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Research Article

Pharmacology for drug discovery

Cardio protective efficacy of Tagetes *Erecta* Methanolic extract in doxorubicin induced oxidative cardiac damage.

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Abstract: Doxorubicin (DOX) is a highly prevalent chemotherapy agent used in the management of a wide range of cancer. However, on prolonged clinical use, DOX causes cardio toxicity and it was restricted in the clinical regimen. Tagetes erecta or marigold is a potent medical plant with many biological actions and it contains chief carotenoid known as lutein. So we have evaluated the efficacy of methanolic extract of Tagetes erecta (METE) against DOX induced cardio toxicity. Animals were randomly divided into five groups, such as control group treated with vehicle, DOX group administered with DOX 15 mg/kg, i.p, extract treated group with METE 200 and 400 mg /kg orally and Digoxin standard group for 14 days. After the experimental period, various biochemical markers were analyzed for the assessment of DOX mediated cardio toxicity. DOX intoxicated rats showed significant ($p<0.05$) increase in serum cardiac markers (creatine kinase, lactate dehydrogenase and Cardiac troponin-I) , lipid peroxidation and protein carbonyl content and reduced antioxidants (SOD, CAT, Gpx and GSH) level in heart tissue. Treatment with METE (200 and 400mg/kg, p.o.) restored the altered biochemical level to normalcy. Thus, the results of the study suggest that Tagetes erecta showed promising cardio protective activity in DOX induced cardiac damage mediated by its membrane stabilizing and antioxidant effect.

Keywords: Doxorubicin, cardio toxicity, oxidative stress, antioxidants, lipid peroxidation, Tagetes erecta

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1. INTRODUCTION

Doxorubicin (DOX) is widely used chemotherapy medication for the management of wide range of cancers and malignant tumours[1]. However, its clinical utility in the treatment of cancer is limited due to the development of cardio-toxicity such as cardiomyopathy and congestive heart failure[2, 3].Previous research studies have shown that DOX mediated cardio-toxicity is primarily due to the high generation of free radicals[4,5], which may further lead to mitochondrial dysfunction[6] and apoptosis[7], but the exact mechanism is still under obscure. However, mounting evidence underscores that the major pathway in the DOX mediated cardiac damage is a generation of reactive oxygen species (ROS) [4, 5]. DOX is an anthracycline class of antibiotics which has the basic quinine nucleus. This quinone structure is involved in redox reactions as an electron acceptor and thus converted to a semiquinone free radical. This unstable semiquinone radical can cause oxidative damage to DNA and converted to quinone, which in turn generates more amounts of ROS [8, 9]. This extreme ROS can lead to oxidative stress and causes an imbalance state between free radical production and the body antioxidant defense mechanism. Recently wide range of plant products gained major attention with significant cardioprotective potential among the researchers [10]. This plant derived products can be instructed to be used along with the chemotherapeutic agents to minimise the adverse effects and drug resistance. *Tageteserecta*Linn.commonly known as Marigold belongs to the family Asteraceae. In folklore practice the plant is used to treat inflammation rheumatism, cold and bronchitis [11]. Previous studies report the antioxidant activity of *Tageteserecta*(*T.erecta*)in various stress models[12,13]. In this backdrop, the present study was undertaken to evaluate the methanolic extract of *Tageteserecta* on DOX induced oxidative cardiotoxicity.

2. MATERIAL AND METHODS

Drugs and Chemicals DOX was procured from Sigma Chemical Co., St.Louis, MO, USA. The other required reagents needed were of highest purity and of analytical grade.

2.1 Collection, Identification and extraction of T.erecta

The entire plant of *T.erecta* was collected from the various gardens and nurseries of Palvancha, Bhadradri district, Telangana, India. The collected plant was authenticated by botanistDr.K.MadhavaChetty, Assistant Professor, Sri Venkateswara University, Chitoor district, Andhra Pradesh. Then the plant materials were dried under shade and coarsely powdered, using pulverizer and packed in sealed containers. The powdered plant material was extracted using methanol by maceration method (72 hours).

2.2 Phytochemical screening

On preliminary screening, the methanol extract of *T.erecta* showed positive reaction for triterpenoids, Shinoda test for flavonoids, steroids, tannins, saponins and alkaloids[20].

Table:1 Phytochemical screening of methanolic extract of T.erecta.

Phytochemicals	Present/Absent
Alkaloids	Present
Flavonids	Present
Tannins	Present
Saponins	Present
Terpenoids	Present
Carbohydrate	Absent

2.3 Animals

All animal studies were performed as per the guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC). CPCSEA Reg. No: 1641/PO/E/S/14/CPCSEA. The standard experimental protocols and procedures adopted in this biological evaluation are described below.

2.4 Acute toxicity studies

The acute toxicity studies were performed as per the OECD guideline No. 425 by using Wistar rats. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

2.5 Doxorubicin induced cardiotoxicity

The study was conducted on male Wistar rats (150 ± 10 g). Animals were obtained from the Animal House, Browns College of Pharmacy. Animals were fed with commercially available standard rat pellet feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided ad libitum. The animals were deprived of food for 24 hours before experimentation but allowed free access to tap water. The rats were housed under conditions of controlled temperature (25 ± 2 °C) and were acclimatized to 12 hours light: 12 hours dark cycles.

2.6 Study Design

The experimental animals were randomized into five groups of six rats each as follows:

Group 1: Control rats received vehicle 2% gum acacia suspension for 14 days. (1 ml/ kg b.wt.), orally for 14 days
Group 2: Rats received vehicle 2% gum acacia suspension for 14 days and received DOX (15mg/kg b.wt; i.p) on 13th and 14th day.
Group 3: Rats received Digoxin for 14 days and received DOX (15mg/kg b.wt; i.p) on 13th and 14th day.
Group 4: Rats received 200mg of methanolic extract of *T.erecta*(METE) suspended in 2% gum acacia orally for 14 days and received DOX (15mg/kg b.wt; i.p) on 13th and 14th day 1 hour after the administration of extract.
Group 5: Rats received 400mg of methanolic extract of *T.erecta*(METE) suspended in 2% gum acacia orally for 14 days and received DOX (15mg/kg b.wt; i.p) on 13th and 14th day 1 hour after the administration of extract.

After the final doses of extract and DOX, the access to food was restricted overnight and on 15th day the animals were anaesthetized using phenobarbital sodium (35mg/kg; i.p) and sacrificed by cervical decapitation. The blood was collected from jugular vein in heparanized tubes and the serum was separated for the measurement of cardiac marker enzymes. The heart tissue was excised, cleaned from adherent tissues, washed in ice cold saline and dried. Then a 100mg weighed tissue was homogenized (10% w/v) in chilled Tris-HCl buffer and used for the analyses of various biochemical markers in DOX induced cardiac damage.

2.7 Estimation of cardiac markers[16]

The creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in serum were estimated using commercial biochemical kits from Pathozyme, India. Further the serum cardiac troponin (cTn) cTn-I was estimated by ELISA method using the kits obtained from Life Technologies (India) Pvt. Ltd

2.8 Measurement of Lipid peroxidation and protein carbonyl content[16]

The lipid peroxidation (LPO) marker, malondialdehyde (MDA) and protein carbonyl content (PCC) was measured according to the instructions provided in the kit obtained from Span Diagnostics Ltd, Gujarat, India

2.9 Measurement of antioxidants[16]

The cardiac level of antioxidants catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) were estimated according to the instructions provided in the kit obtained from Span Diagnostics Ltd, Gujarat, India.

3. STATISTICAL ANALYSIS

The data were represented as mean ± Standard error mean (SEM). The data were analysed by one way analysis of variance followed Tukey's comparison using SPSS version 18.0. $p < 0.05$ was considered as statistically significant.

Table 2. Effect of Tageteserecta and DOX on serum cardiac markers

Groups	LDH (IU/L)	CK-MB (IU/L)	cTnI (µg/ml)
Control	146.43 ± 6.52	106.65±5.12	1.12±0.32
DOX	556.76 ± 9.45 ^{a*}	434.28±9.85 ^{a*}	3.87±0.48^{a*}
Digoxin + DOX	150.67±4.87 ^{b*}	110.54±4.65 ^{b*}	1.32±0.32 ^{b*}
METE (200mg/kg) + DOX	225.54±6.24 ^{b*}	128.65±5.45 ^{b*}	1.76±0.44 ^{b*}
METE (400mg/kg) + DOX	178.54±4.25 ^{b*}	108.42± 6.12 ^{b*}	1.38±0.42 ^{b*}

The values were expressed as mean ± SEM (n=6). Analyses were done by one way analysis of variance (ANOVA) with Tukey's post-hoc test comparison procedure ^{a*} p<0.05, compared to control; ^{b*} p<0.05, compared to Cd. LDH: Lactate Dehydrogenase; CK-MB: Creatine kinase; cTnI: Cardiac Troponin T.

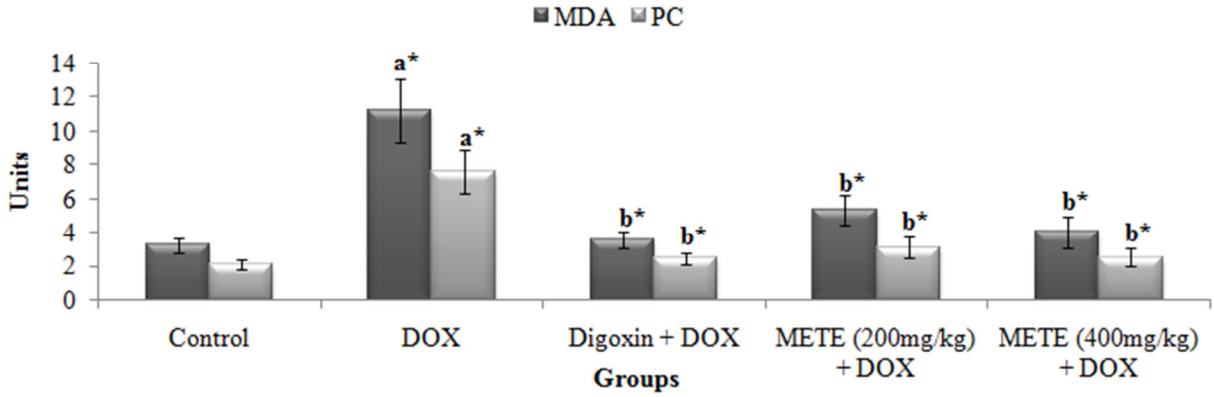

Fig 1.Effect *Tageteserecta*on lipid peroxidation and protein carbonyl in DOX induced cardiotoxicity.

MDA –Malondialdehyde; PC- Protein carbonyl content. Units: MDA- nmole/mg tissue; PC-µmoles/mg protein.The results were shown as mean ± SEM (n = 6). ^{a*} p<0.05, compared to Control; ^{b*} p<0.05, compared to DOX.

Table 3. Effect of Tageteserecta and DOX on antioxidants levels in heart homogenate

Groups	SOD	CAT	GPx	GSH
Control	11.52±0.78	7.24±0.41	19.26±1.35	15.75±1.65
DOX	5.35±0.52 [a*]	3.24±0.32 [a*]	12.45±1.35 [a*]	6.85±0.76 [a*]
Digoxin + DOX	10.86±0.87 [b*]	7.12±0.42 [b*]	18.65±1.45 [b*]	14.58±1.34 [b*]
METE (200mg/kg) + DOX	7.98±0.65 [b*]	5.12±0.56 [b*]	15.89±1.23 [b*]	9.98±0.85 [b*]
METE (400mg/kg) + DOX	9.12±0.85 [b*]	6.32±0.76 [b*]	17.45±1.12 [b*]	13.65±1.28 [b*]

The values were expressed as mean ± SEM (n=6). Analyses were done by one way analysis of variance (ANOVA) with Tukey's post-hoc test comparison procedure. [a*] p<0.05, compared to Control; [b*] p<0.05, compared to DOX. Units- SOD: U/mg protein; CAT: µmoles/H_2O_2/min/mg protein; GPx: µmoles NADPH oxidized /min/mg protein; GSH: nmol/mg protein.

4. RESULTS

4.1 Effect of T.erecta administration on cardiac markers

DOX intoxicated rats showed significant (p<0.005) elevated levels of cardiac markers CK-MB, LDH and cardiac troponin I (cTn-I) in serum. However, treatment with METE at the dose of 200 and 400mg/kg significantly (p<0.05) restored the elevated cardiac markers to normal (Table 2)

4.2 Effect of T.erecta on cardiac lipid peroxidation and protein carbonyl levels

DOX injected rats showed significant (p<0.05) elevation of lipid peroxidation as evident by increased MDA content and protein carbonyl content (PCC). Further, METE administration at the dose of 200 and 400mg/kg reversed the increased level of MDA and PCC to normal as compared to DOX intoxicated rats. The results are shown in Fig 1.

4.3 Effect of T.erectaon antioxidant levels in DOX induced cardiac oxidative stress

In this study, decreased levels of SOD, CAT, GPx and GSH were observed in DOX intoxicated rats and found to be significant when compared to the control group (p<0.05). Meanwhile, METE treatment at the dose of 200 and 400mg/kg significantly (p<0.005) increased the decreased level of antioxidants as that of DOX alone insulted rats (Table 3).

5. DISCUSSION

Doxorubicin is the clinically recommended chemotherapy drug employed for the management of a wide range of tumors. However, many oncologists limited its clinical utility due to systemic toxicities affecting various organs precisely heart leading to cardiotoxicity[14].The cardio toxicity of DOX is acute which shows array of pathological features such as ventricular and atrial arrhythmia and congestive heart failure[14]. This study aimed to evaluate the protective effect of *T.erecta*methanolic extract against DOX induced cardio toxicity in animal model. DOX mediated cardio toxicity is generally as a result of free radicals generation which has toxic effects on the heart. Due to this, endothelial damage occurs with extravasation of plasma proteins into the myocardial interstitium and muscle cells leading to myocardial cell damage [15]. So as a result of this, cardiac enzymes (CPK and LDH) and cardiac specific proteins cardiac troponin I leak from the damaged heart to the serum, which are important markers to analyse the cardiac damage. In our study, DOX mediated cardio toxicity revealed elevated concentration of LDH, CK, and cTn-I in serum. *T.erecta* treatment displayed marked reduction in the levels of cardiac markers as compared to the DOX intoxicated rats which is incorporated with the previous reports[16]. The major mechanism in DOX mediated ROS production is via enzymatic conversion of DOX quinine moiety to semiquin one radical by NADH dehydrogenase and cytochrome P450 reductase[17]. In our study, DOX intoxicated rats displayed increased levels of malondialdehyde (MDA) a marker of lipid peroxidation and protein carbonyl content (PCC). Further lipid peroxidation induced by DOX causes decline in antioxidants such as GSH, SOD, CAT and Gpx. However, *T.erecta* treatment significantly increased the antioxidant levels to normalcy and thus improved the antioxidant status in rats. The cardio protective effect of *T.erecta* might be due to the presence of carotenoid, lutein in the plant [18]. Previous studies showed the cardio protective action of lutein against doxorubicin induced oxidative cardiac damage [19].

6. CONCLUSION

We found that *T.erecta* suppressed DOX induced cardio toxicity mediated through its membrane stabilization effect, antioxidant and anti lipidper oxidative effect. The plant on phytochemical screening found it containAlkaloids, Flavonoids, Tannins, Saponins, Terpenoids, Sterols and Phenols. The cardio protective activity might be due to the presence of carotenoids and lutein.

7. AUTHORS CONTRIBUTION STATEMENT

All authors discussed the methodology and results and contributed equally to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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