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RESEARCH ARTICLE

Effects of alcoholic extract of Terminalia Chebula dried fruit on blood biochemical profile in diabetic rats

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Abstract

Background A considerable amount of attention has been recently paid to the discovery of effective natural antidiabetic drugs. Terminalia chebula is considered as the mother of herbs, with reported antidiabetic activity. This study aims to evaluate the renal and hepatic protective profile of its antidiabetic therapeutic doses.

Methods To achieve the aim of the study, a total of 66 adult male rats of Sprague-Dawley species weighing about 180–200 g (weighed using a digital scale) were used. Type 2 diabetes mellitus (T2DM) was induced in using streptozotocin (STZ), rats were given a 5% dextrose solution for the next 24 h. Subjects received oral treatment of Terminalia chebula ethanolic extract at different doses (200, 400, and 600 mg/kg body weight) for 28 days. Measurements of fasting blood glucose level, change in body weight, lipid profile, serum liver enzymes, serum renal parameter, and histopathology of liver and kidney were carried out.

Results Higher doses of Terminalia chebula (600 mg/Kg) were shown to have a potential therapeutic effect as well as the most prominent antidiabetic, antilipidemic activity, hepatoprotective and renoprotective profiles when compared to lower doses.

Conclusion The use of Terminalia chebula alone or in combination with conventional antidiabetic drugs may be beneficial as a new advent therapy for diabetes.

Keywords T2DM · Terminalia chebula · Streptozotocin

Introduction

Diabetes mellitus (DM) is acknowledged as one of the most significant fatal diseases, and a prominent cause of death worldwide [1]. According to the International Diabetes Federation (IDF), a population of approximately 424.9 million adults (aged 20–79) worldwide in 2017 have been reported to have DM, and it is apparent that the rates will continue to increase evidently [2]. Interestingly, males and females have comparable rates [3]. Globally, DM is the 7th leading cause of death [4–6]. Insulin insensitivity and/or dysfunctional insulin secretion are perceived as the main causes of DM, leading to a prolonged rise in blood sugar level [7], due to destruction of pancreatic β-cells or dysfunctional β-cell and insulin resistance [8, 9], with collateral disturbance in the metabolism of carbohydrates, lipids, and proteins [10–12]. Hyperglycemia, hyperlipidemia, and oxidative stress are reported to be the major risk factors associated with DM micro- and macro-vascular complications that could be life-threatening [11, 13, 14]. Proper glycemic control suppresses the development or progression of diabetic complications but does not completely restore impaired functions [15, 16].

Traditional therapies originated from herbal sources with antidiabetic activity showed a vital role in the control of DM [17]. Terminalia chebula retz is usually called black- or chebulic myrobalan, ink tree, or chebulic myrobalan [18], and is also known as "King of medicine" due to its wide spectrum of pharmacological activity and its popular use in folk medicine [19]. It is found throughout the greater parts of India as a moderate-sized, or large deciduous tree, attaining 15–24 m in height with ovate or elliptic leaves with a

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pair of large glands on the petiole's tip. Its flowers are all hermaphrodite, 4 mm. Across sessile, dull-white, or yellow, with an offensive smell. Its fruits are obovoid or ovoid, yellow to orange-brown, and hard when ripe, 3-5 cm long, 5 ribbed on drying with hard and pale-yellow seeds. The major chemical constituents are Tannins, which on hydrolysis give chebulic acid and a D – galloyl glucose [20].

The fruits of Terminalia chebula have been extensively used in Ayurveda, Unani, Siddha, and homeopathic medicine [21, 22]. The antidiabetic activity of its ethanolic extract was reported in few studies [23, 24]**.** The ethanolic pulp extract of Terminalia chebula fruit has significant anti-diabetic activity probably due to insulin-like action of its constituents and promotion of insulin release. [25].

However, the optimal therapeutic dose is still unknown. The aim of this study was to evaluate the renal and hepatic protective profile of its antidiabetic therapeutic doses and determine the optimal antidiabetic therapeutic dose of T. chebula.

Materials and methods

Drugs and chemicals

Streptozotocin (STZ) was obtained from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. It was stored at −20 °C. All additional chemicals used were of analytical grade and were commercially purchased. Kits used for analysis of lipid, hepatic and renal profiles were obtained from Randox Laboratories (UK).

Extract preparation of Terminalia chebula fruit

Terminalia chebula dried fruits (received from Indore, Madhya Pradesh, India, date of collection is June 2019) were ground to a powder by an electrical grinder. The finely obtained powder (0.5 kg) was drenched in 75% ethanol by cold maceration process for 7 days. Filtration of the Mixture was made by using Whatman No. 2 filter paper to remove peel particles. The filtrate was concentrated by Rotavapor (a rotary evaporator) at 40–50 °C under reduced pressure to remove the organic solvent till obtaining a semisolid viscous mass which was dried on water bath at 50 °C for 48 h until concentrated viscous extract was obtained then it was exposed to air till became completely dried powder, pending its use for the treatment. [26].

Animals

A total of 66 adult male rats of Sprague-Dawley species weighing about 180–200 g (weighed using a digital scale) were used in the present experimental research work. All animal procedures were accordant with the guidelines and recommendations of the Faculty of Pharmacy, Cairo University's Experimental Animal Ethics Committee for the use and proper care of animals (BT 1198).

Experimental design

Animals were treated according to the following scheme (each group consisted of 11 rats):

Group I: Control group (C).
Group II: Diabetic group (STZ group): induced by STZ 60 mg/kg without treatment.
Group III: Diabetic group treated with Terminalia chebula alcoholic extract (200 mg/kg) (STZ+ Term 200 mg).
Group IV: Diabetic group treated with Terminalia chebula alcoholic extract (400 mg/kg) (STZ+ Term 400 mg).
Group V: Diabetic group treated with Terminalia chebula alcoholic extract (600 mg/kg) (STZ+ Term 600 mg).

Groups three, four and five received the treatment as an oral administration daily for 28 days.

Group VI: Diabetic group treated with reference drug metformin (STZ + Metformin) (150 mg/kg) as an oral administration daily for 28 days. Metformin is one of the most widely used antihyperglycemic agents as the first-line drug therapy for the management of T2DM [27, 28]. The metformin tablets were crushed and suspended in water to prepare oral doses of 150 mg/kg body weight [29].

Male Sprague-Dawley rats fasted for 12–14 h, and weights were recorded before the induction of diabetes. Experimental diabetes was induced by a single intraperitoneal injection of 60 mg/ kg of STZ, freshly dissolved in 0.1 M citrate buffer (pH = 4.5) [30]. Then the solution was immediately administered i.p. to each rat. Thirty minutes after the injection, the rats were admitted to free access to food and water. After 6 h of STZ injection, rats were given a 5% dextrose solution for the next 24 h [31, 32]. The development of diabetes was detected after 10 days of the STZ injection, blood samples taken from the lateral tail vein were monitored with a glucometer, rats with fasting blood glucose level (BGL) greater than 200 mg/dl were classified as diabetic and were selected for the experiments [33, 34]. Rats with BGL outside this range were excluded.

Serum collection from blood samples and processing

The collection of blood samples was carried out directly from the heart. Whole blood was obtained then centrifuged

at 3000 rounds per minute (rpm) for 10 min to separate the serum. The sera were carefully withdrawn with a sterilized pipette into labeled Eppendorf tubes. After that, Sera was quickly stored at −20 °C for biochemical analysis.

Histology

At the end of the 28th day, the animals were anesthetized using intraperitoneal injection of ketamine 50 mg/ kg and xylazine 5 mg/ kg then were decapitated. Necropsy samples were taken from the liver, kidney, and spleen of rats in different groups and fixed in 10% formol saline for twenty-four hours. Samples were washed with tap water followed by dehydration with serial dilutions of alcohol (methyl, ethyl, and absolute ethyl). Specimens were cleared in xylene and immersed in paraffin at Fifty-Six (56) degrees in a hot air oven for twenty-four hours. Slidge microtome was used to make paraffin bees wax tissue blocks for sectioning at 4 µm thickness. The attained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin for routine examination using light electric microscope [35].

Biochemical assays

Fasting blood glucose (FBG) of normal and experimental animals was estimated at days 0, 7th, 14th, 21st, and 28th from treatment by using one-touch glucometer (Accu-Chek sensor) of Roche Diagnostics, Germany. A Clinical chemistry analyzer for measuring cholesterol [36] and triglycerides [37] by using a reagent kit obtained from Randox Laboratories (UK) was used for measuring antilipidemic activity. Analysis of liver enzymes including Aspartate transaminase (AST) [38, 39], Alanine transaminase (ALT) [38, 39], and alkaline phosphatase (ALP) [38] was done using a reagent kit obtained from Randox Laboratories (UK). Kidney parameters including levels of creatinine [40] and urea [41] were determined by using a reagent kit obtained from Randox Laboratories (UK).

Statistical analysis

All results were expressed as mean ± standard error of the mean (S.E.M.). Statistically significant differences between experimental groups were determined by one-way analysis of variance (one-way ANOVA) and two-way analysis of variance (two-way ANOVA) with post-hoc Dunnett's t-test using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). P values less than 0.05 were considered statistically significant for the experimental analysis.

Results

Effects of ethanolic extract of Terminalia chebula on fasting blood glucose levels (FBG)

The FBG of the normal and experimental animals was estimated ten days after induction of diabetes and at days 7th, 14th, 21st, and 28th after treatment initiation. Throughout the study, normal control rats FBG levels were remarkably similar. On contrary, the STZ induced diabetic rats demonstrated a significant increment in the level of FBG as compared to normal control rats (Supplementary Fig. 1). After ten days from induction of diabetes then after the first, second, third, and fourth weeks, the FBG levels of the untreated group (STZ group) showed a significant increase ($p < 0.05$) as compared with the control group (Table 1). Treatment with T. chebula extract at different doses (200, 400, 600 mg/ kg) showed a significant decline in FBG levels ($p < 0.05$) after two, three, and four weeks of the treatment respectively, in comparison with the untreated group (STZ group). Moreover, treatment with T. chebula at the dose 600 mg/ kg showed a decline in FBG that was close to the decline noticed in STZ + metformin group.

Effect of different doses of Terminalia chebula ethanolic extract on the change in body weight

Weekly assessment of the body weight of animals gave us an overview of the changes in their body weight. A decrease in body weight was observed in STZ induced diabetic rat group after a period of 28 days. It may be due to muscle wasting and loss of tissue proteins upon induction of diabetes with STZ.

After second, third- and fourth-weeks treatment with Terminalia chebula ethanolic extract at different doses (200, 400, 600 mg/kg), exhibited a non-significant decrease in comparison with STZ group (Table 2).

Effect of different doses of Terminalia chebula ethanolic extract on biochemical parameters

I-lipid profile

After four weeks, cholesterol and triglycerides levels of the untreated diabetic group (STZ group) were significantly higher than the control group ($p < 0.05$) by 49 and 50% respectively. Treatment with alcoholic extract of Terminalia chebula at 200 and 400 mg/kg orally for four weeks induced an improvement in serum cholesterol values, causing a significant decrease ($p < 0.05$) compared to untreated group, whereas cholesterol and triglycerides levels are still

Table 1 Effects of ethanolic extract of Terminalia chebula on fasting blood glucose levels in STZ- induced diabetic rats

Groups	Day 0 (mmol/L)	One week after treatment (mmol/L)	Two weeks after treatment (mmol/L)	Three weeks after treatment (mmol/L)	Four weeks after treatment (mmol/L)
Control	6.88 ± 0.158	7.08 ± 0.147	7.16 ± 0.132	7.21 ± 0.12	7.2 ± 0.068
STZ	32 ± 1.201*	33 ± 1.012*	31 ± 1.034*	31 ± 0.023*	32 ± 1.012*
STZ + TC 200 mg/kg	30 ± 0.956*	29 ± 0.932*#Δa	26 ± 0.654*#Δa	26 ± 0.123*#Δa	25 ± 0.674*#Δa
STZ + TC 400 mg/kg	33 ± 1.021*	26 ± 1.231 *#Δ	24 ± 1.043 *#Δa	21 ± 0.876 *#Δ	20 ± 0.567 *#Δa
STZ + TC 600 mg/kg	33 ± 0.876*	24 ± 1.012 *#	20 ± 0.698 *#	17 ± 0.198 *#	15 ± 0.123 *#
STZ + Metformin 150 mg/kg	31 ± 0.786*	20 ± 0.756 *#	15 ± 0.765 *#	12 ± 0.564 *#	10 ± 0.213 *#

Data are represented as Mean ± SEM, n = 11.

*P < 0.05 vs Control

# P < 0.05 vs STZ.

Δ P < 0.05 vs STZ + metformin.

a P < 0.05 vs STZ + TC 600 mg/kg.

STZ: Streptozotocin; TC: Terminalia chebula.

Table 2 Effects of ethanolic extract of Terminalia chebula on the change in body weight in grams.

Groups	Day 0	One-week after treatment	Two-weeks after treatment	Three-weeks after treatment	Four-weeks after treatment
Control	177.4 ± 3.89	191.1 ± 4.9	205.5 ± 5.27	221. ± 6.45	233.1 ± 5.32
STZ	159.7 ± 3.67	153.4 ± 3.14 *	148.5 ± 3.22*	139.7 ± 2.4 *	133.7 ± 2.19*
STZ + TC 200 mg/kg	153.9 ± 2.93*	150.6 ± 2.93*	142.6 ± 3.02*	138.7 ± 2.91*	135.400 ± 3.138294*
STZ + TC 400 mg/kg	151.7 ± 4.24	121. ± 20.4*	114.3 ± 19.25*	112.8 ± 19.02*	107.3 ± 18.12*
STZ + TC 600 mg/kg	150.1 ± 3.35	138.3 ± 15.75*	140.5 ± 16.07*	140.5 ± 16.07*	145.4 ± 16.58*
STZ + Metformin 150 mg/kg	145.1 ± 2.93#*	147.7 ± 3.13*	143 ± 3.67*	141.2 ± 4.07*	133 ± 4.09*

Data are represented as Mean ± SEM, n = 11.

*P < 0.05 vs Control

#P < 0.05 vs STZ.

STZ: Streptozotocin; TC: Terminalia chebula.

showing significant elevation ($p < 0.05$) in comparison with the control group. On the other hand, treatment with alcoholic extract of Terminalia chebula at the dose of 600 mg/kg induced recovery in lipid profile values, causing a significant decline ($p < 0.05$) in serum cholesterol and triglyceride levels in comparison with untreated group. Their levels that are back to normal values with a significant difference when compared to STZ+ metformin group, suggested that treatment with the 600 mg/kg alcoholic extract of Terminalia chebula has an efficient antihyperlipidemic property (Table 3).

II-liver enzymes activities

STZ treatment has a considerable role in the alteration of liver functions. The activities of serum AST, ALT and ALP levels of the STZ group were significantly higher than normal values ($p < 0.05$) which indicates an ongoing active liver cell necrosis. The activities of serum AST, ALT, and ALP levels of Terminalia chebula alcoholic extract (200 mg/kg) treated group showed a significant elevation ($p < 0.05$) compared to the control group indicating that this dose did not prevent deterioration of liver functions associated with diabetes. However, treatment with a 400 mg/kg dose resulted in a significant reduction in the levels of the enzymes ($p < 0.05$) as compared to the STZ group. However, their values showed a significant rise ($p < 0.05$) in comparison with the control group. This indicates that the dose level of 400 mg/kg helped in the improvement of liver function but does not provide full protection. On the other hand, the dose of 600 mg/kg showed a significant decline ($p < 0.05$) in serum ALT, AST, and ALP compared to the untreated group. Interestingly, Terminalia chebula alcoholic extract (600 mg/kg) decreased AST levels back to normal values

Table 3 Serum cholesterol, triglycerides, AST, ALT, ALP, creatinine, and urea levels in normal, untreated diabetic control, metformin-treated group, and diabetic groups treated with 200,400 and 600 mg/kg Terminalia chebula fruit ethanolic extract

Groups	Cholesterol (mmol/L)	Triglycerides (mmol/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mmol/L)	Urea (mmol/L)
Control	1.47 ± 0.534	0.34 ± 0.011	93.05 ± 0.645	39.25 ± 0.123	121.8 ± 0.621	33.03 ± 0.412	4.98 ± 0.012
STZ	2.19 ± 0.015*	0.51 ± 0.009*	156 ± 2.097*	112 ± 2.101*	971.62 ± 2.145*	45 ± 0.019*	19 ± 0.021*
STZ + TC 200 mg/kg	1.73 ± 0.213 #*	0.46 ± 0.009*	149 ± 2.856 * a	108.75 ± 2.987* a	911.4 ± 2.873 * Δ a	39.27 ± 0.534#*	15.56 ± 0.765#*
STZ + TC 400 mg/kg	1.29 ± 0.675 #Δ	0.42 ± 0.013 #*	126 ± 1.876 #* a	87 ± 1.012 #* a	813 ± 2.189 #* Δ a	33.46 ± 0.123# Δ	8.81 ± 0.102 #* Δ
STZ + TC 600 mg/kg	1.26 ± 0.019 #Δ	0.35 ± 0.007 # Δ	91.5 ± 0.654 # Δ	52.16 ± 0.876#* Δ	552.57 ± 1.876#* Δ	30.9 ± 1.12 #Δ	8.05 ± 0.413 #* Δ
STZ + Metformin 150 mg/kg	2.2 ± 0.018*	0.47 ± 0.012*	145 ± 1.867*	118 ± 1.012*	345 ± 2.187#*	42 ± 0.613*	18 ± 1.087*

Data are represented as Mean ± SEM, n = 11.

*P < 0.05 vs Control

#P < 0.05 vs STZ.

ΔP < 0.05 vs STZ + metformin.

a P < 0.05 vs STZ + TC 600 mg/kg.

STZ: Streptozotocin; TC: Terminalia chebula.

whereas, ALT and ALP levels were still significantly rising ($p < 0.05$) compared to the control group indicating better liver protection than other lower doses. Generally, liver enzymes activities results were the best values after treatment with (600 mg/kg) Terminalia chebula alcoholic extract showing the non-toxic as well as tissue-protective effects against the hepatotoxicity produced by STZ-induced T2DM (Table 3).

III-Renal parameters

After four weeks, serum creatinine and urea levels of the untreated diabetic group (STZ group) showed a significant elevation ($p < 0.05$) as compared to the control group. The observed increase might be due to the STZ-induced metabolic disturbances. Terminalia chebula alcoholic extract at the dose of 200 mg/kg recorded a significant decrease ($p < 0.05$) in serum creatinine and urea levels in comparison with the untreated group. However, their levels had shown a significant rise ($p < 0.05$) in comparison with the control group. This indicates that the mentioned dose cannot provide full protection from renal impairment induced by diabetes. Serum creatinine and urea levels of Terminalia chebula alcoholic extract (400 mg/kg) showed a significant decrease ($p < 0.05$) in their levels in comparison with the untreated group. These results reported lowering creatinine levels back to normal values. However, they are still showing a significant increase ($p < 0.05$) in urea levels compared to the control group indicating improving renal function, however, the renal impairment did not fully resolve yet. Ultimately, it has demonstrated a better value than that with (200 mg/kg) treatment dose. Serum creatinine and urea levels of Terminalia chebula alcoholic extract (600 mg/kg) treated group reported a significant decline ($p < 0.05$) in their serum levels compared to untreated group. Rather, the mentioned dose lowered creatinine levels back to the typical values of the normal group, however, urea levels still recorded a significant increase ($p < 0.05$) in comparison with normal control group. It is worth mentioning that protective effects were potentiated by increasing the dose to 600 mg/kg (Table 3).

Histopathological examinations of liver sections

Microscopic examinations of liver sections of male rats in the control group revealed no histological alteration and the normal histological structure of the central vein, as well as the portal area and the surrounding hepatocytes in the parenchyma, were recorded in (Fig. 1-A). Microscopic examinations of liver sections of male rats in the STZ group observed dilatation and congestion in the central and portal veins as well as sinusoids were recorded in (Fig. 1-B1&B2). The STZ treated group with Terminalia chebula alcoholic extract (200, 400, and 600 mg/kg) for 28 days showed no histopathological alteration as recorded in (Fig. 1C, D, E1 & E2). The STZ treated group with metformin for 28 days revealed no histopathological alteration as recorded in (Fig. 1-F).

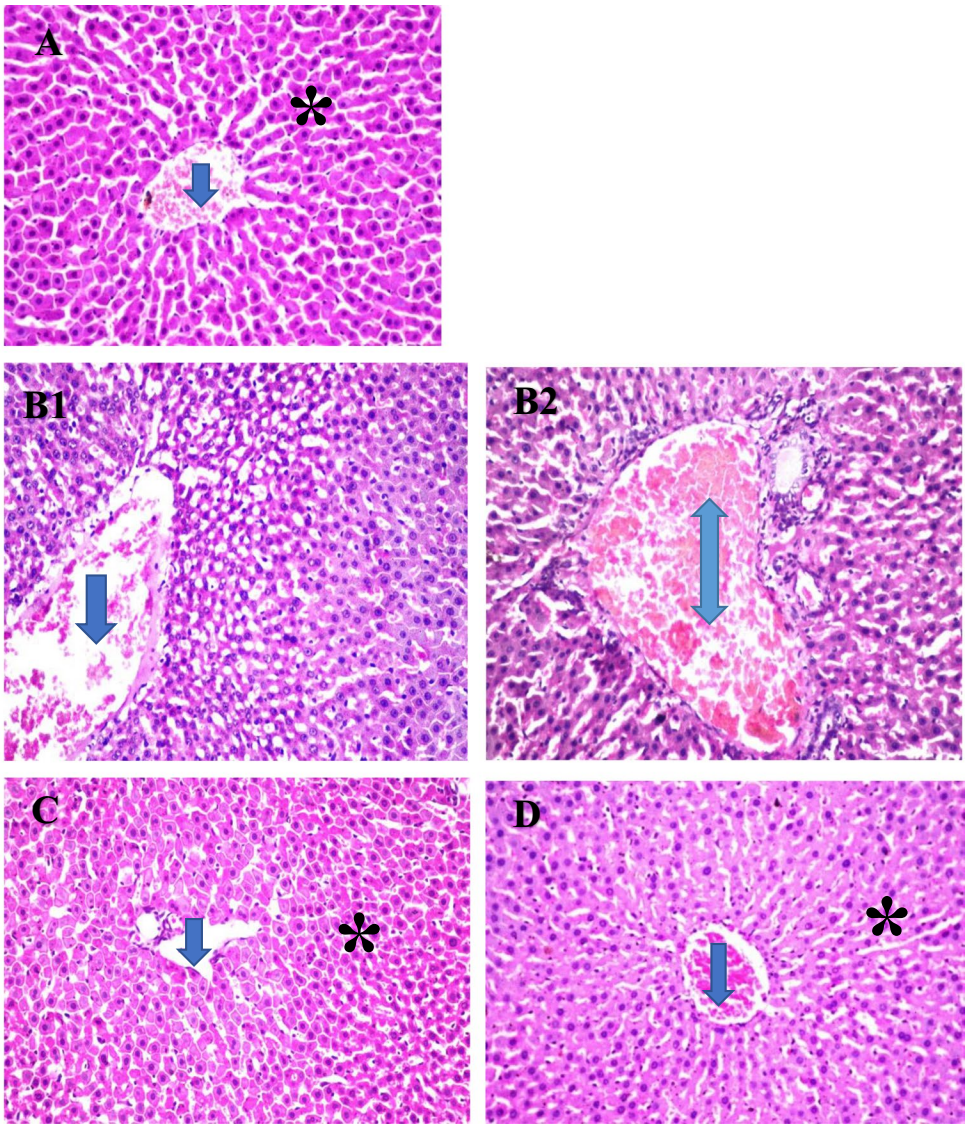
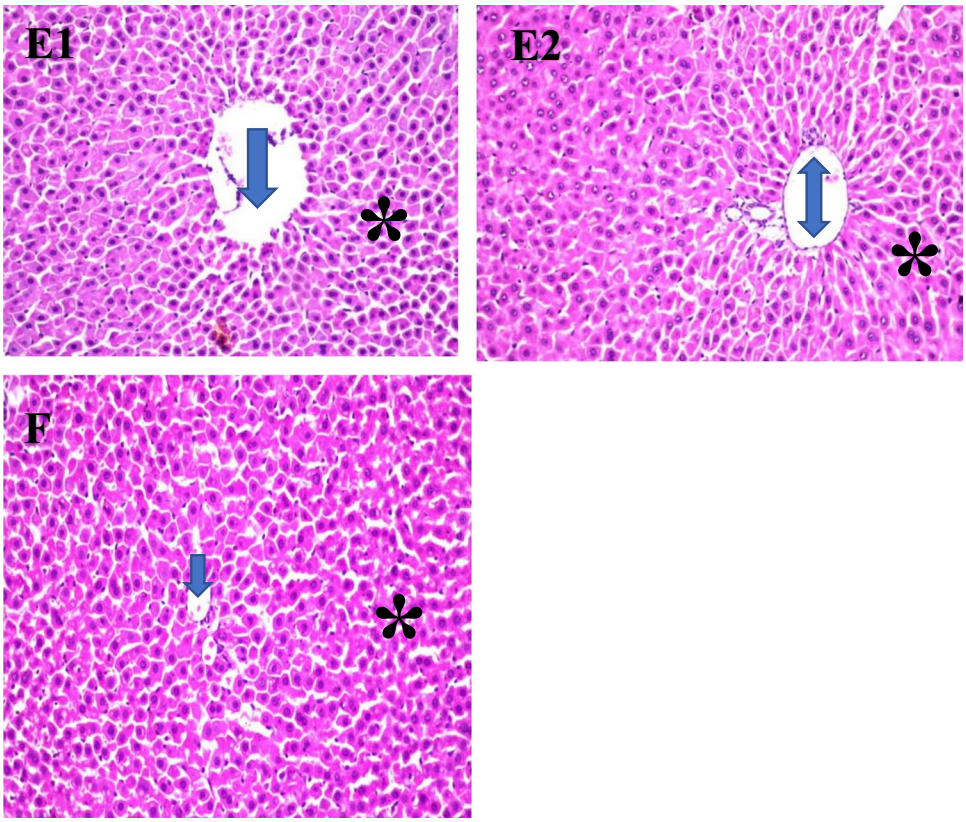


Fig. 1 Histopathological assessments of the liver tissue samples stained using hematoxylin and eosin in the different experimental groups. Control group (**A**), STZ group (**B1 & B2**) observed dilatation and congestion in the central and portal veins as well as sinusoids, STZ+ 200 mg/kg Terminalia chebula alcoholic extract treated group (**C**), STZ + 400 mg/kg Terminalia chebula alcoholic extract treated group (**D**), STZ + 600 mg/kg Terminalia chebula alcoholic extract treated group (**E1&E2**), STZ + metformin treated group (**F**) (H&E, 40×). Central vein (arrow), portal vein (two headed arrow), hepatocytes (*)

Histopathological examinations of kidney sections

Microscopic examinations of kidney sections of male rats in the control group revealed no histopathological and the normal histopathological structure of the glomeruli and tubules at the cortex were recorded in (Fig. 2-A). Microscopic examinations of the STZ group observed swelling and vacuolization in the lining endothelium of the glomerular tufts associated with swelling in the epithelial cells lining the tubules at the cortex (Fig. 2-B1&B2). The corticomedullary portion showed degeneration in the lining epithelium (Fig. 2-B3&B4). Microscopic examinations of kidney sections of male rats in the treated group with Terminalia chebula alcoholic extract (200 mg/kg) showed epithelial cells lining the tubules of the cortical portion (Fig. 2-C1) while the tubules at the corticomedullary portion showed degenerative change (Fig. 2-C2). In addition, microscopic examinations of kidney sections of the treated group with Terminalia chebula alcoholic extract (400 mg/kg) showed no histopathological alteration in the glomeruli and tubules at the cortex (Fig. 2-D1) while the corticomedullary portion showed degeneration in the tubular lining epithelium (Fig. 2-D2). While microscopic examinations of kidney sections of male rats in the Terminalia chebula 600 mg/kg group detected swelling and vacuolization in the endothelial cells lining the tufts of the glomeruli associated with swelling in the epithelial cells lining the tubules at the cortex (Fig. 2-E1). The corticomedullary portion showed focal haemorrhages in between the degenerated tubules (Fig. 2-E2). Liver sections of male rats in the treated group with metformin revealed swelling in the tubular lining epithelium of the cortical portion (Fig. 2-F1) while the tubules at the corticomedullary portion showed degenerative changes (Fig. 2-F2).

Fig. 1 (continued)



Discussion

Diabetes mellitus in rats was manifested via intraperitoneal administration of streptozotocin. STZ-induced hyperglycaemia is useful experimental model for studying antihyperglycemic activity. Streptozotocin is an antibiotic drug produced by Streptomyces achromogenes where it has detrimental effects on the β-cells of the islets of Langerhans [42–44]. Streptozotocin could be a severe poison to the islets of Langerhans and could induce extreme diabetes [45] . This induced diabetes mellitus is described as a significant increase in plasma glucose levels and reduction in insulin secretion [46]. Ethanolic extract of Terminalia chebula fruits was screened for toxicity by oral toxicity studies according to Organization for Economic Cooperation and Development (OECD) guidelines. A dose of 2000 mg/kg body weight was found to be non-toxic (Category 5 / Unclassified) so three test doses 200 mg/kg, 400 mg/kg, and 600 mg/kg were selected for our experiment. Our results were compared with a standard antidiabetic drug which is metformin, which is considered the first-line drug therapy for the management of T2DM [27, 28]. Our results confirmed others' reported antidiabetic activity of T. chebula extract [23, 47] manifested by the significant reduction in FBG (as shown in supplementary material Fig. 1 and in Table 1).

Murali et al. and Lee et al. indicated that aqueous extract of Terminalia chebula (200 mg/kg) improves glucose tolerance and brings down fasting blood glucose in diabetic rats [48, 49]. Rubaiat Nazneen Akhand et al. study indicated that administration of the ethanolic extracts (prepared in 80% ethanolic solvent) at the dose of 1.25 g/kg body weight of dried Terminalia chebula mature fruits improved glycaemic status in T2DM male rats [24]. Another relative study of Daniyal Kazmi et al. observed that treatment of alloxan-induced diabetic Wistar rats with aqueous extract of Terminalia chebula (500 mg/kg body weight) resulted in a significant decrease in blood glucose level [50]. Our study exhibited that the ethanolic extract is considered to have good antihyperglycemic active principles which were achieved by increasing the dose, as the dose of 400 mg/kg showed a better hypoglycaemic effect than the dose of 200 mg/kg. The most potent dose of the ethanolic extract regarding hypoglycaemic activity was 600 mg/kg.

Diabetes mellitus is accompanied by increased biochemical activities like glycogenolysis, lipolysis, and gluconeogenesis resulting in muscle wasting and loss of tissue protein [51]. The loss of body weight is attributed to the increase of blood glucose, protein loss, and increase of muscle wasting in STZ induced diabetic animals [17, 52]. As shown in fig. 2 and Table 2, treatment with T. chebula ethanolic extract especially at a dose of 600 mg/kg, is considered to have a good protective effect against weight loss complications due to hyperglycaemia.

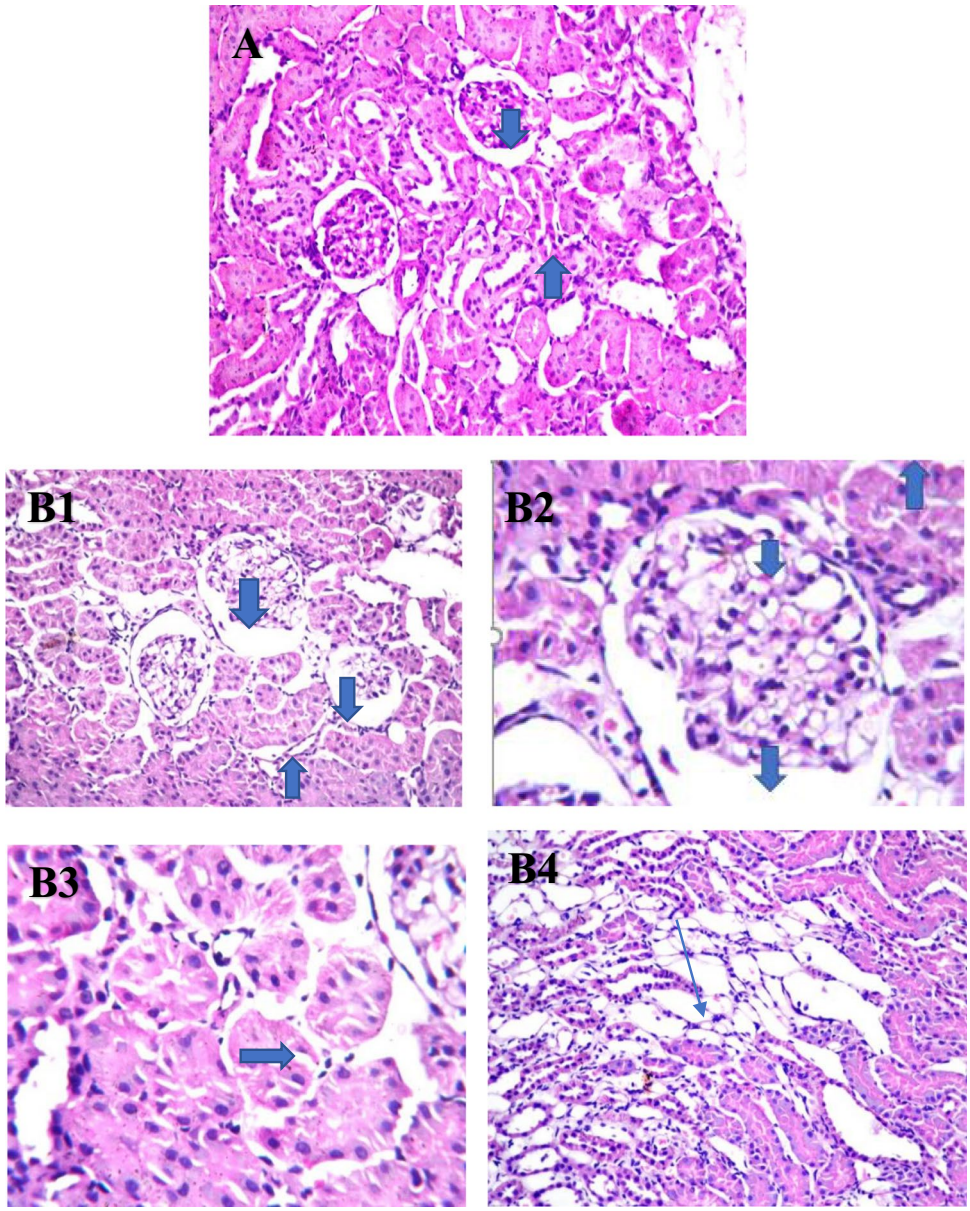
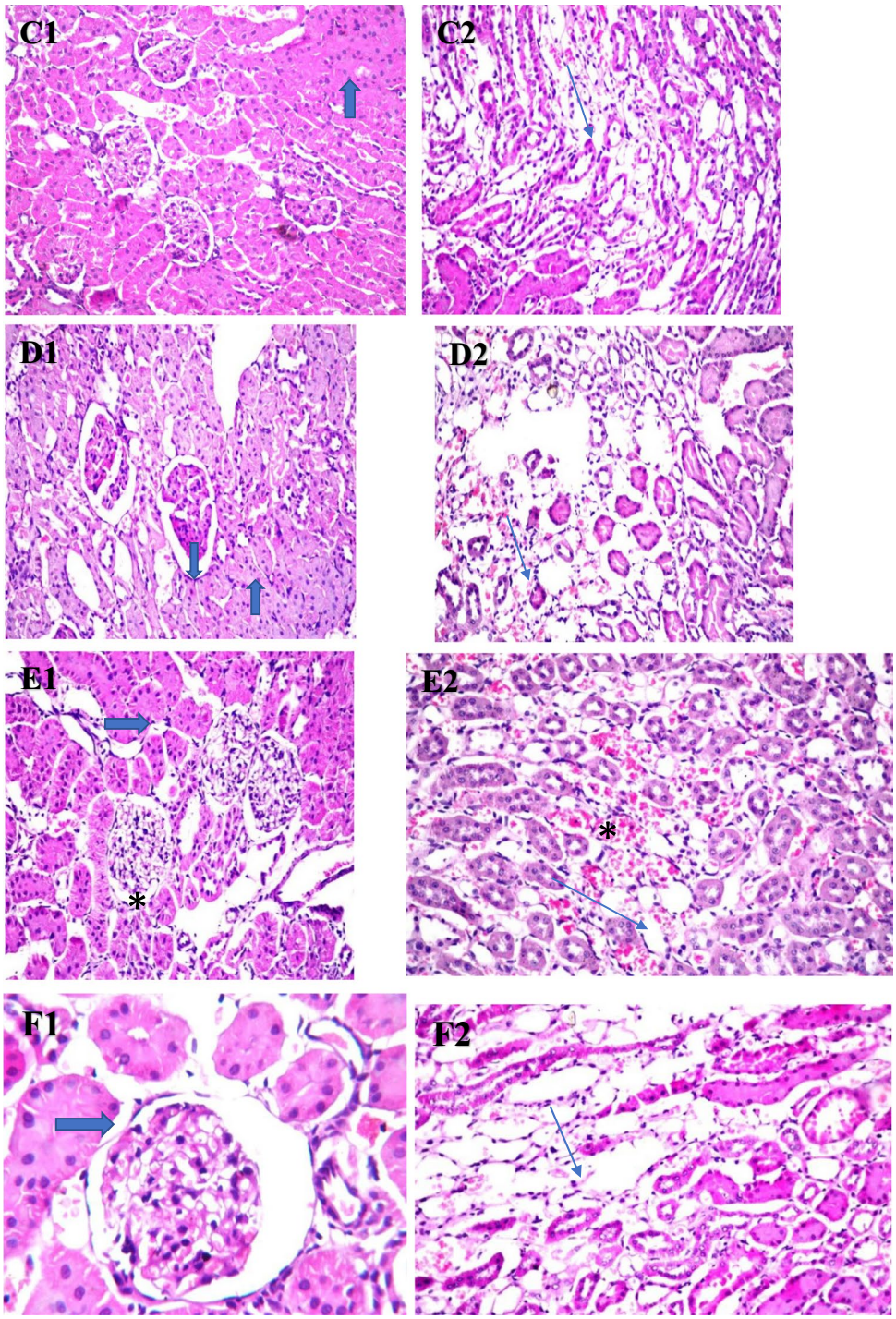


Fig. 2 Histopathological assessments of the renal tissue samples stained using hematoxylin and eosin in the different experimental groups. Control group (**A**), STZ group (**B1–4**), STZ+ 200 mg/kg Terminalia chebula alcoholic extract treated group (**C1–2**), STZ + 400 mg/kg Terminalia chebula alcoholic extract treated group (**D**1–2), STZ + 600 mg/kg Terminalia chebula alcoholic extract treated group (**E1–2**), STZ + metformin treated group (**F1–2**) (H&E, 40 × &80x). Glomeruli (downward arrow), tubules (upward arrow), focal hemorrhages (*), swelling in the epithelial cells (right hand arrow), degenerative changes (thin line head arrow)

Diabetes is associated with profound alteration in the plasma lipid and lipoprotein profile and therefore, is accompanied by an increased risk of coronary heart disease. Under normal circumstances, insulin stimulates the enzyme lipoprotein lipase which degrades triglycerides. Insulin deficiency results in failure of enzyme activation, thereby resulting in hypertriglyceridemia [53]. The increase of blood cholesterol and triglycerides levels is a key indicator of dyslipidaemia [54, 55] which if left uncontrolled, increases the risk of coronary heart disease occurrence [56, 57]**.** We have reported that treated groups with alcoholic extract of Terminalia chebula (600 mg/kg) for four weeks induced recovery in lipid profile values, causing a significant decline ($p < 0.05$) in serum cholesterol and triglyceride levels. Their levels that are back to normal values suggested that treatment with 600 mg/kg alcoholic extract of Terminalia chebula has an efficient antihyperlipidemic property. The extract may inhibit the pathway of cholesterol synthesis in diabetic rats. These results are in concordance with those of Rathore et al., which approved that Terminalia chebula has a hypocholesterolemic effect in animals fed with an atherogenic diet [58]. Our results also ran in parallel to Rubaiat Nazneen Akhand et al., However, their study indicated that administration of the ethanolic extracts of dried Terminalia chebula mature fruits (1.25 g/kg body weight) [24], which is double our effective dose, to improve lipidemic status in T2DM male rats.

The liver plays a vital role in controlling carbohydrate metabolism by keeping glucose levels within normal ranges over both short and long periods of time. Glucose

Fig. 2 (continued)



is produced in the liver by glycogenolysis and gluconeogenesis. Glycogen is the primary intracellular storage form of glucose and its availability in various tissues is a direct demonstration of insulin action, as insulin facilitates intracellular glycogen deposition by activation of glycogen synthase, and inhibition of glycogen phosphorylase [59]**.** Moreover, the liver helps in the detoxification of drugs, exogenous toxins, and therapeutic agents; as well as the bioregulation of proteins, amino acids, fats, carbohydrates, blood coagulation, and immunomodulation [60]**.** Elevated Liver enzymes in the blood including aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) are used to measure the extent of hepatic damage as they are released into the bloodstream due to hepatocellular injury [61, 62]. ALP serum levels elevation is observed in cholestasis; hence it is used as a

marker to determine biliary obstruction and cholestasis. In this study we reported that Terminalia chebula alcoholic extract (600 mg/kg) decreased AST levels back to normal values whereas, ALT and ALP levels were still significantly rising ($p < 0.05$); the recorded changes were 32.9 and 353.7 respectively, compared to control group indicating better liver protection than other lesser doses. Generally, liver enzymes activities results were the best values after treatment with (600 mg/kg) Terminalia chebula alcoholic extract showing the non-toxic as well as tissue-protective effects against the hepatotoxicity produced by STZ-induced T2DM.

Creatinine is produced due to the breakdown of phosphocreatine during the metabolism of muscles and dietary proteins, and it is cleared by the kidneys. The increase in serum creatinine levels is one of the key indicators of renal dysfunction. In our study, an elevation of serum urea and creatinine levels was observed in diabetic untreated rats in comparison with the control group, which are significant markers of renal dysfunction [63]. As mentioned in the results section before, serum creatinine and urea levels of T. chebula alcoholic extract recorded a significant decrease in comparison with the untreated group. However, their levels had shown a significant rise in comparison with the control group. This indicates that the mentioned dose cannot provide full protection from renal impairment induced by diabetes. These results are consistent with those of Gandhipuram Periasamy Senthilkumar et al., who suggested that administration of T. chebula ethanolic extract (200 mg/kg) to diabetic rats significantly decreased the level of blood urea and creatinine [47]. Our results are in accordance with Ramakrishnan thiruchelvi et al. as well, who suggested that the treatment of T. chebula fruit aqueous extract (200 mg/kg body weight) might be helpful to alleviate the cadmium-induced toxicity in the kidney [64]. However, Shaimaa et al., revealed that the greatest improvement of renal functions was achieved at the dose of 400 mg/kg of T. chebula extract, which helps alleviate the renal injury induced by cadmium chloride [65].

We reported that serum creatinine and urea levels of the group treated with T. chebula alcoholic extract (400 mg/kg) were significantly lower in their levels in comparison with the untreated group. These results reported lowering creatinine levels back to normal values. However, they are still showing a significant increase in urea levels compared to the control group indicating improving renal impairment but still not fully resolved. Moreover, we reported a significant decline in serum creatinine and urea levels of T. chebula alcoholic extract (600 mg/kg) compared to the untreated group. Our results run in parallel with Aamina Muneer et al., who indicated that co-administration of ethanolic extract of T. chebula (500, 1000 mg/kg) significantly decreased the renal damage induced by Doxorubicin in rats [66].

Conclusion

The use of Terminalia chebula alone or in combination with conventional antidiabetic drugs may be beneficial as a new advent therapy for diabetes. In addition, the dose of 600 mg/kg seems to represent the ultimate therapeutic dose level.

Limitations of the study

More doses of T. chebula extract should have been evaluated to confirm the ultimate therapeutic dose of T. chebula. In addition, different routes of drug administration should have been compared. Moreover, more antidiabetic tests should be carried out (glucose absorption and gluconeogenesis).

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Declarations

Conflict of interest The authors declared that they have no conflict of interest.

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