

Original Research Article

Anti-hyperglycaemic Activity of *Tribulus terrestris* L Aerial Part Extract in Glucose-loaded Normal Rabbits

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Abstract

Purpose: To investigate the anti-hyperglycaemic activity of methanol extract of *Tribulus terrestris* L. Zygophyllaceae in glucose-loaded normal rabbits.

Methods: The animals were randomly assigned to 4 groups ($n = 5$) and treated with a single oral dose. Group 1 served as normal control group and received distilled water; group 2 served as hyperglycaemic control; group 3 was treated with glibenclamide (5 mg/kg, aqueous suspension) and served as reference standard; group 4 received methanol extract of *Tribulus terrestris* L. (250 mg/kg). Groups 3 and 4 were orally treated with glucose (5 g/kg) after 1 h of drug and extract administration, respectively. Fasting blood glucose (FBG) was determined prior to (0 h) and at 30 min, 1, 2 and 3 h after dosing for acute toxicity study.

Results: On comparing within groups, a single dose of the methanol extract of *Tribulus terrestris* L. lowered FBG to levels comparable to that of glibenclamide (36 vs. 55 %), and reaching the initial level (0 h) at 2 h. FBG were significantly ($p < 0.05$) lowered at 2 and 3 h in both glibenclamide (45.5 and 56.9 %) and extract (45.7 and 52.7 %) groups as compared with their respective glucose levels at 0.5 h. On the other hand, on comparing between groups, both glibenclamide and methanol extract significantly ($p < 0.05$, $p < 0.001$) lowered the rise in blood glucose at 1 h (33.9 and 22.5 %), 2 h (62.8 and 59.16 %), and 3 h (64.6 and 57.1 %) with respect to the hyperglycaemic control group.

Conclusion: The methanol extract of the aerial parts of *Tribulus terrestris* L. possesses potential anti-hyperglycaemic activity in glucose-loaded normal rabbits. Further studies on various organic solvents fractions and isolated compounds from this plant are required.

Keywords: *Tribulus terrestris* L., Zygophyllaceae, Anti-hyperglycaemic activity, Fasting blood glucose, Acute toxicity

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INTRODUCTION

Tribulus terrestris L. is an annual herbaceous plant from Zygophyllaceae family that grows worldwide, in dry climate regions especially in the Mediterranean region and the subtropical area of Asia, Africa and America [1]. *T. terrestris* also known as puncture vine or small caltrops and is

used in traditional medicine of India (Ayurveda), China, Bulgaria, Turkey and other countries as strong aphrodisiac, antihypertensive [2], and diuretic [3]. *T. terrestris* is often used in the treatment of infertility, decreased libido, and erectile dysfunction [4]. It is also used among athletes to increase muscle resistance and improve performance in sports, although

scientific evidence to support this effect is scarce [5].

Several pharmacological studies have revealed its cardioprotective [6], hepatoprotective [7]; antitumor [8], and anti-proliferative activity on mouse carcinoma [9-16] and breast cancer [10]. *T. terrestris* extracts also induce cell growth arrest and apoptosis by down-regulating NF- κ B signaling in liver cancer cells, and exhibits weak cytotoxic effects to normal cells compared to cancer cells [11]. Recently, terrestrosin D a major steroidal saponin of *T. terrestris* has been reported to have an antiangiogenic property, which may suggest that its anticancer effect is also due to inhibition of tumor angiogenesis [12]. It also possess anti-atherosclerotic [13], anti-arthritis [14], anti-oxidant [15], antimicrobial [16], analgesic and anti-inflammatory properties [17].

Recently, the ethanolic extract of *T. terrestris* fruits and rhizomes was reported to be effective in treating streptozotocin-induced hyperglycemia *in vivo* and inhibiting α -glucosidase and aldose reductase *in vitro*. [18]. Therefore, the present study was aimed to investigate the potential anti-hyperglycaemic activity of methanol extract of the aerial parts of *Tribulus terrestris* L. as well as its acute toxicity.

EXPERIMENTAL

Plant material

The aerial part (leaves, stems and flowers) of *T. terrestris* was collected during the flowering stage from Al-Mahweet area, Yemen (November - December 2012) and identified by Dr. Z. Masdos, Department of Botany, Faculty of Science, Sana'a University, Yemen. A voucher specimen was deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University (voucher no. 120).

Extraction and fractionation

The air dried and powdered aerial part of *T. terrestris* (1500 g) was extracted by maceration with 85 % methanol (5 x 2L) at room temperature. The combined obtained methanol extract was dried and concentrated, using rotary evaporator, to give a dark green crude residue (160 g, MEA), from which 137 g was suspended in water and partitioned successively several times with petroleum ether, chloroform, ethyl acetate and n-butanol to provide the corresponding extracts. Each fraction was dried over anhydrous sodium sulphate and evaporated to dryness to yield fractions PET (petroleum

ether, 16.5 g) CET (chloroform, 12.5 g), fraction EET (ethyl acetate, 5.6 g), fraction BET (n-butanol, 38.3 g).

Preliminary phytochemical screening

A preliminary phytochemical analysis of the *Tribulus terrestris* L. obtained extracts was carried out using thin layer chromatography (TLC) plates coated with silica gel 60 F254 for TLC. The mobile phase ethyl acetate: methanol: water (30:5:4) was used. Micro-drops of the concentrated solutions of the fractions (methanol, petroleum ether, chloroform, ethyl acetate, and n-butanol) were spotted on pre-coated Silica gel plates. The chromatogram after complete development was air dried and visualized with different chemical reagents to detect the presence of flavonoids, phenols, tannins, alkaloids, amino acids, saponins, steroids, triterpenes glycosides, and anthraquinones.

Experimental animals

Rabbits with an average weight of 1000 g were used for the anti-hyperglycaemic activity study and Swiss Wistar albino mice with an average weight of 25 g were used for the acute toxicity study. All animals were fed with standard animal feed and water. Animals were acclimatized to the laboratory conditions for 3 weeks prior to experimentation. All experiments carried out were approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana'a University (no. 810-15/03/2013) and were conducted according to the standard guideline for the use of laboratory animals [19].

Acute toxicity study

Ten mice were used and randomly assigned to control or treatment group (5 animals per group). Animals were deprived of food but given water 16 h prior to dosing. Methanol extract at a dose of 2 g/kg were then given orally to test group, while the control group received water at the same volume. Body weight, signs of toxicity (general behaviour, motor activities, aggressiveness, reaction to noise, reaction to pinch, state of tail and state of excrement) and mortality were observed after administration at the third hour on the first day, and throughout the following 48 h and then daily thereafter for 14 days.

Determination of anti-hyperglycaemic activity

For determination of the anti-hyperglycaemic activity of *Tribulus terrestris*, 20 rabbits (900 -

1000 g) were used and randomly assigned to 4 groups (5 animals per group). All rabbits were treated orally with a single dose. Group 1 served as normal control group and received distilled water; group 2 served as hyperglycaemic control; group 3 served as standard treated with glibenclamide as an aqueous suspension (5 mg/kg); groups 4 received methanol extracts of *Tribulus terrestris* L. at a dose of (250 mg/kg). After 1 h of drug and extract administration the rabbits of groups 2-4 were orally treated with glucose (5 g/kg). Blood samples were collected, from the ear vein just prior to (0 h) and at 30 min, 1, 2, 3 h after dosing for acute study, into plain dry tubes and centrifuged at 3,000 rpm for 10 min and glucose was estimated.

Biochemical analysis

The serum samples obtained were transferred into Eppendorf tubes and analyzed by Cobas C 311 analyzer (Roche Diagnostics - GmbH, D-68298 Mannheim, Germany) for serum glucose using a commercially available kit (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Statistical analysis was performed using Social Package of Social Sciences (SPSS) version 11.5 (SPSS Inc, Chicago, IL, USA). The results are presented as mean \pm standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) using independent t-test to compare between groups, and paired t-test to compare within a group. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical examination of the different fractions (Table 1) revealed the presence of flavonoids, anthraquinone, and phenolic compounds in total methanol, petroleum ether, and chloroform extracts of *T. terrestris*.

Alkaloids were not detected in all fractions; whereas persisting foam was formed in all of the extracts suggesting the presence of saponins being more prominent in the methanol extract.

There were no toxic symptoms or mortality observed in any animals, which lived up to 14 days, following the administration of the methanol extract of the aerial parts of *T. terrestris* at a single dose level of 2 g/kg body weight. Moreover, the observed behavioral changes and toxicological signs (general behaviour, motor activities, aggressiveness, reaction to noise, reaction to pinch, state of tail and state of excrement) showed no obvious differences between the treated and control animals (Table 2). The behavioral patterns of animals as well as breathing were also normal with no disturbance in food intake, water consumption or sleep.

Table 3 shows the anti-hyperglycaemic activity of a single dose of the methanol extract of *T. terrestris* when administered 1 h prior to glucose loading. On comparing within groups, fasting blood glucose (FBG) in animals exposed to either glibenclamide or extract were lowered to their corresponding initial levels (at zero time) at 2 h. When compared with their corresponding peak glucose levels at 30 min, both glibenclamide and extract significantly ($p < 0.05$) lowered FBG by 46 % at 2 h and by 53 - 57 % at 3 h. However, on comparing FBG at 3 h and that of zero time, only glibenclamide significantly ($p = 0.002$) lowered blood glucose by 31.7 %; with a non-significant decrease in the extract group by 14.6 %. On the other hand, on comparing between groups, both glibenclamide and methanol extract of *T. terrestris* significantly ($p < 0.05$; $p < 0.001$) lowered the rise in blood glucose at 1 h (33.9 % and 22.5 %), 2 h (62.8 % and 59.16 %), and 3 h (64.6 % and 57.1 %) after glucose administration with respect to the hyperglycaemic group (group 2).

Table 1: Phytochemical profile of aerial parts of *Tribulus terrestris* L

Chemical constituent	Chemical test	Extract				
		MET	PET	CET	EET	BET
Flavonoids	AlCl ₃	+	+	+	-	+
Anthraquinones	KOH	+	+	+	-	-
Phenols + tannins	FeCl ₃	+	+	+	+	-
Saponins	Froth test	+	+	+	+	+
Alkaloids	Dragendorff's Reagent	-	-	-	-	-
Steroids/triterpenes	Liebermann – Burchard test	+	+	+	-	-
Amino acids/protein	Xanthoproteic acid test					

Key: AlCl₃, aluminum chloride; KOH, potassium hydroxide; FeCl₃, ferric chloride; MET, methanol; PET, petroleum ether; CET, Chloroform; EET, ethyl acetate; BET, n-butanol

Table 2: Behavioral observations and toxicological signs of both fresh and dried extracts for control and treated groups

Observation	Control group	Fresh extract (2 g/kg)	Dried extract (2 g/kg)
Motor activity	N	N	N
Aggressiveness	N	N	N
Reaction to noise	N	N	N
Reaction to pinch	N	N	N
State of tail	N	N	N
Excrement	N	N	N
Salivation	N	N	N
Convulsion	N	N	N
Mortality (within 48 h)	NM	NM	NM

Key: N = normal; NM = no mortality

Table 3: Effect of a single dose of *Tribulus terrestris* L. methanolic extract on fasting blood glucose of normal rabbits

Group	Fasting blood glucose (mg/ dl)				
	0	0.5 h	1 h	2 h	3 h
Control	94.6 ± 15.56	96.4 ± 15.17	99.2 ± 18.89	107.4 ± 10.21	101.6 ± 5.89
Glucose	90.2 ± 10.92	168 ± 12.92	256.4 ± 50.19	219.6 ± 25.86	182.4 ± 25.54
Glibenclamide	94.6 ± 3.04	149.8 ± 19.97	169.4 ± 34.83 [‡]	81.8 ± 8.95 [‡]	64.6 ± 11.46 [‡]
MET	91.6 ± 8.84	165.4 ± 41.67	198.8 ± 54.37 [‡]	89.8 ± 12.93 [‡]	78.2 ± 10.57 [‡]

Key: * within groups; ‡ between groups; p < 0.5; MET, methanol extract

DISCUSSION

Diabetes mellitus is a chronic metabolic disease with life threatening complications, characterized by hyperglycaemia and alterations in carbohydrate, lipid and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. The treatment of hyperglycaemia in diabetic patients is directed towards achieving euglycemia and minimizes chronic complications by administering oral hypoglycaemic agents. Over 400 plants as well as 700 recipes and compounds have been scientifically evaluated for Type 2 DM treatment [20]. In light of this we made an attempt to study the anti-hyperglycaemic effect of *T. terrestris* aerial part in glucose loaded normal rabbits.

In this study, a single dose of the methanol extract (250 mg/kg) of *T. terrestris* significantly lowered FBG to their corresponding initial levels (at zero time) at 2 h. The methanol extract significantly lowered the FBG by 22.5 – 59.1 % with respect to the hyperglycaemic animals; which is comparable to that of glibenclamide treated group (33.9 – 64.6 %). Recently, treatment with the *T. terrestris* extract (50 mg/kg) was reported to have a significant effect on both fasting and postprandial hyperglycemia in the

diabetic rats, with a significant improvement in the insulin level and no improvement in the expressions of glucose transporter isoforms GLUT 2 and GLUT4 in liver and muscle, respectively [21]. Moreover, an ethanol extract of *Tribulus terrestris* L. flowers and rhizomes exhibited 70 % inhibition of α -glucosidase at 500 μ g/ml using maltose as the substrate and 100 % inhibition of aldose reductase at a dose of 30 μ g/ml using dl-glyceraldehyde as the substrate [18]. Inhibition of α -glucosidase and aldose reductase is useful in treatment of both postprandial hyperglycemia and hyperinsulinaemia, and thereby in improving insulin sensitivity.

This observed potential anti-hyperglycaemic activity might be due to the large presence of saponins in *T. terrestris* [22]. Saponin from *T. terrestris* reported to possess hypoglycemic properties and produced protective effect in streptozotocin-induced diabetic rats by inhibiting oxidative stress [23]. Some of the main active compounds of *T. terrestris* identified and purified include: steroidal furostanol and spirostanol saponins - protodioscin, terrestrinins A and B, gitogenin, hecogenin, diosgenin and others [22]. The saponin characterized as tigogenin-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-

(1→3)-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-xylopyranoside showed potent anti-hyperglycemic activity in STZ-induced diabetic rats [24]. Moreover, harmaline and norharmaline (β-carboline alkaloids) obtained from *T. terrestris* have been proposed to account for the hypoglycemic property of the extract by stimulating insulin secretion in a glucose-dependent manner [24].

In addition, the presence of flavonoids in *T. terrestris* [1] might contribute to the observed anti-hyperglycaemic activity. Previous investigations have demonstrated a promising role for flavonoids as anti-diabetic agents [25]. Flavonoids such as kampferol and quercetin have been reported to affect the pancreatic β-cells leading to their proliferation and secretion of more insulin [26]. The long-term consumption of quercetin appears to control blood glucose levels in streptozotocin (STZ)-induced diabetic animals [27]; and has also been suggested that quercetin protects the pancreas against oxidative stress in STZ-treated animals, improving hyperglycemia [28]. A recent study revealed that the blood glucose lowering activity of flavonoid compounds: quercetin, and rutin may be by stimulating β-cells to release more insulin [29]; and suggested that the pronounced activity of these compounds could be due to enhanced peripheral glucose utilization by skeletal muscle in addition to that of β-cell stimulation. In addition, quercetin has been reported to inhibit α-glucosidase activity *in vitro* [30]; and its use on glucose absorption in obesity, and obesity with type 2 diabetes patients on oral glucose tolerance test has been investigated [31].

CONCLUSION

The present investigation revealed that the methanol extract of the aerial parts of *Tribulus terrestris* L. possesses a potential anti-hyperglycaemia activity in glucose loaded normal rabbits. This has important therapeutic implications in the treatment of diabetes mellitus. Further studies are required for structural elucidation of the active component(s) involved in the anti-hyperglycaemic activity of *Tribulus terrestris* L.

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