 indicates the stability of the method for 24 hours. In degradation studies drug was exposed to various stress conditions. From the chromatograms of stressed samples, it was found that no interference from degradants was observed at the retention time of Dolutegravir. The summary of results was tabulated in table-2.

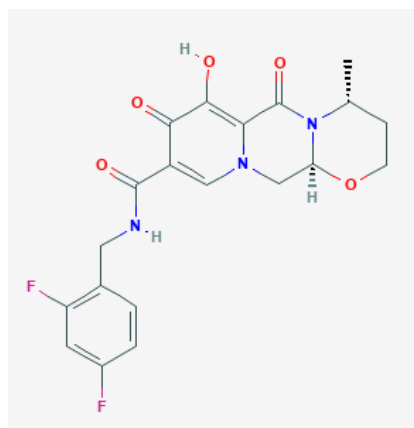


Figure 1. Dolutegravir

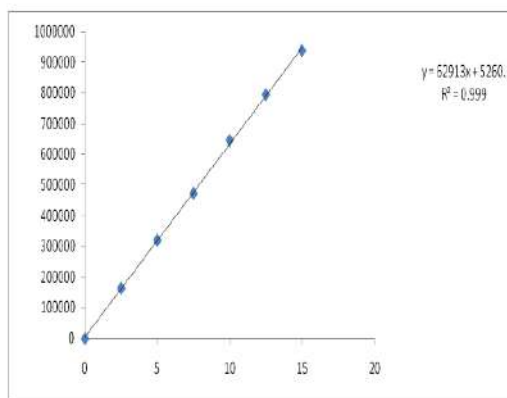
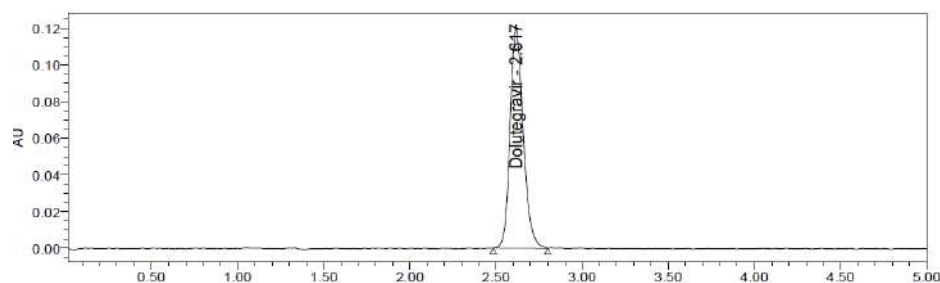
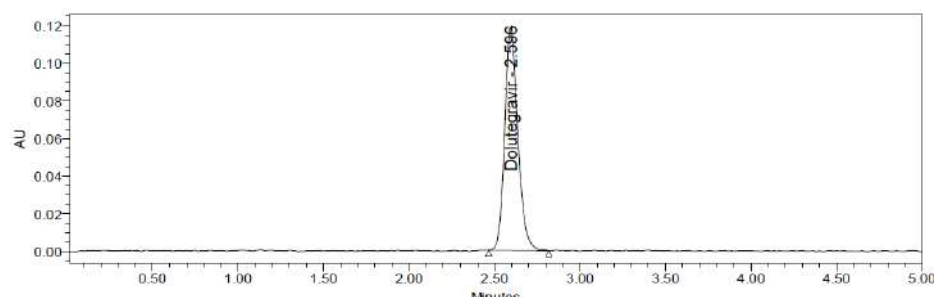


Figure 2 : Linearity Plot

Table 1 :Optimized Chromatographic conditions

Mobile phase	Flow rate	Column	Detector wave length	Column temperature	Injection volume	Run time	Retention time	Diluent
0.1% OPA Buffer : ACN (45:55)	1.0ml/min	Waters C18 150 x 4.6mm, 5µm.	257.0 nm	30°C	10µL	5min	2.617 mins	Acetonitrile : Water (50:50)

**Figure 3 :Chromatogram of Dolutegravir standard****Figure 4: Chromatogram of Dolutegravir sample**

Parameters	Dolutegravir
Calibrationrange(µg/ml)	2.5-12.5
Optimizedwavelength	257nm
Retentiontime	2.617min
Regressionequation(Y)	y=62913x+5260
slope	62913
intercept	5260
Correlationcoefficient(r ²)	0.999
Precision (%RSD*)	<2.0
% Assay	99.28
LOD(µg/ml)	0.013
LOQ(µg/ml)	0.039

Table 2: Summary Table

CONCLUSION

The developed stability indicating RP-HPLC method was found to be rapid, simple, specific, sensitive, linear, accurate, precise and economical for the estimation of Dolutegravir in bulk and formulation. Hence, it can be used for the routine quality control of Dolutegravir in bulk and formulation.

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AN UPDATED OVERVIEW ON HELIOTROPIUM INDICUM

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ABSTRACT

Heliotropium indicum is used in the traditional medicine for many diseases. *Heliotropium indicum* belongs to boraginaceae family. It has been used widely for centuries to treat inflammation and tumours. *Heliotropium indicum* linn showed significant activity in several experimental tumor systems. The active principle is isolated and shown to be the N-oxide of the alkaloid, indicine previously isolated from this plant. External application to promote wound healing and to fight infections seems to be less hazardous and further study will be continued.

KEYWORDS: *Heliotropium indicum* Linn, Traditional, Pyrrolizidine alkaloids, Bioactivity, Pharmacognosy

INTRODUCTION

Heliotropium indicum, commonly known as Indian heliotrope in english, Naga Danti in telugu, Hatisundha in Oriya, Hathi-sundhara in gujarati, Hathajori in hindi, chelubalada Gida in kannada, Thekkada in Malayalam, Bhrundi in Marathi is an annual, hirsute plant that is a common weed in waste places and settled areas. It is native to Asia commonly found in India and some parts of Africa and Bangladesh, but also found in other countries. It is widely used in native medicine in Tamil Nadu, India. It is an annual, erect, branched hirsute plants, 15 to 50 cm high. It is herb, with long taproot. Leaves are alternate or sub-opposite, simple, ovate upto 10cm in length and 7.5-10.0 cm in width blackish, green, hispid-pubescent, spikes mostly leaf opposed, 2.5-20 cm in length. Flowers are calyx green, 3.5 mm in diameter. Stamens 5, inserted on the corolla tube, included. Ovary completely or imperfectly 4-celled, 4 ovules, style terminal or leaf opposed, 3 to 10 cm long, flowers all in one side, and the lower ones opening first. The flowering season is very long and new flowers develop apically within the cyme. Fruits are dry 2- to 4lobed of 2 or 4 nearly free, more or less united nutlets, 4 to 5 mm long. Mature nutlets are already present at the base of the inflorescence. mainly whole plant, roots, leaves, seeds are used.



Fig. 1: *Heliotropium Indicum* Linn.

Taxonomic classification

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae
Class: Dicotyledonae
Order: Boraginales
Family: Boraginaceae
Genus: Heliotropium
Species: Heliotropium indicum

PHARMACOLOGICAL PROPERTY

Heliotropium indicum showed different bioactivities in animal model

Anti-tumor Property

The methanolic extract of aerial parts of the plant contains a significant level of the anti-cancer property. Various extracts of Heliotropium indicum resulted in substantial effects in many tumor systems in the laboratory. Most of the alkaloids which were identified from the plant are hepatotoxic, and therefore internal use is not recommended to date. Ethanol extract showed anti-proliferative property against human breast adenocarcinoma cell line using MTT assay.^[1,2,3,4,5,6,7,8,9,10]

Anti-inflammatory Property

The anti-inflammatory and antinociceptive activities of the plant were showed significantly high in carrageenan-induced hind paw edema and cotton pellet granuloma inflammation models in chloroform leaf extract in an animal model. In another study, various extracts of Heliotropium indicum has been reported significant anti-inflammatory activity in acute and sub-acute models of inflammation.^[11, 12, 13]

Anti-bacterial and Anti-fungal Property

Anti-fungal property of the water crude extract of leaves was tested against Fusarium oxysporum, and anti-bacterial effects of methanolic extract were tested against, Klebsiella spp., Pseudomonas mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis and Escherichia coli.^[14,15,16,17,18]

Anti-plasmodialProperty

Property Heliotropium indicum is ethnomedicinally used for curing malaria. In an experimental study, the plant extract has not shown any effects on malaria diseases directly. Though the plant is used for hyperthermia or colics, which are two significant symptoms of malaria, this could explain its remedial use measure for malaria. So the research study showed indirect anti-plasmodial activity.^[19]

Anti-tuberculosis Property

From the aerial parts of the plant volatile oil was isolated by hydro distillation method and characterized by a combination of GC and GC-MS. The principal constituent of the volatile oil was phytol, 1-dodecanol, and beta-linalool and which histamine, and oxytocin used on the smooth muscle preparations in the said experiment. The extract was prominently stable to plasma cholinesterase. The receptor activity of the plant parts suggests its ethnomedicinal uses like abdominal pain relief, hypertension, impotence and sexual obstructions.^[20]

CONCLUSION

From the above review study and explanation, it's revealed that *Heliotropium indicum* Linn. has been used as an crucial phytochemical source for many diseases in various parts of the world, as it is briefly discussed in the review article. The review also have the different ethnomedicinal, biological, taxonomical and other important biological uses of *Heliotropium indicum* Linn. It is mandatory for detail pharmacological research of this plant to know about its ethnomedicinal utility throughout the world.

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SP-150

AN UPDATED OVERVIEW ON SALACIA FRUTICOSA

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ABSTRACT

Salacia fruticosa heyne ex lawsol one of the red listed medicinal plants belongs to the family Celastraceae which is categorised as threatened by IUCN due to unsustainable and indiscriminate harvesting for commercial purpose. It is an endemic species was studied for the reproductive biology as this species showed reduced fruit set and natural regeneration. The stigma-anther proximity, an extremely low number of pollen grains, a short period of pollen viability, a sparse incidence of pollinators, protandrous

KEYWORDS: *Salacia fruticosa*. Hepatoprotective, Antioxidant, Hippocrateacea.

INTRODUCTION

Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life. With the emerging worldwide interest, in adopting traditional practices, in the healthcare systems by exploiting their potential, the evaluation of the botanicals in these systems of medicine in India is utmost important. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional heritage, but also to rationalize the use of natural products in the healthcare. Standardization is to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect. *Salacia fruticosa* is used as astringent, bitter, anti-inflammatory, liver tonic, and stomachic. It is useful in vitiated conditions of diabetes, hemorrhoids, skin diseases, amenorrhea, dysmenorrhea, wounds and ulcers¹. The present research work is focused with microscopical evaluation of aerial parts of *Salacia fruticosa* to establish its quality parameters which includes physicochemical, Phytochemical evaluation.

TAXONOMICAL /SCIENTIFIC CLASSIFICATION

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Celastrales

Family: Celastraceae

Genus: *Salacia*

Species: *reticulata*; *oblonga*; *campestris*; *hainanensis*; *madagascariensis*; *chinesis*; *petensis*; *Kraussii*; *fruticosa*; *macrocarpa*.



Figure 1: Salacia Fruiticosa flowers



Figure 2: Salacia Fruiticosa leaves and fruits

MACROSCOPIC CHARACTERS

The general macroscopical characteristics of the plants of the genus are as follows, scandent or sarmentosa shrub or small tree. Leaves usually opposite, petioled, coriaceous, shining above, exstipulate. Flowers small, axillary or extraaxillary, fascicled or cymose, rarely solitary or 2-Nate. Calyx small, 5-partite. Petals 5, spreading, imbricate. Stamens 3 (very rarely 2 or 4), inserted on the disk, free or connate with the ovary; filament conniving at the apex, recurved; anthers small, dehiscing extrorsely, adnate, 2- celled, lobes divaricating at the base. Disk thick, sinuate, ovary sunk in the disk, conical, 3-celled; ovules 2, 4 or more in each cell, affixed to the axis, 1 or 2 seriate; style usually very short; stigma simple or 3 lobed. Fruit baccate, edible, 1-3 celled; cells 1-4 seeded; rind coriaceous or sub woody; pulp mucilaginous. Seeds large, angular; testa rather thick, coriaceous or fibrous; cotyledons thick, usually conferruminate ²⁻⁴.

PHYTOCHEMISTRY

Sl No.	Species	Constituents	Isolated Parts	Ref.
1.	<i>S.reticulate</i>	Mangiferi	Root	9
2 .	<i>S.reticulata</i>	Kotanlolol	Root and stem	10
3.	<i>S. fruticosa</i>	Friedl-1-en-3-one, Amyrin, Sitosterol	Root and bark	21

Table 1. Phytoconstituents And Species

PHARMACOLOGICAL ACTIVITY

ANTIDIABETIC

The aqueous decoction of 40 plants were investigated for their hypoglycemic activity in Sprague-Dawley rats by Karunanayake and coworkers for their ability to lower the fasting blood glucose level and improve the glucose tolerance in animals. Maximum reduction in blood glucose level (30%) was observed 3 hours after administration of *S.Fruiticosa* aqueous decoction and which persisted up to 5 hours suggesting its hypoglycemic potential.

ANTIOXIDANT

The antioxidative activity of mangiferin, (-)-4'- O-methylepigallocatechin and (-)-epicatechin- (4b---8)-(-)-4'-O-methylepigallocatechin from the roots of *S.reticulata* were examined. They showed potent scavenging

activity of DPPH radicals and their IC₅₀ values were 5.9, 10 and 3.2 μ M respectively.

ANTIMICROBIAL

Antimicrobial activity of chloroform and methanolic extracts of *S. reticulata* were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escheria coli* and fungal strains viz., *Cryptococcus neoformans*, *Candida tropicalis*, *Candida albicans* and *Epidermophyton floccosum* using zone of inhibition and MIC was reported by Choudhary and coworkers. It was observed that both extracts have inhibitory effects towards all microorganisms used in the test. However, chloroform extract was more effective than methanolic extract⁷. Samy reported the antimicrobial activity of the methanolic extract of *S. macrosperma*. It was found to be very effective against the test microorganism⁸.

ANTI INFLAMMATORY

The anti-inflammatory activity of *S. oblonga* root bark powder was evaluated in male albino rats using carrageenan-induced rat paw oedema (acute inflammation) and cotton pellet granuloma (chronic inflammation) methods at a dose of 1000 mg/kg was studied by Ismail and coworkers. The increased acid and alkaline phosphatase activity and decreased serum albumin in cotton pellet granulomatous rats were normalized after treatment. The drug exerts their activity by anti proliferative, antioxidative and lysosomal membrane stabilization¹⁴.

ANTI OBESE

Salacia is marketed as a Starch Blocker suggesting that it might help to reduce the body weight. The effects of a mixture of the *S. reticulata* aqueous extract and cyclodextrin (SRCD) on the development of obesity were examined by Kishino and coworkers by evaluating the effects of SRCD on the elevation of plasma triacylglycerol levels induced by oral administration of a high-fat (HF) liquid diet to male Sprague-Dawley rats. The plasma triacylglycerol concentration was significantly lower in the SRCD treated rats than in the control rats 4 h after HF diet administration. In another study female C57BL/6 mice that consumed a solid HF diet containing 0.5% SRCD ad libitum for 8 week showed decrease in body weight and visceral fat mass than those fed with HF diet. In addition, the energy efficiency and the plasma leptin and adiponectin concentrations were lower in the mice that were administered SRCD than in those fed the HF diet alone. The inhibitory effects of SRCD on HF diet-induced obesity may be attributed to the inhibition of carbohydrate and lipid absorption from the small intestine.¹⁵

HEPATOPROTECTIVE

The hepatoprotective effect of the hot water (SRHW) and methanolic (SRM) extracts from the roots and stems of *S. fruticosa* were examined by Yoshikawa and coworkers using an oxidative stress-induced liver injury model. Both SRHW and SRM extracts (400 mg/kg, p.o.) significantly suppressed the increase in glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in carbon tetrachloride (CCl₄) treated mice. These extracts also inhibited CCl₄ induced thiobarbituric acid reactive substance (TBARS) formation which indicates increased lipid peroxidation in the liver suggesting its hepatoprotective activity^[6]. Rong and coworkers have demonstrated that an aqueous extract of *S. oblonga* root at the doses of 100, 300 and 900 mg/Kg, p.o. once daily for 28 days induced gender dependent hepatic hypertrophy in rats which is attributed to the sex hormones in both male and female rats. There was dose dependent increase in liver weight in both male and female rats but less predominant in female rats. It was found to activate PPAR-alpha in human hepatoma derived HepG2 cells by up regulation of PPAR-alpha and acyl-CoA oxidase in RNA expression⁹.

CONTRAINDICATIONS

A safety evaluation in animal models suggests that Salacia extract (both *S. reticulata* and *S. oblonga*) in doses 10 times what is suggested for humans for 14 days has no significant adverse reaction on blood chemistry, hematology or organ weights^{10,16,17}. Shimoda and coworkers showed no adverse effect on food intake, body weight, blood chemistry, organ weight or histopathological at doses upto 1000 mg/Kg for 13 weeks of continuous intake¹¹. Animal study suggests only *S. fruticosa* might adversely affect pregnancy with enhanced post-implantation losses, pups with low birth weight. However, the root extract was non-teratogenic. It can be concluded that the *S. fruticosa* root extract can be hazardous to successful pregnancy in

women and should not be used in pregnancy complicated by diabetes as there are reports that mugs made of *S. fruticosa* woods are available to be used routinely by diabetic patients to drink water¹⁰.

ADVERSE REACTIONS

Orally, *Salacia* can cause flatulence and distention. Flatulence is more significant with a 1000 mg dose compared to a 500 mg dose. Drinking *Salacia* tea can cause dyspepsia and loose stool⁵.

INTERACTION WITH DRUG

Salacia is thought to lower glucose levels. Combining *Salacia* with other antidiabetic drugs (glimepiride, glyburide, insulin, pioglitazone, rosiglitazone) that also lower blood glucose might have additive effect but increases the risk of hypoglycemia^{12,13}.

CONCLUSION

From the above review shows that *salicia fruticosa* has been used for phytomedical sources for many diseases like hepatoprotective, antioxidant, antidiabetic, antimicrobial, anti-inflammatory.

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MOLLUGO PENTAPHYLLA: NEW NUTRACEUTICAL USE AS ANTIDIABETIC**V. RAMANJANEYACHARI*, N.SOJANA, SOMNATH DE, J.N.SURESH KUMAR**

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ABSTRACT

Nutraceuticals are the emerging era in the treatment of diabetes mellitus, one of the seriously problematic due to leading the causes of death in all over the world. The newer anti-hyperglycemic drugs continue searching because the existing synthetic drugs have several limitations. Traditional medicinal plants are used in the treatment of diabetes mellitus more than a century, but only a few of these have proofed their safe and efficacious. The aim of this review article is focused *Mollugo pentaphylla* one of the medicinal plants used for antioxidant activities. It contains several kinds of alkaloids, flavonoids, saponins, tannin, diterpenes, triterpenoids, glycosides and phenols. Many researchers have evaluated that these phytochemical substances have the major impact on diabetes mellitus. This review focuses on the hypoglycemic activity of this plant and clears that it has the potential to be considered as a candidate for preparing the new treatment of diabetes mellitus.

KEYWORDS: *Diabetes mellitus, Hypoglycemia, Nutraceutical, Plant, Mollugo pentaphylla*

INTRODUCTION

Diabetes mellitus (DM) is a major heterogeneous endocrine and metabolic disorder, characterized by altered metabolisms of carbohydrate, lipid and protein, which not only lead to hyperglycaemia but also cause many complications, such as hyperlipidaemia, hypertension and atherosclerosis^{1,2,3}. Antioxidants have been shown to prevent the destruction of β -cells by inhibiting the peroxidation chain reaction and thus, may provide protection against the development of diabetes. On the other hand, plants contain natural antioxidants (tannins, flavonoids, vitamins C and E, etc.) that can preserve β -cell^{4,5} function and prevent diabetes induced ROS formation and many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatment of DM. Despite this, few traditionally used antidiabetic plants have received proper scientific screening. The World Health Organization (WHO) has recommended that this area warrants further evaluation. Ethnomedical Information on *Mollugo pentaphylla* cited the folkloric use of the plant as an emmenagogue on female human adult in India and Indonesia⁶. Hot H₂O extract of dried entire plant in India used for whooping cough and in cases of atrophy in human. Decoction of dried entire plant used to treat hepatitis in Taiwan. *M. Pentaphylla* is a component in an important folk medicine named "Peh-Hue-Juwa-Chi-Cao" in Taiwan, which is used as an anticancer, antitoxic and diuretic agent. Eaten as a pot-herb; it is also used medically for mouth infections. The original scientific studies on the plant reported to possess active antifungal activity, antibacterial activity, spermicidal and spermiostatic effect, anti-inflammatory and hepatoprotective activity and antioxidant activity. The plant is reported to contain Flavones such as Apigenin and Mollupentin, Mollugogenol Sitosterol Beta.

NOMENCLATURE AND MORPHOLOGICAL CHARECTERS

Mollugo pentaphylla Linn, commonly known as carpet weed (English), Pitta saga (Oriya) is a perennial herb found throughout India, Ceylon, Malacca, China, Japan, Fiji etc. Roots are creeper and adventitious, leaves are trifoliate small oval shape; flowers are white, pentameric and bisexual. The urban people used this plant medicinally in paste form orally and externally for treatment of skin allergic condition, antimicrobials etc. *Mollugo* is a genus in the flowering plant family Molluginaceae. It comprises a few dozen *species* of herbaceous plants, including *Mollugo verticillata*, carpetweed or green carpetweed. Highly esteemed by Hindus as a bitter vegetable which they eat occasionally on account of its stomachic, aperient and antiseptic properties⁷.

Mollugo pentaphylla L. Terrestrial, annual, tufted to erect herb, up to 35 cm tall. Taproot whitish or brown. Stems erect, rounded, solid, glabrous.



Fig. 1: *Mollugo pentaphylla* small branched tree, leaves, stems, and flowers.

PHYTOCHEMICAL CONSTITUENTS

The Qualitative analysis revealed the presence of carbohydrates, protein, saponins, tannins, terpenoids, flavonoids, steroids, phenols, proteins, alkaloids and glycosides, volatile oils, in the whole plant of *Mollugo pentaphylla* L.



Fig. 1: *Mollugo pentaphylla* flowers.

TRADITIONAL USES

Ethnomedical Information on *Mollugo pentaphylla* cited the folkloric use of the plant as an emmenagogue on female human adult in India⁸, which is used as an anticancer, antitoxic and diuretic agent. Eaten as a pot-herb; it is also used medically for mouth infections. The original scientific studies on the plant reported to possess active antifungal activity⁹ antibacterial activity, spermicidal and spermiostatic effect anti-inflammatory and hepatoprotective activity and antioxidant activity. The plant is reported to contain Flavones such as Apigenin and Mollupentin, Mollugogenol A, an antifungal triterpenoid, Mollugogenol B, Mollugogenol D, Oleanolic acid and a steroid–Sitosterol Beta.

ANTI DIABETIC ACTIVITY

Medicinal plants have gained huge interests from researchers around the world because of their positive bioactivity effects. However, there are still not many data available about the anti-diabetic activity of this medicinal plant,. *Mollugo pentaphylla* during the review searches were done on the scientific databases i.e., Science Direct, Springer Link, PubMed, Google Scholar and etc. Moreover internet searches were undertaken on the search engine. Different combinations of keywords as well as synonyms for keywords were used during the searches.

Hypouricemia activity

In addition, a recent research showed that fructose-induced hyperuricemia plays a pathogenic role in metabolic syndrome^{10,11}. Thus, lowering uric acid may be a novel treatment target for preventing diabetes.

The levels of urea, serum creatinine and uric acid were restored to near normal level by treatment with *Molluga pentaphylla* leaves extract¹².

Antioxidant properties

Many studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models as well as reducing the severity of diabetic complications¹³⁻¹⁵. The elevated oxidative stress marker and diminished antioxidant status were normalized indicating the antioxidant potential of this plant¹⁶. Zarena et al.¹⁷ reported the 29 kDa protein from *Molluga Pentaphylla* leaves named agathi leaf protein (ALP) which possesses antioxidant and cytoprotective activities. Additional, the level of lipid peroxidative markers (thiobarbitric acid reactive substances and lipid hydroperoxides) was significantly reduced on treatment with *Molluga Pentaphylla*, the levels of both enzymatic and non-enzymatic antioxidants were also found to be restored on treatment with this plant¹⁸.

Increased hepatic metabolism

The hypoglycemic activity is thought to be due to increased hepatic metabolism. Aqueous homogenate of this plant administered orally to animal model significantly increased hepatic glycogen and free amino acid content, decreased blood glucose, and triglyceride levels¹⁹.

Insulin elevation

The hypoglycemic activity is thought to be due to stimulation of synthesis and/or release of insulin from pancreatic beta cells and/or insulin sparing effect²⁰. In conclusion, the aim of this review is to focus on the potential utilization of phytochemical constituents which could contribute more effectively to antidiabetic activity of *Molluga Pentaphylla*. It aims to explore a proposed of this plant for complementary alternative medicine and especially for human consumption.

CONCLUSION

This study clearly demonstrates that the aqueous extract of aerial parts of *Mollugo pentaphylla*, exhibited strong antihyperglycaemic activity which could be attributed to its possible action on pancreatic and extra-pancreatic site of glucose and lipid metabolism as evidenced by insulinotropic and antioxidant defense properties. Currently, we are carrying out studies to isolate, and chara bioactive compounds to evaluate the antihyperglycaemic properties of *M. pentaphylla*. This may further clarify the specific properties of the plant.

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SYNTHESIS, CHARACTERIZATION, ANTI MICROBIAL AND *IN VITRO* ANTI TUBERCULAR ACTIVITY OF (Z)- ETHYL-3-(SUBSTITUTED AMINO) BUT-2-ENOATE DERIVATIVES

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ABSTRACT

Enaminones (E1-6), versatile organic synthesis intermediates have been synthesized from ethylacetoacetate and various amines (2a-f), characterized by IR, ¹H NMR, mass. The synthesized enaminones were evaluated for anti microbial, *in vitro* anti tubercular activities using the strains *Bacillus subtilis* (NCIM 2698), *Micrococcus varians* (NCIM 2913), *Staphylococcus aureus*, *Escherichia coli* (NCIM 2344), *Klebsiella pneumonia* (NCIM 2957), *Pseudomonas aeruginosa*, *Serratia marcescens* (NCIM 2919), *Aspergillus niger* (NCIM 548) *Aspergillus flavus* (NCIM 536) and *Mycobacterium tuberculosis* H37 RV strain (ATCC 27294). The compounds E5(R= (4-hydroxyphenyl) acetic acid) have significant anti bacterial activity and *in vitro* anti tubercular activity while, E6(R= dioxoisindolyl), E2(R= mercaptopropionic acid) have better anti bacterial activity on all tested bacteria when compared with standards (Amoxycylav, Gentamycin, Fluconazole, Pyrazinamide, Ciprofloxacin and Streptomycin).

KEYWORDS: *Enaminones, β-ketoester, ceric ammonium nitrate, anti bacterial activity, anti tubercular activity.*

INTRODUCTION

Enaminones or β-amino vinyl ketones¹ are very versatile readily obtainable reagents². β-enaminones are reactive valuable reagents as they have conjugated system N-C=C-C=O³ which act both as electrophile and nucleophile⁴. Enaminones are important synthetic intermediates in heterocyclic chemistry⁵: as raw materials for synthesis of alkaloid like scaffolds⁶, cation locating agents, good chelating ligands for transition metals⁷ and as ligands for diastereoselective synthesis⁸. Condensation of 1,3-diketone or β-ketoester with various amines produce enaminones in presence of catalysts like THF, N₂⁹, I₂⁸, DMF-acetal¹⁰, tin tetrachloride¹¹, PPA³, CAN¹², tungstophosphoric acid¹³, silica sulfuric acid¹⁴, KHSO₄¹⁵, gold (I)/ silver (I)¹⁶, 3-nitrophenyl boronic acid¹⁷, ionic liquid¹⁸, AgNO₃¹⁹, Zn[amino acid]₂⁴, silica supported Antimony (III) chloride²⁰, Microwave²¹ and Ultrasound⁴.

Experimental section

The chemicals used for the present investigation were purchased from Merck and used in unpurified forms. Melting points of the synthesized compounds were determined by open capillary method. TLC of the synthesized compounds were performed by plates of Merck, TLC silica gel 60 F₂₅₄ 20X20 cm and visualized under UV light. IR spectra of the compounds were recorded by BRUKER FTIR, ¹H NMR in DMSO by BRUKER spect 5mm BBO BB-1H was recorded at 400 MHz and chemical shifts were reported as δ (ppm) with coupling constants, mass by Tandem Mass Spectroscopy Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)

General experimental procedure for synthesis of enaminones (E1-6)²²

A mixture of amines (0.01mol) (2a-f) and ethyl acetoacetate (0.01mol) (1) were reacted in ethanol with 5mol % Ceric ammonium nitrate (CAN) as a catalyst. The reaction was carried out at room temperature for 1.5-4 hours. Then the product was extracted thrice with 10ml of dichloromethane and then the extracted product was washed with water and recrystallised from ethanol to get the desired product.

Synthesis of isoindoline-1, 3-dione (5)²³

A mixture of phthalic anhydride (0.01mol) **3** and urea (0.01mol) **4** were placed in motor, grounded to form homogenous mixture. The mixture was transferred into a beaker and heated to 130⁰-135⁰c until it converts to oil, continued heating till it forms a solid. Then the mixture was cooled, added 50ml of water and filtered. The residue was washed with cold water and recrystallized from hot water as crystals.

Preliminary Screening of Antimicrobial activity

Anti microbial activity has been performed using agar disc diffusion method²⁴. Antimicrobial activity has been studied with three gram positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus variens*, four gram negative organisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens* and two fungal strains *Aspergillus niger* and *Asperigillus flavus*. The antibacterial and antifungal activities were compared with standards Amoxyclav, Gentamycin and Fluconazole respectively. 24 hr subcultures of the organisms were used as inoculum. Synthesized enaminone solutions of concentration of 10, 20, 30, 40, 50, 75 and 100µg/ml were prepared with DMSO. Discs soaked in dilutions of synthesized compounds were placed on inoculated media in the Petri dish and incubated for 24-48 hr.

In vitro Anti Tubercular activity²⁵

The *in vitro* anti tubercular activity of the synthesized compounds was assessed using *Mt* H37 RV strain (ATCC 27294) by MABA. The concentrations of synthesized compounds used for the test were 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100µg/ml. Serial dilutions of the synthesized compounds were made directly in the 96-well sterile microtitre plate, then 200µl of deionised water, 100µL of Middlebrook 7H9 broth were added to the well and the plate was covered with parafilm and incubated for five days. After five days, 25µL of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24 hours. After 24 hours plates were observed for color change against the standards pyrazinamide, ciprofloxacin and streptomycin.

RESULTS AND DISCUSSION

The synthesis of (*E*)-4-ethoxy-4-(substituted amino) but-3-en-2-one (E1-6) derivatives is outlined in the scheme I while, isoindoline-1, 3-dione (**2**) in scheme II. Amination of phthalic anhydride by direct condensation of phthalic anhydride and urea at 130⁰-135⁰ C produced isoindoline-1, 3-dione **2** in 80% yields. (*E*)-Ethoxy-4-(substituted amino) but-3-en-2-one derivatives (E1-6) were obtained in good yields by direct condensation of β-ketoester and amines using ceric ammonium nitrate (CAN) as catalyst in ethanol at room temperature. All the compounds synthesized were characterized by TLC, spectroscopic techniques like IR, ¹HNMR and mass. The IR spectrum of the synthesized compound display bands at ~1680-1620 cm⁻¹, ~1190-1130 cm⁻¹ due to the presence of α, β- unsaturated carbonyl system and secondary C-N. The peaks at ~7.0-7.2 ppm, ~4 ppm ¹ HNMR spectra of the synthesized compounds indicate the protons of enaminones functionality. All the spectra are in accordance with the depicted structures.

2-((Z)-1-(ethoxycarbonyl)prop-1-en-2-yl amino)acetic acid: yellow liquid, b.p. 121⁰C, yield:70%, IR: 3453.23, 2984.29, 1706.23, 1642.87, 1177.41cm⁻¹, ¹HNMR: 1.393-1.399(d,3H, *J*=2.4Hz), 1.855-1.861(d,3H, *J*=2.4 Hz), 2.511(s,1H), 3.576-3.597(t,2H, *J*=8.4Hz), 4.18-4.204(t,2H,*J*= 16 Hz), 4.594-4.617(q, 1H, *J*=9.2 Hz), 8.121 (s, 1H), m/e: 189.09(M+2).

2-((Z)-1-(ethoxycarbonyl)prop-1-en-2-ylamino)-3-mercaptopropionic acid: Brown Liquid, b.p. 130⁰ C, yield: 80%, IR:710.25, 1641.42, 1708.22, 2985.05, 3437.41 cm⁻¹. ¹H NMR 1.29-1.306(d, 3H, *J*=604Hz), 1.454(s, 1H), 1.909-1.923(d, 3H, *J*=5.6Hz), 2.18(s, 1H), 3.152-3.225(m, 2H, *J*=11.2Hz), 3.576-3.619(m, 1H, *J*=17.2Hz), 4.281-4.317(t, 2H,*J*=14.4Hz), 4.607-4.624(m, 1H, *J*=6.8Hz), 7.238(s, 1H), m/e: 234.2(M+1)

(Z)-ethyl-3-(piperazin-1-yl)but-2-enoate: Brown Liquid,b.p.128⁰C, yield:80%; IR: 775, 860, 932.2, 1374.45, 1637.68, 1712.70, 2983.69, 3327.23 cm⁻¹¹H NMR: 1.414-1.429(d,3H, *J*=6Hz), 1.620-1.635(d,3H,*J*=6Hz), 2.25(s,1H), 3.15-3.183(t,8H, *J*=13.2Hz), 4.104-4.114(t,2H, *J*=4Hz), 4.31-4.37(q,1H,*J*=15.9Hz), m/e: 197.0(M-1)

(Z)-ethyl-3-(morpholino)but-2-enoate: Brown Liquid, b.p.152⁰C, yield:90%, IR:1296.03, 1639.35, 3422.40 cm⁻¹, ¹HNMR: 1.206-1.23(d,3H,*J*=9.6Hz), 1.759-1.799(d,3H, *J*=16Hz), 2.140(s,2H), 3.014-3.023(dd,4H, *J*= 3.6Hz) ,3.661-3.67(dd,4H, *J*=3.6Hz), 4.301(m,1H, *J*=6.8Hz),m/e:197.0(M-1)

2-((Z)-(ethoxycarbonyl)prop-1-en-2-ylamino)-2-(4-hydroxyphenyl)acetic acid: brown liquid, b.p. 112⁰ C ,yield:88% ; IR: 735.94, 823.98, 906.87, 1632.61, 1720.36, 3228.73 cm⁻¹H NMR: 1.442-1.452(d,3H, *J*=4Hz), 1.973-1.99(d,3H, *J*=6.8Hz), 2.633(s,1H), 3.351-3.406(q,1H, *J*=11.2Hz), 4.05(s,1H), 4.62(m,1H, *J*=6.8Hz), 6.65-6.67(dd,2H, *J*=8Hz), 6.982-7.002(dd,2H, *J*=8Hz), 8.242(s, 1H), m/e: 252.1(M+1)

(Z)-ethyl-3-(1,3-dioxoisindolin-2-yl)but-2-enoate: orange solid, m.p.86⁰ C yield: 85%, IR: 1375.39, 1636.24, 1717.84, 3243.20 cm⁻¹H NMR: 1.180-1.248(d,3H, *J*=26.4Hz), 1.911-1.977(d,3H, *J*=26.4Hz), 2.44-2.62(q,2H, *J*=25.4 Hz), 4.021-4.425(t,2H,), 7.03-7.293(t, 2H) 7.819(s,2H), m/e:261.09 (M+2)

Preliminary anti-microbial screening

Anti microbial screening of the synthesized compounds (E1-6) was done by disc diffusion method against three gram positive, four gram negative and two fungal strains like *Bacillus subtilis*(*B.s*)(NCIM 2698), *Micrococcus variens*(*M.v*) (NCIM 2913), *Staphylococcus aureus*(*S.a*), *Escherichia coli*(*E.c*) (NCIM 2344), *Klebsiella pneumonia*(*K.p*) (NCIM 2957), *Pseudomonas aeruginosa*(*P.a*), *Serratia marcescen*(*S.m*) (NCIM 2919), *Aspergillus niger*(*A.n*) (NCIM 548) and *Aspergillus flavus*(*A.f*) (NCIM 536) respectively. The results were tabulated in the table I. From the table it was observed that the tested compounds (E1-6) have less significant antibacterial activity when compared against the antibacterial standards amoxyclav, gentamycin. The compounds were inactive against the fungal strains like *A. flavus* and *A.niger*. Among the synthesized compounds E6 (R= dioxoisindolinyl) have shown antibacterial activity on all tested bacteria, E5 (R= (4-hydroxyphenyl)acetic acid) also have activity on all bacteria except *Staphylococcus aureus*. While, E2(R= mercaptopropionic acid), E3(R= piperazinyl) and E4 (R= morpholino) among the synthesized compounds have moderate anti bacterial activity and E1(R= methylamino) shown anti bacterial action on gram negative bacteria *Klebsiella pneumonia*, *Serratia marcescens*. Structurally, the compounds with aromatic system such as E5, E6 have shown better activity and compounds with heterocyclic system like E3, E4, E2 have good activity while with aliphatic system have least activity.

In vitro anti-tubercular activity

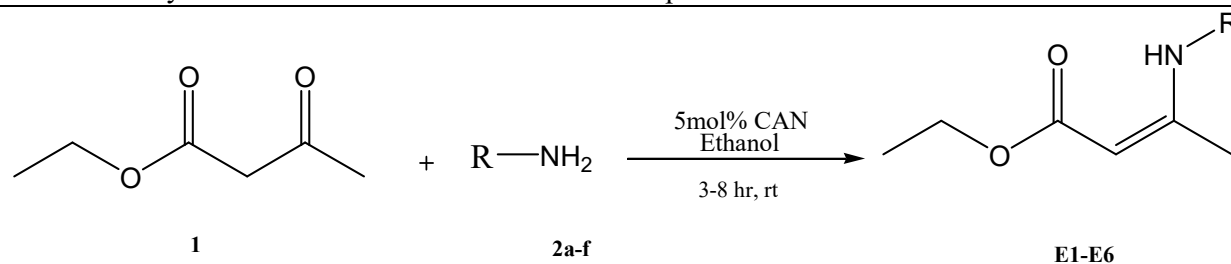
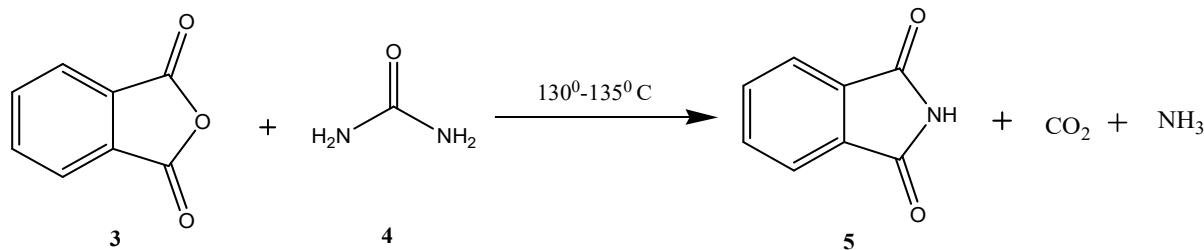
The anti tubercular activity of the synthesized compounds (E1-6) was assessed by *in vitro* Microtitre Alamar Blue Assay (MABA) using *Mycobacterium tuberculosis* (*Mt*) H37RV (ATCC 27294) strain against the standards Pyrazinamide, Ciprofloxacin and Streptomycin. The organism was incubated with the serial dilutions of the synthesized compounds for five days and then the susceptibility of the organism to the compounds was detected by incubating them in freshly prepared 1:1 mixture of Alamar blue and 10% tween 80 for 24 hours in a microtitre well. Growth of the organism in the well was visualized by the pink color while death by blue color indicating resistance and sensitivity of strain towards the synthesized compounds respectively. Compounds E2(R= mercaptopropionic acid), E5 (R= (4-hydroxyphenyl) acetic acid) have better anti tubercular activity and the minimum inhibitory concentration (MIC) was found to be 1.6µg/ml. The MIC of Pyrazinamide, ciprofloxacin and streptomycin was 3.12µg/ml, 3.12µg/ml and 6.25µg/ml respectively. Hence, compounds like E2, E5 having aromatic and heterocyclic systems have better anti tubercular activity than standard streptomycin. So further optimization may produce a better lead with anti tubercular activity.

Table I: Results of preliminary anti microbial Screening of the synthesised compounds									
Compounds	Gram +ve bacteria			Gram -ve bacteria				Fungi	
	<i>B.s</i>	<i>S.a</i>	<i>M.v</i>	<i>K.p</i>	<i>E. c</i>	<i>S. m</i>	<i>P.a</i>	<i>A.n</i>	<i>A. f</i>
E1	-	-	-	+	-	+	-	-	-
E2	+	-	+	+	-	-	-	-	-
E3	+	-	+	-	+	+	-	-	-
E4	+	+	-	+	+	-	-	-	-
E5	+	-	+	+	+	+	+	-	-
E6	+	+	+	+	+	+	+	-	-
Amoxyclav	++	++	++		-				

Gentamycin	++	++	+	++	++	++	++		
Fluconazole								++	++
“-“ : indicate the absence of anti microbial activity “+” : indicate the presence of anti microbial activity (15-20 mm) “++” : (20-40 mm)									

Table II: Results of *in vitro* anti tubercular activity by MABA of the synthesised compounds

Compound	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
E1	S	S	S	S	S	R	R	R
E2	S	S	S	S	S	S	S	R
E3	S	S	S	S	R	R	R	R
E4	S	S	S	S	S	R	R	R
E5	S	S	S	S	S	S	S	R
E6	S	S	S	S	S	R	R	R
Pyrazinamide	S	S	S	S	S	S	R	R
Ciprofloxacin	S	S	S	S	S	S	R	R
Streptomycin	S	S	S	S	S	R	R	R
“R”: Resistance of the <i>M. tuberculosis</i> to the tested compounds								
“S”: Sensitivity of the <i>M. tuberculosis</i> to the tested compounds								

**Scheme I****Scheme II**

CONCLUSION

Condensation of ethylacetoacetate (β -ketoester) and various amines gave the enaminones derivatives (E1-6) and structures of the same were confirmed by spectral analysis like IR, ^1H NMR and mass. These compounds were evaluated for anti microbial activity with the bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus variens*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and fungal strains *Aspergillus niger*, *Aspergillus flavus* with reference to the standards Amoxyclav, Gentamycin and Fluconazole. Compounds E5, E6 showed significant anti bacterial activity while E4, E3 and E2 showed moderate anti bacterial activity. All the synthesized compounds are inactive on tested fungi. Compounds were also evaluated for *in vitro* anti tubercular activity using *Mycobacterium tuberculosis H 37 RV* strains. The compounds E2, E5 among others have showed remarkable anti tubercular activity than standards Pyrazinamide and Ciprofloxacin.

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FORMULATION AND EVALUATION OF ROSUVASTATIN (ORAL DISINTEGRATING) TABLETS BY USING NATURAL POLYMER

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ABSTRACT

Hyperlipidemia involves an imbalance of cholesterol levels, including low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in the blood. Rosuvastatin is used to decrease the amount of cholesterol such as low-density lipoprotein (LDL) cholesterol ('bad cholesterol') and triglycerides in the blood. Mouth disintegrating tablets of rosuvastatin were prepared by using the natural banana powder. Mouth disintegrating tablets are most accepted and exploited for the drug delivery for patients who are having difficulty with swallowing that is mainly in paediatrics and geriatrics. Rosuvastatin is an anti hyperlipidemic agent, which acts by inhibition of HMG-CoA reductase. In the present study mouth disintegrating tablets of Rosuvastatin were prepared by direct compression method. A total of 8 different formulations have been prepared and among them the 4 formulations have been prepared using the Croscarmellose sodium and another 4 formulations have been prepared using the natural banana powder. The Banana powder used formulations have shown better release profile than compared with other formulations. F-6 was known because the best formulation among all the opposite formulations.

KEYWORDS: *Anti hyperlipidemic agent, Croscarmellose sodium, triglycerides, Hyperlipidemia, banana powder.*

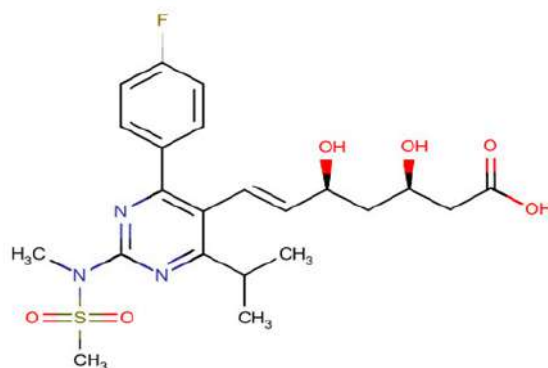
INTRODUCTION

An ideal dose programme within the drug medical care of any unwellness is that the one, that in real time attains the need medicine concentration of drug in plasma (or at the positioning of action) and maintains it constant for the whole period of treatment. This is attainable through administration of typical dose type in a very explicit dose and at a specific frequency. This drug may be administered by a variety of routes in a variety of dosage forms.¹ Drugs are more frequently taken by oral administration. Although many medicines taken orally square measure meant to be dissolved inside the mouth, the vast majority of drugs taken orally are swallowed. Compared with alternate routes, the oral route of drug administration is that the most well-liked and has been with success used for typical delivery of drug. It is thought of most natural, uncomplicated, convenient, safe suggests that of administering medication, larger flexibility in indefinite quantity kind style, simple production and low value. Tablets and laborious gelatin capsules constitute a major portion of the drug delivery systems that are currently available.² Many old persons face difficulties in administering typical oral dose forms thanks to hand tremors and dysphasia. Swallowing drawback is common in kids thanks to their underdeveloped muscular and nervous systems. In some cases like ailment, sudden episodes of allergic attack or coughing, and during unavailability of water, swallowing conventional tablets is difficult. To full fill these medical needs, kindulators have devoted goodly efforts for developing a unique style of dose form for oral administration referred to as mouth dissolving tablets (MDT).³

2.DRUG PROFILE ROSUVASTATIN

Description: Rosuvastatin is an antilipidemic agent that competitively inhibits hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in sterol synthesis. Rosuvastatin belongs to a category of medicines known as statins and is employed to scale back plasma cholesterol levels and forestall disorder.⁶

Structure



IUPAC NAME: (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methyl methanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-hydroxy hept-6-enoic acid

Chemical formula: C₂₂H₂₈FN₃O₆S

Solubility: Soluble in water (< 1 mg/ml at 25° C), chloroform, DMSO (100 mg/ml at 25° C), methanol, and ethanol (< 1 mg/ml at 25° C).

Molecular weight: 481.539 g/mol

Melting point: 173-185°C.

Absorption: Bioavailability is approximately 20%. Peak plasma concentrations were reached three to five hours following oral dosing.

Protein binding: half a mile absolute to plasma proteins (mostly albumin). Binding is reversible and independent of plasma concentrations.

Metabolism: Not extensively metabolized. Only ~10% is excreted as metabolites.

Route of elimination: Following oral administration, rosuvastatin and its metabolites are primarily excreted in the feces (90%). After associate degree endovenous dose, approximately 28% of total body clearance was via the renal route, and 72% by the hepatic route.

Half-life: 19 hours⁶.

PREPARATION OF ORAL DISINTEGRATING TABLETS

Direct compression method

Mouth disintegrating tablets of Rosuvastatin were prepared by direct compression method. All the ingredients were powdered separately and passed through # 40 mesh sieve separately. The drug and directly compressible excipient were mixed by adding tiny parts of every at a time and mixing it to urge a regular mixture and unbroken aside. Then the opposite ingredients were mixed in geometrical order, in associate inflated polythene pouch metallic element stearate and talc were another and mixed for additional 2 minutes and therefore the tablets were compressed exploitation six metric linear unit flat spherical punches to urge tablets of a hundred mg weight.^{3,4}

Table 1. Formulation of Mouth Disintegrating Tablets of Rosuvastatin

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8
Rosuvastatin	10	10	10	10	10	10	10	10
CCS	10	20	30	40	-	-	-	-
Banana powder	-	-	-	-	10	20	30	40
MCC	125	115	105	95	125	115	105	95
Peppermint flavour	1	1	1	1	1	1	1	1
Sodium lauryl sulphate	2	2	2	2	2	2	2	2
Mg. stearate	2	2	2	2	2	2	2	2
Total wt. (mg)	150	150	150	150	150	150	150	150

RESULTS AND DISCUSSIONS

Construction of Standard Calibration Curve of Rosuvastatin in 6.8 phosphate buffer

10 tablets were weighed and fine, a quantity of powder equivalent to 100 mg of Rosuvastatin was transferred to a 100 ml volumetric flask and 10 ml methanol is added. The drug is extracted in fuel by smartly shaking the closed flask for quarter-hour. Then the volume is adjusted to the mark with 6.8 phosphate buffer and the liquid is filtered. The absorbance of the solution was measured at 257nm, using UV spectrometer with 0.1N HCL as blank. The values are shown in table. A graph of absorbance Vs Concentration was planned that indicated in compliance to Beer's law within the concentration vary two to ten µg/ml.⁷

Table 2. Standard Calibration curve Values of Rosuvastatin in 6.8 phosphate buffer

Concentration (µg/ml)	Absorbance
0	0
2	0.153
4	0.267
6	0.382
8	0.505
10	0.632

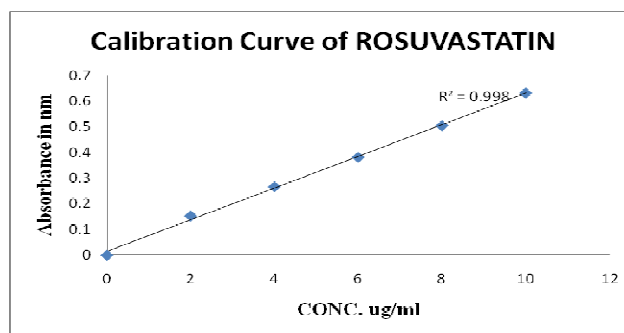


Figure-1. Standard Calibration Curve of Rosuvastatin in

6.8 phosphate buffer.

EVALUATION OF BLEND

A) Pre Compression studies

Table -3. Pre Compression Studies of Rosuvastatin Oral Disintegrating Tablets.

Formulation code	Bulk density (Kg/cm ³)	Tapped density (Kg/cm ³)	Car's index	Hausner's ratio	Angle of repose (°)
F1	0.40	0.48	16	1.2	32.73
F2	0.39	0.48	18	1.23	34.96
F3	0.50	0.58	13	1.16	28.58
F4	0.44	0.50	12	1.1	27.92
F5	0.37	0.41	9.75	1.1	25.35
F6	0.37	0.41	9.75	1.1	33.14
F7	0.36	0.39	7.6	1.0	27.03
F8	0.41	0.45	8.8	1.0	31.85

The pre compression studies were performed and the values were within permissible limits.⁵

B) Post Compression Studies-**Table-4.Post Compression Studies for Oral Disintegrating Tablets of Rosuvastatin.**

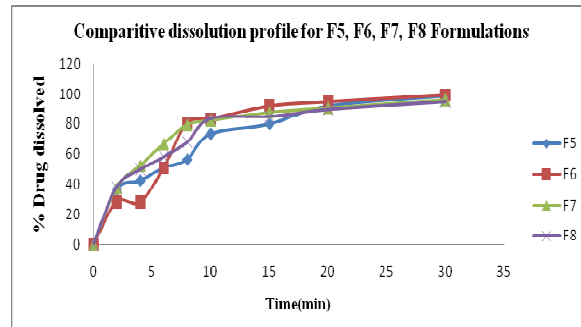
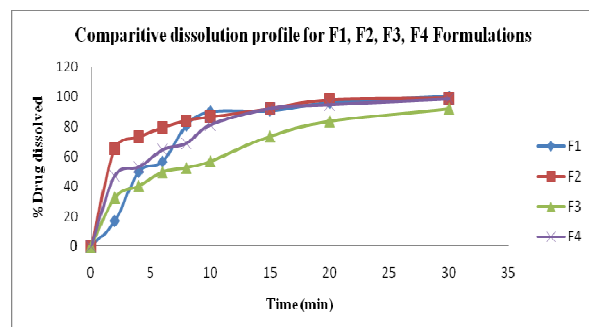
bat ch	Hard ness (kg/c m ²)	Friabil ity (%)	Drug Conte nt (%)	Thick ness (mm)	Disintegr ation Time (sec)	Wettin g Time (sec)	In vitro dispersi on time	Weight variatio n	Water a bsorptio n ratio
F1	3.1	0.45	99.12	2.5	30	45	29	pass	61.3
F2	2.9	0.62	100.73	2.8	25	42	34	pass	69.8
F3	3.3	0.71	99.74	2.6	20	35	25	pass	73.4
F4	2.5	0.32	98.98	2.5	31	31	32	pass	86.2
F5	2.8	0.51	99.67	2.6	27	36	31	pass	84.12
F6	2.8	0.52	99.83	2.8	25	43	33	pass	93.4
F7	2.9	0.38	101.32	2.8	31	41	36	pass	64.3
F8	3.2	0.48	100.87	2.5	26	36	33	pass	74.8

INVITRO DISSOLUTION STUDIES OF ROSUVASTATIN TABLETS

900 cubic centimeter of half-dozen.8 phosphate buffer was placed within the vessel and therefore the USP-II equipment (Paddle method) was assembled. A pill was placed within the vessel and was covered; the equipment was operated up to 30mins at fifty rev. At definite time intervals, 5 metric unit of measurement of dissolution medium was withdrawn; filtered and all over again replaced with 5 metric unit of measurement of recent medium to stay up sink conditions. Appropriate dilutions were through with dissolution medium and were analysed spectrophotometrically at $\lambda_{\text{max}} = 257 \text{ nm}$ employing a UV-spectrophotometer (Lab India).⁷

Table-5.Dissolution Data of Oral Disintegrating Tablets of Rosuvastatin.

Time(min)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
2	17.1	65.1	32.7	47.2	36.8	28.4	37.7	38.3
4	49.7	73	40.5	53	42.6	38.3	52.5	49.7
6	56.6	79.4	49.6	64.6	51	51	66.8	58.2
8	80.7	83.9	52.5	68.9	56.6	80.8	79.3	67.9
10	90	86.5	56.9	81.2	73.6	83.5	82.2	83.6
15	90.7	92.1	73.6	92.4	80	92	87.7	84.9
20	95.9	97.8	83.5	94.8	92.1	94.8	90.6	89.7
30	100	99	92.1	98.6	98.8	99.2	95.8	94.8



○

(B)**Figure-2.(A)comparitive dissolution profile for F1,F2,F3,F4 Formulations.(B)comparitive dissolution profile for F5,F6,F7,F8 Formulations.**

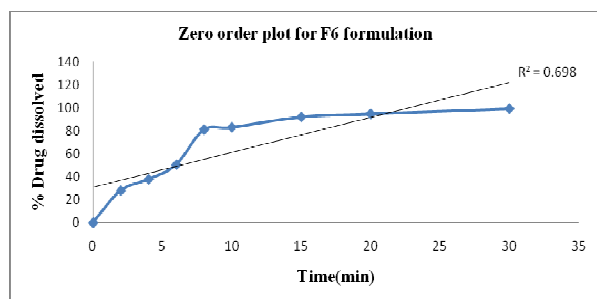


Figure-3.zero order plot for F6 formulation.

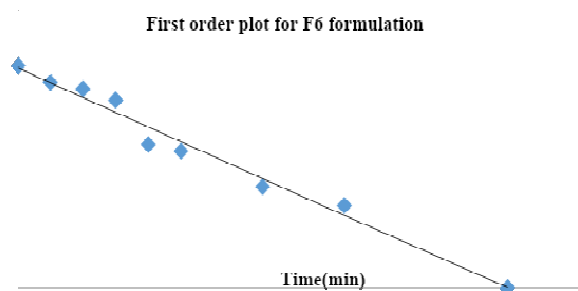


Figure-4.first order plot for F6 formulation.

SOLUBILITY

The prepared formulation of Rosuvastatin was found to be soluble in some organic and inorganic solvents.

Soluble in- Sulphuric acid (CONC), Sodium hydroxide (CONC),phenol.**Insoluble in** – Acetone, Alcohol, Chloroform, Carbon tetrachloride, Ammonia, Formic acid.¹⁰

IR SPECTROSCOPIC ANALYSIS



Figure-5.Banana powder.

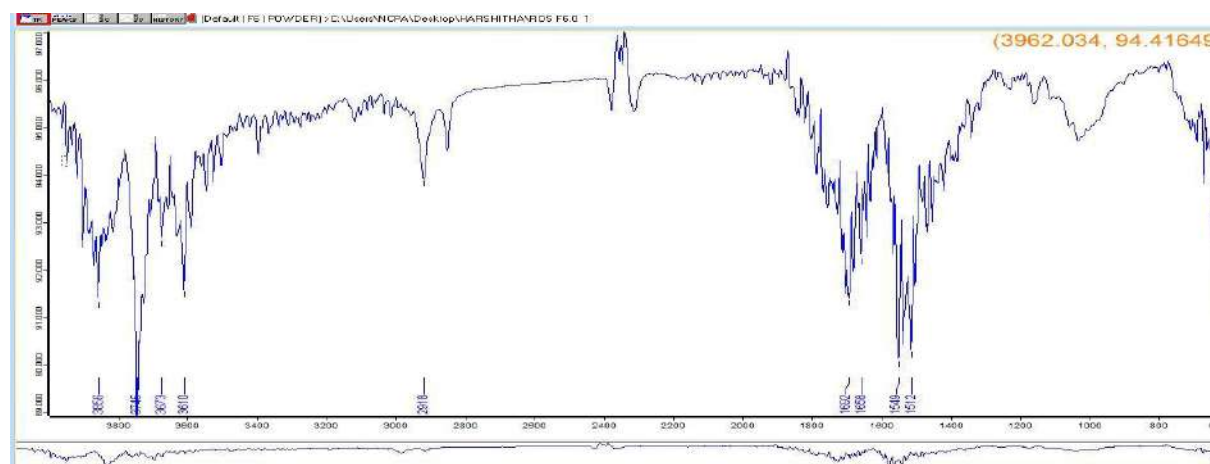


Figure-6.F6 ROSUVASTATIN.

Mouth disintegrating tablets of Rosuvastatin were successfully prepared using Banana powder and Croscarmellose sodium by the direct compression method. Evaluation parameters like hardness⁹, friability⁵, weight variation⁸ and drug content indicate that values were within permissible limit for all formulations. In vitro drug unharshness study was administered and supported the results F-6 was known because the best formulation among all the opposite formulations. The Banana powder used formulations have shown better release profile than compared with other formulations.

SUMMARY AND CONCLUSION

- Suitable analytical method based on UV-Visible spectrophotometer was developed for Rosuvastatin. λ_{max} of 257 nm was known in zero.1N HCL.
- Direct compression method was established to manufacture mouth disintegrating tablets of Rosuvastatin.
- Mouth disintegrating tablets of Rosuvastatin were successfully prepared using Banana powder and Croscarmellose sodium.
- In the present study, mouth disintegrating tablets were prepared by direct compression method.
- Evaluation parameters like hardness, friability, weight variation and drug content indicate that values were at intervals permissible limit for all formulations.
- In vitro drug release study was carried out and based on the results; F-6 was identified as the best formulation among all the other formulations and In vitro release profiles was 99% within 30 minutes.
- The Banana powder used formulation has shown better release profile than compared with other formulations.
- The mouth disintegrating tablets of Rosuvastatin developed in this investigation releases drug within 30 minutes. Thus, we are able to achieve our objective of preparing mouth disintegrating tablets of Rosuvastatin with minimum excipients and simple method of manufacture.

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MODERN BIOMATERIALS FOR ORTHOPAEDIC IMPLANTS

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ABSTRACT

At present, strong requirements in orthopedics are still to be met, both in bone and joint substitution and in the repair and regeneration of bone defects. In this framework, tremendous advances in the biomaterials field have been made in the last 50 years where materials intended for biomedical purposes have evolved through three different generations, namely first generation, second generation and third generation. With the increasing use of orthopaedic implants worldwide, there continues to be great interest in the development of novel technologies to further improve the effective clinical performance of contemporary treatment modalities, devices. Continuing research interest also exists in developing novel bulk biomaterials (e.g., polycarbonate urethanes, silicon) or novel formulations of existing but less widely used biomaterials (e.g., poly aryl ether ketones, poly ether ketone). In terms of tissue engineering, more recent developments have focused on basic engineering and biological fundamentals to use cells, signalling factors, and the scaffold material itself to better restore tissue and organ structure and function. There has also been recent controversy with the use of injectable as a nonsurgical approach to treat joint disorders, but more attention is being directed toward the development newer formulations with different molecular weights. The industry has also continuously sought to improve coatings to supplement the function of existing implants, with the goal of improving their Osseo integrative qualities and incorporating antimicrobial properties. These include the use of bone morphogenetic protein, bisphosphonates, calcium phosphate, silicon nitride, and iodine. Further development of bioactive glass, ceramic materials, and porous titanium particles.

KEYWORDS: *coatings, scaffolds, bio absorbable, bone graft, injectable*

INTRODUCTION

With the increasing use of orthopaedic implants worldwide, there continues to be great interest in the development of novel technologies to further improve the effective clinical performance of contemporary treatment modalities and devices. The design of an orthopaedic device includes aspects of the bulk material and coatings. Novel bulk biomaterials or novel formulations of existing biomaterials are being considered to improve their wear characteristics and longevity, as well as interaction with the surrounding biological environment. There is also growing focus on customizing the material properties of bioabsorbables and composite materials with fillers for non-permanent devices. Non-permanent devices may also include the use of cells, signalling factors, or scaffold material to better restore tissue function.

Poly aryl ether ketones

Poly aryl ether ketones (PAEKs) have been increasingly used as biomaterials for orthopaedic, trauma, and spinal implants, following confirmation of their biocompatibility in the 1980s.¹ PAEKs are a family of high-temperature thermoplastic polymers that contain an aromatic backbone molecular chain with interconnected ketone and ether functional groups. Growing interest in this family of polymers was originally due to the development of "iso elastic" hip stems and fracture fixation plates with stiffness properties comparable to bone.

Polycarbonate urethanes

Polyurethane (PU) biomaterials have been explored for 2 decades for their potential as compliant orthopaedic-bearing materials. They have lower modulus values than UHMWPE and have been hypothesized to operate under a micro elastic hydrodynamic lubrication regime, which leads to reduced

wear.¹ Third generation PU biomaterials, called segmented polycarbonate urethanes (PCUs), have improved oxidative stability relative to poly(ether urethanes

Silicon

Silicones, which are synthetic polymers, comprised of silicon (Si), oxygen, and frequently carbon and/or hydrogen, are widely used in health care and are also of interest in orthopaedic applications. Silicones are traditionally known for their properties of biocompatibility and biodegradability². The most common orthopaedic applications of silicone are hand and foot joint implants, such as the silicone finger joint implants developed by Swanson. Similar implants were developed for the foot and hand. Even now, silicone remains the most prevalent type of small joint implant.

Bio absorbable

The applications of bio absorbable implants in orthopaedics are largely derived from the need to eliminate implant removal operations³. As a newer technology in orthopaedic surgery, bio absorbable is still frequently changing and evolving. Effort in bio absorbable research has focused on developing new materials with fewer adverse effects. The first study of implantable bio absorbable materials was performed by Kulkarni et al,² who studied the biocompatibility of poly-L-lactic acid (PLLA) in animals (guinea pigs and rats) and found that PLLA was nontoxic and gradually degradable (Figure 1) are also widely in use.



Figure 1 A variety of bio absorbable implants for use in spine applications.

Copolymers

PLA and PGA have been widely used among orthopaedic surgeons, and most commercially available implants are made from these two materials and their copolymers. However, recently, other bio absorbable materials such as poly (Orto esters), poly (glycol ide-*co*-tri methylene carbonate), poly (p-dioxanone), poly(ϵ -caprolactone) (PCL), poly(b-hydroxybutyrate) (PHB), and PHB hydroxyvaleric acid have come into use. One of the most popular copolymers currently in use, particularly in oral and maxillofacial surgery, is poly-L/D-lactide 70/30 both in simple and self-reinforced forms. However, some concerns exist with these copolymers as materials for bio absorbable implants⁶.

Scaffolds Bone

The most effective biomaterials for bone regeneration are bio ceramics; two of the most commonly used materials are HA and TCP. To improve the efficacy of HA Cap scaffolds used in bone tissue engineering, several different approaches have been studied, such as modification of scaffold chemistry, seeding of bone marrow stem cells, and incorporation of growth factors, such as transforming growth factor- β (TGF- β), bone morphogenetic protein (BMP), and vascular endothelial growth factor (VEGF) into the scaffold⁵.

Cartilage

Complete repair of cartilage is extremely difficult to achieve because it has a minimal vascular network and has little metabolic activity. There are inherent difficulties associated with current treatments such as viscosupplementation, chondrocyte transplantation, and the use of auto grafts or allografts, therefore, the use of scaffolds for cartilage tissue engineering has gained traction.¹ Many of these scaffolds rely on surface modification techniques to promote functionality. For example, various surface peptides have been cross-linked to PCL or polyethylene oxide/chitosan-based scaffolds to enhance mesenchymal stem cell recruitment, improve adhesion and proliferation of chondrocytes, and stimulate chondrogenesis with enhanced quantities of glycosaminoglycan's and collagen both in vitro and in vivo.

Spinal cord

Spinal cord injury severity depends on the type and intensity of the injury. The primary mechanical injury leads to damage of nerve fibre pathways in the white matter, while secondary degeneration includes apoptosis, bleeding, ex cito toxicity, free radical production, inflammation, ischemia, oedema, scarring, and cystic cavitation, which all also contribute to tissue loss. Other natural processes such as intervertebral disc degeneration can also lead to back pain.

Coatings

The industry has continuously sought to improve coatings to supplement the function of existing implants, with the goal of improving their Osseo integrative qualities and incorporating antimicrobial properties. The following should be considered for these coatings: 1) biocompatibility, 2) osteoconductive abilities, 3) osteoinductive abilities, and 4) adequate mechanical strength of the coating–implant interface.

BMP coatings

Biological coatings with growth factors, such as TGF- β 2 and BMP-2, have been incorporated on metallic implants to help improve their osteoinductivity. TGF- β helps stimulate chemotaxis and promotes the proliferation of osteoprogenitor cells and osteoblasts, while BMP-2 (which is normally secreted by osteoblasts and osteoprogenitor cells) facilitates osteoblastic differentiation of mesenchymal stem cells. Of these, BMP is the most commonly used growth factor to enhance Osseo integration of metallic implants, and other advanced coatings that incorporate BMP into various biomaterials and orthopaedic applications may also be able to take advantage of its osteoinductive properties.

Bisphosphonates

Bisphosphonates are commonly used to treat osteoporosis by inhibiting osteoclastic bone resorption and promoting net bone deposition. The use of bisphosphonates as an implant coating has also been explored via an interposing layer of CaP or fibrinogen. Some researchers have suggested that the relative effectiveness of the bisphosphonate-loaded coatings is dependent on the type of bisphosphonate based on a rat study. The effectiveness of bisphosphonate-loaded implants may not only be limited to an osteoporotic bone environment as these types of implants have been shown to provide improved Osseo integration in non-osteoporotic healthy bone in animal studies. Bisphosphonates released from surface coatings appear to be a viable method to increase per prosthetic bone density and improve overall implant stability.

Calcium phosphate

CaP-like HA constitutes about 50%–60% weight of bone and also forms an integral part of natural apatite bone minerals, thus providing a natural choice as a coating material. CaP-based coatings can exist in various phases depending on the concentration of soluble CaP. Specifically, HA is relatively insoluble, while TCP and brushite are relatively more soluble.¹ Hence, the morphology and chemical composition of the CaP may be modified to maximize its osteoinductive potential. Increased amounts of TCP in TCP–HA combinations can also improve their osteoinductivity due, at least in part, to TCP's ability to introduce pores as it dissolves, as well as to promote bioactive apatite deposits on the coating.

Antimicrobial

Efforts have been made to develop anti-infective biomaterials as a primary means to prevent medical device-associated infections. They may not only possess anti-infective bioactive properties, i.e., serve as a barrier for bacterial adhesion, but may also be employed in the local delivery of antimicrobials or anti-infective medical substances. Antifouling coatings, such as Si₃N₄ and silk sericin-functionalized Ti surfaces, which aim to reduce bacterial adhesion, are undergoing development. While antifouling surfaces have their advantages, these coatings could possibly inhibit tissue adhesion and integration of orthopaedic implant devices that rely on tissue integration. Silver, zinc, and copper, some polymeric materials, such as chitosan and its potentiated derivatives, and various bioactive glasses¹

Bioactive glass

In the late 1960s and early 1970s, Professor Larry Hench developed a new biocompatible material using silica (glass) as a base material that could be mixed with other materials such as Ca to treat bone fractures. Professor Hench's 45S5 Bioglass[®] was the first synthetic material found to chemically bond with bone,

which helped launch the field of bioactive ceramics (or bio ceramics). It is composed of sodium oxide, CaO, phosphates, and silicates and has been in clinical use since 1985. These are minerals that occur naturally in the body (Si dioxide [SiO_2], Ca, sodium oxide [Na_2O], hydrogen [H], and phosphorous [P])

Silicon nitride

Si_3N_4 is currently being introduced as a biomaterial because it is a hydrophilic negative charged ceramic, which means that blood with nutrients and proteins attach to the material, facilitating bone–cell adherence and incorporation of the material in the surrounding bone. Recently, Si_3N_4 has been evaluated as an alternative to existing coated implants. A porous form of Si_3N_4 , cancellous-structured ceramic, has been developed as a non resorbable, partially radiolucent porous structure that can be bonded to orthopaedic implants made of Si_3N_4 to facilitate skeletal attachment. Bone ingrowth into the porous structure was shown as a viable method for achieving skeletal attachment in a sheep model.

Iodine

To reduce the risk of infections, several biomaterial surface treatments, such as iodine coatings, have been proposed. It is believed that iodine-containing surfaces may have antibacterial activity, biocompatibility, and no cytotoxicity. Iodine-supported Ti implants have been compared against stainless steel and Ti implants serving as controls in a rabbit femora study. Fewer signs of infection and inflammatory changes were observed in conjunction with the iodine-support Ti implants in the presence of *Staphylococcus aureus* or *Escherichia coli*. Bone formation was also observed around the iodine-supported Ti and Ti implants, while little osteoid formation was found around the stainless steel implants.

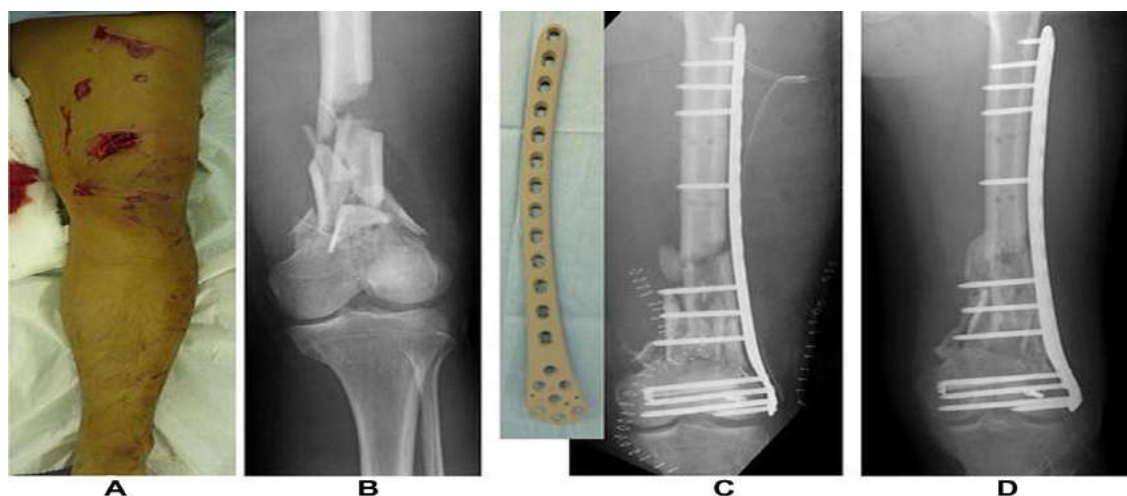


Figure 2 Case of a 42 year-old patient who was treated with an iodine-coated internal fixation plate. Notes: A Gustilo grade IIIa open fracture (A: photograph; B: X-ray) treated with an iodine-supported titanium plate (C). Bony union was established with good callus formation at 4 months' post-surgery, with no signs of infection (D). Reproduced from Tsuchiya H, Shirai T, Nishida H, et al. Innovative antimicrobial coating of titanium implants with iodine. *J Orthop Sci.* 2012.

Bone graft material

Due to the increasing use of bone grafts and the challenges with biological grafts, synthetic bone graft substitutes are expected to play a vital role in bone regeneration. It has been stated that approximately 60% of the currently available synthetic bone graft substitutes incorporate some form of ceramics, such as Ca sulphates, HA, TCP, or combinations thereof. Recent developments in bone substitutes have explored the use of antibacterial or BMPs. On the other hand, limited indications for synthetic bone grafts, along with the drawbacks and potential complications related to the use of allo- and autograft, have facilitated the progress toward a biologic alternative.

Bioactive glass

Because bioactive glass is available in multiple forms such as particulate, pellets, powder, mesh, and cones, it can be used for different applications. Bioactive glasses are composed of SiO_2 , sodium oxide (Na_2O), CaO, and phosphorous pentoxide (P_2O_5). By varying the proportions of sodium oxide, CaO, and Si dioxide, a range of forms from soluble to nonresorbable ones can be prepared.

Ceramic products

Calcium phosphate

CaP ceramics have been used for several decades due to their excellent biocompatibility, bioactivity, osteoconductivity, and mechanical strength. A limitation with current CaP bone graft materials is that they exist in large granular form, which affects its handling ability during surgery. To improve handling ability and to better fill defects of complex geometric shapes, injectable and moldable forms of bone substitute material have been developed recently. An ovine critical-sized femoral condyle defect model has examined the use of silicate-substituted CaP bone substitute materials with various granule sizes.

Collagen–HA

Collagen–HA scaffolds are composed of collagen and HA, which are the two main constituents of bone, making it biocompatible, osteoconductive, osteoinductive, and bio absorbable, with nontoxic degradation products. The combination of these materials also helps offset the limitations in each standalone material. For example, the ductile properties of collagen help to increase the relatively poorer fracture toughness of HA. The addition of collagen to a ceramic structure can also provide other advantages such as shape control and increased particle and defect wall adhesion.

Porous Ti particles

It is believed that porous Ti possess mechanical characteristics favorable for impaction grafting, such as good handling, impactability, and resistance to compressive loading. Porous Ti particles coated with either silicated CaP consisting of a sol–gel coating of HA and TCP crystals (HA:TCP ratio of 60:40) embedded in a silica layer or a coating consisting of carbonated apatite have been examined in an unloaded goat model. The osteoconductive properties of impacted porous Ti with a CaP coating have been shown to be comparable to impacted allograft bone and impacted biphasic ceramics. Under loaded conditions, CaP-coated porous Ti particles have also been evaluated in goats to reconstruct AAOS type-III defects in the acetabulum.

CONCLUSION

There continues to be great interest in developing new biomaterials for orthopaedic implants, using either novel material, altering the formulations of existing materials, or finding new applications for existing materials. Despite the effective clinical performance of contemporary orthopaedic implants, with the expected growth in the use of orthopaedic implants and increasing demands placed on the devices by the patients, improving the performance of the devices through the use of “new” biomaterials may aid in addressing these needs. The ability to translate in vitro evaluations, animal studies, and pilot clinical studies to larger scale use will help determine the viability of many of these biomaterials from a safety and effectiveness standpoint, but also from a commercial standpoint in terms of being able to produce these biomaterials on a large scale and in a cost effective manner.

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RECENT TREATMENT APPROCHES IN TARGETTING MALARIA

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ABSTRACT

Malaria is a parasitic infection of global importance. Although rather common in developed countries, the place the sickness takes place often in travellers WHO have come returned from endemic regions, it stays one of the most widely wide-spread infections of human world wide.in endemic regions, malaria may be a important rationalization for morbidity and mortality and creates massive social and monetary burdens. Current efforts to control malaria center of attention on lowering attributable morbidity and mortality. Targeted chemoprophylaxis and use of insecticide-treated mattress nets are palmy in some endemic areas. Prompt analysis of the febrile somebody, a high degree of suspicion of malaria, rapid and accurate diagnosis, and appropriate anti-malarial remedy are indispensable so as to optimize clinical outcomes of contaminated patients. Additional processes to malaria control, along with genetic manipulations of mosquitoes and malarial vaccines, are areas of on-going research. The social and financial burden of malaria in endemic countries is immense. Already a ailment of the poor, malaria similarly contributes to monetary woes by using debilitating people afflicted with the disorder and imposing sizeable financial costs on affected human beings and governments. A calculable US\$12 billion in monetary income is misplaced yearly in Africa due to protozoal infection.

KEYWORDS: *parasitic infection, chemoprophylaxis, genetic manipulations*

INTRODUCTION

Nearly a decade has passed considering the fact that the announcement by the planet Health Organization (WHO) and consequently the Bill & Melinda Gates Foundation of the long-run goal of wipe-out of protozoal infection. Use of existing scientific useful resource over that quantity has helped power a profound minimize in mortality and morbidity of sixty and thirty seventh compared thereto at the starting of the Millennium¹. Clear 'road maps' on the types of current drug treatments have presently been referred to with the aid of the protozoal contamination neighborhood², related an formidable purpose of an greater ninetieth reduction in morbidity and mortality set with the aid of the WHO³. Four years past, a thought was seen for the patterns of molecules [target candidate profiles (TCP)] and medicines [target product profiles (TPP)] required⁴, putting clear desires for complete spanking new clinical care.

Table 1. precis of recently outlined TPPs andTCPs
Overview of newly described TPPs and TCPs

Profile	Intended use
TPP-1	Case management; treatment of acute uncomplicated malaria in children or adults. Uses a combination of two or more molecules with TSP-1 activity, plus TSP-5 for reducing transmission and TSP-3 for relapse prevention, when such molecules become available For severe malaria, a parenteral formulation of a single fast-acting TSP-1 would be appropriate
TPP-2	Chemo protection: given to subjects migrating into areas of high endemicity, or during epidemics. Uses a combination of TSP-4 activity, potentially with TSP-1 support for emerging infections
TSP-1	Molecules that clear asexual blood-stage parasitemia
TSP-2	(profile retired, see body of text)

Profile	Intended use
TSP-3	Molecules with activity against hypnozoites (mainly <i>P. vivax</i>)
TSP-4	Molecules with activity against hepatic schizonts
TSP-5	Molecules that block transmission (targeting parasite gametocytes)
TSP-6	Molecules that block transmission by targeting the insect vector (endectocides)

Malaria is an infectious disease, and resistant parasite lines will usually emerge, requiring the chronic era of new molecules.⁵

Insights into new drug treatments for malaria indications

The ideal drug projected in 2011 was one encounter radical therapy and post-treatment bar (SERCaP).² This would incorporate a minimal of 2 energetic molecules, preventing the emergence of resistance in blood schizonticides. (The post-treatment bar is assumed to be for the most phase from the blood schizonticides preventing new infections, then the time period is simplified to SERC). Over the remaining 4 years there has been increasing readability on the function of various categories of drugs in elimination and wipe-out, each from WHO³ and the UN Special Envoy for Malaria's Aspiration to Action, that is summarized

The single-exposure therapy dilemma: effectuality versus security within the wider population

Compounds that square measure utilized in combination to affect a single-encounter radical remedy must be distinctly amazing and very nicely tolerated. When imparting the entire dose into one administration with extended length of high-quality exposure, there is an enlarge in the maximum plasma concentration, which places extra protection constraints

The need to prioritise chemical scaffolds and combos that don't seem to be vulnerable to resistance generation

Although one will rank compounds supported their potential to get resistant mutants in vitro, the understanding of how this interprets to scientific resistance and the transmission of resistance is too limited to be used for choice making. Recent research with compounds from the open-access protozoal infection Box have recognised many chemo types in opposition to that resistance cannot be generated inside the laboratory.⁶

Compounds ideally have to be compelled to goal pretty one Plasmodium life-cycle stage

Although most of this portfolio was in the beginning recognized from screening towards nonsexual blood schizonts, many compounds have been shown to have recreation in mobile assays for transmission-blocking (preventing the production of stage V gametocytes) similarly as for chemo safety (inhibition of growing inside organ schizonts). Any new mixture ought to both have transmission-blocking effectuality itself, or be well matched with low-dose (0.25 mg/kg) primaquine, which is the WHO-approved regimen for transmission blockade. Considering the relapse of dormant sporozoan or Plasmodium ovale, no new scaffolds past 8-aminoquinolines have been recognized with anti-hypnozoite activity and potential for development. In medical test studies, the 8-aminoquinoline tafenoquine has demonstrated high endeavor as a single dose.⁷

Insights into new medicines for reducing the incidence of protozoal contamination cases

The sizeable discount within the incidence of typical protozoal contamination over the last fifteen years has been executed by shielding the at-risk populations, particularly with insecticide-treated mattress nets and spraying with insecticides and larvicides. Vaccination represents another attainable strategy, and even though recent consequences with the RTS, S immunizing agent square measure encouraging, they're at present eliminated from the extent needed to force wipe-out alone.⁸

Chemo protection

A SEC pills would optimally be a mixture of 2 compounds, with minimal suited profile of causal liver stage activity, plus workable advantage from recreation in opposition to asexual blood stages, which want now not

be fast-onset. The definition of this TPP, TPP-2, used to be conceptually engineered around atovaquone-proguanil (causal liver stage activity, and a profile tolerance for gradual onset of action towards apomictic blood stages).

Chemo prevention

Chemoprevention is described specially in the context of current wondering on SMC¹⁰ and related strategies. SMC is in modern times outlined due to the fact the intermittent administration of complete package complete kit and boodle guides of an anti-malarial capsules during the protozoal infection season, to prevent malarial illness. The objective is to take care of preventative anti-malarial drug concentrations within the blood in the course of the quantity of best protozoal contamination risk.

Severe malaria

In severe protozoal infection, sufferers are regularly unconscious or in any other case too ill to take oral medication. At this stage of disease, the sequestration of massive numbers of parasites in the Microvasculature of the Genius leads to intelligence swelling, and in some instances affecting the lungs and choice very necessary organs, which may additionally swiftly lead to death, whereas in young youth the foremost complications may additionally result from severe anaemia. Any new pills would for that reason ought to free up the microvasculature, either by means of disengaging or killing the parasites; the drug's speed of onset of exercise is consequently of paramount concern.¹¹

Linking the TPPs to man or woman molecules and TCPs

Both high-level TPPs need a combination of organic activities, and every of these can be described via a TCP. The significance is that one molecule are capable to do over one communications protocol, for instance having vegetative blood-stage activity (TSP-1) and transmission interference (TSP-5).

Insights into the evolution of TCPs

Proposed adjustments to the nomenclature

Although this review's description of TCPs helped increase a common language in the improvement of leads and preclinical candidates, there are many areas where it can be improved.

TSP-4: general considerations	Minimum essential	Ideal
Dosing regimen; adult/pediatric dose	Oral, once per week; <500 mg/<100 mg in infants. Simple oral formulation Injectable: subcutaneous or intra-muscular, once per month, with injection volumes <0.5 ml for infants via a 25 gauge or smaller needle	Oral, once per month; <100 mg, Injectable: subcutaneous or intramuscular once per 6 months
Susceptibility to loss of efficacy due to acquired resistance	No fit drug-resistant parasites identified in controlled human challenge model; no cross-resistance with partner drug in combination	Very low; no cross-resistance with partner drug in combination; independent mechanism to those of treatments used in geographical area

TSP-3: concentrated on Plasmodium hypnozoites

The definition of a radical therapy is the removal of all types of the parasite from a contaminated individual, no longer simply the asexual ranges that purpose symptoms. Whilst this definition covers all non-asexual blood stages, the focus of TSP-3 is on the shape that is the hardest to kill: the dormant liver stages that follow new P. vivax and P. ovale infections.

TSP-4: focused on hepatic schizonts

The terminology here has frequently been confusing, and it is important to repeat the definitions made earlier. Chemo protection, as part of the SEC, is the use of drug treatments to protect subjects getting into high-transmission zones from a place besides malaria transmission, or to defend those in malaria-free areas in risk of epidemics inside the remaining tiers of protozoal contamination demolition. (This is awesome from chemoprevention, as in SMC, which is the use of full courses of blood schizonticides to prevent medical malaria in populations that live in endemic areas). This assessment uses the time period TSP-4 endeavor to explain interior organ schizonticide activity.

TSP-5: transmission blocking

One of the key points anywhere the parasite lifecycle may be broken is to stop transmission from the infected human host to the two-winged bugs vector. ACT does no longer block transmission, so the modern WHO recommendation⁹ is to use a single, low dose of 0.25 mg/kg primaquine to decrease transmissibility of dealt with *P. falciparum* infections in low-transmission areas.

Direct impact on the insect vector (potential TSP-6)

Studies with the endectocide ivermectin, used to deal with onchocerciasis (river blindness), lymphatic filariasis and strongyloidiasis, have verified a enormous effect on these parasites as nicely as the morbidity and mortality of insects feeding on topics with plasma concentrations of the drug.

Portfolio concerns for choosing aggregate components

One of the most difficult problems with managing the portfolio is navigating the maze between the early research (up to medical proof of notion in volunteers or patients) and the later stages, the place compounds are used exclusively in combination. Selection of that molecules to mix, and how, depends in the major on 4 factors: effectivity, safety, propensity to get resistance, and ability to be co-formulated:

Table 3
Success quotes (%) in improvement 2009–2014 for MMV in contrast to benchmark data, by phase

S.NO	MMV				CMR ^a		PBF ^b	
	Excluding LCM ^c		Including LCM		Per phase	Cumulative	Per phase	Cumulative
	Per phase	Cumulative	Per phase	Cumulative				
Preclinical	50	8	50	14	60	5	40	3
Phase I	70	16	70	27	56	9	54	7
Phase II A	75	23	78	39	36	16	34	13
Phase II B	60 ^d	30	75	50	60 ^d	45	60 ^d	38
Phase III	50	50	67	67	84	75	70	64
Registration	100	100	100	100	89	89	91	91

LCM: Life cycle management; these are the drugs that had been delivered into the MMV portfolio when it was already clear that they are nicely tolerated and effective, but the project was once to generate new formulations or co-formulations

CONCLUSION

The shift from control to removal of malaria set ambitious desires for treatment and chemoprevention of malaria, at a time when solely a handful of new molecules entered preclinical development over the previous decade. The final four years has considered the persevered arrival of new molecules, a deepening perception of the molecules in the pipeline, a extra profound understanding of how to mix these molecules, and, of course, classes learned from attrition in the pipeline. The use of early clinical research permitting for longer term follow-up (to 28 days) or managed human malaria contamination research lets in characterization of

the key performance parameters such as the PRR and the MIC and MPC for every molecule, and in combination.

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SP-156

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY FOR SIMULTANEOUS DETERMINATION OF EMTRICITABINE, BICTEGRAVIR AND TENOFOVIR ALAFENAMIDE IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC.

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ABSTRACT

A simple, accurate, quick, economical, precise method was developed for the simultaneous estimation of the Emtricitabine, Bictegravir and Tenofovir Alafenamide in solid dosage form by using RP-HPLC. Chromatogram was run through BDS C8 150x4.6mm, 5 μ . Mobile phase was composed with Buffer and Acetonitrile in the ratio of 60:40 v/v was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.01N KH₂PO₄ buffer at PH adjusted to 3.5 with dil. Orthophosphoric acid solution.. Temperature was maintained at 30°C. Optimized wavelength for Emtricitabine, Bictegravir, and Tenofovir Alafenamide was 272.0 nm. Retention time of Emtricitabine, Bictegravir and Tenofovir Alafenamide were found to be 2.396 min, 3.426min and 4.263 min. %RSD of method precision for Emtricitabine, Bictegravir and Tenofovir Alafenamide were found to be 0.5 , 0.6 and 0.6 respectively. % recovery was Obtained they were 99.97%, 100.03% and 99.89% for Emtricitabine, Bictegravir and Tenofovir Alafenamide respectively. LOD, LOQ values are obtained from regression equations of Emtricitabine, Bictegravir and Tenofovir Alafenamide were 0.21ppm, 0.24ppm, 0.47ppm and 1.43ppm, 0.42ppm, 1.28 ppm respectively. Regression equation of Emtricitabine was $y = 8064.x + 1063$, Bictegravir was $y = 8047x + 2253$ and of Tenofovir Alafenamide was $y = 8145.x + 2172$. The developed method was optimum, precise, economical and it can be used for industrial purpose for estimation of Emtricitabine. Bictegravir, Tenofovir Alafenamide in combined dosage forms.

KEYWORDS: *Emtricitabine. Bictegravir, Tenofovir Alafenamide, RP-HPLC*

INTRODUCTION

The analytical techniques were developed to determine the exact amounts of the identified substances. A simple, accurate, precise method was developed for the simultaneous estimation of the Emtricitabine, Bictegravir and Tenofovir Alafenamide in solid dosage form¹. There are RP-HPLC methods where two drugs or combination with other drugs are there for above drugs². An attempts was made to develop simultaneous estimation of Emtricitabine, Bictegravir and Tenofovir Alafenamide by RP-HPLC³.

MATERIALS AND METHODS

Experimental procedure: All the materials and solvents used were from Sigma Aldrich and they are AR/LRgrade. Drug sample were obtained from Spectrum pharma research solutions pvt Ltd. The instruments used were Waters HPLC2695 series with quaternary pumps,

Sample Processing

Diluents: Based up on the solubility of the drug diluents was selected, the drugs were primarily dissolved in methanol and diluted with Acetonitrile and Water.

Preparation of Standard stock solutions: 50mg of Emtricitabine, 12.5 mg of Bictegravir and 6.25mg of Tenofovir Alafenamide were weighed and transferred into three 25ml volumetric flasks separately. 10ml of

diluent was added to flasks and sonicated for 20mins. Flasks were made up with water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3. From the standard stock solutions the remaining solutions such as standard working solution, sample stock solution, sample working solution were prepared and 0.1% OPA buffer, 0.01N KH_2PO_4 Buffer is also prepared.

RESULTS AND DISCUSSION

Method development: Method development was performed by changing various columns, mobile phase ratios, buffers and its pH etc. Emtricitabine, Bictegravir and Tenofovir Alafenamide were eluted at 2.396 min, 3.426min and 4.263 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated Fig no:4. Drugs were eluted with good retention time, resolution and all the system suitable parameters like Plate count and Tailing factor were within the limits. The optimum parameters were in the table no:1, fig 1.

Validation: System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Emtricitabine, Bictegravir and Tenofovir Alafenamide and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results were not more than 2%.

Specificity: Checking of the interference in the optimized method. There is a no interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Plate count of the Emtricitabine was 3974 ± 136 , Bictegravir was 5418 ± 136 , and of Tenofovir Alafenamide was 6402 ± 136 , tailing factor of Emtricitabine was 1.37 ± 0.3 , Bictegravir was 1.31 ± 0.2 , and of Tenofovir Alafenamide was 1.24 ± 0.2 , resolution between Emtricitabine and Bictegravir was 8.0 and resolution between Bictegravir and Tenofovir Alafenamide was 3.3.. All the system suitable parameters were passed and were within the limits Table no:2. Other validation parameters such as accuracy, precision, linearity (fig no 2,3,4), robustness, LOD, LOQ are studied the results are with the specified limits. The % Recovery for each level should be between 98.0 to 102. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

Assay: Biktarvy (200+50+25) the label claim Bictegravir 200mg, Emtricitabine 50mg, Tenofovir Alafenamide 25mg per unit formulation, Assay was performed with the above formulation. Average % Assay for Emtricitabine, Bictegravir and Tenofovir Alafenamide. Obtained was 99.82%, 99.95% and 100.48% respectively.table no2.

Degradation studies: Degradation studies were performed under various conditioned such as acid, base, degradations. Oxidation by hydrogen peroxide, thermal degradation, UV, neutral degradation.(table no 3,4,5)

Table 1. Optimised conditions

Column Used	BDS C8 150 x 4.6 mm, 5μ .
Buffer	0.01N KH_3PO_4
Mobile phase	buffer: Acetonitrile (60:40 v/v)
Flow rate	1.0ml/min
Diluent	First dissolved in Methanol and made up with water (50:50 v/v).
Wavelength	272.0 nm
Temperature	30°C
Injection Volume	10 μl

Table 2. Assay data

S.NO	Assay Data of Emtricitabine	Assay Data of Bictegravir	Assay Data of Tenofovir Alafenamide
1	99.35	99.32	99.97
2	99.38	100.59	100.50
3	99.93	100.17	101.01
4	100.47	100.75	99.64
5	99.60	99.63	100.70
6	100.19	99.24	101.05
Avg	99.82	99.95	100.48
Stdev	0.454	0.65	0.568
%RSD	0.5	0.6	

Table 3. Degradation Data of Emtricitabine

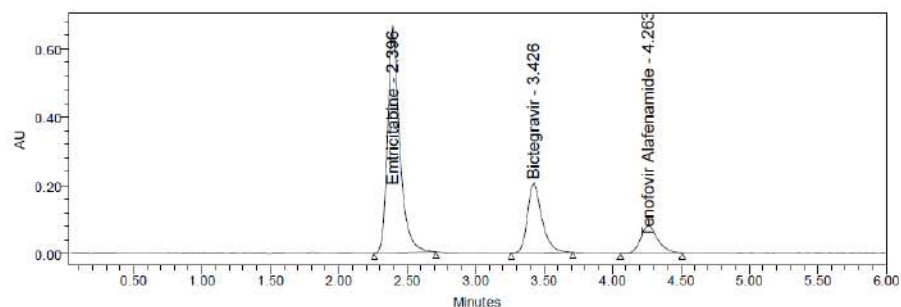
S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.20	0.839	1.514
2	Alkali	4.10	0.687	0.822
3	Oxidation	3.54	0.778	1.486
4	Thermal	2.68	0.658	1.426
5	UV	1.31	0.832	1.411
6	Water	0.92	0.773	1.417

Table 4. Degradation Data of Bictegravir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.96	0.353	0.514
2	Alkali	4.79	0.634	0.719
3	Oxidation	3.66	0.411	0.474
4	Thermal	2.46	0.299	0.413
5	UV	1.69	0.339	0.404
6	Water	0.96	0.298	0.397

Table 5. Degradation Data of Tenofovir Alafenamide

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.68	0.510	0.749
2	Alkali	5.95	0.528	0.754
3	Oxidation	4.75	0.446	0.683
4	Thermal	2.64	0.379	0.592
5	UV	1.75	0.331	0.568
6	Water	0.54	0.367	0.556

**Figure 1. Optimized chromatogram**

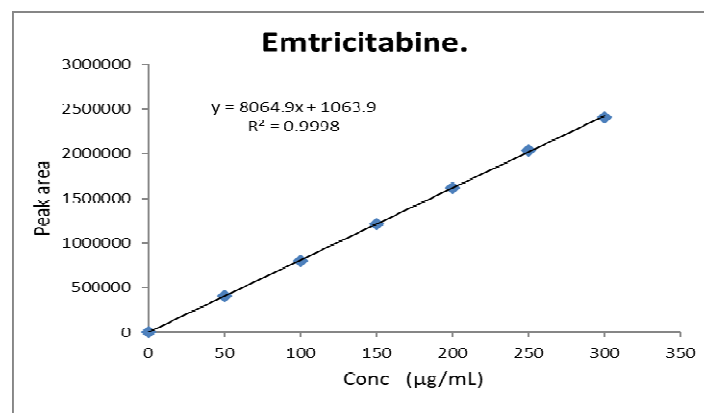


Figure 2. Calibration curve of emtricitabine

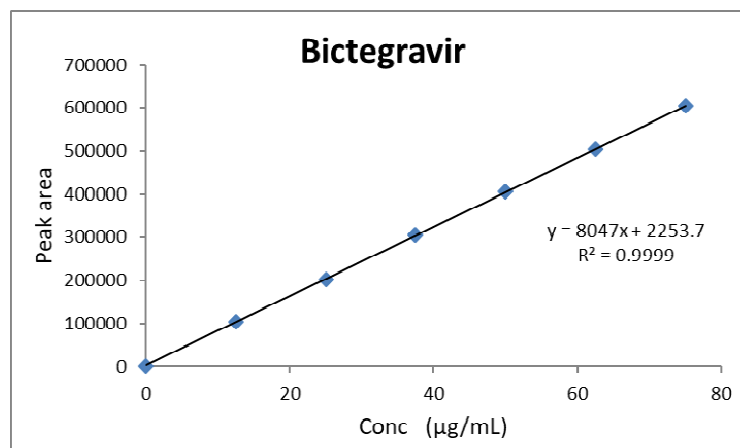


Figure 3. Calibration curve of Bictegravir

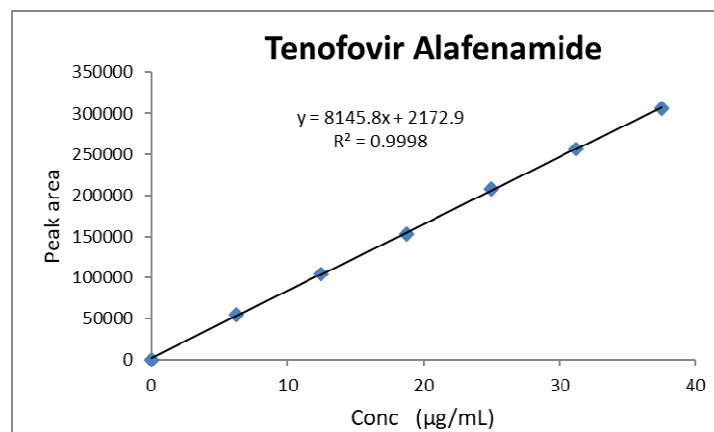


Figure 4. Calibration curve of Tenofovir

CONCLUSION

Emtricitabine, Bictegravir and Tenofovir Alafenamide were estimated by using RP-HPLC method in Tablet dosage form. Retention time of Emtricitabine, Bictegravir and Tenofovir Alafenamide were found to be 2.396 min, 3.426 min and 4.263 min. %RSD of system precision for Emtricitabine, Bictegravir and Tenofovir Alafenamide were and found to be 0.5, 0.5 and 0.7 respectively. %RSD of method precision for Emtricitabine, Bictegravir and Tenofovir Alafenamide were and found to be 0.5, 0.6 and 0.6 respectively. % recovery was obtained as 99.97%, 100.03% and 99.89% for Emtricitabine, Bictegravir and Tenofovir Alafenamide respectively. LOD values were obtained from regression equations of Emtricitabine, Bictegravir and Tenofovir Alafenamide were 0.21ppm, 0.47ppm, 0.42ppm and LOQ values are obtained from regression equations of Emtricitabine, Bictegravir and Tenofovir Alafenamide were 0.24ppm, 1.43ppm

,1.28 ppm respectively. Regression equation of Emtricitabine was $y = 8064.x + 1063$. Bictegravire was $y = 8047x + 2253$ and of Tenofovir Alafenamide was $y = 8145.x + 2172$. Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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SP-157

DUAL ACTING DRUG FOR OSTEOPOROSIS

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ABSTRACT

Osteoporosis is a massive and growing public ill health. Once considered an inevitable consequence of ageing, it's currently eminently preventable and treatable. Ironically, despite tremendous therapeutic advances, there is an increasing treatment gap for patients at high fracture risk. Bone fracture could be a continual concern for individuals with osteoporosis. Until recently, osteoporosis patients were treated with either ANTIRESORPTIVE or ANABOLIC MEDICATION. ANTIRESORPTIVE MEDICATIONS facilitate slow bone loss although they do not stimulate bone formation. ANABOLIC MEDICATIONS stimulate bone formation although they can stimulate bone resorption additionally a replacement dual-acting drug, ROMOSUZUMAB, combines the advantages of earlier medication, each increasing bone formation and decreasing bone resorption. ⁹Approved by the FDA agency in 2019, ROMOSUZUMAB builds additional bone than any previous osteoporosis drug. Studies showed that ROMOSUZUMAB stimulates new bone formation on trabecular and cortical bone surfaces by stimulating osteoblastic activity, resulting in increase in trabecular and cortical bone mass and enhancements in bone structure and strength. One dose of the drug consists of 2 injections and also the injection solution consists of dosage 105/1.17ml, one instantly following the other, given once a month by a healthcare professional. Medical aid is interrupted when twelve doses (one year of treatment) as a result of the bone-forming impact of drug decreases. If osteoporosis therapy is required after the twelve doses, patients ought to begin an osteoporosis treatment that reduces bone breakdown. ^{1a}

KEYWORDS: *Romozumab, Osteoporosis, Anabolic, Antiresorptive Medication, Post-Menopausal Woman.*

INTRODUCTION

Osteoporosis causes bones to become weak and brittle .So brittle that a fall or maybe delicate stresses like bending over or coughing will cause a fracture. Osteoporosis-related fractures most ordinarily occur within the hip, wrist or spine. Bone is living tissue that's perpetually being dampened and replaced. Osteoporosis occurs once the creation of new bone does not sustain with the loss of old bone. Osteoporosis affects men and women of all races. Oestrogen deficiency during menopause increases osteoclast activity, increasing bone restoration more than formation. Men are at lower risk when compared to women for developing osteoporosis and osteoporotic fractures because of larger bone size, greater bone mass, increase in bone width with aging, fewer falls and shorter life expectancy .Male osteoporosis occurs from aging and secondary causes. Age- related osteoporosis occurs from hormone, calcium, vitamin D deficiencies leading to osteoblast formation. Drug-induced osteoporosis may result from systemic corticosteroids, thyroid hormone replacement, antiepileptic drugs like phenytoin and phenobarbitone and other agents. There generally aren't any symptoms within the early stages of bone loss. however once your bones are weakened by osteoporosis, you may have signs and symptoms that include: Back pain, caused by a broken or folded vertebra, Loss of height over time, A unerect posture, A bone that breaks way more simply than expected. Things that make osteoporosis more likely include: age, gender, family history, bone structure and body weight, broken bones, ethnicity some drugs tobacco and alcohol.

DIAGNOSIS OF OSTEOPOROSIS

Components of the evaluation include a bone density test, osteoporosis-directed medical history and physical exam, laboratory studies, and possibly skeletal imaging. A bone density test with dual-energy X-ray absorptiometry (DXA) helps with diagnostic classification, assessment of fracture risk, and provides a baseline for monitoring the skeletal effects of treatment. Physical exam may show skeletal deformities due

to unrecognized fractures (e.g., loss of height, kyphosis, or diminished rib-pelvis space), identify possible secondary causes of skeletal fragility (e.g., blue sclera with osteogenesis imperfecta, urticarial pigmentosa with systemic mastocytosis, or bone tenderness with osteomalacia), and help to recognize patients with poor balance and frailty that might lead to falls. Laboratory studies may show potentially reversible abnormalities (e.g., vitamin D deficiency, hypocalcemia, or impaired kidney function) that must be assessed and corrected.

MANGEMENT OF OSTEOPOROSIS

Osteoporosis can be managed with the help of calcium and vitamin D, exercise, and smoking cessation. These components have been examined primarily as preventive measures for Bone Mass Density (BMD) loss and fracture occurrence. Adults older than 50 years are to consume at least 1200 mg/d of calcium, ideally through calcium-rich foods. Calcium supplements should be prescribed if needed to achieve recommended daily levels. The least expensive preparation is calcium carbonate. Adverse effects, such as intestinal gas and constipation, are minimized by taking calcium with food.¹⁰ Calcium citrate causes less frequent gastrointestinal adverse effects and may be better absorbed.⁷ Vitamin D is less widely available in the diet than calcium, and supplements may be necessary to provide the recommended level of 400 to 600 IU/d for all adults older than 50. All women received calcium and vitamin D, but the protein supplement group had less BMD loss at 12 months. Weight-bearing exercise has been associated with an improvement in BMD and a reduction in hip fracture risk in postmenopausal women. Smoking accelerates bone loss, in part due to increased metabolism of oestrogen. Moreover, cigarette smokers tend to be thinner and undergo earlier menopause.^{1d}

PHARMACOLOGIC THERAPY

Most available drugs for treatment of osteoporosis are antiresorptive. They alter the balance of bone remodelling by inhibiting bone breakdown to a greater extent than bone formation, thereby preventing a decline in BMD and decreasing fracture risk.^{1e}

1. **ESTROGEN:** reduces bone turnover by inhibiting osteoclast recruitment and activity. The primary outcome was coronary heart disease, with fractures as a secondary outcome. Hormone therapy was found to reduce the risk of vertebral, hip, and other osteoporotic fractures. While HT is effective in preventing osteoporosis and reducing fracture risk, it can no longer be recommended as a first-line therapy. Hormone therapy should be limited to women with persistent menopausal symptoms or women who are intolerant of other agents for osteoporosis treatment.^{1a,15}

2. **CALCITONIN:** Calcitonin is produced by the C cells of the thyroid gland, and it slows bone loss by inhibiting osteoclast formation and attachment. Injectable salmon calcitonin was approved for therapy of osteoporosis, but was not widely used because of discomfort of subcutaneous injections, high cost, and limited tolerance.^{1a,15}

3. **BISPHOSPHONATES:** Bisphosphonates inhibit osteoclast activity and promote osteoclast death, thereby reducing bone resorption. Alendronate sodium was approved for treatment of postmenopausal osteoporosis because of its positive effect on BMD and a reduction in fracture risk. Risedronate sodium is a bisphosphonate which is also used in osteoporosis treatment. The main adverse effects of the oral bisphosphonates are gastrointestinal, specifically oesophageal irritation.^{1a,15}

4. **SELECTIVE ESTROGEN RECEPTOR MODULATORS:** Raloxifene hydrochloride is a mixed oestrogen receptor agonist-antagonist and the first selective oestrogen receptor modulator to be approved for treatment of post-menopausal osteoporosis. It acts as an agonist in bone, reducing turnover by inhibiting osteoclast recruitment and activity and as an antagonist in the breast and uterus. Raloxifene increases BMD and reduces the risk of vertebral fractures, although to a lesser degree than the bisphosphonates, it is a valuable second-line agent for the treatment of postmenopausal osteoporosis. In addition, raloxifene has been associated with favourable changes in lipids and with a potential decrease in the risk of cardiovascular events and breast cancer.^{1a,15}

5. **PARATHYROID HORMONE:** Produced by the parathyroid glands, PTH normally helps control calcium exchange between the bones and the bloodstream. Although continuous exposure to high levels of

PTH elicits a catabolic response and a decrease in BMD, as seen in primary hyperparathyroidism, intermittent low-dose synthetic human PTH causes an anabolic response, increasing the number and action of osteoblasts. A synthetic form of PTH containing its biologically active portion was used for the treatment of osteoporosis in postmenopausal women and men at high risk for fracture. However, it requires a daily injection, is expensive, and may be associated with bone malignancy. It is indicated for postmenopausal women and men with osteoporosis who are at high risk for fracture, such as individuals with prior osteoporotic fractures.¹⁵

ROMOSUZUMAB FOR THE TREATMENT OF OSTEOPOROSIS: The discovery of sclerostin as a key inhibitor of bone formation was made by groups evaluating patients with 2 rare autosomal recessive syndromes associated with high bone mass². Sclerostosis is a disorder characterized by very high bone mass due to inactivating mutations of the SOST gene on chromosome 17q21, the gene that codes for sclerostin. Excess bone growth during childhood results in frontal bossing, cranial and basilar stenosis, cranial nerve entrapment, and mandibular hypertrophy³. Patients with Van Buchem disease, a somewhat less severe disorder, have a separate noncoding deletion of a gene required for normal transcription of the SOST gene.⁴ Heterozygous cases of both disorders have moderately high bone mass without other phenotypic or clinical features. Sclerostin is most highly expressed in osteocytes.⁵ Binding of sclerostin to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) prevents activation of canonical Wnt signalling in bone, resulting in decreased bone formation. These findings stimulated interest in exploring the potential of antisclerostin therapy as a strategy to increase bone formation and to restore skeletal architecture in patients with osteoporosis.¹⁵

PHARMACOLOGY OF ROMOSUZUMAB: Absorption: Peak plasma time: 5 days, Peak plasma concentration: 22.2 mcg/mL. AUC: 389 mcg·day/mL. Steady-state achieved by 3 months following monthly injections. Development of antibodies to romosozumab led to decreased mean concentrations up to 22%.

Distribution: Volume of distribution: ~3.92 L

Metabolism: IgG2 expected to be degraded into small peptides and amino acids via catabolic pathways similar to endogenous IgG

Elimination-Half-life: 12.8 days, Clearance: 0.38 mL/hrs./kg 10%

DOSAGE AND STRENGTH: Injectable solution: 105mg/1.17mL (single-use prefilled syringe). Osteoporosis: Indicated for osteoporosis treatment in postmenopausal women at high risk for fracture, defined as a history of osteoporotic fracture or multiple risk factors for fracture; also indicated for patients in whom other available osteoporosis therapy has failed or who are intolerant of other available osteoporosis therapy-210 mg SC Month x 12 month^{6f}.

NOTE: Adequately supplement patient with calcium and vitamin D during treatment

Dosage Modifications: Renal impairment: No dose adjustment required. Severe renal impairment (eGFR 15-29 mL/min/1.73 m²) or receiving dialysis: Greater risk of developing hypocalcemia; monitor calcium concentrations and adequately supplement calcium and vitamin D in this patients.^{1d}

Dosing Considerations: Limitations of use: Anabolic effect wanes after 12 monthly doses; therefore, duration of use should be limited to 12 monthly doses. If osteoporosis therapy remains warranted, consider continued treatment with an antiresorptive agent.

STORAGE: Refrigerate at 2-8°C (36-46°F) in the original carton to protect from light. Do not freeze. Do not shake. If removed from refrigerator, can be kept at room temperature up to 25°C (77°F) in original carton and must be used within 30 days; if not used within 30 days, discard. Do not expose to temperatures >25°C (77°F)

ADVERSE EFFECT: Arthralgia, Headache, Hypersensitivity, Injection site reactions, Muscle spasms, Asthenia, Peripheral oedema, Neck pain, Insomnia, Hypocalcemia

MAJOR ADVERSE CARDIAC EVENT (MACE): Study 1: Myocardial infarction (MI), Stroke, Cardiovascular (CV) death. Study 2: MI, Stroke, CV death.^{1d}

OTHER SEVERE ADVERSE EFFECTS: Osteonecrosis of the jaw (ONJ), atypical femoral fracture^{7f}

Black Box Warnings

May increase the risk of MI, stroke, and CV death.

Do not initiate in patients who have had an MI or stroke within the preceding year.

Consider whether the benefits outweigh the risks in patients with other CV risk factors.

If a patient experiences an MI or stroke during therapy, discontinue drug immediately.

Table 1. Contraindication

Contraindications: Hypocalcemia; pre-existing hypocalcemia must be corrected before initiating, Systemic hypersensitivity (e.g., angioedema, erythema multiforme, urticaria).

Cautions: A higher rate of MACE, a composite endpoint of CV death, nonfatal MI, and nonfatal stroke was observed in a randomized controlled trial in postmenopausal women treated with romosozumab compared with alendronate.^{1a,f} Hypersensitivity reactions reported including angioedema, erythema multiforme, dermatitis, rash, and urticaria; discontinue drug and initiate appropriate treatment if anaphylaxis or other clinically significant allergic reaction occurs.^{1a,f} Hypocalcaemia reported; correct hypocalcemia before initiating; monitor for signs and symptoms of hypocalcemia; ensure adequate supplementation with calcium and vitamin D during therapy. Atypical low-energy or low-trauma femoral shaft fractures reported; many patients report prodromal pain in the affected area, usually presenting as dull, aching thigh pain, weeks to months before a complete fracture occurs.²⁴

CONCLUSION

ROMOSUZUMAB is an exciting potential therapy for osteoporosis, it's very effective for the treatment of postmenopausal women and others with osteoporosis that are at high risk for fracture as it increases bone formation and decreasing bone resorption.¹⁵

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A BRIEF REVIEW ON SELECTED HPLC QUICK SEPARATION AND APPLICATION GUIDE

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ABSTRACT

HPLC is one of the most utilized analytical techniques among all the different chromatographic methods. HPLC is the most versatile, safest, and fastest chromatographic technique for quality control of drug components. HPLC is a method for the separation of various elements in plant extracts that resemble a specific and sensitive process. HPLC is required demonstrate the specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance and helps in the education of the structure of the degradation product. HPLC is checking the purity of a compound, presence of impurities, determination of a mixture of drugs, isolation of identification of drugs, isolation of identification of mixture. High-performance liquid chromatography is used to biogenic substances, medical products, food products, environmental samples, and organic industrial products. This review article provides an overview of the essential analysis parameters of a large number of combinations of pharmaceutical formulations which will both inspire and inform you of your lab's progress and accomplishment in the analysis.

KEYWORDS: *HPLC application quick guide, Pharmaceutical quality control, Stationary phase, Pharmaceutical combination formulations, Validation.*

INTRODUCTION

In the modern pharmaceutical industry, HPLC is the vital and integral analytical tool applied in all stages of drug discovery, development, and production. During method development³ except for the small influence from the change of the mobile phase flow rate. Approximately 80 % of all divorces is done on 5-10 μ m reverse phase C₁₈ silica columns. Table 1 shows the essential pharmaceutical applications with HPLC. They are listed according to combination of drugs. These separations are intended as a guide. Some times conditions will vary from compound to compound and from column to column.

Table 1. Selected HPLC quick separation and applications

Drug	Column	Mobile phase	Flow rate	Detector (nm)
5- Quinolones antibiotics Nalidixic acid, Norfloxacin Ofloxacin, Ciprofloxacin & Lomefloxacin	C ₁₈	Acetonitrile with tetrabutylammonium acetate, Sodium dodecyl sulphate and citric acid pH-3.4	1ml\min	UV 235
Isoniazid , Rifampicin Pyrazinamide	ODS	Methanol-tetra butyl ammonium hydroxide, pH 3.0 (80:20 v\ v)	1 .5ml\min	UV 265
Pyrimethamine, Sulphadoxine	C ₁₈	Methanol- Water (50:50,v\ v) 2% acetic acid	1.0 ml\min	UV 221
Trimethoprim, Nitrofurantoin	C ₁₈	Water- Acetonitrile Triethylamine (65:35:0.1v\ v) pH- 6.8 with dilute orthophosphoric acid	1ml\min	UV 270
Levo, Pruli, Gati, Sapr, Moxi and Balofloxacin.	C ₁₈	10mM phosphate buffer pH- 3.1 : Acetonitrile 70:30 v\ v	1ml\min	UV 293

Cardiovascular system: Atorvastatin calcium ⁴ Amlodipine besylate	C ₁₈	0.02 M KH ₂ PO ₄ - Acetonitrile MeOH (30:10:60 v\ v), pH 4.0 with 0.1M O- H ₃ PO ₄	1ml\min	UV 240
Atenolol hydrochloride, Metoprolol succinate Hydrochlorothiazide	C ₁₈	10mM Phosphate buffer pH-3.0 adjusted with triethylamine : acetonitrile (50:50 v\ v)	1.0 ml\min	UV 235
Telmisartan, Losartan, Valsartan, Olmesartan, Irbesartan, Atenolol	C ₁₈	Phosphate buffer with pH-3.3 and acetonitrile 50:50 % v\ v	1ml\min	UV 230
Hydrochlorothiazide, Nebivolol hydrochloride	C ₁₈	0.1M Sodium phosphate buffer (pH2.5 adjusted with ortho phosphoric acid):Methanol, 25:75 v\ v	1ml\min	UV 284
Telmisartan, Ramipril ⁵	C ₁₈	0.01 M potassium dihydrogen phosphate buffer (adjusted to pH 3.4 using orthophosphoric acid): methanol: acetonitrile (15:15:70 v\ v\ v)	1ml\min	UV 210
Atenolol, Amlodipine	C ₁₈	10mM phosphate buffer pH-3.0 : acetonitrile (50:50 v\ v)	1.0 ml\min	UV 235
Musculoskeletal Disorders: Paracetamol , Aceclofenac	C ₁₈	20mM phosphate buffer, pH – 5.0- Acetonitrile(40:60v\ v)	0.8ml\min	UV 265
Paracetamol , Valdecocixib	C ₁₈	20mM octane sulphonic acid pH 3.0 with O- H ₃ PO ₄ – acetonitrile (50:50 v\ v)	1ml\min	UV 250
Paracetamol , Etoricoxib	C ₁₈	Acetonitrile – methanol- water (60:15:25 v\ v)	1ml\min	UV 236
Paracetamol , Piroxicam	C ₁₈	MeOH- Water pH 4.0 with formic acid (70:30v\ v)	1ml\min	UV 227
Paracetamol, Diclofenac sodium, Chlorzoxazone	C ₁₈	Phosphate buffer (pH- 6.65) : acetonitrile (60:40 % v\ v)	1ml\min	UV 280
Tizanidine, Aceclofenac, Chlorzoxazone, ⁶ Paracetamol	C ₁₈	Phosphate buffer (pH-6.65): Acetonitrile(60:40 % v\ v)	1.0 ml\min	UV 280
Alimentary system : Rabeprazole, Itopride	C ₁₈	Acetonitrile – phosphate buffer, pH- 7.2 (50:50 v\ v)	1ml\min	UV 268
Omeprazole, Domperidone	C ₁₈	0.1 M ammonium acetate buffer- methanol (85:15 v\ v)	1.5 ml\min	UV- 302
Pantoprazole sodium , Mosapride citrate	C ₁₈	Buffer pH -5.0 – Acetonitrile 1.4 ml of orthophosphoric acid in 500 ml of water, pH adjusted to 5.0 with TEA.	1ml\min	UV 276
Gatifloxacin, Ornidazole	C ₁₈	Acetonitrile – methanol-water	1ml\min	UV 280
Levofloxacin, Ornidazole	C ₁₈	0.05 M KH ₂ PO ₄ buffer pH 6.8 –methanol-acetonitrile	1.5 ml\min	UV -295
Respiratory system : Dextromethorphan hydrobromide, Phenylephrine HCL	ODS - 80	Phosphate buffer, pH- 4.7-acetonitrile (40:60v\ v), containing 5mM heptane sulphonic acid Sodium, pH-adjusted to2.7 with acetic acid	2ml\min	UV-214
Bromhexine HCL, Amoxicillin	C ₁₈	methanol and glacial acetic acid (50:50 v\ v)	1.0ml/min	UV 254

Central nervous system: Fluoxetine, Olanzapine	C ₁₈	Buffer – Acetonitrile- glacial acetic acid (55:44:1v\ v)	1ml\min	UV 230
Alprazolam, Fluoxetine HCL	C ₁₈	Acetonitrile- water pH - 2.75 with 0.1 % H ₃ PO ₄ (75:25 v\ v)	1ml\min	UV- 235
Escitalopram oxalate, Clonazepam	C ₁₈	buffer and acetonitrile in a ratio of (50:50 v/v)	1ml\min	UV- 240
Analgesic drugs: Antipyrine Hydroxy antipyrine, Acetaminophen	C ₁₈	0.0 5M KH ₂ PO ₄ pH – 3\ Acetonitrile	1.0 ml\min	UV 204
Anti viral drugs : Acyclovir ⁷	C ₁₈	0.1 % phosphoric acid and methanol, acetonitrile	0.8 ml\min	UV 254
Androgen drugs: Testosterone Testosterone Propionate ⁸	ODS	Water\Acetonitrile	1 ml\min	UV254,234
Antianginal drugs: Verapamil ⁹	C ₁₈	0.025 M KH ₂ PO ₄ pH – 3\ Acetonitrile	0.6 ml\min	FLD, 228\312
Antiarrhythmic drugs : Quinidine, Disopyramide Procainamide ¹⁰	RP - 18	0.05 KH ₂ PO ₄ pH – 2.5\ Acetonitrile	1 ml\min	UV220 nm, 254 nm, 204 nm
Antiasthmatic drugs: Caffeine, Theophylline Theobromine	C ₈	water-THF (0.1% THF in water, pH 8) acetonitrile (90:10, v/v)	0.8 ml\min	UV 273
Antibacterial drugs : Penicillin like : Ampicillin, Amoxicillin, Penicillin–G, Penicillin – V	C ₁₈	0.025 KH ₂ PO ₄ pH – 3\ Acetonitrile	0.8 ml\min	UV 204 nm
Tetracyclines: Minocycline , Tetracycline Doxycycline	C ₁₈	0.025 KH ₂ PO ₄ pH – 3\ Acetonitrile	0.5 ml\min	UV 350 nm
Anticoagulant drugs : Warfarin	C ₁₈	0.025 KH ₂ PO ₄ pH – 3\ Acetonitrile	1.0 ml\min	204 nm, FLD 272\355
Anti-convulsants: Valproic acid,Phenytoin	C ₁₈	acetonitrile and 0.05 M phosphate buffer (pH 3.0) 45:55 v/v	1.0 ml\min	UV 360 nm to 210 nm
Anti depressant drugs Bupropion ¹¹ , Trazodone Maprotiline, Fluoxetine	C ₁₈	0.025 KH ₂ PO ₄ pH – 3\ Acetonitrile	1.0 ml\min	UV 210 nm
Antiepileptic drugs: ¹² Caffeine, Phenytoin Methylphenylsuccinide	ODS	Water\Acetonitrile	1.0 ml\min	UV 204 nm
Antiestrogen drugs : Tamoxifen	RP- 18	0.025 KH ₂ PO ₄ pH – 3\ Acetonitrile	0.7 ml\min	UV 204 nm
Anti -histaminic drugs Tetracaine , Promethazine Chlorpheniramine ,	C ₁₈	0.025 KH ₂ PO ₄ pH – 3\ Acetonitrile	1.0 ml\min	UV 204 nm
Antihypertensive drugs: Enalapril, Captopril	C ₁₈	0.025M KH ₂ PO ₄ pH – 2\ Acetonitrile	1 ml\min	UV 204 nm
Anti-inflammatory drugs		0.025M KH ₂ PO ₄ pH – 3\ Acetonitrile	1ml\min	UV 230 nm

Naproxen	C ₁₈			
Antiprotozoal drugs: Metronidazole	C ₁₈	0.025M KH ₂ PO ₄ pH – 3\ Acetonitrile	1.2 ml\min	UV 320 nm
Antitumor drugs : Paclitaxel (Taxol)	ODS	Water\Acetonitrile	1.2 ml\min	UV 204 nm
Antitussive drugs : Dextromethorphan	C ₁₈	0.025 M KH ₂ PO ₄ pH – 3\ Acetonitrile	1 ml\min	UV204 nm
Anti-anxiety: Buspirone , Lorazepam Diazepam-	C ₁₈	Water:acetonitrile:methanol (45:35:20 v\ v)	1.0 ml\min	UV 210nm
Anticancer : Gemcitabine HCL Capecitabine HCL	C ₁₈	Acetonitrile:Water:triethylamine (70:28:2 v\ v)	1 ml\min	UV 260
Capecitabine	C ₁₈	Methanol:Acetonitrile:Water (50:30:20:v\ v) pH 4.6 using Triethylamine	1.0 ml\min	UV 245
Angiotensin converting enzyme (ACE) : Enalapril, Captopril	RP-18	Methanol–water 50:50%v\ v pH- 3.2 with orthophosphoric acid	1 ml\min	UV 218 nm
Anti-bacterial : Trimethoprim	C ₈	Potassiumhydrogenphosphate acetonitrile, methanol and water pH 6.2	1 ml\min	UV 260 nm
Anti-fungal : Ketoconazole	C ₁₈	Methanol and water (90:10 v\ v) adjusted to pH 8.90 with a phosphate buffer.	1ml\min	UV 274 nm
Levamisole¹³ and Albendazole	C ₁₈	Buffer pH 3.5 and acetonitrile (70:30 v\ v)	1.0 ml\min	UV 224
β – blockers : Atenolol , Amlodipine	C ₁₈	0.1% trifluoroacetic acid Methanol\ Water (50:50,v\ v)	1.0 ml\min	UV 276 nm
Bronchodilators: Albuterol	C ₈	Potassium dihydrogen ortho- phosphate, 1-pentane sulphonic acid sodium salt monohydrate (pH 4.0) and acetonitrile (70:30 v\ v)	1.0 ml\min	UV 276 nm and 220 nm
Catecholamines : Norepinephrine, Epinephrine Dihydroxy benzylamine Dopamine	C ₁₈	0.025M KH ₂ PO ₄ 0.3mM heptane- Sulfonic acid pH – 3 Acetonitrile	0.3 ml\min	UV 204 nm
Calcium channel blockers: Verapamil, Diltiazem	C ₁₈	50mM ammonium phosphate: acetonitrile 70:30 v\ v pH – 4.5	1ml\min	UV 280 nm
Cardiac glycosides: Digoxin	C ₁₈	water–acetonitrile (72:28, v\ v)	1.1 ml\min	UV 220 nm
Benzodiazepines: Clonazepam	C ₁₈	Acetonitrile: Methanol (60:40 v\ v).	1.0 ml\min	UV 254 nm
Diuretics: Amiloride ,Triamterene Furosemide	C ₁₈	50 Mm phosphate buffer solution: acetonitrile (50:50 v\ v, pH 3.0).	1.0 ml\min	UV 283 nm
Glucocorticoid drugs: Beclomethasone, Prednisolone	ODS	Water\Acetonitrile	1.0 ml \min	UV 254 nm
H₂ – Antagonists Ranitidine , Cimetidine	C ₁₈	0.025M KH ₂ PO ₄ pH – 3\ Acetonitrile	1ml\min	UV 225 nm
Hypnotic drugs :		Water\Acetonitrile		

Barbital, Allobarbitol Phenobarbital,	ODS		1.5 ml\min	UV 204 nm
Keratolytic drugs : Salicylic acid, Phthalic acid, Benzoic acid	C ₁₈	0.05M NH ₄ OAC pH – 2.2 \ Acetonitrile	0.2 ml\min	UV 204 nm, 230 nm
Muscle relaxing drugs Papaverine	C ₁₈	0.025 M KH ₂ PO ₄ pH – 3\ Acetonitrile	1 ml\min	UV 254 nm
Sedative drugs : Diazepam , Oxazepam Clonazepam, Flunitrazepam	C ₁₈	0.025 M KH ₂ PO ₄ pH – 3\ Acetonitrile	1.0 ml\min	UV 210 nm
Sulfa drugs : Sulfanilamide, Sulfadiazine, Sulfathiazole	ODS	Water\Acetonitrile	1.0ml\min	UV 254 nm
Steroidal: Betamethasone	C ₁₈	Methanol-acetate buffer-acetonitrile (33:27:40, v/v)	1 ml\min	UV 254nm
Therapeutic drugs : Angiotensin II , Oxytocin Angiotensin I , Insulin ,	C ₁₈	0.1 % TFA\ Acetonitrile	0.5 ml\min	UV 210 nm
Tricyclic antidepressant drugs : Protriptyline , Imipramine Nortriptyline, Doxepin ,	C ₁₈	0.025M KH ₂ PO ₄ pH – 7\ methanol	1.0 ml\min	UV 210 nm
Vitamins : Fat-soluble : Vitamins A, D, E	C ₁₈	Water\ methanol	1.0 ml\min	UV 210 nm
Water soluble : Aminobenzoic acid , Biotin , Folic acid , Niacinamide	C ₁₈	0.05 M KH ₂ PO ₄ pH – 2.5\Acetonitrile	1.0 ml\min	UV 204 nm
Abbreviations: KH₂PO₄ , Potassium dihydrogen phosphate; TFA , Trifluoroacetic acid; NH₄OAC , Ammonium acetate; TEA , Triethanolamine; H₃PO₄ , Phosphoric acid; MeOH , Methanol; ACN , Acetonitrile.				

CONCLUSION

HPLC is just the leading technique for the trace analysis of organic and inorganic substances. Estimation of trace compounds is very vital in pharmaceutical, biological, toxicology, and environmental studies since even a trace substance can be dangerous or poisonous. The prominent role of HPLC in the pharmaceutical industry is essential particularly in preformulation research, process development, throughout formulation development and drug discovery, and to verify drug purity. All the work which has been done in pharmaceutical substances, Preparation of pure compounds, trace analysis, food safety where we have to analyze for pesticides and toxic chemicals founds in food and food products all of these things are done routinely and daily rapidly by high-performance liquid chromatography or GC. So HPLC is the best separation technique for quantitative trace analysis of toxic chemicals, impurities, Manufacturing of high purity products, medicinal uses, and research purpose.

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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF EMPAGLIFLOZIN USING HYDROTROPIC AGENT BY UV SPECTROSCOPY

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ABSTRACT

A new, simple, precise and accurate UV spectroscopic method has been developed for the estimation of Empagliflozin. The standard and sample solutions were prepared using Urea solution (6M) as solubilizer and solvent. The drug was quantitatively determined at the wavelength of 232 nm (λ_{max}). The linearity concentration was in the range of 5-65 $\mu\text{g/ml}$ with the correlation coefficient of 0.9988. Precision studies showed that % Relative standard deviation was within acceptable limits with the mean percentage recovery of 90%. The Proposed method was validated according to ICH guidelines. Assay for Empagliflozin tablets was found to be 103%. Hence the developed method can be used for routine analysis.

KEYWORDS: *UV Spectroscopy, Empagliflozin, Method development and Validation, ICH Guidelines.*

INTRODUCTION

Empagliflozin (EMPA) (1,5-anhydro-1-(4-chloro-3 tetrahydrofuran-3-yloxy)D-Glucitol) (Fig 1) is an anti diabetic agent, sodium glucose co transporter-2 (SGLT2) inhibitor. SGLT2 co transporters are responsible for the reabsorption of glucose from the glomerular filtrate in the kidney. EMPA act by blocking the SGLT2 reduces blood glucose levels by blocking reabsorption in the kidney.¹ few methods have been reported for the estimation of EMPA either alone or in combination. These include UPLC with UV detection^{2,3} and LC-MS⁴. The drug is poorly water soluble, therefore present study was aimed to develop a simple, safe and cost effective spectrophotometric technique for determination of EMPA by using a hydrotropic agent.

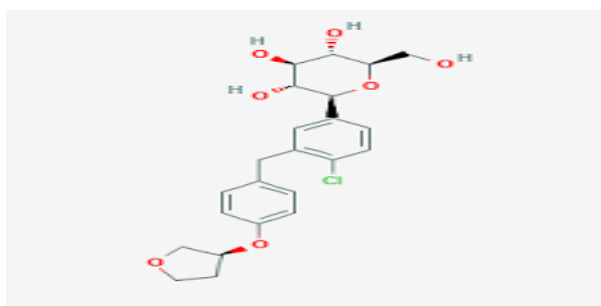


Fig1: Chemical structure of Empagliflozin

MATERIALS AND METHODS

Instrumentation: UV-Visible spectrophotometer with matched Quartz cells, SHIMADZU (Model: UV-1800).

Reagents and chemicals: Empagliflozin, Urea solution (6M), Marketed formulation: The tablet containing 10MG empagliflozin were obtained from market.

Preparation of standard solution: The standard stock of EMPA was prepared by dissolving 10mg of API in 10ml of diluent to produce 1mg/ml solution (stock solution). From this 1ml of aliquot was taken in 10ml volumetric flask diluted with urea solution (diluent) to prepare solution of 100 $\mu\text{g/ml}$.

Preparation of diluent: Concentrated urea solution (6M) was prepared by dissolving 72gm of urea in 200ml of distilled water.

RESULTS AND DISCUSSION

Development of UV method:

Selection of wavelength: Standard solution of EMPA was scanned in the UV range of 200-400nm for the determination of $\lambda_{\max}$ using an appropriate blank. The $\lambda_{\max}$ was found to be 232nm for EMPA. (Fig 2)

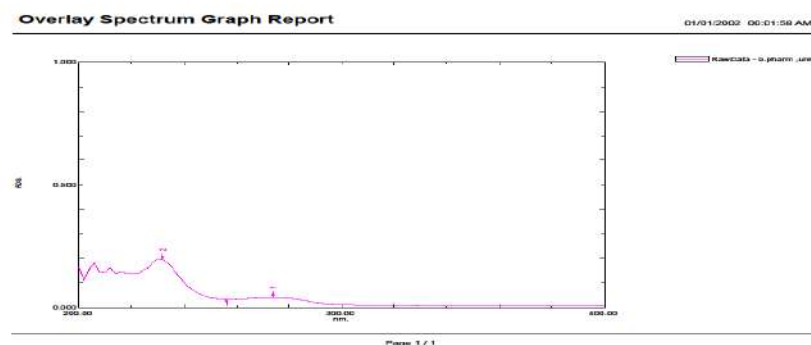


Figure 2: UV Spectrum of EMPA

Method validation

Linearity and Range: The linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration range. The concentration and absorbance values were plotted to get a standard calibration curve. The regression equation was $y=0.015x+0.004$ with correlation coefficient 0.998 (Table 1, Figure 3).

S.NO	Concentration ($\mu\text{g/ml}$)	Absorbance
1	5	0.102
2	15	0.222
3	25	0.373
4	35	0.525
5	45	0.689
6	55	0.825
7	65	0.998

Table 1: Conc. Vs Abs. table for linearity study.

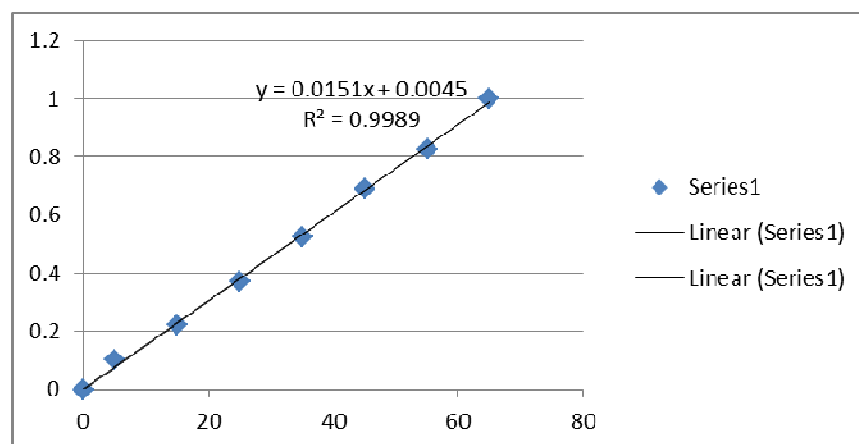


Figure 3: Calibration curve of EMPA

Accuracy: The accuracy of the method is the nearness of the results obtained to the true value. Accuracy was established by recovery experiments. The recovery was performed by adding EMPA working standard to placebo in the range of concentration 80%, 100%, 120% and expressed as percent recovered (%). The percent recovery at each level was found to be 92.5%, 90%, 87.8%. Results are shown in table 2.

Amount of drug formulation (µg/ml)	Amount of pure drug added (µg/ml)	% added	Amount found (µg/ml)	*% recovery	% RSD
30	24	80	50	92.5%	0.870
30	30	100	54	90%	0.931
30	36	120	58	87.8%	0.059

*Average of three determinations

Table 2: Results of accuracy method of EMPA

Precision

Precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. The precision of a method is verified by repeatability (intraday) and intermediate precision studies. Repeatability was performed by analysis of concentration of working standard solution, intermediate precision was performed on different days under similar experimental conditions. The results were expressed in table 3,4.

S.NO	Concentration	Absorbance	
		Day 1	Day 2
1	30	0.554	0.557
2	30	0.557	0.560
3	30	0.558	0.559
4	30	0.553	0.554
5	30	0.542	0.556
6	30	0.550	0.569
	%RSD	1.05	0.94

Table 3: Intermediate precision results of EMPA

S.NO	Concentration(µg/ml)	Absorbance		
		Morning	Afternoon	Evening
1	30	0.449	0.448	0.446
2	30	0.445	0.446	0.443
3	30	0.438	0.437	0.438
4	30	0.430	0.440	0.436
5	30	0.438	0.437	0.439
6	30	0.511	0.430	0.435
	%RSD	1.49%	1.50%	0.96%

Table 4: Intraday precision results of EMPA

Ruggedness: it is the reproducibility of results when the method is performed under actual use condition. This includes different analysts, laboratories, instruments.

Analyst 1:

S.NO	Concentration (µg/ml)	Absorbance
1	30	0.540
2	30	0.546
3	30	0.535
%RSD=1.02		

Analyst 2:

S.NO	Concentration (µg/ml)	Absorbance
1	30	0.548
2	30	0.538
3	30	0.552

%RSD= 1.32		
Analyst 3:		
S.NO	Concentration (µg/ml)	Absorbance
1	30	0.573
2	30	0.560
3	30	0.563
%RSD= 1.20		

Table no 5: Evaluation data of ruggedness**Limit of detection and Limit of quantification:**

The limit of detection(LOD) and Limit of quantification (LOQ) of EMPA were calculated using the standard deviation of responses (N) and slope(S) of respective calibration curves using Signal-to-Noise Ratio (S/N). The results were shown in table 6.

$$\text{LOD} = 3.3 \times \text{S/N}$$

$$\text{LOQ} = 10 \times \text{S/N}$$

S.NO	Parameters	*EMPA
1	LOD	0.033 µg/ml
2	LOQ	0.099 µg/ml

Table 6: LOD and LOQ values of EMPA

Robustness: Robustness is the measure of its capacity to remain unaffected by small but deliberate changes in method parameters. The results were shown in table no 7.

S.NO	Concentration (µg/ml)	λ_{max}	Absorbance
1	30	227	0.359
2	30	228	0.355
3	30	229	0.351
4	30	230	0.354
5	30	231	0.352
6	30	232	0.350
7	30	233	0.358
8	30	234	0.352
9	30	235	0.358
10	30	236	0.354
11	30	237	0.359
%RSD = 0.95			

Table 7: Evaluation data of Robustness study**CONCLUSION**

The proposed analytical method was validated ad per ICH guideline and it meets the acceptance criteria. It is concluded that the analytical method was simple, specific, precise, accurate, robust and cost effective. The present analytical method can be used for its intended purpose.

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NANOBOTS ARE THE FUTURE MEDICINE

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ABSTRACT

Nanorobots (Nanobots) have the advantages of small size, low weight, large thrust-to-weight ratio, high flexibility, and high sensitivity. Due to the characteristics distinguishing from macroscopic robots, nanorobots have stimulated the research interest of the scientific community and opened up numerous application fields such as drug delivery and disease diagnosis. In the past 30 years, research on nanorobots has made considerable progress. This review article aims at giving an overview of the present status of nanorobotics with the aid of biotechnology, molecular biology (as engineered organism) and molecular medicine can develop fully self-sufficient nanorobots/nanobots. The nanorobotics considered as a wonderful vision of medicine in the future are an advanced submicron device generally made of bio-nanocomponents. It has an eminence future in the drug delivery technology target in cancer, the disease that leading cause of death among youngster than 45 years. Nanorobots could carry and deliver large amounts of anti-cancer drugs into cancerous cells without harming healthy cells, reducing the side effects related to current therapies like damage of the conventional chemotherapy. This review presents a comprehensive overview of the development of nano-robots. Further, it also provides an insight to the future scope in this field of study.

KEYWORDS: *Nanorobots, nanobots, cancer therapy, applications.*

INTRODUCTION

1. Overview of Nanotechnology

Most of the substantial things done and made in this world start from an idea. Scientists have dreamed for long of miniature robots that can be controlled and navigated inside human body, to help the medical doctors to diagnose and treat the diseases. As shown by the old classic movie *Fantastic Voyage* released in 1966, a submarine with a group of crews was shrunk to the micro-scale so that it can navigate inside the human blood vessel for the removal of a blood clot. To explore the possibility to realize that wild idea, scientists have proposed and developed many types of miniature machines and robots, some of which are aimed to adapt various kinds of physiological environments for diagnosis and disease treatment. A variety of nano-scale robots have been developed among the past decade, which offer promise for diverse biomedical applications.^{1,2,3,4,5,6} The original idea of nanotechnology can be attributed to a Physicist, Richard Feynman in 1959.⁷ Feynman's vision of nanotechnology became known to all in his classic talk on December 29th 1959 at the annual meeting of the American Physical Society at Caltech, which was first published in the February 1960 issue of Caltech's *Engineering and Science*. Feynman went on to propose miniaturizing computers to a nano scale. This of course, would require a different manufacturing process. He came up with miniaturization through evaporation and the applications of small moveable machines to. Do assembly. This idea of moveable machines has given the widely used term, 'nanorobots.' He suggested a way to make tiny mechanisms through the use of slave "hands," which are controlled to perform a specific task. Yet, Feynman thinks beyond this point explaining his concept of having hundreds of tiny "hands" that manufacture copies of it doing the same function. Though the word self-replication was not used in his talk, this was definitely the concept that scientists relate to that topic. Nanorobots are promising devices for biomedical and environmental applications. The past few years have witnessed rapid developments in this field. This short review intends to address recent progress on magnetically driven nanorobots by different research groups.

*2 What are Nanobots?*⁸

Nanobot is a nano-technological robot machine, also called a nanite, which is a mechanical or electro

chemical device whose dimensions are measured in nanometers. Since nanorobots would be microscopic in size, it may be necessary for very large numbers of them to work together to perform macroscopic tasks. These nanobots don't have microprocessors in them that think and act according to a program. They don't swim intelligently to a desired target in a straight line path instead they float around exhibiting simple behaviors such as releasing a chemical agent, attaching to something or destroying something based on their geometries and chemical constituents.

3. Ideal Characteristics⁹

- Nanorobots must have size between 0.5 to 3 microns large with 1-100 nm parts. Nanorobots of larger size than the above will block capillary flow.
- They prevent themselves, from being attacked by the immune system by having a passive, diamond exterior.
- It will communicate with the doctor by encoding messages to acoustic signals at carrier wave frequencies of 1-100 MHz.
- It might produce multiple copies of it to replace worn-out units, a process called self-replication.
- After the completion of the task, it can be retrieved by allowing it to exfuse themselves via the usual human excretory channels or can also be removed by active scavenger systems.

4. Advantages¹⁰

- Use of nanorobot drug delivery systems with increased bioavailability;
- Targeted therapy such as only malignant cells treated.
- Fewer mistakes on account of computer control and automation.
- Reach remote areas in human anatomy not operable at the surgeon's operating table.
- As drug molecules are carried by nanorobots and released where needed the advantages of large interfacial area during mass transfer can be realized.
- Non-invasive technique.
- Computer controlled operation with knobs to fine tune the amount, frequency, time of release.
- Better accuracy.
- Drug inactive in areas where therapy not needed minimizing undesired side effects.

5 Disadvantage

- The initial design cost is very high.
- The design of the nanorobot is a very complicated one.
- Electrical systems can create stray fields which may activate bioelectric-based molecular recognition systems in biology.
- Electrical nanorobots are susceptible to electrical interference from external sources such as *rf* or electric fields, EMP pulses, and stray fields from other *in vivo* electrical devices.
- Hard to Interface, Customize and Design, Complex
- Nanorobots can cause a brutal risk in the field of terrorism. The terrorism and anti-groups can make use of nanorobots as a new form of torturing the communities as nanotechnology also has the capability of destructing the human body at the molecular level.

6 Challenges Faced by Nanorobots¹¹

While designing nanorobots in nanoscale dimensions there should be a better understanding of how matter behaves on this small scale. Matter behaves differently on the nanoscale than it does at larger levels. So the behavior of the nanorobots must be taken care so that they do not affect us both inside and outside the body. Other challenges apply specifically to the use of nanostructures within biological systems. Nanostructures can be so small that the body may clear them too rapidly for them to be effective in detection or imaging. Larger nanoparticles may accumulate in vital organs, creating a toxicity problem. So we need to consider these factors as they anticipate how nanostructures will behave in the human body and attempt to create devices the body will accept.

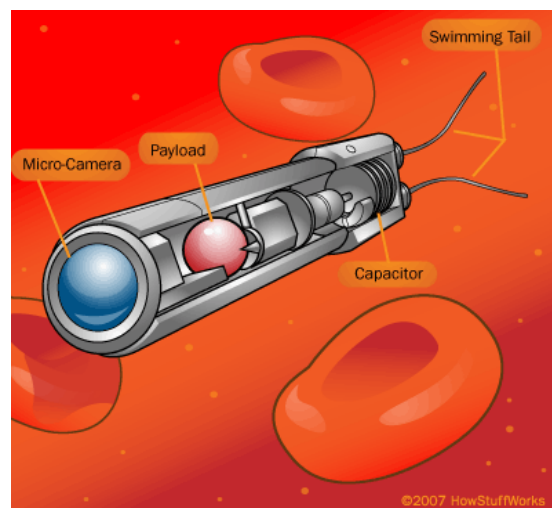
7 Components of Nanobots¹²

The various components in nanorobot include power supply, fuel buffer tank, sensors, motors, manipulators, onboard computers, pumps, pressure tanks and structural support. The substructures in a nanorobot include (Figure 1):

- a) Payload- This void section holds a small dose of drug/medicine. The nanorobots could transverse in the blood and release the drug to the site of infection/injury.
- b) Micro camera- The nanorobot may include a miniature camera. The operator can steer the nanorobot when navigating through the body manually.
- c) Electrodes- The electrode mounted on the nanorobot could form the battery using the electrolytes in the blood. These protruding electrodes could also kill the cancer cells by generating an electric current, and heating the cells up to death.
- d) Lasers- These lasers could burn the harmful material like arterial plaque, blood clots or cancer cells.
- e) Ultra sonic signal generators- These generators are used when the nanorobots are used to target and destroy kidney stones.
- f) Swimming tail- The nanorobot will require a means of propulsion to get into the body as they travel against the flow of blood in the body.

The nanorobot will have motors for movement and manipulator arms or mechanical leg for mobility. The two main approaches followed in construction of nanorobots are Positional assembly and Self-assembly. In self-assembly, the arm of a miniature robot or a microscopic set is used to pick the molecules and assemble manually. In positional assembly, the investigators will put billions of molecules together and let them automatically assemble based on their natural affinities into the desired configuration. Nanorobot Control Design is the software developed for simulating nanorobots in environment with fluids which is dominated by Brownian motion. The nanorobots have chemical sensors which can detect the target molecules.¹³

Figure 1: How nanobot look like and its components



8 Actuation of Nanorobots

Instead of building a single complex molecule to identify multiple features of a cell surface, Dr. Stojanovic and his colleagues at Columbia used a different, and potentially easier, approach based on multiple simple molecules, which together form a robot. To identify a cell possessing three specific surface proteins, Dr. Stojanovic first constructed three different components for molecular robots. Each component consisted of a piece of double-stranded DNA attached to an antibody specific to one of the surface proteins. When these components are added to a collection of cells, the antibody portions of the robot bind to their respective proteins. On cells where all three components are attached, a robot is functional and a fourth component initiates a chain reaction among the DNA strands. Each component swaps a strand of DNA with another, until the end of the swap, when the last antibody obtains a strand of DNA that is fluorescently labeled. At the end of the chain reaction- which takes less than 15 min in a sample of human blood-only cells with the three surface proteins are labeled with the fluorescent marker. Actuation methods of nanorobots are categorized into physical methods, chemical methods, biological methods and hybrid actuation methods.¹⁴

8.1 Physical actuation: In general, physical motion actuation is realized by magnetism, electricity, light, thermotics, acoustics, and piezoelectricity, among other mechanisms.

8.1.1 Magnetically driven Nanorobots: Magnetic actuation is widely used for nano-robots power due to the advantages of strong penetration and remote drive, and magnetic actuation can be used to power the motion of living biological materials in a non-toxic manner.¹⁵ They are Helical swimmers, Flexible swimmers and Surface walkers. Nanorobots can be propelled by rotating or oscillating magnetic fields. Rotation of these machines around their helical axis generates a translational cork screw-like movement. This locomotion strategy is widely adopted by several microorganisms such as bacteria, which rotate their flagella to swim at low Reynolds number regimes. Helical structures have been manufactured by several methods such as self-scrolling¹⁶, direct laser writing (DLW)^{17,18}, glancing angle deposition (GLAD)¹⁹, template-assisted electrode position (TAE)²⁰, and biotemplating synthesis (BTS).^{21,22} Nelson's group, Oscillating magnetic propelled nanorobots that include wagging propulsion, surface walker propulsion, and scallop propulsion.²³ The propulsion mechanisms include, (1) wagging propulsion; (2) surface walker; and (3) scallop propulsion are described below.

- *Wagging Propulsion:* Inspired by the swimming mechanisms of fish or bacterial flagellum, researchers proposed wagging propulsion as an efficient propulsion mode for micro-/nanomotors. Moreover, in an oscillating magnetic field, the magnetic part of a micro-/nanomotor tends to change its status and follow the change of the field strength. Therefore, wagging propulsion has been widely applied to drive micro-/nanomotors in past studies. Dreyfus et al.²⁴ used a magnetic 24- μm -long filament to transport red blood cells and record their movement with a fast camera.
- *Surface Walker:* In 2008, Tierno et al.²⁵ reported a new propulsion mechanism: the combination of special colloidal particles linked by DNA was observed to be able to rotate while clinging to a float flat, like a surface walker. This propulsion mode allowed micro-/nanomotors to swim efficiently and even overcome obstacles.
- *Scallop Propulsion:* The results of past research have proved that movement in a low-Re fluid generally requires the nonreciprocal actuation of the nanoswimmers. If this is not achieved, the net displacement of the periodic motion will be zero. Qiu et al. discovered a new method to meet this requirement.²⁶

8.1.2 Electric field driven Nanorobots: Electric field is typically the preferred mechanism after the magnetic field for the propulsion principle of nanorobots. Fan et al.²⁷ have showed that gold nanowires with a length of 6 μm and diameter of 300 nm conjugated with cytokine such as tumor necrosis factor- α (TNF α) can be transported along any prescribed trajectory or orientation using electrophoretic and dielectrophoretic forces to a specific location with subcellular resolution. Additionally, the integration of DEP and microfluidic systems offers numerous applications for the separation, trapping, assembling, transportation, and characterization of nano particles.²⁸ Nanowires with a large aspect ratio that are particularly suitable for use in electric tweezers have also been developed for patterning, assembling, and manipulation with the aid of DC and AC electric fields.²⁹

8.1.3 Light driven Nanorobots: Light-driven nanorobots have attracted increasing attention.^{30,31} Many light-driven nanotools operate in a similar manner as the corresponding macro tools. For example, Villangca et al.³² developed a new type of light robot that is capable of loading and unloading cargo such as silica and polystyrene beads using photothermally induced convection currents within the body of the tool. The light-driven nanocar is a synthesized molecule containing a unidirectional molecular motor with the size of 1.7 nm $\times$ 1.38 nm actuated by a certain wavelength of light. Ikuta et al.³³ developed optically trapped nanorobots with multiple degrees of freedom and realized remote manipulation of a single cell in liquid with simultaneous reaction force sensing.

8.1.4 Thermally driven Nanorobots: Thermal actuation is another common actuation method. By heating the liquid environment where the nanorobot is located, the nanorobot can be driven. The changing temperature can make the molecule-derived nanocars begin to move in two dimensions through a combination of both translation and pivoting.³⁴ Rotary nanomotors for which the rotational frequency can be adjusted by varying the temperature have also been developed.³⁵ However, that thermal actuation can cause

undesired negative effects; for example, such actuation may cause fatal harm to living organisms or irreversible damage to the materials, making them challenging to recover.

8.1.5 Acoustic driven Nanorobots: Acoustic actuation driving force and can achieve more efficient manipulation of nanorobots. For example, artificial nanoswimmers have been proposed that show high propulsive forces.³⁶ During acoustic excitation of the nanoswimmer, the tail structure oscillates, leading to large amplitude of propulsion in traveling waves. Additionally, Ultrasonic (US) propulsion provides a large propulsion force that can overcome the low Reynolds number regime dominated by viscosity, while US imposes little harm on biological samples at MHz frequencies.³⁷

8.1.6 Piezoelectric driven Nanorobots: Piezoelectric provides an alternative and attractive method of propulsion generation.³⁸ Jain et al.³⁹ developed a microgripper using piezoelectric actuator for handling the miniature parts and carried out the design and analysis of a piezoelectric actuator created by using a combination of two piezo ceramic layers and one resistive layer between them where the voltage (± 60 V) is supplied and controlled through a proportional-integral-derivative (PID) controller. Piezoelectric actuation is typically used for slightly larger robots (such as microrobots) and is rarely used for nanorobots.

8.2 Chemical driven Nanorobots: Nanorobots also use chemical methods for propulsion, which is one of the typical actuation methods.^{40,41} Powered nanorobots can actuate themselves by the energy from chemical reactions. The chemical propulsion is suitable for a wide variety of nanorobots with different shapes⁴² which can meet the needs of various application scenarios. For example, researchers have developed nanowires,^{43,44} asymmetric Pt,⁴⁵ nanorods,⁴⁶ different biomolecules,^{47,48} and combined architectures such as a combination of a biomolecule and a nanorod.

8.3 Biologically actuated Nanorobots: To address the actuation of nanorobots, another strategy is the application of biological motors.⁴⁹ In other words, in addition to synthetic nanorobots, biological organisms can be used as components of nanorobots. The biological organism can act as a driving part of the biohybrid nanorobot⁵⁰ by combining biology and robotics; alternatively, it can constitute the robot itself.⁵¹ These biological organisms integrate their inherent characteristics (which are well beyond the state of the art in engineering, i.e., small size, natural structural advantages, high energy efficiency, high sensitivity and favorable environmental adaptability) into robots that can respond to external stimuli in real time, which can greatly improve their performance.

8.4 Hybrid actuation of Nanorobots: Some nanorobots use a combination of the abovementioned drive modes. Hybrid actuation of nanorobots has thus been a major topic of research. Park et al.^{Error! Bookmark not defined.} proposed a hybrid actuated microrobot predominantly actuated by an electromagnetic field to overcome the flow of the bloodstream in large blood vessel and actuated by the bacterial chemotactic response and self-motility in the small blood vessels. That is, different driving methods can be adopted in different application scenarios. Furthermore, the research team led by Professor Martel successfully developed a nanorobot based on magnetic/oxygenotrophic bacteria. With the guidance of an external magnetic field and the bacteria's perception of hypoxic regions, this nanorobot can effectively carry drugs to the depth of a tumor and to anoxic areas. The movement of the bacteria combines the guidance of external magnetic fields, the self-propagation of bacteria, and the bacteria's perception of anoxic regions.⁵²

9 Fabrication of nanorobots: Research and design of drug-carrying nanorobots is not new. Scientists have already created nanobot prototypes by using advanced molecular design software to create nanostructures that can store various molecular cargo. Using a method known as 'DNA origami', pioneered in 2006 by scientist Paul Rothemund from Caltech University in the U.S., scientists have been able to manipulate DNA material into specific shapes and even program the 3-D DNA structures to carry out very basic robotic tasks, such as fusing to other cells and operating within other DNA material. However, the DNA nanorobots created so far have faced challenges in movement, activation and targeting of drug release. Although DNA nanorobots have already been programmed to carry cargo and work in conjunction with other nanorobots, this new study is the first time that structural techniques have been exploited by advanced computing functions to securely deliver treatment to specific diseased cells. Nanorobot fabrication techniques employed

are electrodeposition, self-assembly, electron beam evaporation, and three-dimensional (3D) direct laser writing.

9.1 Electrodeposition: Electrodeposition can easily be realized without expensive instruments and strict experimental conditions, and enables the fabrication of a variety of structures with different materials. Therefore, this technique is a widespread approach applied to micro-/nanorobots driven by oscillating magnetic fields. In 2007, Mirkovic et al.,⁵³ first utilized this technique to make flexible metal multilink nanorods. This fabrication method allows researchers to change the length and material of each segment, so it has great versatility.

9.2 Self-Assembly: The self-assembly technique can help researchers combine various disordered elements to prepare an organized structure through a spontaneous reorganization process. This process can be simply divided into layer-by-layer (LbL) self-assembly and the assembly of micro-/nanoparticles. LbL is a manufacturing technique involving the deposition of alternating layers of oppositely charged materials to fabricate a multilayer structure.⁵⁴

9.3 Electron Beam Evaporation: Electron beam evaporation is a type of conventional physical vapor deposition technique which can incorporate desired materials into micro-/nanorobots to perform special functions. It is mostly used to coat nonmagnetic particles with a magnetic material layer. Li et al., applied it to fabricate a Janus microdimer surface walker. This is a type of new micro-/nanorobot which includes two Janus microspheres that are magnetized so as to be propelled by oscillating magnetic fields.⁵⁵

9.4 Three-Dimensional Direct Laser Writing: Three-dimensional (3D) direct laser writing (DLW) is a mature technique which is used to fabricate complex structures. Therefore, it is applied by some researchers to manufacture micro-/nanorobots or their molds. The study of Qiu et al., introduced a microrobot which is similar to a scallop, which was readily constructed by 3D printing and the micro-molding technique.

10 Types of Nanobots

10.1 Respirocytes

Respirocytes are the nanorobots designed as artificial mechanical red blood cells which are blood borne spherical 1 μm diameter sized. The outer shell is made of diamondoid 1000 atm pressure vessel with reversible molecule-selective pumps. Respirocytes carry oxygen and carbon dioxide molecules throughout the body. The respirocyte is constructed of 18 billion atoms which are precisely arranged in a diamondoid pressure tanks that can store up to 3 billion oxygen and carbon dioxide molecules. The respirocyte would deliver 236 times more oxygen to the body tissues when compared to natural red blood cells. The respirocyte could manage the carbonic acidity which will be controlled by gas concentration sensors and an onboard nano-computer.^{56,57}

10.2 Microbivores

Microbivores are the nanorobot which functions as artificial white blood cell and also known as nanorobotic phagocytes. The microbivore is a spheroid device made up of diamond and sapphire which measures 3.4 μm in diameter along its major axis and 2.0 μm diameter along minor axis and consists of 610 billion precisely arranged structural atoms. It traps in the pathogens present in the blood stream and break down to smaller molecules. The main function of microbivore is to absorb and digest the pathogens in the blood stream by the process of phagocytosis.

10.3 Clottocytes

Hemostasis is the process of blood clotting when there is damage to the endothelium cells of blood vessels by platelets. These platelets can be activated by collision of exposed collagen from damaged blood vessels to the platelets. The whole process of natural blood clotting can take 2-5 min. The nanotechnology has shown the capabilities of reducing the clotting time and reducing the blood loss. In certain patients, the blood clots are found to occur irregularly. This abnormality is treated using drugs such corticosteroids. The treatment with corticosteroids is associated with side effects such as hormonal secretions; blood/platelet could damage lungs and allergic reactions.⁵⁸

11 Applications of nanorobots^{59,60}

11.1 Cancer Treatment

According to World Health Organization, the main cause for deaths now-a-days is cancer. The traditional methods used in the treatment of cancer are Radio therapy and Chemo therapy. But the patient undergoing this treatment has to bear lots of pain and side effects like fatigue, hair loss, weight loss etc. So, to avoid this researchers have now come up with an idea of using Nanorobots in treatment of cancer. So by using Nanorobots a cancer patient can be cured completely without any side effects. This work presents an innovative approach to build, control, and monitoring medical Nanorobot in diagnosis of cancer for its early stage before metastasis. The proposed work uses biomedical flows, E-cadhering signal analyses, and RF CMOS, allied with mobile phones, for the Nanorobot architecture development.

11.2 Nanodentistry

Nano diagnostic devices are able to be used for early infection recognition at the cellular and molecular levels. From an in vivo perspective, nano-devices might be placed in into the body to recognize the timely occurrence of a disease in order to recognize and enumerate toxic molecules, tumour cells and so forth. This indicator has being deliberated with the help of atomic force microscopy which utilize nanoparticles. The pasture of dentalnanomedicine involve the usage of three modules of molecular arrangement they are nanomaterials, non-biological nanoparticles, materials and mechanism based on biotechnology and non-biological instruments including nanorobotics needed for diagnosis andtherapy purposes.

11.3 Nanorobots performing operations on Blood cells

Nanorobotic phagocytes called microbivores could patrol the bloodstream, seeking out and digesting unwanted pathogens including bacteria, viruses or fungi. Each nanorobot could completely destroy one pathogen in just 30 seconds - about 100 times faster than natural leukocytes or macrophages - releasing a harmless effluent of amino acids, mononucleotides, fatty acids and sugars.

11.4 Treatment of AIDS

AIDS by itself is not a killer disease. The cause of AIDS is the HIV virus that is capable of destroying the immune system. Thereby the host system is vulnerable to small diseases which will turn into a fatal one but actually it is not a fatal disease. The HIV virus attacks the WBC's by converting them into the HIV. Thereby all the WBC's are converted into HIV, so the immune system will fail. This is the reason for the death of the patient. Our idea is to convert the AIDS affected WBC's back into the original form of the WBC by using a nanorobot; thereby the patient is made to have a constant amount of immune system. Nanorobot performs the inverse process of the HIV.

11.5 Treatment of Arteriosclerosis

Arteriosclerosis occurs when plaque builds along the walls of the arteries which will block the blood flow through them. Nanorobots could be used to cut away all the plaques and clear the arteries and capillaries and hence reducing the chances of heart attack.

11.6 Bypass Surgery

People with coronary artery disease are treated by doing heart bypass surgery. This is done to increase the flow of blood to the heart muscles. More than one artery may be bypassed during the operation but this method is not without side effects. Instead a Nanorobot can be used to operate. This Nanorobot contains two regions an outer region and an inner region .This Nanorobot is attached with an electric motor for its circulation. Along with the electric motor an artery thermometer, a microprocessor, a camera and a revolving needle is incorporated into the Nanorobot.The microprocessor will control the complete operation. A radioactive material is infused into the outer region, which will help to trace the movements of the Nanorobot. The nanorobot can be switched anytime on and off anytime using a magnetic switch. The Nanorobot can be injected into femoral artery in the leg which is one of the largest arteries in the body. The induced Nanorobot with the help of the diamond chip can cut out the plaque and grind them into micro particles. The Nanorobot is removed by directing the Nanorobot to anchor to a blood vessel that is easily accessible from outside.⁶¹

11.7 Gene Therapy

Medical nano robots can readily treat genetic diseases by comparing the molecular structures of both DNA and proteins found in the cell to known or desired reference structures. In some cases, chromosomal replacement therapy is more efficient than in CY to repair. Floating inside the nucleus of a human cell, an assembler built repair vessel performs some genetic maintenance. Stretching a super coil of DNA between its lower pair of robot arms, the nano machine gently pulls the unwound strand through an opening in its prow for analysis. Upper arms, meanwhile, detach regulatory proteins from the chain and place them in an intake port. The molecular structures of both DNA and proteins are compared to information stored in the database of a larger nano computer positioned outside the nucleus and connected to the cell-repair ship by a communications link.

11.8 Nano Robot for Brain Aneurysm

The nano robot for brain aneurysm prognosis, they are using computational nanotechnology for medical device prototyping. This is consisting of three main Equipment (a) prototyping (b) the manufacturing approach and (c) inside-body transduction. It is the computational nanotechnology provides a key tool for the fast and effective development of nano robots, and that is supports of investigation to address major aspects on medical instrumentation and device prototyping.

11.9 Nanorobots Treatment of Diabetes

Glucose carried through the blood stream is the most important role to maintain the human metabolism working for health, and its correct level is a key issue in the diagnosis and treatment of diabetes. Intrinsically related to the glucose molecules, the protein hSGLT3 has an important influence in maintaining proper gastrointestinal cholinergic nerve and skeletal muscle function activities, regulating extracellular glucose concentration. The hSGLT3 molecule can serve to define the glucose levels for diabetes patients. The most interesting aspect of this protein is the fact that it serves as a sensor to identify glucose. At a typical glucose concentration, the nanorobots try to keep the glucose levels ranging around 130 mg/dl as a target for the Blood Glucose Levels (BGLs).

12 Challenges in nanorobots development

With the development of nanotechnology, robotics, biomedicine, and electromechanical science, research on nanorobots has made considerable progress. Previous research has achieved the movement and control of nanorobots and explored their promising applications in the biomedical field. However, to accommodate the ever-increasing actual demand, some challenges associated with nanorobots must be addressed by follow-up research. First, nanorobots should be multifunctional. Constrained by the nano size, existing nanorobots have a single function, and it is challenging to integrate multiple functions (i.e., actuation, perception, assessment, and intelligence) into one robot. In practical applications, it is important for intelligent nanorobots to incorporate signal perception, acquisition, processing and transmission. Proper feedback mechanisms are essential for preventing the nanorobot from losing contact with external controls. Second, nanorobots should solve the problems encountered in clinical applications and cross the gap between scientific research results and market demand, and one of grand challenges is to realize nanorobotics devices for the clinical application.⁶² Application efforts regarding nanorobots in vivo should consider the biocompatibility, reliability and biodegradability. Third, nanorobots need new energy conversion mechanisms, more robust wireless drive and control methods, and more reasonable fabrication technologies. The existing driving methods have drawbacks in all these areas. Fourth, nanorobots need innovative materials. Bio-syncretic robots have the advantages of both living components and nonliving components⁶³ and the bio-syncretic nanorobot has been an important part of the nanorobots field. Moreover, some living microorganisms can be directly used to assemble nanorobots. Living organisms can act as sensing or driving elements for these types of robots. Relative to inorganic materials, biomaterials have advantages such as higher sensitivity. Finally, precise cluster control of nanorobots deserves further study. Faced with complex tasks, a single nanorobot cannot be in two places at once. At this point, clusters of nanorobots can complete tasks through collaborative cooperation. How to implement synchronous or independent control of the nanorobots in the cluster is therefore another important research direction. The ideal cluster control strategy should involve the clear division of labor of individual robots within the cluster and information exchange between the robots so that all robots work together in an orderly manner to finish the task. Clearly, the achievement of this goal will require much research effort.

CONCLUSION

The paper is just a theoretical justification. But the recent advancement in the field of nanotechnology gives the hope of the effective use of this technology in medical field. This paper starts by giving an introduction to nanorobots and its importance as recognized by various other technocrats. Nanotechnology as a diagnostic and treatment tool for patients with cancer and diabetes showed how actual developments in new. The field of medical nanorobotics has achieved considerable advances. However, several issues and challenges must be addressed before micro/nanorobots could have real-world clinical applications. The goal of the *in vivo* model is not only to evaluate the therapeutic efficiency of the platforms, but to identify the clinical risk, as evaluating off-target effects of nanorobots is as essential as evaluating efficacy.⁶⁴ Manufacturing technologies are enabling innovative works which may help in constructing and employing nano robots most effectively for biomedical problems. Consequently they will change the shape of the industry, broadening the product development and marketing interactions between Pharma, Biotech, Diagnostic and Healthcare industries. Furthermore, proper standards should be established to clarify the advantages of micro/nanorobot therapies over traditional methods which already fulfill FDA standards. The principal future direction in the biomedical nanorobots field is to design biocompatible nanorobots that can be fabricated efficiently and at a large scale, as well as functionalized by easy and low-cost approaches. Future healthcare will make use of sensitive new diagnostics for an improved personal risk assessment.

CONFLICT OF INTEREST

Conflict of interest declared none.

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ARTIFICIAL INTELLIGENCE IN PHARMACEUTICAL INDUSTRY: THE FUTURE IS HERE

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ABSTRACT

Artificial Intelligence (AI) is the simulation of human behavior in relation to the processes of intelligence involved in problem solving. Such mechanisms include human cognitive science reading, observation, preparation, interpretation, reasoning, correction, speech recognition, linguistics, and other sources. AI simplifies tasks by making machines learn from past experiences, mapping efforts and actions to results, identifying errors, correcting them, adjusting to new and random input values, and effortlessly performing human-like tasks through in-depth scenario analysis. AI simplifies work by analyzing, filtering, sorting, predicting, scoping, and determining large data volumes to follow the best implementation procedures for producing an optimal solution. The major pharmaceutical industry applications of AI as on 2019 are; Discovery and development of new drugs; AI is helping big pharma create cures for complex and rare diseases such as Alzheimer's and Parkinson, Drug-Adherence and Dosage; Using AI to make sense of clinical data and to produce better analytics; Finding more reliable patients faster for clinical trials; Introducing automated robot pharmacies to fill prescriptions and dispensing; and marketing, logistics and supply chain. AI is the future of pharma but the technology is available now. Artificial Intelligence can cut costs down, create new, effective treatments and above all else, help save lives. So biotech companies should start making use of the advantages of AI at the earliest. The industry therefore has a lot to gain from embracing solutions to AI and machine learning. It can be used to create a strong, sustainable pipeline of new medicines to good effect. Using the power of modern supercomputers and machine learning would make it possible for us to produce medicines faster and at reduced costs. This article reviews exhaustively the present status and future prospects of Artificial Intelligence in pharmaceutical sciences with specific attention to pharmaceutical industry. The literature has been collected from Pubmed, Google Scholar and commercial websites related to this field. More emphasis has been given to commercial application of AI in pharmaceutical industry in future. In conclusion, the future lies in cooperation between humans and machines, and alongside technological advances, human clinical experts will need to adapt, learn and grow. Although potential experts will have to be both medical and technology experts, it is evolution of medicine, not extinction.

KEYWORDS: *Artificial Intelligence, AI, Machine learning, Deep learning, Neural networks, Pharmaceutical Sciences, Pharma Industry, Startups, Drug discovery*

INTRODUCTION

To meet the needs of society and clinicians in the 21st century, the current drug discovery process needs to shift drastically. In particular, artificial intelligence and machine learning offer the pharmaceutical industry a real opportunity to do R & D differently, so that it can operate more efficiently and significantly improve the early stages of drug development success.¹ Artificial Intelligence (AI) is the simulation of the human behavior with regard to intelligence processes involved in problem-solving. Such mechanisms include human cognitive science reading, observation, preparation, interpretation, reasoning, correction, speech recognition, linguistics, and other sources. AI simplifies tasks by making machines learn from past experiences, mapping efforts and actions to results, identifying errors, correcting them, adjusting to new and

random input values, and effortlessly performing human-like tasks through in-depth scenario analysis.² For this, AI uses Natural Language Processing (NLP) in order to understand natural human communication and translates it internally to a code that machines comprehend. AI also depends on Deep Learning (DL) to accomplish this task. Through the application of these technologies, AI trains computers to perform specific tasks with the least amount of human intervention by processing large data volumes and recognition of familiar or new patterns in data.³ At Dartmouth college conference the term "Artificial Intelligence" was coined in 1956 by a scientist, John McCarthy who is considered as father of artificial intelligence. The term Artificial Intelligence has gained its importance today because of larger data volumes, advanced algorithms and programming, and advancements in computer storage and power.⁴ Recently and largely because of the developments in deep learning that demonstrated incredible precision in image recognition and autonomous driving.⁵ The use of artificial intelligence, and in particular the deep-learning subtype, has been allowed across all sectors through the use of marked big data, together with significantly enhanced computing power and cloud storage.⁶

Differences between machine learning and artificial intelligence

"Machine learning" (ML) is the branch of AI where the goal is defined and the steps to achieve this goal are fed into the system along with the rules and alternative actions to be considered. This information is automatically learned by the system that improves itself based on experience. The program that generates the algorithm integrates the input and the output for efficient performance. The main aim is to produce accurate results than to produce the desired ones. It does not involve randomization based on changing parameters; rather it is based on fixed values. The goal of ML is to learn from a predetermined set of data and produce mechanical solutions for better machine performance, no decision-making is involved. It is completely algorithm-based with structured data formats for inputs and outputs. It involves knowledge building without the discretion of what is right or wrong. The important functionality of ML involves pattern recognition in huge data sets and acting on them. Artificial Intelligence (AI) is based on the ability of a machine to acquire knowledge and apply it skillfully based on various real-life scenarios and real-time data. In AI the goal is to act instead of a human in a human-like manner in order to execute specific independent or interdependent tasks. AI may incorporate multiple programming integrations, validations, and pattern recognition methods to behave in an expected way. The main aim of AI is to produce a result through intelligent data analysis, mining, and deeper understanding. It involves producing results based on smart real-time processing and automation where the parameters are dynamic. The goal of AI is to simulate human intelligence in producing realistic solutions to complex problems through better decision-making. It is based on the machine's response to circumstances and complex problems without a fixed algorithm in place. It involves multiple levels and forms of analysis to seek an optimal solution. It leads to the wisdom of learning using intelligence and self-imposed discretion of right and wrong. AI involves smart learning by a computer through awareness of past iterations and application of alternative information processing and cognitive analysis that demand distinct capabilities.

Objectives of AI⁷

- *Creation of Expert Systems:* It involves the creation of automated systems that exhibit intelligent behavior and advice humans on the right course of action.
- *Implementation of Human Intelligence in Computers⁸:* It will help create identical cognitive patterns in computers which will help them behave like humans and take appropriate actions to solve complex problems. This will enable automated processes and reduced human workload through the application of algorithms.
- *Multi-Domain Application:* AI will help in multiple domains of implementation like Computer Science, Cognitive Science, Statistics, Psychology, Medical Science, Engineering, Ethics, Natural Sciences, Healthcare, Space Technology, Logic, Linguistics, E-commerce, and more.
- *Applications in Computer Science:* AI helps in developing a number of mechanisms to solve many difficult problems in the field of computer science like Search and Optimization, Logic, Control Theory, Language Analysis, Neural Networks, Classifiers, and Statistical Learning Methods, and Probabilistic Methods for uncertain reasoning.

Pros and Cons of Artificial Intelligence⁹

AI simplifies work by analyzing, filtering, sorting, predicting, scoping, and determining large data volumes to follow the best implementation procedures for producing an optimal solution. Performing mundane tasks is faster and effective with reduced errors when performed efficiently by AI systems. Accurate results can be obtained by the implementation of highly-responsive AI systems in technology-driven complex tasks and difficult/uncertain explorations. They function in all environments without being bound physically, environmentally, or emotionally. Simulations are worked on in real-time by AI systems as if they are real-life scenarios for better and realistic results. The Security and Protection of data and crucial information is maintained by AI systems and loopholes are automatically fixed or notified about High-Quality analysis and results are delivered by AI systems that are well-integrated with various technology systems. The associated costs are high due to the complex programming capabilities of AI systems Repair and maintenance are not only expensive but also complex and demand high levels of expertise AI systems lack human judgment during various critical scenarios like target picking during fighter plane routines or investment calls during stock value changes AI systems will lead to loss of jobs for humans in the future since they will take over especially in fields like banking and retail These systems have the scope of being misused or altered either due to internal glitches or external system breach AI systems are not capable of improvement in functionality or infrastructure until these are developed by humans which may leave them obsolete at certain situations.

Types of Artificial Intelligence

As per Arend Hintze, the AI technology based upon their existence as follows:¹⁰

Type 1: (a) Weak AI or Narrow AI which is focused on *one narrow task*, not too intelligent to do their own work. Eg., *poker game* in which *all rules and moves* are fed into the machine. (b) Strong AI: The machines that can actually can think.

Type 2: Based on functionalities it is classified as; (a) "Reactive Machine" which is one of the basic forms of AI which doesn't have past memory and cannot use past information to information for the future actions. Eg., IBM chess program that beat Garry Kasparov in the 1990s. (b) "Limited Memory" in which the AI systems can use past experiences to inform future decisions. Some of the decision-making functions in *self-driving cars* have been designed this way. Observations used to inform actions happening in the not so distant future, such as a car that has changed lanes. These observations are not stored permanently and also *Apple's Chatbot Siri*. (c) Theory of Mind: This type of AI should be able to understand people's *emotion, belief, thoughts, and expectations* and be able to *interact socially*. (d) Self-awareness: An AI that has its own conscious, super intelligent, self-awareness and sentient or in simple words act as a complete human being.

Technologies used in AI

Natural language processing (NLP): program computers to process and analyze large amounts of natural language data.

Support vector machine (SVM): Given a labeled training data (supervised learning), the algorithm outputs an optimal hyperplane which categorizes new examples.

Heuristics: mental shortcuts that ease the cognitive load of making a decision. Eg., using a rule of thumb, an educated guess, an intuitive judgment, a guesstimate, profiling, or common sense.

Artificial neural networks (ANN): Started in way back in 1940s, is an information processing model that is inspired by the way biological nervous systems, such as the brain, process information. An artificial neuron is a mathematical function. ANN takes data samples rather than entire data sets to arrive at solutions, which saves both time and money. ANNs have three layers that are interconnected (PYTHON). Neural networks learn things in exactly the same way as the brain, typically by a feedback process called back-propagation (backprop). ANNs are used in Self Driving cars, Character Recognition, Image Compression and Stock Market Prediction. It is depicted schematically in Figure 2. Software of artificial neural networks (ANNs) models the pattern recognition capacities of brain neural networks. Like a single neuron in the brain, artificial neuron system receives feedback from many external sources, processes it, and makes decisions.

Interestingly, ANN simulates the biological nervous system and uses adaptive biological neurons analogues.¹¹ ANN represents a promising modeling technique, especially for data sets having non-linear relationships which are frequently encountered in pharmaceutical processes. The various applications of ANNs can be summarized into classification or pattern recognition, prediction and modeling (Figure 1). Based on their pattern of association ANNs are classified as supervised 'associating networks, Unsupervised feature-extracting networks and Non-adaptive unsupervised networks. The potential applications of ANN methodology in the pharmaceutical sciences range from interpretation of analytical data, image recognition, drug and dosage form design through biopharmacy to clinical pharmacy.^{12,13}

Figure 1: Typical illustration of artificial neural network

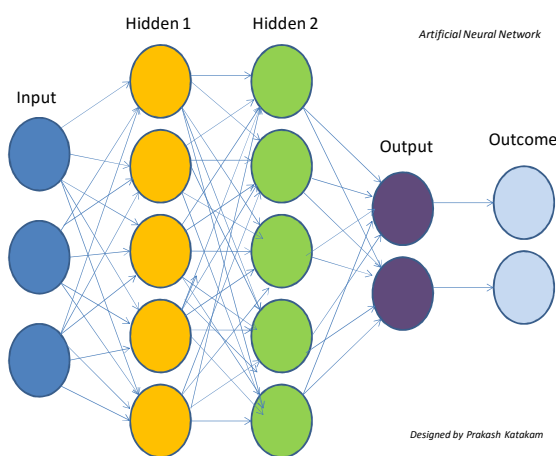
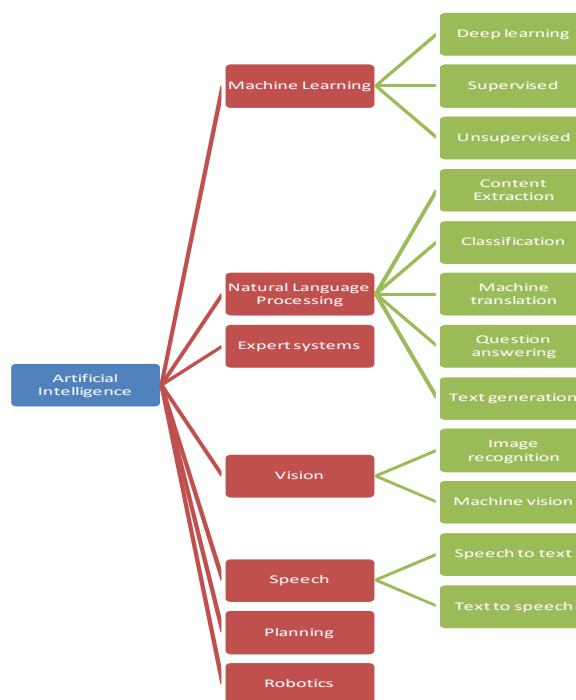


Figure 2: Ways and techniques of achieving AI



Achieving AI

AI is achieved using the following tools and techniques and are shown in Figure 2.

Machine Learning (ML)

It is a method where the *target* (goal) is defined and the *steps to reach* that target are learned by the machine itself by *training* (gaining experience). For example to identify a simple object such as an apple or orange. The target is achieved not by explicitly specifying the details about it and coding it but it is just as we teach a child by showing multiple different pictures of it and therefore allowing the machine to define the steps to identify it like an apple or an orange.

Natural Language Processing (NLP)

Natural Language Processing is broadly defined as the automatic manipulation of natural language, like speech and text, by software. One of the well-known examples of this is email spam detection as we can see how it has improved in our mail system.

Vision

It can be said as a field which enables the machines to see. Machine vision captures and analyses visual information using a *camera*, analog-to-digital conversion, and digital signal processing. It can be compared to *human eyesight* but it is not bound by the human limitation which can enable it to see through walls (now that would be interesting if we can have implants that can make us see through the wall). It is usually achieved through machine learning to get the best possible results so we could say that these two fields are interlinked.

Robotics

It is a field of engineering focused on the design and manufacturing of robots. Robots are often used to perform tasks that are *difficult for humans to perform or perform consistently*. Examples include car assembly lines, in hospitals, office cleaner, serving foods, and preparing foods in hotels, patrolling farm areas and even as police officers. Recently machine learning has been used to achieve certain good results in building robots that interact socially. *Sophia* is a social humanoid robot developed by Hong Kong based company Hanson Robotics.

Autonomous Vehicles

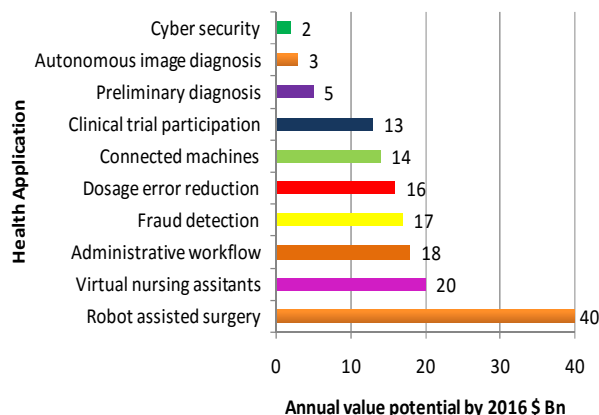
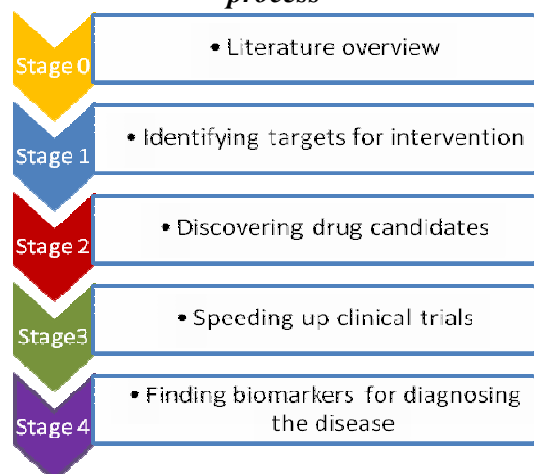
This area of AI has gathered a lot of attention. The list of vehicles includes cars, buses, trucks, trains, ships, submarines, and autopilot flying drones, etc.

AI Market in Health care¹⁴

According to venture capital firm Rock Health, 121 health AI and machine learning companies raised \$2.7 billion in 206 deals between 2011 and 2017. The value of 10 promising AI applications and found that they could create up to \$150 billion in annual savings for U.S. health care by 2026. Reducing health costs by *Image processing, safeguarding patients' personal records against cybercriminals and assisting in surgeries*. AI-enabled workflow assistants are aiding doctors free up their schedules, reducing time and cost. AI helps pathologists in analyzing tissue samples and thus, in turn, making more accurate diagnosis. As per Harvard Business Review and Accenture, there are 10 potential applications of AI which could change the health care industry (Table 1 and Figure 3).

Table 1: Ten potential health applications and potential motivation for adoption

Health Application	Motivation for adoption
Robot assisted surgery	Technological advances in robotic solution for more types of surgery
Virtual nursing assistants	Increasing pressure caused by medical labor shortage
Administrative workflow	Easier integration with existing technology infrastructure
Fraud detection	Need to address increasingly complex service and payment fraud attempts
Dosage error reduction	Prevalence of medical errors, which leads to tangible penalties
Connected machines	Proliferation of connected machines/devices
Clinical trial participation	Patent cliff; plethora of data; outcomes-driven approach
Preliminary diagnosis	Interoperability/data architecture to enhance accuracy
Autonomous image diagnosis	Storage capacity, greater trust in AI technology
Cyber security	Increase in breaches; pressure to protect health data

Figure 3: 10 AI applications that could change health care**Figure 4: Stages of drug development process³⁰**

AI in Pharma Industry

An enormous amount of estimated US\$ 2.6 billion over more than 10 years is spent on developing a drug. In spite of this spending nine out of ten candidate therapies fail between phase I trials and regulatory approval.¹⁵ As described in the report by Codrin Arsene, CEO of Digital Authority Partners, Artificial intelligence in Pharma refers to the use of automated algorithms to perform tasks which traditionally rely on human intelligence. Over the last five years, the use of artificial intelligence in the pharma and biotech industry has redefined how scientists develop new drugs, tackle disease, and more¹⁶. Machine learning and other technologies are expected to make the hunt for new pharmaceuticals quicker, cheaper and more effective. A report by the HIMSS Analytics 2017 Essentials Brief shows that less than 5% of healthcare organizations are currently using or investing in AI technologies.¹⁷ Leading pharma companies are progressing towards using AI for certain strategic applications. AI will help the pharma companies to increase the success rates of new drug discovery meanwhile decreasing operational costs.¹⁸ McKinsey predicts that better decision-making, optimized innovation, enhanced testing performance, clinical trials, and new tool development could produce up to \$100b in pharmaceutical and medicine annually with the aid of big data, AI and machine learning.¹⁹ AI in pharma covers the following expectations that are shown in Table 2.

Table 2: Expectations and strategies of using AI in pharma industry

Company	Strategy of using AI
<i>Expectation 1. Development of new drugs and biomarkers²⁰</i>	
Novartis	Novartis is using machine learning to classify digital images of cells that are treated with new compounds (drugs) for grouping the compounds having similar effects. To speed up the screening process images from machine learning algorithms to predict which untested compounds might be useful and do worth exploring more details.
Berg	Developed an AI model to identify previously unknown cancer mechanisms in more than 1000 cancer and healthy cell samples.
Wuxi NextCODE	Develops drugs and assess them in clinical trials by sophisticated pattern recognition.
Verge Genomics	It is developing new drugs by automating data gathering and analysis to create solutions to some of the most complex diseases known today, including ALS and Alzheimer's predict the effect of new treatments using AI.
Bayer and Merck and Co	Developed artificial intelligence software that aims to support clinical decision making of chronic pulmonary hypertension (CTEPH), asthma and COPD which uses AI algorithms.
Cyclica and Bayer	Created cloud based technology known as "Ligand express" which is AI-augmented integrated network. It uses AI to determine polypharmacological

	profiles to discover drugs faster, safe, and cheaper.
<i>Expectation 2. Tackling of diseases which were difficult to treat earlier</i>	
Tencent Holdings and Medopad	AI facilitated smartphone apps will monitor how a patient opens and closes their hands and captures the movements to determine the severity of their Parkinson's disease symptoms.
Mission Therapeutics	Using AI to develop Deubiquitinase (DUB) inhibitors in the fight against Alzheimer's and Parkinson's.
Healx	Uses AI based platform HealNet that enables scientists to increase production in disease drug discovery while simultaneously reducing time, cost and risk.
<i>Expectation 3. Drug adherence among volunteers during clinical trials</i>	
AiCure and AbbVie	Uses AI based mobile SaaS platform for image recognition to improve drug adherence.
CURATE	An AI based platform built by National University of Singapore, to stop the advanced cancer disease progression by optimizing drug dosage at an individual patient level.
AstraZeneca and Alibaba	The AI platform and smart health services will help patients find and keep using the correct medicine along with automated cancer diagnostics
<i>Expectation 4. Better analysis and utilization of clinical data</i>	
IBM Watson	Uses a deep natural language processing and reasoning algorithm which helps clinicians to identify patient's health attributes against clinical trial requirement, thereby helps clinicians to find potentially eligible patients for clinical trials.
BenevolentBio	AI platform, into which the data from sources such as research papers, patents, clinical trials and patient records are fed to produce knowledge graphs
Apple's ResearchKit	An open source framework that allows researchers to create apps dedicated to medical care. It uses AI to screen children for autism by collecting data from its iPhone and Apple watch products to improve health care.
GNS Healthcare and Genentech	GNS's unique hypothesis free approach of Reverse Engineering and Forward Simulation (REFS) technology that can turn large and diverse patient data streams into mechanistic computer models which show new pathways, novel targets and diagnostic markers that may lead to the discovery of personalized cancer treatments.
<i>5. Finding the correct patients for clinical trials and reducing turnaround times</i>	
Antidote	It uses NLP reads unstructured data entered to screen patients for drug trial enrollment by simplifying the complexity of the inclusion/exclusion criteria in clinical trials. Earlier, this was traditionally near impossible for a normal computer to understand the unstructured data.
Deep 6	About 86% of clinical trials fail to recruit sufficient patients for studies slowing down research. Deep-6 uses AI to analyze unstructured and structured clinical data, doctors' notes and other text documents. The key elements are separated from Clinical data and multi-dimensional profiles are created. These profiles are used to find suitable candidates for clinical trials with quick and better precision. ²¹
Santen and twoXAR	Developed AI based drug discovery platform to discover, screen, and prioritize novel drug candidates for ocular problems.

Pharmaceutical Industry Applications of AI as on 2019^{22,23}

- Discovery and Development of New Drugs
- AI is helping big pharma create cures for complex and rare diseases, Alzheimer's, Parkinson etc.
- Drug-Adherence and Dosage
- Using AI to Make Sense of Clinical Data and to Produce Better Analytics
- Finding More Reliable Patients Faster for Clinical Trials
- Introducing Automated Robot Pharmacies to fill prescriptions and dispensing
- Marketing, Logistics and Supply Chain

AI in Drug Discovery²⁴

There are five stages of drug development process where AI and ML could be employed (Figure 4). Machine learning and artificial intelligence are transforming the process of scientific discovery by allowing scientists to see deeply into vast research data sets and augment scientific intuition. BenevolentAI used this technology to identify several compounds, including compound A, which may have therapeutic potential in (Amyotrophic Lateral Sclerosis) ALS.²⁵ BenchSci and The Science Academy conducted survey in December 2017 and found that the stages where AI is used in drug discovery process include: Target identification and validation, Safety tests, Compound discovery, Lead optimization, Preclinical studies, Formulation, Clinical studies including clinical trial recruitment, Approval, Marketing and Post-marketing surveillance²⁶ There are many AI drug discovery startups offering solutions for every phase of the process, from research to publishing. As on November 13, 2019 there are about 167 startup companies using AI for drug discovery and an exhaustive list of these startups are published with hyperlinks for convenience of the reader (Table 3).²⁷ Based on the importance and gaining popularity of AI in drug discovery, Journal of Medicinal Chemistry of ACS publications is planning to publish a special issue on "Artificial Intelligence in Drug Discovery".²⁸ The road map from clinical data generation to natural language processing data enrichment; to machine learning data analysis, to clinical decision making has been shown in Figure 5.29 The leading 10 disease types considered in the artificial intelligence (AI) literature through searching the disease types in the AI literature on PubMed are Neoplasm, Nervous, Cardiovascular, Urogenital, Pregnancy, Digestive, Respiratory, Skin, Endocrine and Nutritional.

Automation of Community and Hospital Pharmacies

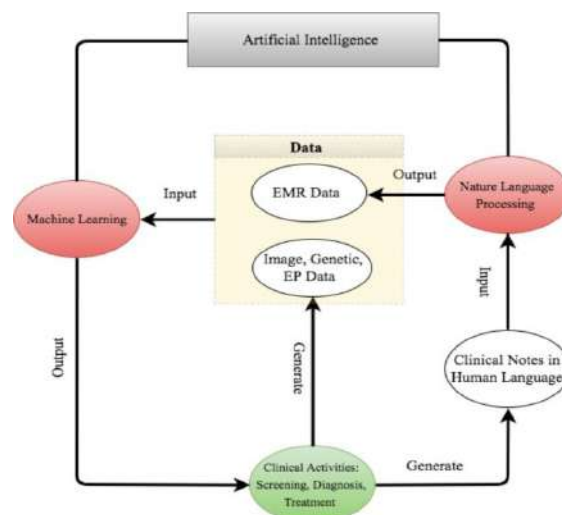
Robots may already be better at doing this than humans, according to KQED Science. Actually, "it's a perfect job for a robot: a boring and repetitive mission," the author wrote. However, many doctors are optimistic about the potential for automation that allows pharmacists to reinvent themselves as a profession rather than being afraid. Unless pharmacists are hoping to survive AI's growth, they need to find a way to use it for their benefit without allowing robots to completely replace them—a truly delicate balance. The fact that the robots did not actually take jobs away from pharmacy technicians is important to note here, instead they created an opportunity for the technicians to concentrate on other more important work.³²

Table 3: Startups involved in various stages of drug discovery process

Stage of drug discovery	Startup company names with hyperlinks
Aggregate and synthesize information	Amplion, BioSymetrics; Biorelate; Causaly; Data2Discovery; Data4Cure; Elucidata Corporation; Evid Science; Genialis; HelixAI; Innoplexus; Intellegens; InveniAI; Iris.ai; Kyndi; LabVoice; LabTwin; Linguamatics; Meta; Mozi; Owkin; PatSnap; Percayai; Plex Research; Quertle; Researchably; Sparrho; ThoughtSpot; nference
Understand mechanisms of disease	Aiforia; Cambridge Cancer Genomics; CytoReason; Euretos; FDNA; OccamzRazor; Phenomic AI; Precisionlife; ReviveMed; Sensyne Health; Structura Biotechnology
Establish biomarkers	Ariana Pharma; Biodesix; Biotrillion; Biotx.ai; Evoke Neuroscience; Glympse Bio; HistoIndex; Syntekabio
Generate data and models	TARA Biosystems
Repurpose existing drugs	Acurastem; BioXcel Therapeutics; Biovista; Healx; Lantern Pharma; Pharnext; Qrativ; Recursion Pharmaceuticals; Standigm
Generate novel drug candidates	A2A Pharmaceuticals; AI Therapeutics; Alphanosos; Antiverse; Arbor Biotechnologies; Arzeda; Atomwise; Auransa; BenevolentAI; Berg; BioAge Labs; BlackThorn Therapeutics; Celsius Therapeutics; ChemPass; Cloud Pharmaceuticals; Clover Therapeutics; Cotinga Pharmaceuticals; Datavant; Deep Genomics; Engine Biosciences; Envisagenics; Erasca; Evaxion Biotech; Exscientia; GTN; Globavir; Gritstone Oncology; Iktos; Insilico Medicine; Insitro; LabGenius;

	Micar Innovation; Mind the Byte; Nanna Therapeutics; Neon Therapeutics; NuMedii; Nuritas; Pharos iBT; ProteinQure; Quantitative Medicine; Qulab; Relay Therapeutics; Resonant Therapeutics; SEngine Precision Medicine; SEngine Precision Medicine; Spring Discovery; Systems Oncology; Systems Oncology; Unnatural Products; Verge Genomics; Vyasa; e-Therapeutics; uBiome
Validate and optimize drug candidates	ATOM; Accutar; Acellera; InVivo AI; NetraMark; Reverie Labs; Turbine; VERISIM Life; XtalPi
Design drugs	Cyclica; Fetch Biosciences; MAbSilico; Molecule.one; Molomics; Numerate; Pending.ai; Pepticom; Peptone; Remedium; TeselaGen; Virvio
Design preclinical experiments	BenchSci; Desktop Genetics
Run preclinical experiments	Arctoris; Berkeley Lights; Emerald Cloud Lab; Strateos; Synthace
Design clinical trials	BullFrog AI; GNS Healthcare; Intelligencia; Keen Eye Technologies; PathAI; Perceiv Research; Reveal Biosciences; Trials.ai
Recruit for clinical trials	Antidote.me; Clinithink; Deep 6 AI; Deep Lens; Inato; Massive Bio; Mendel.ai; Notable; Tempus
Optimize clinical trials	AiCure; Athelas; Brite Health; Imagia; Imagia; nQ Medical
Publish data	Deep Intelligent Pharma; sciNote
Analyze real world evidence	Action; Concerto HealthAI

Figure 5: The road map from clinical data generation to natural language processing data enrichment, to machine learning data analysis, to clinical decision making. EMR, electronic medical record; EP, electrophysiologica.³¹



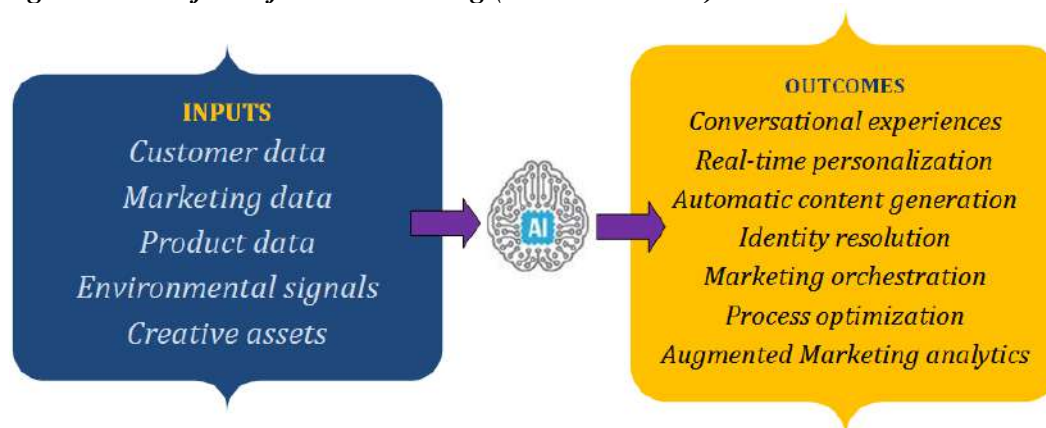
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AI in Pharmaceutical Marketing

Successful pharma brands in 2023 will employ AI in all aspects of their marketing. Every minute of every day, people, machines, sensors and devices generate unimaginable amounts of data. Cloud computing, smart phones and social media platforms are everywhere. Everyone is connected— three billion people online, five billion mobile phones, and six billion devices connected. McKinsey Podcast says that the Real-World Potential and Limitations of Artificial Intelligence – healthcare is likely to be one of the industries where AI has the greatest financial impact, at nearly \$400 billion.³³ It is projected that the data we produce will increase tenfold between 2016 and 2015 to 163 zetta bytes. In the future, data volume will be measured in yottabytes-a number with 24 zeros.³⁴ The General Data Protection Regulation (GDPR) is Europe’s new framework for data protection laws, effective as of May 2018. Any company that stores or processes

personal information about EU citizens must comply with the GDPR. Companies that fail to comply could face steep fines. The way AI manages customer service requests in Pharma Marketing: forecasts, study 2018-2023 reveals that Chatbots handles customer service requests. Hyper-smart marketing technology is leading to greater ROI. Predictive models help us understand the consequences of compliance and drop-off. There are already a lot of AI-powered applications like these. Over the next five years, they will strengthen in number and power, and will be joined by others standing on their shoulders to go even further, especially in decisions on coverage, reimbursement, and treatment. A recent survey by the World Federation of Advertisers found that the greatest skills gap between current and future needs is AI predictive modeling. Simply put, the modern pharma marketers who succeed will be the ones who understand what successful people have always grasped: what tools to work with, how to use them, and how to work with the right people.³⁵ In reality, only 15% of companies used AI this time last year and 31% expected to use it within a year. The technology could turn pharmaceutical sales in 2019, according to Veeva.³⁶ Just as the digital revolution of the 1990s required individuals across the company to build their knowledge and work together, the AI revolution will do the same. Teams across organizations will be tasked with working together to find new and better ways to identify, collect, share, analyze, interpret, and act on data. The process is summarized in Figure 6. The impact of AI on pharmaceutical sales can start before the sales rep meets a customer. Machine learning can separate customers into highly specific segments, allowing sales teams to personalize their activity to greater degree. According to Accenture, AI could help workers to use their time more efficiently, increasing labour productivity by up to 40% –just the sort of boost that sales reps need. Pfizer Australia teamed up with AI provider, Complexica, back in 2017 to simulate sales and marketing strategies and resolve tricky 'what-if' scenarios.

Figure 6: Workflow of AI in marketing (Source: Gartner)Error! Bookmark not defined.



Other applications of AI in Pharma and Healthcare³⁷

- Conducting repetitive tasks such as data entry and laboratory testing, thus clearing time for working on more important or complicated tasks and communicating with patients.
- Management of data, including medical records.
- Healthcare systems analysis to determine errors or inefficiencies.
- Natural language processing. This AI division lets computers understand human speech and writing and interpret it. It can review thousands of detailed electronic medical records and then detail the appropriate steps to identify and treat multi-illness patients.
- Consultations with the medical profession. AI-based systems like Babylon are developed to provide database-based medical advice

Adoption of AI by Pharma Industry

Despite today's high standard of care, drug advancement is becoming increasingly difficult. Overall revenues are therefore generally down and many businesses are looking for innovative treatment solutions to combat this. More effective and automated processes, data-driven decisions, and better predictive analytics tools are needed to boost R & D performance and develop new drugs in order to get out of this situation. It's here that AI comes in.

Nonetheless, due to a lack of AI expertise among many healthcare and pharmaceutical practitioners, it may be difficult to fully embrace AI. There are several approaches to facilitate adoption:

- *Collaboration with, or acquisition of, AI startups and tech companies.* Many pharmaceutical companies reach out to specialist firms and startups that concentrate on drug discovery powered by AI. This enables their expertise and tools to be tapped to create promising drug candidates based on existing hypotheses and experience. Notable examples include collaborations with Bergand companies such as AstraZeneca, Roche, and Sanofi Pasteur, the above-mentioned biopharmaceutical company. In addition, when they recently partnered with IBM Watson for Drug Discovery, Pfizer made headlines, and both Novartis and Johnson and Johnson partnered with IBM Watson Health.
- *Collaboration with academia.* Industry-academia partnerships are expected to keep growing as pharma is starting to embrace AI.
- *Developing internal expertise* and granting employees with the needed resources.
- *Open Science Projects and R & D challenges.* This is a valuable tactic of AI adoption for drug discovery, with less financial risk involved compared to other tactics.

Future of Artificial Intelligence

Companies like Google and Uber are already using AI capabilities to power self-driven cars. AI will have a great bearing on the automated transportation field by aiding handicapped drivers and preventing accidents. More evolved AI systems will support in hazardous factory-based jobs and may replace humans as well. Climatic change predictions can be made by AI systems using data sciences and environmental technologies. Around 80 percent of customer service operations will be handled by effective and timely AI systems. Personalized health management will be made easier through AI systems symptom-identification and medical data processing abilities. Cyborg technology can help patients utilize artificial prosthetics for a better living by communicating with a robotic system. In space technology, AI can study orbital paths during successful launches and suggest actions based on its observations. Coming to Pharma Industry, AI is the future of pharma but the technology is available now. Artificial Intelligence can cut costs down, create new, effective treatments and above all else, help save lives. So biotech companies should start making use of the advantages of AI at the earliest.

Terms of compound design, scope and increase given to us by AI and machine learning will mean that we can tap into a much wider chemical space, giving us a much wider and more diverse range of chemicals to better enable us to pick the best drug discovery molecules. In terms of the industry's choice of patients for clinical trials, the software will also help companies detect any problems with drugs far earlier in terms of efficacy and safety. The industry therefore has a lot to gain from embracing solutions to AI and machine learning. It can be used to create a strong, sustainable pipeline of new medicines to good effect. Using the power of modern supercomputers and machine learning would make it possible for us to produce medicines faster and at reduced costs.

CONCLUSION

Artificial intelligence (AI) of health industry is a set of multiple technologies that allow machines to feel, understand, act, and learn to perform administrative and clinical health care functions. In conclusion, the future lies in cooperation between humans and machines, and alongside technological advances, human clinical experts will need to adapt, learn and grow. Although potential experts will have to be both medical and technology experts, it is evolution of medicine, not extinction. There are various AI and machine learning applications in pharmaceutical applications, including disease identification/diagnosis, personalized treatment/behavioural modification, drug discovery/manufacturing, radiology and radiotherapy, smart electronic health records, prediction of epidemic outbreaks, sales, marketing, predictive analytics, and so on. In addition, AI and ML-based analytics are superior for advertising, particularly because success often needs many ongoing complex decisions with a high degree of judgment. Revenue on the AI health sector is projected to hit \$6.6 billion by 2021, a 40% CAGR, and the medical AI industry is expected to expand more than 10 times in only the next five years.³⁸ AI and machine learning have the potential to enable smarter, faster, and cheaper operations in the near term.

CONFLICT OF INTEREST

Conflict of interest declared none.

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CARDIOMYOPATHIC EFFECT OF METHANOLIC EXTRACT OF *ZIZIPHUS XYLOPYRUS* FRUIT AND ITS ACTIVE FRACTION C IN STZ INDUCED DIABETIC RATS

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ABSTRACT

Diabetic cardiomyopathy is characterized by an early diastolic and later systolic dysfunction with intracellular retention of calcium and sodium and loss of potassium. Deficiency of oxygen supply or glucose supply may cause damage to the myocardial cell membrane leading to permeable and ruptures so that the enzymes leaks out. The present study was investigate that cardiomyopathic effect of methanolic extract of *ziziphus xylopyrus* fruit and its active fraction c on STZ induced diabetic rats. Diabetes was induced in overnight fasted wistar rats (150-200gm) by a single intraperitoneal (i.p.) injection of a freshly prepared buffer (0.1 mol/L citrate, pH 4.5) solution of STZ at the dose of 60 mg/kg body weight. After 72 h of STZ administration, the blood was collect from tail vein to determine fasting blood glucose level with an AccuChek sensor comfort glucometer (comfort). Rats with hyperglycemia (glucose > 200 mg/dL) were considered as diabetic and included in the experiment. After completion of anti-hyperglycemic activity (at the end of 28th day), heart was isolated from all experimental animals, homogenated and collect the supernatant by centrifugation process. Supernatant was subjected for estimation of Troponin-I, Creatine kinase isomerase (CK-MB) and Total Creatine Kinase (CK), using commercial kits (Biosystems) and semi auto analyzer. At the end of 28th day rats were humanely euthanized by cervical dislocation. The cardiac tissue was excised and washed in normal saline and fixed in 10% formalin. The fixed specimens were embedded in paraffin and sectioned at 5 µm thickness. Sections were stained with Heamatoxylin and eosin for texture of myocardium. Cardiomyopathic parameters such as Troponin-I, CK-MB and Total CK in cardiac tissue were examined. Parameters were well rectified with high dose of MEZX (200 mg/kg) and respectively after treatment with MEZX active fraction C at 100 mg/kg.. STZ induced diabetic rats exhibited significantly increase in Troponin-I, CK-MB and Total CK compared with Control group. The study was concluded that MEZX at a dose of 100, 200 mg/kg and its active fraction at a dose of 100mg/kg showed significant reduction of cardio biomarkers in STZ induced diabetic rats.

KEYWORDS: *Diabetic cardiomyopathy, wistar rats, Glucose, Streptozocin and Kinase*

INTRODUCTION

Diabetes mellitus is a chronic disease due to primarily a disorder in carbohydrate metabolism, as a result of deficiency or diminished effectiveness of insulin, resulting in hyperglycemia and glycosuria¹. Diabetes affects more than 100 million people worldwide (6% of the population) and in the next 10 years, projected to affect five times more people than it does now². Herbal medicines and plant components with insignificant toxicity and no side effects are notable therapeutic options for the treatment of this disease around the world³. In recent times many traditionally used medicinally important plants were treated for their antihyperglycemic potential by various investigators in the experimental animals. Most tests have demonstrated the benefits of medicinal plants containing hypoglycemic properties in diabetes management. The most common herbal active ingredients used to treat diabetes are flavonoids, tannins, phenolics and alkaloids⁴. The existence of these compounds implies the importance of the anti-diabetic properties of these

plants. For example, tannin improves the function of pancreatic Beta-cells and increases insulin secretion. Quercetin is an antioxidant that acts in several mechanisms related with the removal of oxygen radicals, so prevents lipid peroxidation and metal ion chelation. In fact, the mechanisms of actions for hypoglycemic plants include: increasing of insulin secretion, increasing of glucose absorption by muscle and fat tissues, prevention of glucose absorption from the intestine, and prevention of glucose production from liver cells⁵. *Ziziphus xylopyrus* fruits contain oleanolic acid, epicatechin and leucocyanidin, 3, 3, 4-tri-o-methyl ellagic acidquercetin⁶. These biological activities are related to their antioxidative effects. These properties were attributed to different formulations, extracts and active principles Working on the same line, an attempt has been made to study on traditional medicinal plant like *Ziziphus xylopyrus* for anti-diabetic activity.

MATERIALS AND METHODS

Collection and Processing of Plant Material

The fruits of *Ziziphus xylopyrus* were purchased from the local market of Guntur, Collected materials were authenticated by Prof K. Ammani, Department of Botany and microbiology, Acharya Nagarjuna University. The *Ziziphus xylopyrus* fruits were shade dried and pulverized. Powdered materials were stored in a desiccator until further extraction.

Physicochemical investigations

Physical parameters determined for the powdered leaves such as a percentage of ash values and extractive values were determined according to the well-established official methods and procedure.^{7, 8} the results were shown in table 1-2 and 3.

Phytochemical investigation

Chemical tests were performed in the preliminary phytochemical screening to identify various secondary metabolites such as alkaloids, glycosides, saponins, flavonoids, sterols using the standard procedure described by Kokate *et al.* and Khandelwal.^{9, 10} the results were shown in table 4.

Fractionation of MEZX using Column Chromatography

Thin layer chromatography and column chromatographic techniques were used for purification of selected active extract¹¹. The solvent proportion used for TLC (silica gel aluminum plate 60F-255, thickness 0.5mm) was Petroleum ether/ ethyl acetate (7:3). The spots were observed under UV light and H₂SO₅ (10%) in methanol. The MEZX was subjected to column chromatography for further purification. The column was stabilized for one hour by petroleum ether at flow rate of 5 mL/min. The sample was (1 g dissolved in methanol) loaded on to the column and observed for fractions separated by varieious solvent system such as Petroleum ether, Ethyl acetate: n-Butanol: Water (5:4:1), Chloroform: Ethyl acetate (7:3),Methanol: Ethyl acetate (6:4),Petroleum ether: Ethyl acetate (6:4), Petroleum ether: Chloroform (7:3), Chloroform: Petroleum ether (9:1). Above yielded products were pooled into four fractions based on TLC and checked for their anti-diabetic activity.the results were shown in table 5-7.

Experimental Animals

Wistar albino rats (150-200 gm) were used for the study. The animals were purchased from Mahaveer enterprises (1656/PO/b/12/CPSEA), Hyderabad. All the rats were housed in standard plastic rat cages with stainless steel cover lids and bedding material. The animals were maintained at the animal house, Chebrolu Hanumaiah institute of Pharmaceutical Sciences. The animals were housed with standard environmental condition as per CPCSEA guidelines. All the study protocols for animal experimentation were approved by CPCSEA recognized local IAEC [1529/PO/Re/11/CPCSEA] at Chebrolu Hanumaiah institute of pharmaceutical sciences, Guntur, Andhra Pradesh.

Evaluation of Antihyperglycemic Activity

Diabetes was induced in overnight fasted wistar rats(150-200gm) by a single intraperitoneal (i.p.) injection of a freshly prepared buffer (0.1 mol/L citrate, pH 4.5) solution of STZ at the dose of 45 mg/kg body weight. After 72 h of STZ administration, the blood was collect from tail vein to determine fasting blood glucose level with an AccuChek sensor comfort glucometer (comfort). Rats with hyperglycemia (glucose > 200

mg/dL) were considered as diabetic and included in the experiment. Diabetic rats were divided into eight groups with six animals each.¹² the results were shown in table 8-10 and Fig 1-3.

Experimental animal groups with respective administration were as follows:

Groups	Treatment
Group I	: Control
Group II	: Diabetic control (STZ 60 mg/kg)
Group III	: Glibenclamide (10 mg/kg)
Group IV	: MEZX (100 mg/kg.)
Group V	: MEZX (200 mg/kg.)
Group VI	: Fraction A (100 mg/kg)
Group VII	: Fraction B (100 mg/kg)
Group VIII	: Fraction C (100 mg/kg)
Group IX	: Fraction D (100 mg/kg)

Treatment with MEZX and Glibenclamide were given daily for 4 weeks using oral gastric gavage. Blood glucose levels were measured on 30mins, 1st, 3rd, 6th, and 12th hour of the first day for acute effect of MEZX and active fractions. Further, fasting blood glucose levels were measured on 7th, 14th, 21st and 28th day respectively for chronic effects of MEZX and active fractions.

The percentage of change of glucose from each group was estimated in relation to initial (0 hr) level, according to:

$$\% \text{ glucose change} = G_0 - G_t \times 100 / G_0$$

Procedure for Cardiomyopathic effect of MEZX and its active fraction C in STZ induced Diabetic Rats:

After completion of anti-hyperglycemic activity (at the end of 28th day), heart was isolated from all experimental animals, homogenated and collect the supernatant by centrifugation process. Supernatant was subjected for estimation of Troponin-I, Creatine kinase isomerase (CK-MB) and Total Creatine Kinase (CK), using commercial kits (Biosystems) and semi auto analyzer.

Histological analysis of Cardiomyopathic effect of MEZX and its active fraction C on STZ induced Diabetic Rats

At the end of 28th day rats were humanely euthanized by cervical dislocation^[192]. The cardiac tissue was excised and washed in normal saline and fixed in 10% formalin. The fixed specimens were embedded in paraffin and sectioned at 5 µm thickness. Sections were stained with Heamatoxylin and eosin for texture of myocardium. The results of MEZX and fraction C were shown in fig 4.

RESULTS

Physicochemical Characters.

Table 1. Determination of moisture content

Parameter	<i>Ziziphus xylopyrus</i> fruit powder
Loss on drying (%w/w)	11.6

Table 2. Determination of ash values of *Ziziphus xylopyrus* fruit powder

S. No	Parameter	<i>Ziziphus xylopyrus</i> fruit powder (%w/w)
1	Total ash	14.2
2	Acid insoluble ash	0.52
3	Water insoluble ash	1.10

Table 3. Determination of alcohol and water soluble extractive values

S. No	Parameter	<i>Ziziphus xylopyrus</i> fruit powder (%w/w)
1	Alcohol soluble extractives	11.2
2	Water soluble extractives	11.3

Table 4. Preliminary phytochemical screening of MEZX and MECI

S. No	Name of the Test	MEZX
1	Tests for Alkaloids	
	a) Dragendroff's test	+
	b) Mayer's test	+
	c) Hager's test	+
	d) Wagner's test	+
2	Tests for Flavonoids	
	a) Alkaline reagent test	+
	b) Shinoda test	+
3	Test for Steroids	
	a) Salkowski test	+
4	Tests for Phenolic and Tannins	
	a) Ferric chloride test	+
	b) Lead acetate test	+
	c) Gelatin test	+
5	Tests for Saponins	
	a)Forth development test	+
	b) Lead acetate test	+
6	Tests for glycosides	
	a)Legal test	-
	b)Keller-Kiliani test	-
7	Tests for Carbohydrates	
	a) Fehling's test	+
	b) Benedict's test	+
8	Tests for Polypeptides and proteins	
	a) Millon's test	+
	b)Ninhydrin test	+
9	Test for Terpenoids	+

MEZX are the methanolic extracts of *Ziziphus xylopyrus* fruits Whereas, (+) indicates the presence and (–) indicates the absence of respective principle in the extract.

Table 5. Fractions and TLC parameters of MEZX

Solvent System	No of Spots	R _f value	Fractions
Ethyl acetate : <i>n</i> -Butanol : Water (5:4:1)	5	0.5, 0.61, 0.70, 0.90, 0.98	A
Chloroform : Ethyl acetate (7:3)	4	0.4, 0.59, 0.61, 0.98	B
Methanol : Ethyl acetate (6:4)	4	0.6, 0.71, 0.89, 0.90	C
Petroleum ether: Ethyl acetate (6:4)	3	0.3, 0.4, 0.9	D

Table 6. Color characteristics of fractions of MEZX

Fraction	Consistency	Colour of the Fraction
A	Greasy mass	Dark yellow
B	Pasty mass	Dark red
C	Pasty Mass	Light red
D	Pasty Mass	Light yellow

Table 7. Phytochemical analysis of fractions of MEZX

S. No	Name of the Test	A	B	C	D
1	Tests for Alkaloids				
	a) Dragendroff's test	-	-	-	-

	b) Mayer's test	-	-	-	-
	c) Hager's test	-	-	-	-
	d) Wagner's test	-	-	-	-
2	Tests for Flavonoids				
	a) Alkaline reagent test	-	+	+	-
	b) Shinoda test	-	+	+	-
3	Test for Steroids				
	a) Salkowski test	-	-	-	-
4	Tests for Phenolic and Tannins				
	a) Ferric chloride test	-	-	-	-
	b) Lead acetate test	-	-	-	-
	c) Gelatin test	-	-	-	-
5	Tests for Saponins				
	a)Forth development test	-	+	+	-
	b) Lead acetate test	-	-	-	-
6	Tests for glycosides				
	a)Legal test	-	-	-	-
	b)Keller-Kiliani test	-	-	-	-
7	Tests for Carbohydrates				
	a) Fehling's test	-	-	-	-
	b) Benedict's test	-	-	-	-
8	Tests for Polypeptides and proteins				
	a) Millon's test	-	-	-	-
	b)Ninhydrin test	-	-	-	-
9	Test for Terpenoids	+	+	+	-

A,B,C and D represents the fractions of methanolic extract of *Ziziphus xylopyrus* fruits (MEZX). Whereas, (+) indicates the presence and (–) indicates the absence of respective principle in the extract

Table 8.Acute anti-hyperglycemic effect of fractions of MEZX on STZ induced diabetic rats.

Treatment	Blood glucose levels (mg/dL)					
	0 min	30 min	1 st hr	3 rd hr	6 th hr	12 th hr
Control	120.11±1.8	121.12±1.9	110 ±1.5	100.1 ±1.7	99.1±1.6	102.3±1.5
STZ	331.6±21.8	334.6±22.3	332.3±23.6	339.6±23.4	324.2±23.5	328.1±124
Glibenclamide	341 ±22.4	321 ±21.4	287 ±23.4	198 ±3.2	209±21.5	302±18.9
Fraction A	298.2 ±20.4	315.8 ±19.3	300.6±20.5	286.6±15.3	279.2±19.2	297.6±19.2
Fraction B	308.4 ±22.3	277.2 ±10.2	264.8±18.7	255.4±19.4	274.4±18.2	281.8±16.3
Fraction C	293.6 ±19.8	260.6 ±16.4	235 ±19.3	227 ±18.2	248.2±14.7	263.6±16.3
Fraction D	304 ±20.7	287.2 ±17.2	261.6±14.3	254.8±17.3	267 ±14.2	280.6±17.4

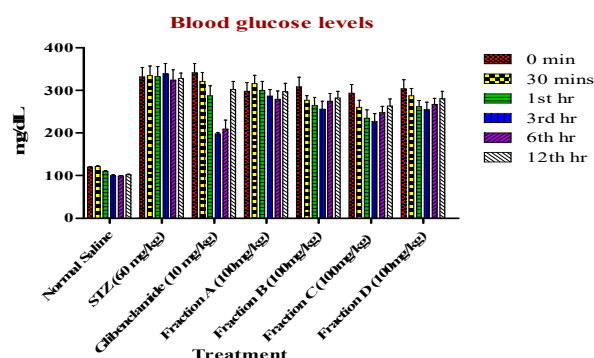


Fig1. Represents the acute anti-hyperglycemic effect of different doses of methanolic extract of *Ziziphusxylopyrus* fruit powder (MEZX) at different time intervals on Streptozotocin (STZ) induced diabetic rats. Streptozotocin and glibenclamide were given at a dose of 60mg/kg and 10 mg/kg respectively. Whereas, fractions of MEZX (A, B, C and D) were administered in 100 mg/kg to the respective groups. Values are expressed as Mean $\pm$ SEM; Significance was done with Dunnett's test by one way ANOVA compared with diabetic animals.

Table 9. Chronic anti-hyperglycemic effect of fractions of MEZX on STZ induced diabetic rats

Treatment	Blood glucose levels (mg/dL)				
	0 day	7 th day	14 th day	21 st day	28 th day
Control	87.6 $\pm$ 1.6	86.6 $\pm$ 1.2	86.2 $\pm$ 0.9	93 $\pm$ 1.2	89.8 $\pm$ 1.2
Streptozotocin	310.4 $\pm$ 15.4	307.2 $\pm$ 21.3	313.8 $\pm$ 21.1	326.4 $\pm$ 19.8	317 $\pm$ 20.1
Glibenclamide	307.2 $\pm$ 14.5	212.2 $\pm$ 18.9	179.2 $\pm$ 14.3	123.4 $\pm$ 1.9	67 $\pm$ 0.9
Fraction A	298.2 $\pm$ 20.4	254 $\pm$ 11.8	234 $\pm$ 12.3	215 $\pm$ 10.5	203 $\pm$ 11.2
Fraction B	308.4 $\pm$ 22.3	260 $\pm$ 12.1	242 $\pm$ 11.2	231 $\pm$ 10.2	220 $\pm$ 14.1
Fraction C	293.6 $\pm$ 19.8	226.8 $\pm$ 13.2	193.8 $\pm$ 9.8	160.2 $\pm$ 5.6	148 $\pm$ 6.4
Fraction D	304 $\pm$ 20.7	220 $\pm$ 11.2	234 $\pm$ 7.4	241 $\pm$ 8.6	238 $\pm$ 7.9

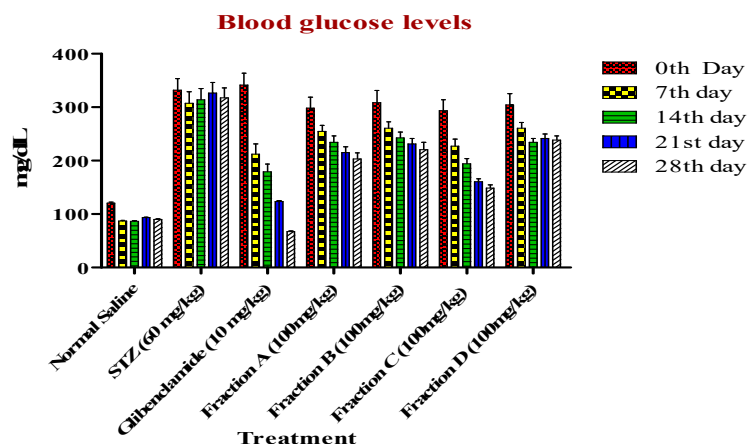


Fig2. Represents the chronic antihyperglycemic effect of different doses of methanolic extract of *Ziziphusxylopyrus* fruit powder (MEZX) at different time intervals on Streptozotocin (STZ) induced diabetic rats. Streptozotocin and glibenclamide were given at a dose of 60mg/kg and 10 mg/kg respectively. Whereas, fractions of MEZX (A, B, C and D) were administered in 100 mg/kg to the respective groups. Values are expressed as Mean $\pm$ SEM; Significance was done with Dunnett's test by one way ANOVA compared with diabetic animals.

Table 10
Effect of MEZX and its active Fraction C on cardiac Troponin-I, CK-MB and Total CK levels

S.No	Treatment	Troponin-I (ng/mL)	CK-MB (U/L)	Total CK(U/L)
1	Control	0.18 $\pm$ 0.02	329 $\pm$ 9.8	64 $\pm$ 3.4
2	STZ (60 mg/kg)	1.32 $\pm$ 0.01	469 $\pm$ 12.4	160 $\pm$ 7.4
3	MEZX (100 mg/kg)	1.05 $\pm$ 0.01	409 $\pm$ 13.2	98 $\pm$ 4.5
4	MEZX (200 mg/kg)	1.02 $\pm$ 0.02	402 $\pm$ 19.3	89 $\pm$ 4.8
5	Fraction C (100 mg/kg)	0.87 $\pm$ 0.02	376 $\pm$ 1.2	76 $\pm$ 1.6

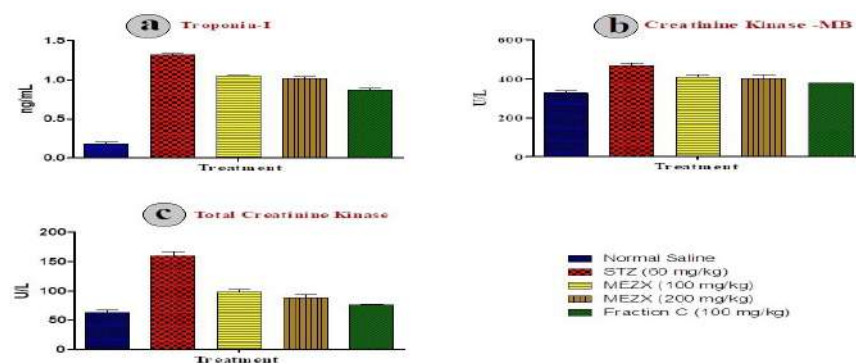


Fig3. a) Represents the levels of Troponin-I, after treatment with different doses of methanolic extract of *Ziziphus xylopyrus* fruit powder (MEZX) on STZ induced diabetic rats. Whereas Creatinine Kinase-MB and total Creatinine Kinase were represented as b and c respectively. MEZX was administered as doses 100, 200 mg/kg respectively and Fraction C with 100 mg/kg to the respective groups. Values are expressed as Mean $\pm$ SEM; Significance was done with Dunnett's test by one way ANOVA, results compared to Diabetic Control.

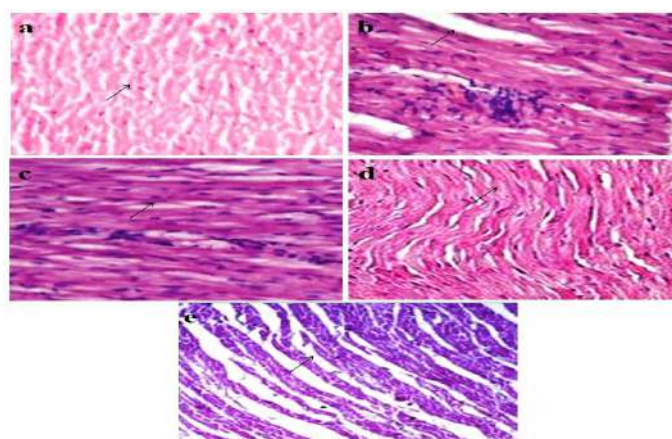


Fig4. Histological studies of methanolic extracts of *Ziziphus xylopyrus* fruit powder (MEZX) and its active fraction C against STZ induced Diabetes in rat myocardium by H&E staining. Morphology of cardiac tissue was examined and images were represented as a for Control and b for Streptozotocin (60 mg/kg) induced diabetic rats. Treatment with MEZX in different doses (100 and 200 mg/kg) was represented as c and d. whereas, myocardial morphology of MEZX active fraction C (100 mg/kg) was represented with e.

DISCUSSION

Plant materials contain a variety of natural products with different polarities and hence different solubility properties^{13,14}. Whereas, traditional healers are known to use mainly crude aqueous plant extracts in their treatment of diseases, the majority of published scientific studies which have investigated the medicinal properties of plant materials have utilized combinations of two or more polar and non-polar solvents to extract bioactive substances from plant materials^{15,16}. Streptozotocin (69%) and alloxan (31%) are most frequently used as diabetic inducers and our model has been useful for screening the anti-diabetic activity¹⁷. Streptozotocin enters the pancreatic cell via a glucose transporter-GLUT2 cause's deoxyribonucleic acid (DNA) alkylation and also activation of poly adenosine diphosphateribosylation and nitric oxide release; resulting pancreatic cells are destroyed by necrosis¹⁸. Present study was reported that the administration of single dose of STZ (45 mg/kg) produced significant hyperglycemia in normal rats and hyperglycemia was significantly ameliorated by treatment of MEZX when compared with standard glibenclamide. Glibenclamide was regulated hyperglycemia by increased release of insulin from pancreatic beta cells¹⁹. Hypoglycaemic effect was observed that the treatment with MEZX (100 and 200 mg/ kg) reduced blood sugar significantly at 120 and 240 mins when compared to untreated rats. Similarly, comparison with Glibenclamide treated rats exhibited significant blood glucose lowering efficacy at all time intervals. Hypoglycaemic effect of MEZX was studied by administration of different doses at 100 mg/kg and 200 mg/kg in laboratory conditions. During the study, we found that Glibenclamide treated rats also showed

significant blood glucose lowering efficacy at all the time intervals at highest of 41.9% in 3rd hour, which was extended up to 78.8% on 28th Day. MEZX at 200 mg/kg and corresponding fractions at a dose of 100 mg/kg produced significant reduction in blood glucose. Among all fractions, Fraction C of MEZX produced 22.4 % reduction in blood glucose at 3rd hr which was extended to 53.3% at the end of 28th day. These results are far better when compared to MEZX at 200 mg/kg in 12th hr (26.3%) which was extended to 64.3% on 28th day in STZ treatment rats. In addition, cardiomyopathic parameters such as Troponin-I, CK-MB and Total CK in cardiac tissue were examined. Parameters were well rectified with high dose of MEZX (200 mg/kg). STZ induced diabetic rats exhibited significantly increase in Troponin-I, CK-MB and Total CK compared with Control group. These particular cardiac myopathic parameters such as Troponin-I, CK-MB and Total CK were decreased from 1.32 to 1.02 ng/mL, 469 to 402 U/L and from 160 to 89 U/L respectively with treatment of MEZX (200 mg/kg) when compared with diabetic rats. This cardiomyopathic marker such as Troponin-I, CK-MB and Total CK were decreased from 1.32 to 0.87 ng/mL, 469 to 376 U/L and from 160 to 76 U/L respectively after treatment with MEZX active fraction C at 100 mg/kg. Haematoxylin and eosin (H&E) stained sections of heart of control rats showing well-arranged cardiac myocytes and normal striations. Section of STZ-induced diabetic rats depicting disarranged cardiac myocytes with destroyed striations. Cardiac section of rats portraying better striations in dose dependant manner after administration with 100 mg/kg and 200mg/kg of MEZX respectively.

CONCLUSION

It was concluded that MEZX at dose of 100, 200 mg/kg and fractions C at 100 mg/kg exhibited significant reduction in blood glucose levels of STZ induced diabetic rats and MEZX at a dose of 100, 200 mg/kg and its active fraction C at a dose of 100mg/kg showed significant reduction of cardio biomarkers in STZ induced diabetic rats. All the above scientific evidences supported that selected plant extract and their specific fraction exhibited potential benefit against diabetes and its complication diabetic cardiomyopathy.

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A STUDY OF MEDICATION ERRORS IN HOSPITALIZED PATIENTS AT A SOUTH INDIAN TERTIARY CARE HOSPITAL

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ABSTRACT

The ultimate goal of medication use is to attain the optimum therapeutic benefits or outcomes for the improvement of the patient's quality of life. About 30% of medical problems that are occurring in hospitals are related to medication errors which are preventable and harming at least 1.5 million people every year. To evaluate and categorize the medication errors that occurred in hospitalized patients of tertiary care hospital, Vijayawada, Andhra Pradesh to improve the patient treatment outcomes. The prospective observational study was carried out in the in-hospitalized patients of tertiary care hospitals from June 2018 to November 2018. Out of 400 prescriptions, medication errors were found in 66 cases, which counted to a total of 80 medication errors, the most frequently occurring medication error was wrong dose error (36, 40%), wrong drug error (14, 17.5%), wrong frequency error (10, 12.5%), omission error (4, 5%) dispensing error (4, 5%), wrong route of administration (2, 2.5%), wrong dosage form error (2, 2.5%) monitoring error (2, 2.5%) High incidence of medication error falls under the category Prescribing error (58, 72.5%). Out of 80 ME'S high incidence of medication errors falls under the Category C (47.5%) followed by Category A (15%), Category E (15%), Category F (10%), Category B (7.5%), Category D (5%). This may be due to an environment that is susceptible to medication errors. The study concluded that the high incidence of medication error occurred in the Neurology department (25%), followed by the Gastroenterology department (22.5%). The majority of errors that occurred to the patients did not cause any harm. There is a need to set up a ME detailing framework by Clinical Pharmacists to diminish its frequency and improve tolerant of patient consideration and safety.

KEYWORDS: *Medication errors, patient care, clinical pharmacists, medicine, Up to Date Prescribing error.*

1. INTRODUCTION

Generally, medication errors represent one of the largest single cause of errors in the hospitals, that may be resulted from the miscommunication among physician, nurse, pharmacist, patient and other healthcare staff, which could result to lack of data, quality of tasks concerned, lack of expertise, the nature of the work environment, handling of high risk drugs and also due to noncompliance of patient to be given instructions. In India, medication errors and medication-related problems are mainly due to the irrational use of medications. A medication error is an episode associated with the use of medication that should be preventable through an effective control system.¹ It is thought of as close to, 30% of problems occurring during hospitalization are related to medication errors. Over the past forty-five years, various terms and definitions have been used to describe medication errors previously, the term (prescription error) alluded to administration blunders as it were. However today, it refers to errors at any stage of the medication use process.² Such events could also be associated with skilled observe, health care products, procedures, and systems together with prescribing, order communication, product labelling, packaging, and word, combining, dispensing, distribution, administration, education; monitoring, and use.³ A similar definition of medication errors was also reported in previous studies.^{4, 5} The first step toward preventing the incidence of medication errors is a proper understanding of the contributing factors for the occurrence of these errors.^{6, 7} The contributing factors may be divided into two subgroups: those caused

by systems and those caused by individual health care professionals.^{8, 9} One of the health care work features is multiple interruptions. Situations like these make the nurses be easily distracted; forget what they were doing, and be more likely to commit errors.^{10, 11} One of the major contributing factors for the occurrence of medication errors in the nursing high workload,^{12, 13} Therefore nurses are more prone to commit medication errors under such stressful events and^{14, 15} the higher the workload the higher the risk for error. The incidence of medication error rates was found to be between 5% and 18% of drugs administered currently the incidence of medication errors is greatly underestimated and under-reported.^{16, 17} According to ASHP (American society of hospital pharmacist) guidelines, a medication error can be categorized into many more types, such as; 1. Prescribing error 2. Omission error 3. Improper dose error 4. Unauthorized drug error 5. Deteriorated drug error 6. Wrong time error 7. Wrong dosage form error 8. Wrong drug preparation error 9. Wrong administration technique error 10. Monitoring error 11. Compliance error.

2. METHODOLOGY

2.1 Study design, study site, and duration of the study:

It is a prospective observational study conducted at tertiary care Super specialty hospital, for a period of six months from June 2018 to November 2018.

2.2. Our study was followed the both criteria's

2.2.1 Inclusion Criteria

All age group patients both genders being admitted to the study site during the study period.

2.2.2 Exclusion Criteria: Out patients departments and Psychiatric patients

2.3 Study material, Study procedure, Data analysis:

All the necessary and relevant data were collected through chart review method during clinical rounds using a data collection form inpatient case records. The patients, prescriptions and dispensing systems were monitored. The medication errors were identified, and those identified medication errors were categorized based on the NCCMERP index. The medication errors were identified by using software like Micromedex, Up To Date [lexicomp], Medscape, drugs.com, etc.

2.4 Statistical analysis

The P-value of less than < 0.05 was considered as statistically significant. Data were analyzed by using Microsoft excel for windows.

2.5 Ethical Approval

The study was approved by the Institutional Ethics committee (IEC) of Nirmala College of Pharmacy, Atmakur, Guntur district, Andhra Pradesh. (PD003/IRB/NRML/2018-2019)

3. RESULTS AND DISCUSSION

The ultimate goal of medication use is to achieve the optimum therapeutic benefits.^[18,19] Medication errors are the most common type of medical errors that occur in hospitalized patients. It increases the morbidity and mortality of the population along with an increase in the cost of the treatment, about 30% of medical problems that are occurring in hospitals are related to preventable medication errors.^[20] Such medication errors may be related to the health care professional practice, healthcare products, procedures, and systems.^[21,22] In India, the irrational use of drugs is more and this has led to antibiotic resistance, medication errors and other drug-related problems.^[23]

3.1 Prescriptions with and without medication errors

A total of 400 patients prescriptions were collected from various departments in the hospital, among them, a total of 80 medication errors were identified, in that 66 prescriptions are with errors (16.5%) and 334 prescriptions (83.5%) are without ME.

3.2 Clinical characteristics of patients with and without medication errors

Table 1: Clinical characteristics of patients with and without medication errors

S. No.	Age group (In years)	No of patients (n=400) (%)	No of patient with medication error(n=66)	No of patient with out medication error(n=334)
1	0-20	36 (9%)	10 (15.15%)	26 (7.78%)
2	21-40	116(29%)	24 (36.36%)	92 (27.54%)
3	41-60	176 (44%)	26 (39.39%)	150 (44.91%)
4	61-80	64 (16%)	6 (9.09%)	58 (17.36%)
5	>80	8 (2%)	0 (0 %)	8 (2.39%)
	Gender			
1	Male	250 (62.5%)	44 (66.66%)	206 (61.67%)
2	Female	150 (37.5%)	22 (33.33%)	128 (38.32%)
	No of medications			
1	02-May	116 (29%)	12(18.18%)	104(31.13%)
2	06-Oct	198(49.5%)	40(60.60%)	158(47.30%)
3	Nov-15	66(16.5%)	8(12.12%)	58(17.36%)
4	>15	20(5%)	6(9.09%)	14(4.19%)
	Length of Stay			
1	02-Apr	136(34%)	12(18.18%)	124(37.12%)
2	05-Jul	118(29.5%)	16(24.24%)	102(30.53%)
3	08-Oct	124(31%)	34(51.51%)	90(26.94%)
4	≥11	22(5.5%)	4(6.06%)	18 (4.19%)

Table 1 explains about age and gender wise description. The demographic reports of the present study showed a higher incidence of medication errors in patients with the age group of 41-60 years 26(39.39%). Out of these 400 patients, a high incidence of ME occurred in adult male i.e. 44(66.66%) patients due to polypharmacy and co-morbidity. Out of 400 patients, no of medications that high incidence of medication error was occurred in 6-10 medication patient i.e. 40(60.60%). Length of stay that high incidence of medication error was occurred in 8-10 days staying patient i.e. 34(51.51%). The study reported that the risk of medication errors increases as the number of medications per prescription increases and as the length of hospital stay increases number of medication errors increases and it may also increase the cost of drug therapy.

3.3 Department wise distribution of medication errors

Out of 80 medication errors, the high incidence of medication error occurred in the neurology department (25%), followed by the gastroenterology department (22.5%) and the infectious department (15%). Most of those ME seen in CVA patients and Epileptic patients.

3.4 Class of drugs and the number of medication errors

In our study distribution of ME's according to the class of drugs involved. Out of 80 medication errors more than 10 class of drugs were involved, in those the drug class having high incidence of medication error was antimicrobials (n=28, 35%) because of the irrational use of antibiotics followed by CNS drugs (10%).

3.5 Categorization of medication error based on underlying causes

S.No	Type of error	No of medication error(n=80)	Percentages %
1	Prescribing error	58	72.50%
	Wrong Drug	14	17.50%
	Wrong Dose	32	40%
	Wrong Route	2	2.50%
	Wrong frequency	10	12.50%
2	Omission error	4	5%
3	Improper dose error	0	0

4	Unauthorized drug error	0	0
5	Deteriorated drug error	0	0
6	Wrong time error	2	2.50%
7	Wrong frequency error	4	5%
8	Wrong dosage form error	2	2.50%
9	Wrong drug preparation error	2	2.50%
10	Wrong route of administration error	2	2.50%
11	Monitoring error	2	2.50%
12	Dispensing error	4	5%

Table 02 Exemplifies the distribution of ME's based on the underlying cause.

A high incidence of medication error falls under the category Prescribing error (58,72.5%). Out of 80 number of medication errors the most frequently occurring medication error in the category of prescribing error was wrong dose error (32, 40%), and the remaining errors were reported in the table 02. In most of the pediatric patient's wrong dose of Augmentin was prescribed. Most of the administration errors occurred in the hospital was by the nursing staff and the majority of administration errors fall under the type of wrong frequency errors. The documented rates of dispensing errors diverse greatly from 0.6% to 48 %, in our study the dispensing errors are 4(5%). Similar results were identified in previous studies. The identified omission errors and monitoring errors result from inadequate drug therapy reviews.

Level of severity	Category	No. of ME(n=80)	Percentage
No error	Category A	12	15%
Error, no harm	Category B	6	7.50%
	Category C	38	47.50%
	Category D	4	5%
Error, harm	Category E	12	15%
	Category F	8	10%
	Category G	0	0
	Category H	0	0
Error, Death	Category I	0	0

Table 03 illustrates the categorization of ME based on the NCCMERP index.

Out of 80 ME'S high incidence of medication errors falls under the Category C(47.5%) followed by Category A(15%), Category E(15%), Category F(10%), Category B(7.5%) and Category D(5%). Based on the NCCMERP severity Out of 80 ME, only 25% of occurred errors resulted in temporary harm to the patient or required initial or prolonged hospitalization. Out of 80 medications errors that occurred in the hospital, 58 (72.5%) were by a physician, 18 (22.5%) were by nurses and 4 (5%) were by a pharmacist. (Table no 3)

3.7 Statistical analysis between study variable

S. No	Correlation	Z value	P-value (<0.05)
1	Age versus the number of medications per prescription	1	0.804
2	Length of stay versus Number of medications	1	0.0001***
3	Number of medications versus errors	0.345	0.049**

Table 04 Statistical analysis (multivariable analysis between different variables using two-way Anova)

In Our study statistically highly significant correlation was observed between the length of stay and no of medication .Our study reports that there is a correlation between the number of medications and the number of errors.

5. CONCLUSION

According to the observed results, medication errors are common in the hospital,we had observed the medication error in hospitalized patients at a rate of 1 per 20 prescriptions. Ourstudy concludes that the overall percentage of medication errors was 20% without any life-threatening events. The present study shows a higher incidence of this study prescribing error (72.5%) was the most commonly observed medication error, rest of types, that creating necessary intervention which reduces the incidence of medication errors. So, Clinical pharmacists will play a major role within the early detection and prevention of medication errors by regular auditing of prescriptions and educating health care professionals and so will improve the quality of care to the patientsat the medicine ward and to develop hospital formulary, drug protocols, and prescription policies within the hospital.

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7. CONFLICT OF INTEREST:

Authors do not have any conflict of interest

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