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Hypoglycemic and hypolipidemic effects of alcoholic extract of *Tribulus alatus* in streptozotocin-induced diabetic rats: A comparative study with *T. terrestris* (Caltrop)

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The extracts of both *T. alatus* and *T. terrestris* significantly decrease fasting glucose level in diabetic rats. After 4 and 6 hr, *T. alatus* extract showed significant reduction in glucose level as compared to *T. terrestris.* After 3 weeks of treatment with *T. alatus* extract, glucose level was significantly decreased to the normal level. Both the extracts also caused a significant decrease in the levels of glycosylated hemoglobin, total cholesterol, triglycerides and LDL-cholesterol. The percent of reduction in rats treated with *T. alatus* extract was significantly higher than that of the rats treated with *T. terrestris.* The results indicate that alcoholic extract of *T. alatus* possesses hypoglycemic activity in type-1 model of diabetes.

Keywords: Alcoholic extract, Diabetic rats, Hypoglycemic, Hypolipidemic, *Tribulus alatus, Tribulus terrestris*

Synthetic hypoglycemic agents in current use for the treatment of diabetes mellitus produce serious side-effects, including hematological coma and disturbances of liver and kidney. In addition, they are not suitable for use during pregnancy[1]. Therefore, emphasis nowadays all over the world is on medicinal plants with less side effects. The plants and some of the compounds purified from them possessing anti-hyperglycemic activity have recently been reviewed[2,3]. WHO recommends the use of medicinal plants for the treatment of diabetes mellitus[4].

The genus *Tribulus* (Zygophyllaceae) comprises about 20 species which grow as shrubs or herbs in subtropical areas around the world[5].

T. terrestris (Caltrop) is extensively used in traditional medicine for the treatment of eye troubles, edema, abdominal distention and morbid leucorrhea as well as vitiligo, and also as diuretic, and anthelmintic[6]. The fruits of *T. alatus* (*Tribulus longipetals viv*.) are used in Pakistan for the treatment of urinary disorders and cough[7]. The hypoglycemic and hypolipidemic activities of *T.terrestris* have been reported[8,9].

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The purpose of the present study is to compare the hypoglycemic and hypolipidemic activities of *T.alatus* with that of *T.terrestris.*

Materials and Methods

Plant material—Samples of *T. alatus* and *T. terrestris* were collected from Al Azhar University, Nasr-city, Cairo and were identified by Department of Botany, Faculty of Science, Cairo University.

Extraction procedure—The freshly cut aerial part with fruits of both the plants were dried in the drying room with active ventilation at ambient temperature 25°±1°C and packed in paper bags. Approximately 500 g of both *T. alatus* and *T. terrestris* was finely powdered and macerated separately in 70% methanol till exhaustion. The methanol extracts were combined and evaporated to dryness under vacuum. The residue of dried extract was weighed.

Test animals—Male Swiss albino rats (30) weighing between 150-200 g each were used. The animals were housed in a temperature (25°±1°C), humidity controlled room and a 12 hr light-dark cycle (lights on at 0600 hr). Rats were allowed free access to tap water and standard pellet diet.

Twenty four rats fasted for 18 hr, were made hyperglycemic by intraperitoneal injection of

Streptozotocin (Sigma) dissolved in citrate buffer (*p*H 4.5), at a dose of 55 mg/kg body weight. After 72 hr of streptozotocin injection, the rats were fasted for 6 hr and their plasma glucose levels were estimated. Rats, having plasma glucose levels above 250 mg/dl[10] were considered diabetic. The remaining 6 rats were injected with equal volume of 10% physiological saline and these were used as healthy control rats (Group I). The 24 diabetic rats were randomly divided into following 4 groups of 6 each:

Group II: diabetic rats maintained on unrestricted standard diet and water *ad libitum* and served as untreated diabetic rats.
Group III: diabetic rats treated orally with *T. alatus* extract (50 mg/kg body wt.)
Group IV: diabetic rats treated orally with *T. terrestris* extract (50 mg/kg body wt.)
Group V: diabetic rats received orally glibenclamide (10 mg/kg body wt).

Treatments for 21 days in all groups were started 4 days after streptozotocin injection. The bodyweight of the animals was recorded after the termination of the experiment.

Blood samples were collected from overnight fasted animals at 0, 2, 4, 6 hr and 21 days after treatment. Whole blood was used for the estimation of hemoglobin (Hb) and glycosylated hemoglobin (HbA1c).

Hb[11] and glycosylated hemoglobin (HbA1c)[12] blood glucose[13], serum triglyceride (TAG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-c)[14] were estimated. Low density lipoprotein cholesterol (LDL-c) was estimated by the equation of Friedewald *et al*[15]:

$$\text{LDL-c} = \text{TC} - \text{HDL-c} - \text{TAG}/5.$$

Histopathological study—The animals were killed, the pancreas was dissected out and used for histopathological studies. Pancreatic tissues were fixed in 10% neutral buffered formalin, dehydrated with 50-100% ethanol solutions, and embedded in paraffin. Sections (4-5 μm thick) were cut and stained with hematoxylin-eosin.

Statistical analysis—The data are represented as mean±SE, and statistical significance between treated and control groups was analyzed using Student's *t*-test.

Results

Bodyweight—A significant decrease in bodyweight was observed in the untreated diabetic group (Group II; 155±6.6 g) as compared to the control group (Group I; 175±6.1 g). The administration of the extracts (Groups III and IV) resulted in a significant increase in bodyweight (197±4.9, 194±7.3 g respectively) compared to Groups II and I.

Blood glucose level—The results of blood glucose (Table 1) show that all rats of Groups II-V, injected with streptozotocin developed severe diabetes with very much higher initial blood glucose level of about 500-511 mg/dl when compared to the blood glucose of healthy (Group I) control group (90±4.8). Feeding of diabetic rats with 50 mg/kg body wt of alcoholic

Table 1—Anti-diabetic activity of *T. alatus* and *T. terrestris* extracts in streptozotocin-induced hyperglycemic rats
[Values are mean ± SE from 6 animals in each group. Figures in parentheses are % reduction]

Treatment and dose	Serum glucose level mg/dl Time after treatment (hr)			
	0	2	4	6
Group I Normal Control	90 ± 4.8	95 ± 3.5	93 ± 1.8	97 ± 2
Group II Diabetics	500 ± 4	505 ± 3.0	510 ± 3.2	503 ± 3.5
Group III *T. alatus* (50 mg/kg body wt)	503 ± 3.6	163 ± 3.9*$ (67 ± 6.1)	128 ± 10.7*$ (74 ± 2.2)*	125 ± 5.4*$ (74 ± 1.0) *
Group IV *T. terrestris* (50 mg/kg body wt)	511 ± 4.2	262 ± 4.5$ (55 ± 6.7)	205 ± 2.8$ (59 ± 5.7)	208 ± 2.3$ (58 ± 4.8)
Group V Glibenclamide (10 mg/kg body wt)	510 ± 3.9	388 ± 3.9$ (22.6 ± 0.9)	345 ± 6.0$ (31.2 ± 0.85)	348 ± 6.0$ (31.8 ± 0.8)

*Significant difference between *T. alatus* and *T. terrestris*
$Significantly different from diabetics

extracts of *T. alatus* or *T. terrestris* produced significant decrease in blood glucose level after 2,4 and 6 hr of treatment as compared to untreated diabetic rats. After 4 and 6 hr of treatment, the percent of reduction in blood glucose level produced by *T. alatus* extract was significantly higher (74±2.2), (74±1.0) when compared with that of *T. terrestris* extract (59±5.7), (58±4.8). The percent of reduction in both these groups (III and IV) was higher than that seen in glibenclamide treated group (V) (31.2±0.8 and 31.8±0.8). After 3-weeks of treatment (Table 2) blood glucose level in diabetic rats treated with *T. alatus* and *T. terrestris* extracts significantly decreased (83-84%) to below normal level. Glibenclamide also showed similar results (84% reduction).

Hb and *HbA1c level*—There was a significant decrease in Hb level and increase in HbA1c level in untreated diabetic rats (Group II) as compared to control rats (Group I). After treatment with both the extracts (Groups III and IV) and glibenclamide (Group V), the levels of Hb and HbA1c returned to the normal values (Table 2).

Serum lipid profile—The results of the serum lipid profile (Table 2) show that in streptozotocin induced diabetic rats (Group II) there was not only hyperglycemia but also hyperlipidemia in which serum triglycerides, total cholesterol, HDL-c and LDL-c cholesterols increased significantly when compared to control group(Group I). Treatment of diabetic rats with *T. alatus* (Group III) extract for 3-weeks resulted in significant decrease of serum triglycerides, total cholesterol and LDL-c cholesterols as compared to untreated diabetic rats (Group II) and the values came down significantly below those in the normal healthy control group(Group I). A disadvantage of treatment of diabetic rats with *T. alatus* extract that brought HDL-c cholesterols values significantly below the normal values, (Table 2). On the other hand, treatment of diabetic rats with *T. terrestris* extract (Group IV) resulted in significant decrease in serum triglycerides, total cholesterol and LDL-c cholesterols as compared to untreated diabetic rats (Group II), total cholesterol and LDL-c cholesterols were equal to those in control group (I) and HDL-c cholesterols was kept significantly higher than that of control group (I) and this is an advantage of treatment of diabetic rats with *T. terrestris* (Group IV). The serum triglycerides levels in all treated groups (III-V) was significantly below the normal value (Table 2).

Table 2—Various biochemical parameters of serum and fasting glucose, lipid profile, blood hemoglobin and glycosylated hemoglobin in streptozotocin-induced hyperglycemic rats before and 3-weeks after treatment with *T. alatus* and *T. terrestris* extracts

[Values are mean ± SE from 6 animals in each group. Figures in parentheses are % reduction].

Treatment and dose	Hb (g/dL)	HbA1c (mg/g Hb)	Glucose (mg/dl)	TAG (mg/dl)	TC (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Group I Normal Control	13.2± 0.4	0.35± 0.02	90 ± 4.8	97 ± 5.4	65.5 ± 3	27 ± 2.7	24 ± 0.48
Group II Diabetics	7.75 ± 0.4$^{@}$	0.97± 0.04$^{@}$	505 ± 3.5$^{@}$	119.5± 6.4$^{@}$	97.1 ± 4.1$^{@}$	38.7 ± 1.9$^{@}$	33.7 ± 3.1$^{@}$
Group III *T. alatus* (50 mg/kg body wt)	14 ± 0.3$^{@\$}$	0.3 ± 0.02$^{\$}$	72 ± 4.4$^{@\$}$ (84 ± 0.7)	60 ± 3.2$^{@\$}$* (51 ± 1.4)*	47 ± 3$^{@\$}$* (53 ± 2.8)*	17 ± 1.1$^{@\$}$* (57 ± 3.0)*	17 ± 3.3$^{@\$}$* (67 ± 4.4)*
Group IV *T. terrestris* (50 mg/kg body wt)	13.9 ± 0.3$^{@\$}$	0.32± 0.03$^{\$}$	82 ± 4.1$^{\$}$ (83 ± 0.9)	71 ± 9$^{@\$}$ (36 ± 2.9)	67 ± 6.6$^{\$}$ (32 ±1.6)	36 ± 1$^{@}$ (7.7 ± 1.2)	24 ± 3.3$^{\$}$ (17 ± 1.7)
Group V Glibenclamide (10 mg/kg body wt)	12.8 ± 0.5 $^{\$}$	0.35± 0.02$^{\$}$	76 ± 0.3$^{@\$}$ (84 ± 0.1)	86 ± 1.1$^{@\$}$ (26 ± 6.9)	77 ± 3.2$^{@\$}$ (27 ± 5.4)	38 ± 0.726$^{@}$ (-)	22 ± 2.5$^{\$}$ (18 ± 0.9)

Hb=hemoglobin, HbA1c=glycosylated hemoglobin, TAG=triglycerides, TC=total cholesterol, HDL-c=high density lipoprotein cholesterol, LDL-c=low density lipoprotein cholesterol

$^{@}$Significantly different from control.

$^{\$}$Significantly different from diabetics.

*Significant difference between *T. alatus* and *T. terrestris*.

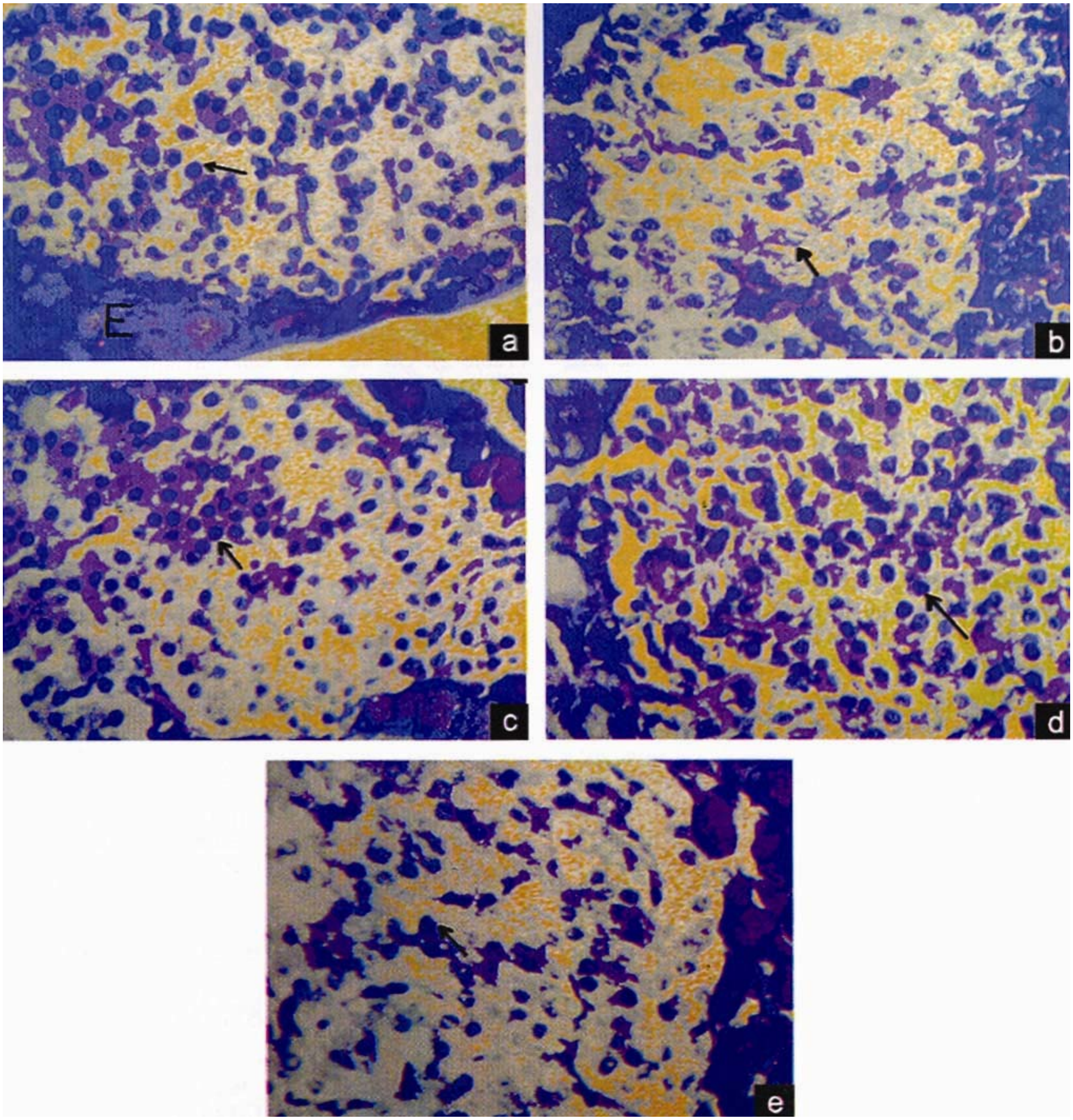

Fig. 1—Histopathological section of islets of Langerhans from pancreas of (a) normal healthy rat (b) an untreated diabetic rat (c) rat treated with *T.alatus* (d) rat treated with glibenclamide (e) rat treated with *T. terrestris*. Arrows indicate β-cells [Figs a-e: H & E × 160]

Histopathological findings—In the normal rats (Group I) histopathological examination showed normal histological structure of β-cells at the central zone in the islet of the Langerhans in the endocrine portion and the normal histological structure of the acini in the exocrine portion were recorded (Fig. 1a). In the untreated diabetic rats (Group II), atrophy and degeneration were observed mostly in the β-cells of the central zone at the islet of Langerhans (Fig. 1b). On the other hand, treatment of diabetic rats by

T. alatus extract (Group III) and glibenclamide (Group V) led to normalization of the affected β-cells, (Figs 1c and d). On the contrary, there were atrophy and degeneration in the cells of the islet of Langerhans in the diabetic rats treated with *T. terrestris* extract (Group IV) but less than that in the diabetic not treated one (Fig. 1e).

Discussion

Both *T. alatus* and *T. terrestris* extracts caused a significant decrease in blood glucose level as well as HbA1c which is more reliable index of diabetic control in STZ-induced diabetic rats which remained elevated in untreated diabetic animals. The decrease in HbA1c naturally resulted in the increase of hemoglobin. The results of *T. terrestris* are in agreement with previous studies[8,9].

Further studies are necessary to find out whether the mechanism of hypoglycemic action of the extract of *T. alatus* is either by increasing the peripheral utilization of glucose or by stimulating the secretion of insulin by the remaining intact β-cell after the destruction of the pancreas or insulin like action[16].

Atrophy and degeneration of mostly β-cells of the central zone of the islet of Langerhans (Fig. 1b) of untreated diabetic rats were normalized in rats treated with *T. alatus* extract and glibenclamide (reference drug) (Figs 1c and d, respectively). This suggests that *T. alatus* extract may act through the mechanisms of glibenclamide by reversing the abnormalities in the pancreatic islets. Phytochemical analysis of *Tribulus* showed that the major chemical constituents were flavonoids, steroidal saponins, alkaloids and lignanamides[17]. Over 150 plant extracts and some of their active principles, including coumarins, flavonoids, terpenoids, and a host of other secondary plant metabolites, including arginine and glutamic acid and flavnoids are known for the treatment of diabetes[18-20].

Both *T. alatus* and *T. terrestris* extracts as well as glibenclamide caused a significant decrease in serum triglycerides, total cholesterol, and LDL-c cholesterols level (P <0.05) in STZ-induced diabetic rats.

A disadvantage of treatment of diabetic rats with *T. alatus* extract was that it brought down HDL-c cholesterols values (17±1.1) to below the normal values, while *T. terrestris* extract had the advantage of keeping HDL-c cholesterols values (36±1) significantly higher than that of control group. It is known that HDL-c plays a key role in the protection against oxidative damage of membranes and lipid metabolism by transporting cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport[21].

Normalization histopathology of β-cells in the islets of Langerhans in pancreas of diabetic rats fed with *T. alatus* extract (Fig. 1c) may explain the both hypoglycemic and hypolipidemic actions of the extract. Hypolipidemia by *T. alatus* extract may be also due to their flavonoids which remove LDL-c from blood by increasing the LDL receptor densities in liver and by binding to apolipoprotein B[22].

Conclusion

T. alatus extract appears to be superior to *T. terrestris* because of restoring the functional β-cells in addition to its hypoglycemic effect and hypolipidemic action in STZ-diabetic animals. However, the disadvantage of *T. alatus* in lowering HDL-c is to be kept in view. Thus, it would be useful to carry out further studies to find out whether *T. alatus* extract can control diabetes and its hyperlipidemic complications.

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