The Protective Effect of *Tribulus terrestris* in Diabetes

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ABSTRACT: ***Tribulus terrestris*** **L (*TT*) is used in the Arabic folk medicine to treat various diseases. The aim of this article was to investigate the protective effects of *TT* in diabetes mellitus (DM). Diabetes is known to increase reactive oxygen species (ROS) level that subsequently contributes to the pathogenesis of diabetes. Rats were divided into six groups and treated with either saline, glibenclamide (Glib), or *TT* for 30 days. Rats in group 1 were given saline after the onset of streptozotocin (STZ)-induced diabetes; the second diabetic group was administered Glib (10 mg/kg body weight). The third diabetic group was treated with the *TT* extract (2 g/kg body weight), while the first, second, and third nondiabetic groups were treated with saline solution, Glib, and *TT* extract, respectively. At the end of the experiment, serum and liver samples were collected for biochemical and morphological analysis. Levels of serum alanine aminotransferase (ALT) and creatinine were estimated. In addition, levels of malondialdehyde (MDA) and reduced glutathione (GSH) were assayed in the liver. The tested *TT* extract significantly decreased the levels of ALT and creatinine in the serum ($P < 0.05$) in diabetic groups and lowered the MDA level in liver ($P < 0.05$) in diabetic and ($P < 0.01$) nondiabetic groups. On the other hand, levels of reduced GSH in liver were significantly increased ($P < 0.01$) in diabetic rats treated with *TT.* Histopathological examination revealed significant recovery of liver in herb-treated rats. This investigation suggests that the protective effect of *TT* for STZ-induced diabetic rats may be mediated by inhibiting oxidative stress.**

KEYWORDS: diabetes; antioxidant; *Tribulus terrestris*; medicinal plants; streptozotocin; oxidative stress

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine diseases in the world that affects more than 6% of the world population.[1] There are an estimated 143 million people worldwide with the disease, almost five times more than estimates 10 years ago. This number will probably double by the year 2030.[2] Hyperglycemia associated with diabetes increases the glucose autoxidation and protein glycation, and the subsequent oxidative degradation of glycated proteins leads to enhanced production of reactive oxygen species (ROS).[3] ROS are through to play a major role in a variety of physiologic and pathophysiological processes in which increased oxidative stress may play an important role in disease mechanisms.[4] Lower endogenous antioxidants and elevated lipid peroxidation (LP) levels are risk factors for the development of diabetic complications, such as atherosclerosis.[5,6]

Tribulus terrestris L (*TT*) is a member of the plant family Zygophyllaceae. It is called "Qutiba" in Bedouin language and is widely distributed in the entire Mediterranean region. It flowers throughout the year and all parts of the plant are used for various purposes in folk medicine. The fruit is regarded as tonic diuretic and aphrodisiac. It is also used to treat urinary disorder, impotency, and heart diseases. The seeds are recommended in hemorrhages, kidney stone, and gout.[7] The extract of *TT* contains protodioscin (PTN), a steroidal saponin, that has been extensively used for the treatment of various ailments, such as urinary, cardiovascular,[8] and gastrointestinal disorders.[9] Administration of *TT* to humans and animals improves libido and spermatogenesis.[10] PTN has been reported to upregulate the levels of testosterone and leuteinizing hormone,[11] dehydroepiandrosterone,[12] dihydrotestosterone, and dehydroepiandrosterone sulfate.[13] *TT* has a proerectile effect.[14] Saponin from *TT* is also known for its hypoglycemic effect.[15]

The purpose of this article was to investigate the protective role of *TT* against the streptozotocin (STZ)-induced diabetes in rats.

MATERIALS AND METHODS

Chemicals

STZ was purchased from Sigma Co. (St. Louis, MO). Sodium lauryl sulfate (SDS), acetic acid, thiobarbituric acid aqueous solution (TBA), n-butanol, pyridine, 1,1,3,3-tetramethoxypropane standard, trichloroacetic acid (TCA), phosphate buffer, 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), and reduced glutathione (GSH) standard, reagents, were obtained from Fluka (Taufkirchen, Germany). All the chemicals used were of analytical grade.

Extraction

TT was obtained from the local stores. The herb was grounded to a fine powder in an electrical grinder. Its dried powder was exhaustively extracted with 70% ethyl alcohol. The extracts were evaporated in a rotary evaporator at 40°C under reduced pressure. The yield was 6.8%.

Animals

Male Wistar rats (220–240 g) used in this study were raised and housed at the Animal Facility of the Faculty of Medicine and Health Sciences, UAE University. Rats were maintained at 22 ± 2°C and were subjected to 12-h light–dark cycle. Rats were housed in standard cages, fed on standard laboratory food, and had free access to water *ad libitum*. This study was conducted according to the animal ethics protocols of UAEU. Rats were fasted for 12 h before the experiments.

Induction of DM

DM was induced by a single dose of intraperitoneal (60 mg/kg body weight) injection of STZ.[16] The rats were considered diabetic if the fasting level values were more than 300 mg/dL.[16]

Experimental Design

Six rats were randomly assigned into six experimental groups. The first, second, and third (diabetic-treated) groups and the fourth, fifth, and sixth groups (normal nondiabetic groups). All rats were treated for 30 days of the experimental period. The first group (the diabetic control) has been orally receiving only 0.9% NaCl (saline) solution. The second group (the diabetic rats) was orally administered glibenclamide (Glib; 10 mg/kg body weight/day) in saline solution. The third group (the diabetic rats) was orally given 2 g/kg body weight of *TT* extract dissolved in saline. The fourth group (the normal rats) was orally receiving only 0.9% NaCl (saline) solution. The fifth group (the normal rats) was only receiving similar dose of Glib. The sixth group (the normal rats) received 2 g/kg body weight of *TT* extract dissolved in saline.

At the end of the experimental period, body weights were determined and rats were sacrificed by cervical decapitation. Blood was collected and the separated serum was used for further estimation. The liver was immediately excised, rinsed in ice-cold saline, dried, weighed, and homogenized in Tris-HCl buffer of pH 7.4 (0.1 M) using a Teflon homogenizer. The tissue homogenate

was then centrifuged in a cooling centrifuge at 4000 rpm for 15 min at 4°C in order to remove the debris, and the supernatant was used for the analysis of biochemical parameters. The tissue homogenate was stored at −20°C until further use.

Biochemical Parameters

Liver and Kidney Functions

Blood samples were processed for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, and the electrolytes calcium and phosphorous were estimated following the manufacturer's instructions.

Estimation of Protein

Protein content, in all samples, was estimated by the method in Reference 17. Bovine serum albumin was used as standard. Enzymes are normalized on the basis of total protein content.

LP

LP was assayed by the method of Reference 18, in which the released malondialdehyde (MDA) serves as an index of LP. 1,1,3,3-Tetramethoxypropane was used as standard. A mixture of 0.2 mL of homogenate, 0.2 mL 8.1% SDS, 1.5 mL 20% acetic acid (pH 3.5), 1.5 mL 0.8% TBA, and 0.6 mL distilled water was made. The mixture was boiled in a tightly closed glass tube in a water bath for 60 min. Immediately, the solution was cooled in ice bath or tap water for 5 min. One milliliter of water and 5 mL of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min at 4°C, the organic layer was taken and its absorbance was measured against reagent blank using a glass cuvette at 532 nm. The level of MDA was expressed as nmol of MDA/mg of liver tissue protein.

Reduced GSH

Reduced GSH was estimated as described in Reference 19. Briefly, 0.5 mL of homogenate was precipitated with equal volume of 5% TCA. The contents were mixed well for complete precipitation of proteins and centrifuged at 4000 rpm for 15 min at 4°C. A mixture of 0.1 mL of supernatant, 1.9 mL of 0.2 M phosphate buffer (pH 8.0), and 2 mL of 0.6 mM DTNB was made,

vortexed, then the absorbance at 412 nm was read against a blank containing TCA instead of sample. A series of GSH standards treated in a similar way as the sample were also run to determine the GSH content. The amount of GSH is expressed as nmol/ mg liver tissue protein.

Histopathology

Liver tissues were fixed in a 10% neutral-buffered formalin solution, embedded in paraffin, and sectioned. Thin sections were then deparaffinized, hydrated, and stained with hematoxlin and eosin and examined using a microscope.

Statistical Analysis

Values are expressed as mean ± SD. One-way analysis of variance (ANOVA) and Student's *t*-test are applied to calculate the statistical significance between the various groups. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Liver and Kidney Functions

After treatment of the STZ-induced and normal rats with 10 mg/kg body weight of Glib and 2 g/kg body weight of *TT* extract, respectively, levels of ALT and AST were decreased in all treated groups compared with the nontreated ones, and the decrement in STZ-induced rats treated with *TT* was significant ($P < 0.05$) compared to the STZ-induced nontreated group (FIG. 1).

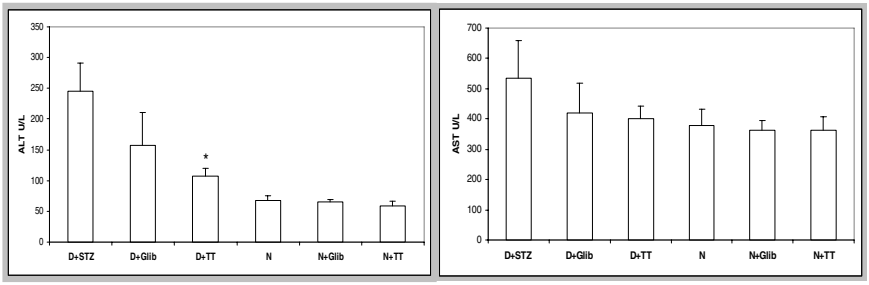

FIGURE 1. Effect of Glib and *TT* on serum ALT and AST of controls and experimental groups of diabetic (D) and nondiabetic (N) rats. Results are expressed as mean ± *SD* ($n = 6$). $^*P < 0.05$. Comparisons are made between nontreated groups and treated groups.

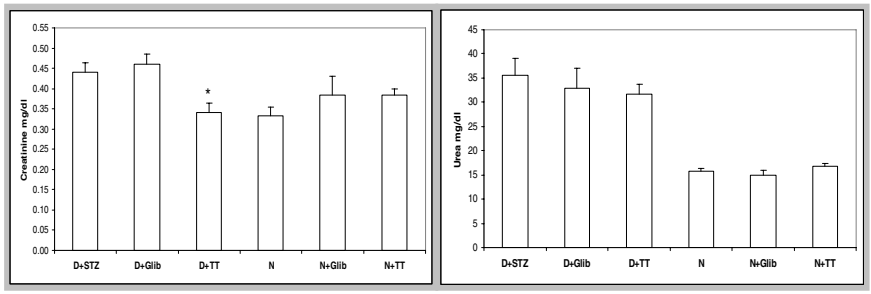

FIGURE 2. Effect of Glib and *TT* on serum creatinine and urea of controls and experimental groups of diabetic (D) and nondiabetic (N) rats. Results are expressed as mean ± *SD* ($n = 6$). $^*P < 0.05$. Comparisons are made between nontreated groups and treated groups.

Oral administration of 10 mg/kg body weight of Glib and 2 g/kg body weight of *TT* extract had shown a significant decrement ($P < 0.05$) of serum creatinine levels in the STZ-induced *TT*-treated group and there were insignificant changes in between rats in the normal groups. However, there was an insignificant reduction in the levels of serum urea in the STZ-induced rats. There were no significant alterations in the levels of serum urea in all normal treated groups (FIG. 2).

In STZ-induced diabetic rats there was a significant increment ($P < 0.05$) of serum calcium under the oral administration of 10 mg/kg body weight of Glib while there was an insignificant decrement of serum calcium after the administration of 2 g/kg body weight of *TT* extract. The effect of 10 mg/kg body weight of Glib and 2 g/kg body weight of *TT* extract on normal rats leads to insignificant increment of calcium levels in all normal groups. On the other hand, treatment with Glib on both STZ-induced and normal rats increases the levels of serum phosphorous insignificantly while *TT* extracts decrease the serum levels of phosphorus (FIG. 3).

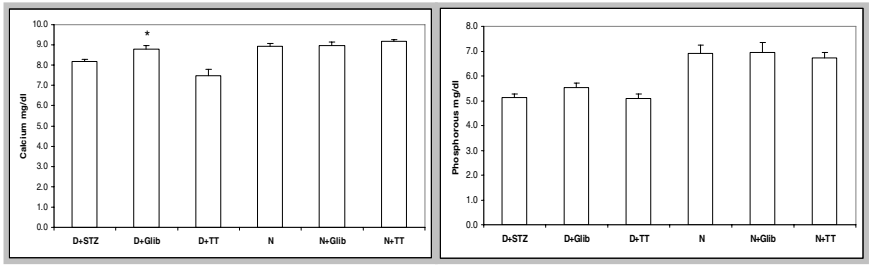

FIGURE 3. Effect of Glib and *TT* on serum calcium and phosphorous of controls and experimental groups of diabetic (D) and nondiabetic (N) rats. Results are expressed as mean ± *SD* (n = 6). $^*P < 0.05$. Comparisons are made between nontreated groups and treated groups.

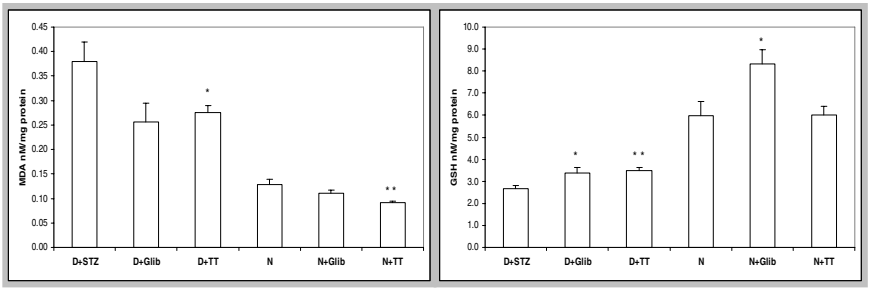

FIGURE 4. Effect of Glib and *TT,* on liver MDA and GSH of controls and experimental groups of diabetic (D) and nondiabetic (N) rats. Results are expressed as mean ± *SD* ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$. Comparisons are made between nontreated groups and treated groups.

Antioxidants

Effects of tested herb on STZ-induced diabetic rats and normal rats were examined by monitoring the levels of MDA. Following treatment with *TT*, levels of hepatic MDA decreased significantly both in the diabetic and nondiabetic groups [($P < 0.05$) and ($P < 0.01$) respectively]. Treatment with Glib also insignificantly decreased the levels of MDA in both STZ-induced diabetic and normal rats. Following Glib treatment, the activity of hepatic GSH was significantly increased ($P < 0.05$) in both diabetic and nondiabetic groups. However, treatment with *TT* increased GSH levels significantly ($P < 0.01$) in diabetic rats and insignificantly in nondiabetic rats (FIG. 4).

TT *Protects against Liver Damage*

In diabetic rats, hepatocytes were disrupted and severe dilatations of liver sinusoids were observed. In addition, *TT* protected against necrotic lesion in the liver. The liver appears near normal after treatment with *TT* (FIG. 5). On the other hand, the liver structure seems to be normal in all the normal rat groups (FIG. 6).

DISCUSSION

Diabetes is one of the world's fastest growing metabolic disorders. While the knowledge of the heterogeneity of this disease increases, so also is the need for more appropriate therapies.[20] Traditional medicinal plants are used throughout the world for a wide range of diabetic presentations. The study of such medicines might offer a natural key to find new antidiabetic drugs.[21]

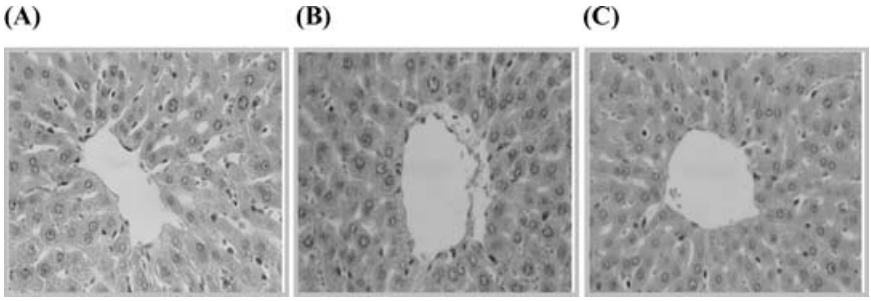

FIGURE 5. Histopathological effects of *TT* and Glib on the liver of diabetic rats. Light micrographs of liver sections. (**A**) Liver section of STZ-induced diabetic rats showing hepatocytes degeneration with dilatation of sinusoids in centrilobular area with some congestion and hemorrhage. (**B**) Liver sections of STZ-induced diabetic rats treated with 10 mg/kg B.W. of Glib showing less degrees of hepatocyte degeneration and nearly no dilated sinusoid in midzonal area. (**C**) Liver section of STZ-induced diabetic rats treated with 2g /kg B.W. of *TT* showing minor degree of degeneration of hepatocytes and dilated sinusoids. Areas with normal hepatic architecture can also be observed. H & E: Magnification: ×400.

Liver enzymes, such as ALT and AST, are used as markers for liver damage. ALT is thought to be a more specific indicator of liver damage. Serum ALT decreased after treatment of the STZ-induced diabetic rats with *TT* and this result indicates the protective effect of the herb. The same effect is shown on the decrement levels of AST. So these results show that *TT* has hepatoprotective effects.

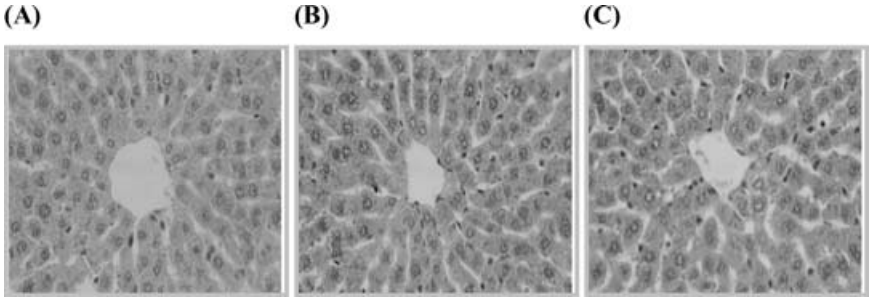

FIGURE 6. Histopathological effects of *TT* and Glib on liver of normal rats. Light micrographs of liver sections. (**A**) Liver sections showing normal hepatic architecture, where the hepatocytes are arranged around the central vein and alternate with blood sinusoids. Each hepatic cell possesses a limiting membrane, centrally placed large nucleus, and prominent nucleoli. (**B**) Liver section of normal rats treated with 10 mg/kg B.W. of Glib showing normal hepatocytes arrangement and normal dilatation of sinusoids in centrilobular area. (**C**) Liver sections of rats treated with 2 g/kg B.W. of *TT* showing similar degrees of hepatocyte architecture with normal dilated sinusoid in midzonal area. H & E: Magnification: ×400.

Increase in creatinine level is indicative of impairment of renal function.[22,23] *TT* restores the kidney functions after the treatment of the rats. Serum creatinine and urea levels decreased after the administration of the herb and this means that *TT* has a beneficial effect on kidney functions.

Hypoinsulinemia in STZ-induced diabetic rats increases the activity of the enzyme fatty acyl Coenzyme A oxidase, which initiates β-oxidation of fatty acids, resulting in LP.[24] Increased LP impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors. Its products (lipid radical and lipid peroxide) are harmful to most cells in the body and are associated with a variety of diseases, such as atherosclerosis and brain damage.[25] In this study, the decreased level of liver MDA ($P < 0.05$) was shown in STZ-induced diabetic *TT*-treated rats compared with nontreated diabetic rats. The extract could significantly reduce ($P < 0.01$) liver MDA levels in normal rats also. Similar reductions were also observed in liver MDA levels after treatment with Glib in diabetic and normal rats but insignificantly. These findings suggest that the extract has antioxidant effects and can protect tissues from LP.

Insulin deficiency in the diabetic pair's glucose utilization, leads to an increase in oxygen-free radicals. The present results show an increased activity of hepatic antioxidant enzyme GSH in STZ-diabetic rats. GSH, as one of the important nonenzymatic antioxidants in the antioxidant defense system, is synthesized mainly in the liver.[26] It is involved in the synthesis of important macromolecules and in the protection against reactive O_2 compounds.[27] The decreased GSH concentration contributes to the pathogenesis of complications associated with the chronic diabetic state. This study has shown that liver GSH levels were significantly increased ($P < 0.01$) in STZ-induced diabetic rats treated with the extract and the increment was insignificant in case of normal rats treated with the *TT* extract, while a significant increase ($P < 0.05$) of GSH levels was observed in Glib-treated diabetic and normal rats. This investigation reports significantly increased levels of liver GSH, in extract-treated diabetic and normal rats and also reduced MDA in extract-treated diabetic and normal rats, which may be attributed to the presence of such antioxidant compounds in *TT*.

Histopathological changes in the liver STZ-induced diabetic rats show dilatation of blood vessels, congestion in the lobules, some hemorrhagic coagulative foci in hepatic parenchyma, and infiltration of mixed inflammatory cells around the necrotic hepatocytes. In this study, there was a pronounced restoration of the normal hepatic architecture after the treatment of STZ-induced diabetic rats with *TT* ethanolic extract.

CONCLUSION

From this study it can be seen that increased oxidative stress is apparent in STZ-induced diabetic rats. The ethanolic extract of *TT* exhibits a significant

antioxidant activity against STZ-induced diabetes. Further studies are needed to explain the mechanism(s) of protective effects of *TT* .

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