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Potent Antidiabetic Activity of Ethanolic Extract of Terminalia Arjuna Fruits on Streptozotocin-Induced Diabetic Wistar Albino Rats

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Abstract: Diabetes mellitus is the most common chronic endocrine and metabolic disorder, impacts the quality of life, and has become the leading cause of death worldwide. Now a day's, herbal drugs are in great demand for the treatment of diabetes because of their traditional acceptability, lesser side effects, and adverse effects compared with allopathic medications. *Terminalia arjuna* fruits were selected for the study due to their abundant availability and based on ethanomedicinal folk claims. The fruits of *Terminalia arjuna* have been evaluated for their antidiabetic and antihyperlipidemic activities using streptozotocin-induced diabetic Wistar albino rats. In the study, two doses of ethanolic extract of *Terminalia arjuna* fruits (TAF) were selected and administered to normal rats for an oral glucose tolerance test. For an antidiabetic study in streptozotocin-induced diabetic rats, the effect of the extract was observed for 28 days for blood glucose alterations. All experimental animals were observed for weight changes, due to STZ-induced diabetes, and were found to be significantly reverted to normal in all animals except the diabetic control group. After the completion of the experimental period, the collected serum samples were subjected to various biochemical parameters studied, including a lipid profile, glycosylated hemoglobin (HbA1c), urea, and creatinine level. An antioxidant study was performed using collected tissues, followed by histopathological studies of the pancreas. The research study revealed that the TAF extract has significant antidiabetic activity and the potential to revert the altered lipid profile and increase HbA1c to normal. TAF extract significantly normalized the urea, creatinine, superoxide dismutase, catalase, and glutathione peroxidase levels. Thus, the study concluded that TAF extract exhibits significant (p>0.05) antidiabetic activity in STZ-induced diabetic rats.

Keywords: Diabetes, Antihyperlipidemic, *Terminalia Arjuna*, Fruits, Streptozotocin, and Antioxidant.

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1. INTRODUCTION

Diabetes, a chronic metabolic disease, leads to severe damage to the heart, blood vessels, eyes, kidneys, and nerves[1]. In addition, due to degenerative changes in β -cells in the pancreatic islets, impaired insulin secretion results in chronic hyperglycemia [2]. leading to various diabetes complications. Apart from this, free radicals play a crucial role in the pathogenesis of diabetes mellitus[3] and its associated complications. Currently, treatment for controlling diabetes includes a range of oral hypoglycemic agents, which have many known side effects[4]. Hence there is a clear need for an effective antidiabetic drug with non-toxic antioxidant potential of herbal origin. Since ancient times, herbal medicines have been highly tested for treatment and therapeutic agents and now have become a part of modern medicine. Therefore, the demand for natural drugs has risen, drastically demonstrating a significant effect on diabetes mellitus[5].

The plant *Terminalia arjuna* (Roxb.) belongs to the family Combretaceae. It has been grown in most parts of India and used in Ayurvedic formulations since ancient times. The plant parts such as stem bark, leaves, roots, and fruits of *Terminalia arjuna* are used in the indigenous system of medicine for different ailments[6] like. Bark, leaves, and roots are reported to have proven antidiabetic activity[7-9]. Hence, to justify the ethnomedicinal claims, research was conducted to assess the antidiabetic effect of *Terminalia arjuna* fruit using streptozotocin-induced diabetic rats and its impact on different biochemical parameters. *Terminalia arjuna* is one of the most accepted and beneficial medicinal plants in the indigenous system of medicine for the treatment of various critical diseases[10]. Based on works of literature on acute toxicity studies of fruit, they are considered safe up to 2000mg/kg b.w. Hence, the study was planned to evaluate the antidiabetic and antioxidant potential of the fruit extracts.

2. MATERIAL AND METHODS

2.1 Chemicals and Equipments

Streptozotocin (Sigma Aldrich, Bengaluru, India), Glibenclamide (Emcure Pharmaceuticals, India), glucometer (Accu-Check active; Roche Diagnostic India Pvt. Ltd), automated hematology analyzer (BC-5000; ASPEN Diagnostics), spectrophotometer (Shimadzu UV-1800), mild anesthesia, EDTA and all other reagents used were of analytical graded.

2.2 Plant Collection and Extract Preparations

The fresh fruits of *Terminalia* arjuna were collected from the RKDF University Campus, Bhopal (M.P.) India. Plants selected for the research study were collected and authenticated by Dr. S.N. Dwivedi at the Department of Botany, Janata PG College, APS University, Rewa M.P. India. Herbarium specimens of each were prepared and deposited with voucher specimen No. JC/B/PAN/483. Fruits were shade-dried and coarsely powdered using a mixer grinder. Accurately weighed 500 g of powdered fruits were defatted with petroleum ether, the marc was removed and air dried, then dried marc was extracted with 95% ethanol using soxhlet apparatus. Ethanolic extract filtrates were collected and evaporated using a vacuum evaporator under reduced pressure and temperature [11-12]. Finally, the obtained extracts were stored in desiccators for the research study.

2.3 Phytochemical Screening

Preliminary phytochemical screening of the extracts was performed for the *Terminalia arjuna* fruits to identify the presence of various phytochemical constituents. [13].

2.4 Experimental Animals

All animal experiments were performed following the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC), Vedica College of B. Pharmacy, RKDF University, Bhopal, M.P. The experimental animals were used with permission number IAEC/VCP/2019/001/6. Adult Wistar male albino rats weighing 150 to 200 g were used for the *in-vivo* antidiabetic study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated, temperature-controlled animal house with a regular 12 hours light/dark schedule. The experimental animals were fed a standard rat pellet diet, and clean drinking water was provided with ad libitum.

2.5 Toxicity Study

An acute oral toxicity study was performed per Organization for Economic Co-operation and Development (OECD)-423 guidelines. The ethanolic extracts were tested for their acute toxicity in male Wistar albino rats weighing 150-200 gm (OECD, 2001). Three rats were taken per Group, and five groups were maintained. The animals were kept fasting overnight and provided only with water, after which the extracts were administered orally at a dose range of 50, 100, 200, 400, and 2000 mg/kg body weight (b.w.) by intragastric tube with the control of 0.5% carboxy methyl cellulose(CMC). After administering plant extracts, the animals were observed to find any changes in grooming, hyperactivity, sedation, corneal reflex, urination, and salivation. All the animals were observed twice daily for any mortality during the experimental period of 14 days.

2.6 Preparation of Doses

All test drugs, extracts, and glibenclamide (GLB) were suspended in 0.5 % w/v carboxy methyl cellulose (CMC) suspension and prepared in distilled water.

2.7 Evaluation of Antidiabetic Activity

2.7.1 Oral Glucose Tolerance Test

An oral glucose tolerance test was performed using overnight fasted normal rats. Animals were separated into four groups, and glucose 2g/kg b.w. was administered orally. Animals in Group I was administered normal saline (0.9% w/v NaCl). Group II animals received the standard drug glibenclamide 5mg /kg b.w [14]. Group III and IV received a dose of 200 and 400 mg/kg b.w. ethanolic extract of *Terminalia arjuna* fruit orally. Blood samples were collected by tail pricking of each animal just after oral glucose administration and then at the regular time intervals of 0, 30, 60,120, and 180 mins for the estimation of the effect of the extract on blood glucose levels of the glucose-loaded animals by Accu-chek glucometer.

2.7.2 Antidiabetic Activity

The animals fasted overnight, and diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (60

mg/kg b.w.) in 0.1 M citrate buffer-pH 4.5[15]. After 72 hrs of STZ induction, fasted rats' blood glucose levels were evaluated, and blood glucose levels greater than 250 mg/dl were considered diabetic and were used for further detailed studies. During experimentation, the animals had free access to a 5% glucose solution to overcome the drug-induced hypoglycemia. Finally, the overnight fasted experimental rats were divided into five groups of six rats each for antidiabetic activity evaluation. These diabetic groups of animals were administered with saline, a standard drug (glibenclamide), and ethanolic extracts of *Terminalia arjuna* once daily for 28 days. The tail pricking method estimated the fasting blood glucose levels on the 0, 7th, 14th, 21st, and 28th days.

2.7.3 Blood Collection and Biochemical Estimations

At the end of the experimental period, *i.e.,* after 28 days, rats were sacrificed by cervical dislocation under mild anesthesia. Blood samples were collected through the arterial jugular with ethylenediamine tetraacetic acid (EDTA). Plasma and serum were separated by centrifugation at 3000 rpm for 10 min, and the supernatant was transferred into labeled sample bottles[16]. The serum was stored in the refrigerator at 4-8 °C before analysis. Samples were analyzed for various biochemical parameters associated with diabetes, such as lipid profile[17], glycosylated hemoglobin (HbA1c) [18] serum urea, and creatinine[19]. Finally, the pancreas of the experimental rats was removed after the autopsy for histopathological studies**,** and a portion of each was stored in formalin for performing the antioxidant assays[18].

2.7.4 Biochemical Estimation in Pancreatic Tissue

The antioxidant assay was performed in pancreas tissues of normal, diabetic control, and TAF 200 and 400 treated rats. Antioxidant activity was determined by measuring superoxide dismutase (SOD) and catalase activities (CAT) and the level of reduced glutathione (GSH) [20-21].

2.7.5 Histopathological Study of Pancreatic Tissue

Histopathological studies of the pancreas isolated from the sacrificed rats were performed. The tissues were washed with normal saline immediately and fixed in 10% formalin for 24hrs. Tissues were dehydrated with alcohol, embedded in paraffin, then cut into 4-5µm-thick sections, stained with hematoxylin-eosin dye, and photo-microscopic observation was performed[18].

3. STATISTICAL ANALYSIS

All the results were expressed as mean ± SD (n = 6) in each experimental Group. Statistically, the data were analyzed using GraphPad Prism version 5.0. The data were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's test. *p*-values <0.05 were considered statistically significant, and $p<0.01$ was very significant.

4. RESULTS AND DISCUSSIONS

In the present study, ethanolic extracts of TAF were evaluated for antidiabetic activity using STZ induced diabetic model. This study was conducted to get the most therapeutically efficacious extract and its role in diabetes-induced alterations associated with lipid and renal profiles. The antioxidant activity of the extract was performed to evaluate its potency against free radicals scavenging.

4.1 Plant Extract Preparations

The fresh fruits of *Terminalia arjuna* were defatted using petroleum ether, and on extraction with 95% ethanol, the extract percentage yield was found to be 13.9%.

4.2 Phytochemical Analysis

Preliminary phytochemical screening of *Terminalia arjuna* revealed the presence of different phytochemical constituents in ethanolic extracts like alkaloids, phenols, glycosides, carbohydrates, flavonoids, steroids, tannins, triterpenes, and saponins. These were also reported by the previous researchers [22].

4.3 Toxicity Study

The toxicity study for the ethanolic extract of TAF was performed using albino rats. The extracts were administrated orally in increasing doses up to 2000 mg/kg. In the study, extract at the dose of 2000 mg/kg neither showed visible signs of toxicity nor mortality during the study. No observed adverse effects were detected at 2000 mg/kg[23]. Hence, the biological doses were fixed at 200 mg/kg b.w (sub-maximal) and 400 mg/kg b.w (maximal dose) for the extract.

4.4 Evaluation of Antidiabetic Activity

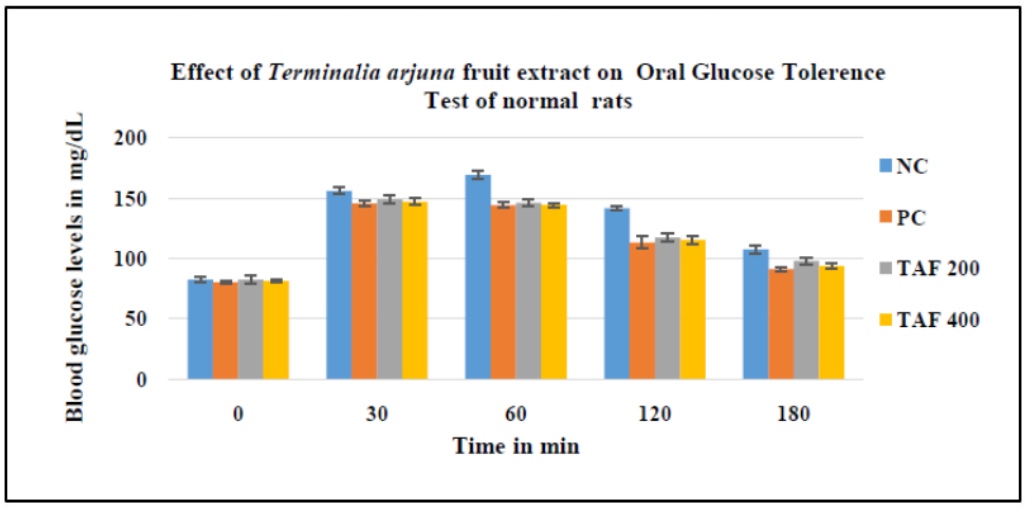
4.4.1 Oral Glucose Tolerance Test

The oral glucose tolerance test (OGTT) is widely used to evaluate apparent insulin release and resistance in various clinical settings[24]. OGTT was performed to determine glucose clearance from blood with time after glucose intake. It is an important parameter to determine diabetic status also[25]. Thus, OGTT was used to evaluate glucose tolerance, indirectly indicating insulin sensitivity and pancreatic β - cell function. In the study, the OGTT was performed on normoglycemic rats.

Table 1: Effect of *Terminalia arjuna* fruit extract on oral glucose tolerance test of normal rats

Groups	Blood glucose level (mg/dL)				
	0 min	30 mins	60 mins	120 mins	180 mins
Normal Control	82.5±1.87	156.16±2.9	169.5±3.50	141.83±1.94	107.5±3.61
Positive Control	80.16±1.47	145.66±2.16	144.34±2.17	113.16±5.03	91.34±1.86**
TAF200	82.5±3.32	149.16±3.86	146.34±2.94	117.67±3.20	98.16±2.63**
TAF400	81.34±1.36	147.34±3.14	144.6±1.75	115.50±3.27	93.83±2.48**

Values are expressed as mean ± SD (n=6); * (p< 0.05) significant; ** (p< 0.01) more significant.



TAF-Terminalia arjuna fruit, NC-Normal control, DC-Diabetic control, PC-Positive control

Fig 1: Effect of *Terminalia arjuna* fruit extract on oral glucose tolerance test of normal rats

After glucose administration, it was observed that the blood glucose levels increased in the first 30 mins in all four groups. It gradually started decreasing after 120 mins and normalized in 180 mins, as shown in Table 1 and illustrated by Figure 1. The OGTT study also revealed that oral administration of TAF 200 and 400 mg/kg significantly (p<0.01) reduced the blood glucose concentrations by 34.19 and 36.32 % to the normal. In contrast, the reduction was 37.29 % in the positive control group. And thus, TAF exhibited significant antidiabetic activity due to the accumulation of their common active constituents or the synergic action of different compounds present.

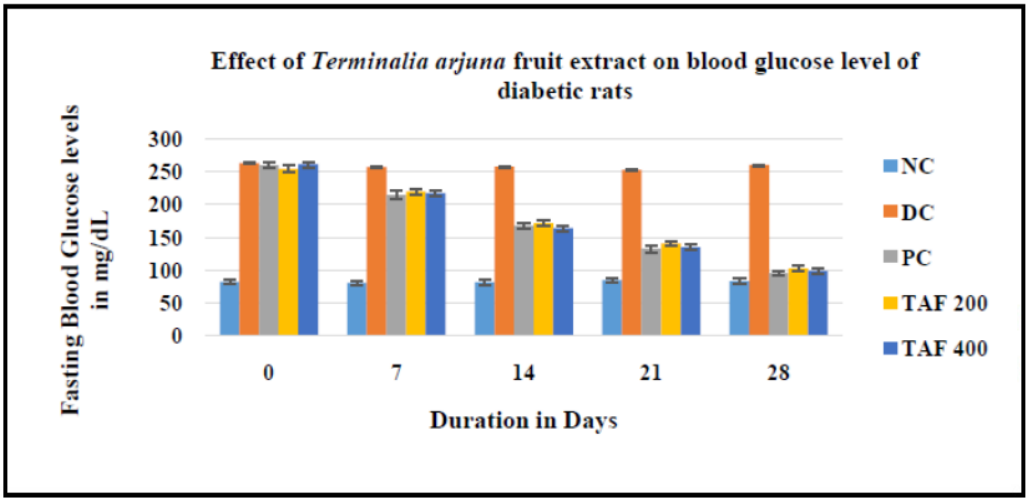
4.4.2 Antidiabetic Activity

The antidiabetic study for TAF ethanolic extract was performed using STZ-induced diabetic rats.

Table 2: Effect of *Terminalia arjuna* fruit extract on the blood glucose level of diabetic rats

Animal Groups	Fasting blood glucose level(mg/dL)				
	0 Day	7 Day	14 Day	21 Day	28 Day
Normal Control	82.37±3.42	80.58±3.25	81.27±3.72	84.5±3.28	83.42±3.88
Diabetic Control	262.94±2.81	256.61±6.78	257.34±5.83	252.58±5.77	259.86±5.67
Positive Control	259.74±4.97	214.52±5.93	167.28±4.64	132.63±5.18**	95.27±3.89**
TAF 200	254.43±25.78	219.64±4.65	171.85±3.38	140.51±4.19*	102.53±4.56*
TAF 400	260.78±4.35	217.23±3.76	163.73±4.67	135.12±4.51**	98.64±3.5**

*Values are expressed as mean ± SD (n=6); * (p< 0.05) significant; ** (p< 0.01) more significant*



TAF-Terminalia arjuna fruit, NC-Normal control, DC-Diabetic control, PC-Positive control

Fig 2: Effect of *Terminalia arjuna* fruit extract on blood glucose level of diabetic rats

In the study, it was observed that blood glucose levels were elevated in STZ-induced diabetic rats by 219.21%, as compared to the standard control. Blood glucose level was significantly reduced on administering TAF doses 200 and 400 mg/kg for 28 days. TAF significantly (p<0.05) decreased blood glucose levels by 59.7 % and 62.18 % toward normal. The

positive control group (glibenclamide treated) showed a 63.32 % reduction, as depicted in Table 2 and Figure 2. Streptozotocin elevated blood sugar levels significantly and developed diabetes in experimental rats. After 28 days of treatment with TAF, blood glucose significantly reduced to near normal as that of the standard drug. The antidiabetic effect of the extract might have been achieved by different possible mechanisms, including inhibiting starch digestion, decreasing glucose absorption from the intestine, enhancing insulin secretion from β -cells by stimulating the damaged/destructed β -cells, inhibiting glucose formation in the bloodstream and suppressing the transport of glucose[26].

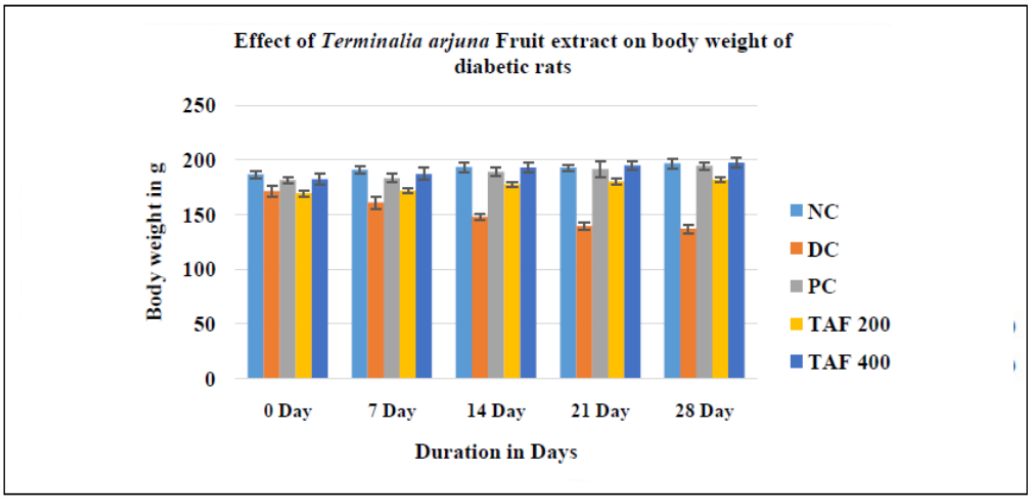
4.4.3 *Effect On the Body Weight of Animals*

All animals ingested an average amount of food and water during the study.

Table-3. Effect of *Terminalia arjuna* fruit extract on body weight of diabetic rats

Animal Groups	Animals Body Weight (g)				
	0 Day	7 Day	14 Day	21 Day	28 Day
Normal Control	186.5±3.21	190.7±2.96	193.54±4.38	192.66±3.2	196.5±4.1
Diabetic control	171.16±4.89	160.83±5.67	147.61±3.17	139.23±3.73	136.86±3.92
Positive control	181.41±3.15	183.16±3.91	188.96±3.79	191.5±6.89**	194.51±3.2**
TAF200	169.18±5.24	171.65±6.45	177.54±5.78	180.16±5.52**	181.63±5.14**
TAF400	182.164.78	187.17±5.63	192.87±4.31	194.73±4.39**	197.28±4.32**

*Values are expressed as mean ± SD (n=6); * (p< 0.05) significant; ** (p< 0.01) more significant*



TAF-Terminalia arjuna fruit, NC-Normal control, DC-Diabetic control, PC-Positive control

Fig 3: Effect of *Terminalia arjuna* fruit extract on body weight of diabetic rats

In the study, slightly reduced body weight due to STZ-induced diabetes was observed and significantly reverted to normal in all groups except the diabetic control, as summarized in Table 3 and illustrated in Figure 3. whereas, in the diabetic control group, body weight reduction was 20.03%. Decreased body weight was due to muscle wasting, which was observed in diabetic rats compared to normal rats, indicating loss of body weight, which may be due to excessive breakdown of tissue protein[27]. Treatment with TAF stopped the progression and reversed the breakdown of tissue protein, thus improving body weight to a certain extent, indicating that control over muscle wasting resulted from glycemic control.

4.4.4 *Effect of Terminalia Arjuna Fruit Extract On Biochemical Parameters*

4.4.4.1 *Estimation of Lipid Profile*

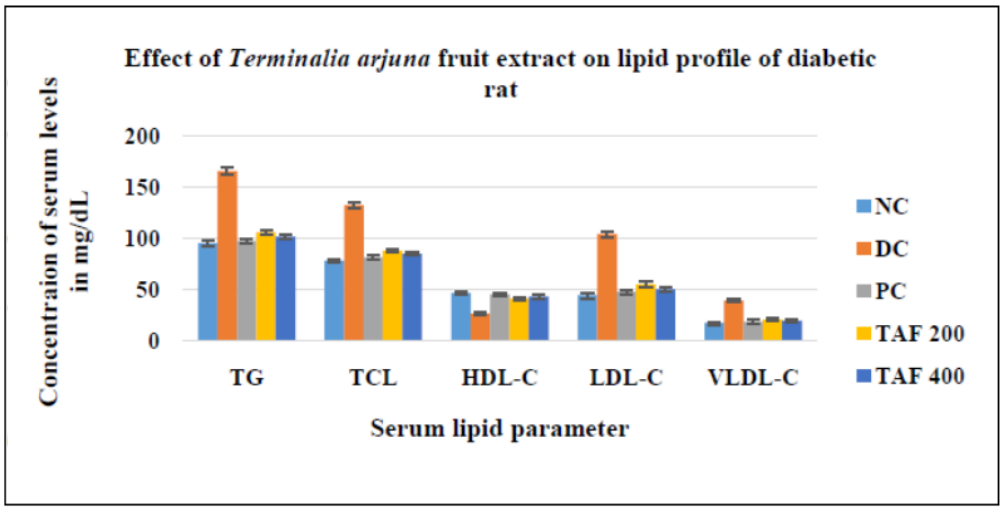
In STZ-induced diabetic rats, there was a significant increase in triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) cholesterol, *i.e.*, by 74.57, 69.62, 132.33 and 135.35 % respectively and significant decrease in high-density lipoprotein (HDL) cholesterol in serum 44.32 % compared with standard control was observed.

Table 4: Effect of *Terminalia arjuna* fruit extract on lipid profiles of diabetic rats

Animal Groups	Serum lipid level on the 28th Day of the study				
	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Normal Control	94.83±2.74	77.74±1.98	46.23±1.63	43.74±2.8	16.53±1.45
Diabetic Control	165.54±3.16	131.86±2.45	25.74±1.78	101.62±3.15	38.48±1.67
Positive Control	97.16±1.83**	80.92±1.73**	44.58±2.10**	47.29±1.96**	17.96±2.31**

TAF 200	105.63±2.74*	87.5±1.04**	40.66±1.63**	54.17±2.83**	20.13±1.98**
TAF 400	101.2±2.48**	84.6±1.69**	42.5±1.51**	49.63±2.21**	18.96±1.13**

*Values are expressed as mean ± SD (n=6); * (p< 0.05) significant; ** (p< 0.01) more significant*



Triglyceride-TG, TCL-Total Cholesterol, HDL- High-density lipoprotein, LDL- Low-density lipoprotein, VLDL-Very low-density lipoprotein, TAF-Terminalia arjuna fruit, NC-Normal control, DC-Diabetic control, PC-Positive control

Fig.4: - Effect of *Terminalia arjuna* fruit extract on the lipid profile of diabetic rats

After treatment with the ethanolic extract, it significantly reverted the disturbed lipid profile parameters. As depicted in Table 4 and illustrated in Figure 4. Diabetes is often associated with dyslipidemia, the main risk factor for cardiovascular diseases[28]. Therefore, serum triglyceride and cholesterol levels are usually elevated in STZ-induced diabetic rats. The study observed an increase in the concentration of cholesterol, triglyceride, and LDL and a decrease in HDL. Continual administration of TAF extract normalized serum lipid profile, i.e., secondary to the diabetic state. Diabetes-induced hyperlipidemia is attributable to excess fat mobilization from adipose due to the underutilization of glucose. The regression of the diabetic state due to continual administration of TAF extract increased glucose utilization, thereby depressing fat mobilization.

4.4.4.2 Estimation of Glycosylated Hemoglobin

In STZ-induced diabetic rats, a significant increase of HbA1c by 82.53 % compared with the normal control was observed.

Table 5: Effect of *Terminalia arjuna* fruit extract on glycosylated hemoglobin levels of diabetic rats

Animal Groups	Glycosylated hemoglobin (HbA1c %)
Normal Control	5.95±0.35
Diabetic Control	10.86±0.45
Positive Control	6.1±0.18**
TAF 200	6.75±0.2*
TAF 400	6.15±0.35**

*Values are expressed as mean ± SD (n=6); * (p< 0.05) significant; ** (p< 0.01) more significant*

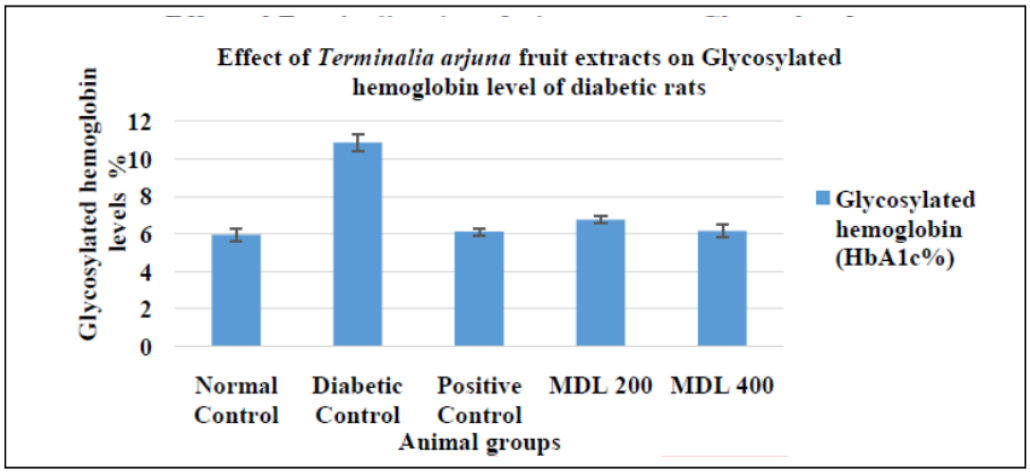


Fig 5: Effect of *Terminalia arjuna* fruit extract on glycosylated hemoglobin level of diabetic rats

By study, it was revealed that ethanolic extract of TAF 200 and 400 reverted the elevated HbA1c profile by 37.84 and 43.37%, compared to diabetic control, which was as significant as a positive control, i.e., 43.84%. Results have been depicted in Table 5 and illustrated in Figure 5. In STZ-induced diabetic rats, a significantly elevated level of HbA1c has been identified as a significant risk factor for coronary heart diseases and stroke in subjects who may have diabetes[29]. If HbA1c is uncontrolled, sugar flow in the blood is high and might affect the kidneys. On treatment with TAF 200 and 400, HbA1c level decreased to near normal values. This might have improved the plasma insulin level and helped to control the blood glucose level and utilization.

4.4.4.3 Estimation of Urea and Creatinine

STZ-induced diabetic rats showed a significant increase in creatinine and urea level by 33.78 and 82.93 % compared with normal control.

Table 6: Effect of *Terminalia arjuna* fruit extract on serum creatinine and urea level of diabetic rats

Animal Groups	Creatinine (mg/dL)	Urea (mg/dL)
Normal Control	0.74±0.036	16.17±0.063
Diabetic Control	0.99±0.054	29.58±0.011
Positive Control	0.77±0.011**	16.18±0.037**
TAF 200	0.81±0.024*	17.29±0.015*
TAF 400	0.79±0.062**	16.31±0.068**

Values are expressed as mean ± SD (n=6); * (p< 0.05) significant; ** (p< 0.01) more significant

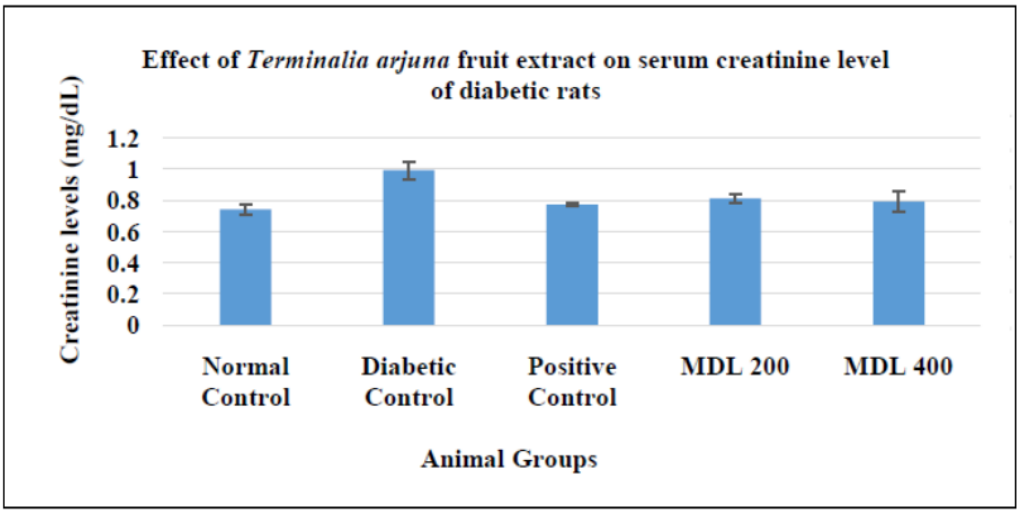


Fig 6: Effect of *Terminalia arjuna* fruit extract on serum creatinine levels in diabetic rats

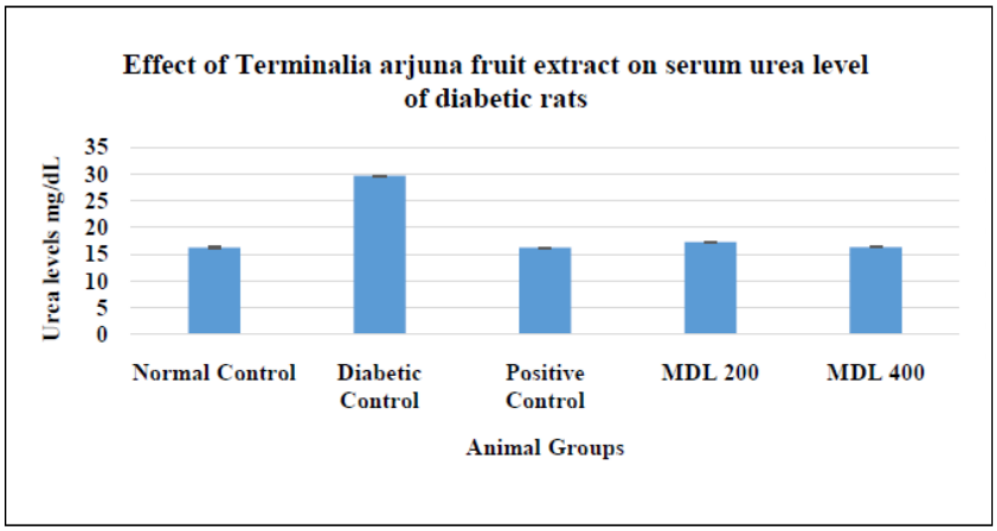


Fig 7: Effect of *Terminalia arjuna* fruit extract on serum urea levels of diabetic rats

By study, it was noted that ethanolic extract of TAF 200 and 400 reverted the elevated creatinine by 18.19 % and 20.21 %, and urea by 41.55 % and 44.86 %, compared to diabetic control, which was as significant as positive control by 22.23 and 45.30 % respectively. Results are depicted in Table 6 and Figures 6-7. STZ has an inherent nephrotoxic potential and showed definite signs of nephrotoxicity and marked renal dysfunction[30], compared to the normal control group. The

elevation of the serum urea and creatinine levels evidenced this. However, TAF treatment of 28 days resulted in the reversal of altered elevation. Studies have shown that increased urea and creatinine concentrations were due to excessive lipolysis in severe diabetes, leading to ketosis and, later, to acidosis. The kidney maintains the optimum chemical composition of body fluid by acidifying the urine and removing metabolic wastes such as urea and creatinine. But in renal function impairments or diseases, the concentration of these metabolites rises in the blood[31].

4.4.5 *Biochemical Estimation in Pancreatic Tissue*

In STZ-induced diabetic rats, there was a significant decrease in SOD, CAT, and GSH by 47.68, 63.19, and 68.50 % compared with normal control. Treatment with ethanolic extract TAF 200 and 400 reverted the reduced SOD, CAT, and GSH significantly as the positive control. SOD level improved by 57.89 and 79.71 %, CAT improved by 144.72 and 157.05 %, and GSH improved by 176.87 and 195.75 %, respectively. Results have been depicted in Table 7 and illustrated in Figure 8.

Table 7: Effect of *Terminalia arjuna* fruit extracts on antioxidant levels in diabetic rats

Animal groups	SOD (µ g/mg tissue)	CAT (µ mol/mg tissue)	GSH (µ mol/mg tissue)
Normal Control	28.46±1.62	50.23±2.4	38.86±2.74
Diabetic Control	14.89±1.28	18.49±1.18	12.24±1.23
Positive Control	26.32±1.47**	49.63±1.39**	37.51±1.69**
TAF 200	23.51±1.78*	45.25±1.71*	33.89±1.2**
TAF 400	26.76±1.22**	47.53±1.42**	36.2±1.31**

Values are expressed as mean ± SD (n=6); * (p< 0.05) significant; ** (p< 0.01) more significant

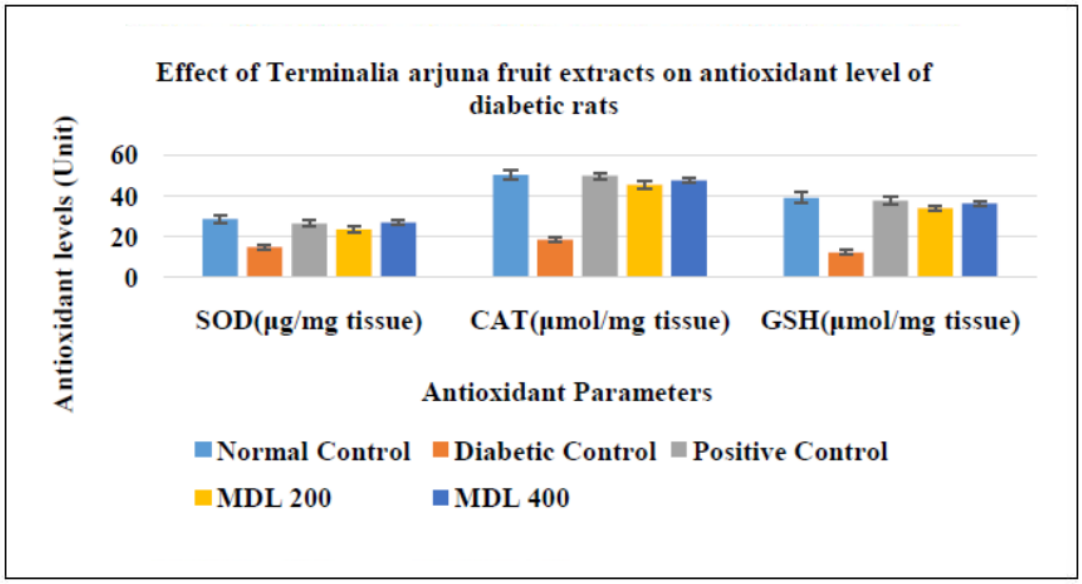
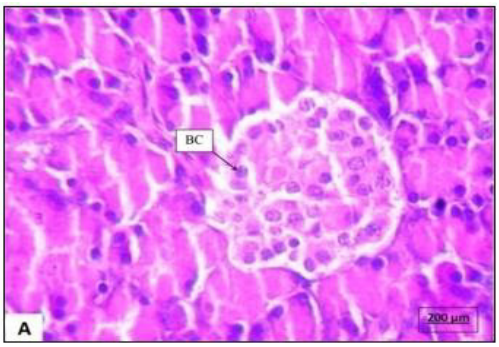


Fig 8: Effect of *Terminalia arjuna* fruit extracts on antioxidant level of diabetic rats

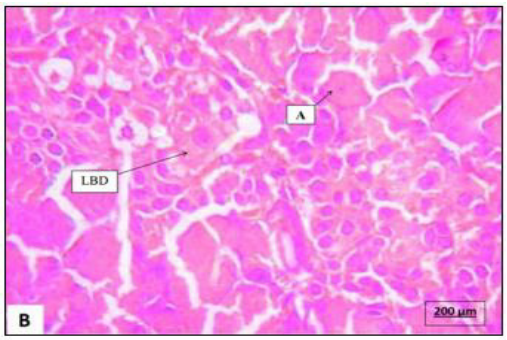
The elevated blood glucose level in diabetes facilitates the free radical's production and the generation of free radicals beyond the scavenging abilities of endogenous antioxidant defenses resulting in macro- and microvascular dysfunction[32]. The SOD, CAT, and GSH are important enzymes that scavenge free radicals and protect the cells against oxidative stress injury[33]. In the present study, a decreased SOD, CAT, and GSH have been observed in diabetic rats indicating a high level of oxidative stress. Administration of TAF significantly improved the SOD, CAT, and GSH levels. This activity might be correlated with supporting phytochemicals such as flavonoids and phenols, which can scavenge free radicals[34]. Furthermore, the reversal of antioxidant enzyme concentrations with the TAF might have regenerated pancreatic β -cells that might have contributed to the antidiabetic activity[35].

4.4.6 *Histopathological Study of Pancreatic Tissue*

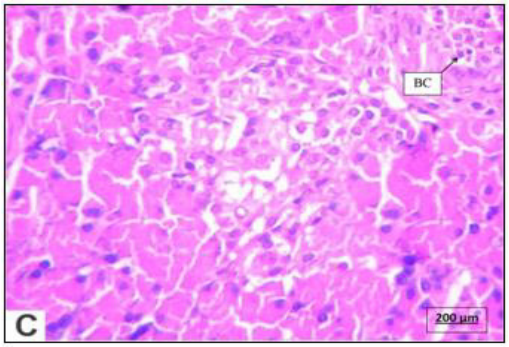
The histopathology of the rat pancreas was performed. Microscopic investigation of pancreas sections of normal control rats showed the normal appearance of islets of Langerhans. The islets appeared more lightly stained than the surrounding acinar cells (Fig. 9A). However, the diabetic control rats showed pathological changes in both exocrine and endocrine components. The acinar cells were swollen, and small vacuoles were observed in almost all acinar cells. Islet β -cells are almost entirely lost in STZ-treated rats (Fig. 9B). Similar findings were obtained in the glibenclamide-treated rats. Both the diabetic control and glibenclamide-treated pancreas was associated with different intensity of eosin as compared to the normal control rats (Fig. 9C). On the other hand, TAF 200 and TAF 400 groups depicted evidence of cellular regeneration among the islets of Langerhans (Fig. 9D and 9E).



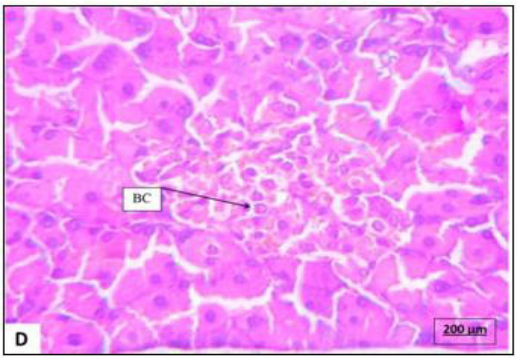
Normal Control



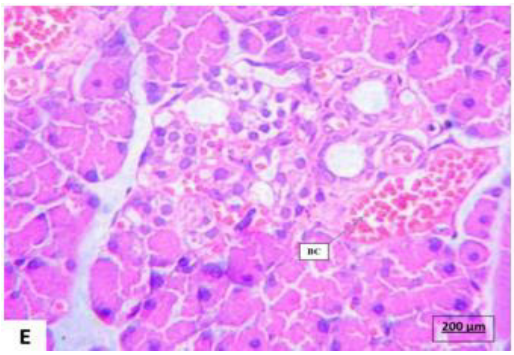
Diabetic Control



Positive Control (GLB 5 mg/kg b.w.)



TAF 200 Treated



TAF 400 Treated

Abbreviations:
Normal: All normal beta cells structure (BC).
Diabetic:

- Loss of beta-cell and degranulations (LBD)
- Atrophy, fibrotic changes, and lesions in the islet of Langerhans
- Destroyed pancreatic lobules and acini cells (A) and reduced pancreatic cell number and size.

Fig.9: Effect of *Terminalia arjuna* fruit extracts on histopathology of pancreatic tissues

Pancreatic histopathological observations revealed that *Terminalia arjuna* fruit (TAF) extract-treated groups exhibited marked improvement in pancreatic β -cell activity. There was a dose-dependent gradual improvement of pancreatic β -cell density compared to diabetic rats. In diabetic rats, the pancreas that showed atrophy of islet cells with inflammatory edema, necrosis, fibrotic changes, and shrinkage of islet cells[36-37] reverted to normal after treatment with TAF extract.

5. CONCLUSIONS

The present study concluded that the ethanolic extract of *Terminalia arjuna* fruits possesses potent antidiabetic activity, with improved body weight and associated altered biochemical parameters. It significantly affects diabetes-induced dyslipidemia, oxidative stress, and antioxidant effect in STZ-induced diabetic rats. As observed by histopathology, the positive effect may be through restoring beta cells, thus improving insulin secretion. Furthermore, due to its antioxidant activity, it might have shown the potential to improve diabetes-induced nephropathy. The positive results of the present study might be due to the presence of phenolic compounds, glycosides, phytosterol, flavonoids, and saponins in the medicinal plant extract. Thus the research study supported the claim of *Terminalia arjuna* fruits in the management of diabetes mellitus as stated in the folklore medicine and will prove to be a milestone in the treatment of diabetes through alternative medicine.

6. ACKNOWLEDGEMENT

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7. AUTHORS CONTRIBUTION STATEMENT

Dr. Rakesh Sagar and Dr. Mohan Lal Kori conceived the presented idea. Pradeep Adlak developed the theory and performed the computations. Dr. Rakesh Sagar and Dr. Mohan Lal Kori verified the analytical methods. Dr. Mohan Lal Kori directed Pradeep Adlak to carry out the research and investigation of the antidiabetic activity, and Dr. Rakesh Sagar supervised the findings of this work. All authors discussed the results and contributed to the final manuscript. Pradeep Adlak wrote the manuscript with support from Dr. Rakesh Sagar and Dr. Mohan Lal Kori.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2013;36(1);Suppl 1:S67-74. doi: 10.2337/dc13-S067, PMID 23264425.
2. Rorsman P, Ashcroft FM. Pancreatic β-cell electrical activity and insulin secretion: of mice and men. Physiol Rev. 2018;98(1):117-214. doi: 10.1152/physrev.00008.2017, PMID 29212789.
3. Mazumder PM, Rathinavelusamy P, Sasmal D. Role of antioxidants in phytomedicine with special reference to antidiabetic herbs. Asian Pac J Trop Dis. 2012;2:S969-79. doi: 10.1016/S2222-1808(12)60303-X.
4. Manandhar Shrestha JTM, Shrestha H, Prajapati M, Karkee A, Maharjan A. Adverse effects of oral hypoglycemic agents and adherence to them among patients with type 2 diabetes mellitus in Nepal. J Lumbini Med Coll. 2017;5(1):34-40. doi: 10.22502/jlmc.v5i1.126.
5. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs are used for the treatment of diabetes. J Clin Biochem Nutr. 2007;40(3):163-73. DOI: 10.3164/jcbn.40.163, PMID 18398493.
6. Chaudhari GM, Mahajan RT. A comprehensive study on pharmacognostic, physico, and phytochemical evaluation of *Terminalia arjuna* Roxb. stem bark. J Pharmacogn Phytochem. 2015;4:186-93.
7. Ragavan B, Krishnakumari S. Antidiabetic effect ofT. arjuna bark extract in alloxan induced diabetic rats. Indian J Clin Biochem. 2006;21(2):123-8. doi: 10.1007/BF02912926, PMID 23105628.
8. Biswas M, Kar B, Bhattacharya S, Kumar RBS, Ghosh AK, Haldar PK. Antihyperglycemic activity and antioxidant role of *Terminalia arjuna* leaf in streptozotocin-induced diabetic rats. Pharm Biol. 2011;49(4):335-40. doi: 10.3109/13880209.2010.516755, PMID 21281245.
9. Singh S, Shukla N, Bhowmick M, Rathi J. Evaluation of antidiabetic activity of *Terminalia arjuna* root extract in alloxan induced diabetic rats. JBPR. 2019;8(1). doi: 10.32553/jbpr.v8i1.566.
10. Amalraj A, Gopi S. Medicinal properties of Terminalia arjuna (Roxb.) Wight & Arn.: A review. J Tradit Complement Med. 2017;7(1):65-78. doi: 10.1016/j.jtcme.2016.02.003, PMID 28053890.
11. Kokate CK, Purohit AP, Gokhale SB. A text book of Pharmacognosy. NiraliPrakashan Pune. 1995;4(40):1-20.
12. Harborne JB. Phytochemical methods. London: Chapman & Hall; 1973. p. 49-188.
13. Trease GE, Evans WC. Drugs of biological origin. Pharmacognosy. 1983:309-540.
14. Soni LK, Dobhal MP, Arya D, Bhagour K, Parasher P, Gupta RS. In vitro and in vivo antidiabetic activity of isolated fraction of *Prosopis cineraria* against

streptozotocin-induced experimental diabetes: A mechanistic study. Biomed Pharmacother. 2018;108:1015-21. DOI: 10.1016/j.biopha.2018.09.099, PMID 30372801.
15. Sharma U, Sahu R, Roy A, Golwala D. In vivo antidiabetic and antioxidant potential of *Stephania hernandifolia* in streptozotocin-induced-diabetic rats. J Young Pharm. 2010;2(3):255-60. doi: 10.4103/0975-1483.66803, PMID 21042481.
16. Erukainure OL, Ebuehi OAT, Adeboyejo FO, Aliyu M, Elemo GN. Hematological and biochemical changes in diabetic rats fed with fiber-enriched cake. J Acute Med. 2013;3(2):39-44. doi: 10.1016/j.jacme.2013.03.001.
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502. doi: 10.1093/clinchem/18.6.499, PMID 4337382.
18. Patra S, Bhattacharya S, Bala A, Haldar PK. Antidiabetic effect of *Drymaria cordata* leaf against streptozotocin-nicotinamide-induced diabetic albino rats. J Adv Pharm Technol Res. 2020;11(1):44-52. doi: 10.4103/japtr.JAPTR_98_19, PMID 32154158.
19. Gupta V, Kori ML. Assessment of hepatic and nephroprotective potential of polyherbal combinations against STZ-induced diabetic liver and kidney complications in Wistar rats. ap. 2022;11(1):311-9. DOI: 10.54085/ap.2022.11.1.32.
20. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alex J Med. 2018;54(4):287-93. doi: 10.1016/j.ajme.2017.09.001.
21. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta. 1979;582(1):67-78. doi: 10.1016/0304-4165(79)90289-7, PMID 760819.
22. Dwivedi S, Chopra D. Revisiting *Terminalia arjuna* - an ancient cardiovascular drug. J Tradit Complement Med. 2014;4(4):224-31. doi: 10.4103/2225-4110.139103, PMID 25379463.
23. Variya BC, Bakrania AK, Madan P, Patel SS. Acute and 28-days repeated dose sub-acute toxicity study of gallic acid in albino mice. Regul Toxicol Pharmacol. 2019;101:71-8. doi: 10.1016/j.yrtph.2018.11.010, PMID 30465803.
24. Chaim-aom N, Chomko S, Talubmook CToxicology and Oral glucose Tolerance Test (OGTT) of Thai Medicinal Plant Used for Diabetes controls, Phyllanthus acidus L. (EUPHORBIACEAE). Pharmacogn J. 2016;9(1):58-61. doi: 10.5530/pj.2017.1.11.
25. Kumar A, Lingadurai S, Shrivastava TP, Bhattacharya S, Haldar PK. Hypoglycemic activity of *Erythrina variegata* leaf in streptozotocin-induced diabetic rats. Pharm Biol. 2011;49(6):577-82. doi: 10.3109/13880209.2010.529615, PMID 21281246.
26. Kasabri V, Flatt PR, Abdel-Wahab YHA. *Terminalia bellirica* stimulates the secretion and action of insulin and inhibits starch digestion and protein glycation in vitro. Br J Nutr. 2010;103(2):212-7. doi: 10.1017/S0007114509991577, PMID 19723351.
27. Abu-Lebdeh HS, Nair KS. Protein metabolism in diabetes mellitus. Baillieres Clin Endocrinol Metab. 1996 Oct;10(4):589-601. doi: 10.1016/s0950-351x(96)80741-5. PMID 9022953.
28. Leon BM, Maddox TM. Diabetes and cardiovascular disease: epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes. 2015;6(13):1246-58. DOI: 10.4239/wjd.v6.i13.1246, PMID 26468341.
29. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in diagnosis and prognosis of diabetic patients. Biomark Insights. 2016;11:95-104. doi: 10.4137/BMI.S38440, PMID 27398023.
30. Evan AP, Mong SA, Gattone VH, Connors BA, Aronoff GR, Luft FC. The effect of streptozotocin and streptozotocin-induced diabetes on the kidney. Ren Physiol. 1984;7(2):78-89. doi: 10.1159/000172927, PMID 6231704.
31. Amartey NA, Nsiah K, Mensah FO. Plasma levels of uric acid, urea and creatinine in diabetics who visit the clinical analysis laboratory (can-lab) at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. J Clin Diagn Res. 2015;9(2):BC05-9. doi: 10.7860/JCDR/2015/10905.5530, PMID 25859443.
32. Bajaj S, Khan A. Antioxidants and diabetes. Indian J Endocrinol Metab. 2012;16(Suppl 2):S267-71. doi: 10.4103/2230-8210.104057, PMID 23565396.
33. Kim JO, Kim KS, Lee GD, Kwon JH. Antihyperglycemic and antioxidative effects of new herbal formula in streptozotocin-induced diabetic rats. J Med Food. 2009;12(4):728-35. DOI: 10.1089/jmf.2008.1195, PMID 19735171.
34. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel, Switzerland). 2018;5(3):93. DOI: 10.3390/medicines5030093, PMID 30149600.
35. Zhong F, Jiang Y. Endogenous pancreatic β cell regeneration: A potential strategy for the recovery of β cell deficiency in diabetes. Front Endocrinol. 2019;10:101. DOI: 10.3389/fendo.2019.00101, PMID 30842756.
36. Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic beta-cell dysfunction in diabetes. Curr Diabetes Rev. 2013;9(1):25-53. doi: 10.2174/157339913804143225, PMID 22974359.
37. Kumar V, Ahmed D, Anwar F, Ali M, Mujeeb M. Enhanced glycemic control, pancreas protective, antioxidant and hepatoprotective effects by umbelliferon-α-D-glucopyranosyl-($2^{I} \rightarrow 1^{II}$)-α-D-glucopyranoside in streptozotocin-induced diabetic rats. Springerplus. 2013;2:639. doi: 10.1186/2193-1801-2-639, PMID 24349947.