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Original Research Article

Anti-diabetic potential of aerial parts of Galium tricornutum (Dandy) Rubiaceae

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Abstract

Purpose: *To evaluate the anti-diabetic potential of methanol extract of the aerial parts of Galium tricornutum (Dandy) in diabetic rats.*
Methods: *The methanol extract of the aerial parts of Galium tricornutum was first subjected to acute toxicity studies. Thereafter, the effect of the extract on oral glucose tolerance was determined. In addition, the effect of the extract on fasting blood glucose, as well as serum lipid profile, urea, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, alkaline phosphatase (ALP) and protein were investigated in alloxan-induced diabetic rats.*
Results: *No acute toxicity were observed in the rats after administration of the plant extract up to a dose of 2000 mg/kg. The effect of the extract on glucose tolerance test was significant from 30 to 180 min after treatment. In the diabetic rats, the extract showed significant ($p < 0.05$) anti-hyperglycemic activity at 400 mg/kg. It also led to significant increases in body weight and HDL-cholesterol, and significant reductions in serum LDL, triglycerides and transaminases ($p < 0.05$).*
Conclusion: *These results indicate that the aerial parts of G. tricornutum possess significant anti-diabetic potential.*

Keywords: *Diabetes, Galium tricornutum, Glibenclamide, Glucose tolerance tes, Lipid profile*

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INTRODUCTION

Diabetes mellitus is one of the most common metabolic disorders. It is caused by inability of pancreatic cells to produce insulin, or defects in insulin utilization. The disease is associated with significant morbidity and mortality, and is considered one of the five leading causes of death in the world [1]. There are no drugs that can effectively treat diabetes mellitus. Moreover, the use hypoglycemic drugs and insulin results in unwanted side effects. Thus there is increasing interest in the management of diabetes through the use of natural products with hypoglycemic potential.

Many medicinal plants have been reported to possess hypoglycemic properties. These include *Ocimum santum* (Tulsi), *Momordica charantia* (bitter round), *Trigonella foenum* (Fenugreek), *Allium sativum* (garlic), *Vinca rosea* (nayantara), and *Azadirachta indica* (neem). However, in severe diabetes, many of these plants are not very effective in lowering blood glucose levels.

Galium tricornutum is an annual herb that belongs to the family Rubiaceae. It has trailing or climbing stems and is found almost all over the world. The plant is used as an anti-scorbutic agent, and as a refrigerant, diuretic and aperient

[2]. It is also used as anti-malarial, antipyretic and emetic [3]. In the northern parts of Pakistan, the plant is used for the treatment of skin infections [4]. However, despite its medicinal importance, no studies have been carried out on the anti-diabetic potential of *Galium tricornutum* to date.

The present study was carried out to investigate the anti-diabetic effects of methanol extract of aerial parts of *Galium tricornutum* in alloxan-induced diabetic rats. Antidiabetic potential and biochemical parameters such as LDL, HDL, cholesterol, urea, creatinine, ALP, bilirubin, triglycerides, protein, ALT and SPT were studied in male Wistar albino rats exposed to two doses of the extract for 21 days.

EXPERIMENTAL

Plant collection and extraction

Galium tricornutum was collected from the outskirts of Bannu district, Khyber Pakhtunkhwa, Pakistan. The plant was identified and authenticated by Professor Abdur Rahman, Department of Botany, Government Postgraduate College, Bannu.

The aerial parts of the plant were shade-dried, and made into coarse powder by pulverization. The coarse powder (200 g) put extracted by shaking with 80 % commercial grade methanol in a beaker for 3 days, and the extract was filtered through qualitative Whatman no. 1 filter paper.

Animals

Wistar albino rats of both sexes (8 - 10 weeks old) weighing 170 – 200 g were obtained from the Animal House of Quaid-i-Azam University, Islamabad. The rats were fed with standard rat feed before and during the experiment, and were randomly assigned to various groups. Prior to commencement of the study, the rats were acclimatized to laboratory conditions for 7 days at room temperature, 12 hour-dark/light cycle and relative humidity.

Acute oral toxicity studies

The experiment was conducted according to the guide lines for experimental animal model [5] approved by the ethical committee of University of Science and Technology Bannu. Two groups of rats (3 rats/group) were treated orally with the *Galium tricornutum* extract at a dose of 5000 mg/kg. A third group received an equivalent volume of normal saline. The dose of 5000 mg/kg did not result in mortality or any signs of toxicity or changes in general behavior.

Oral glucose tolerance test

Four groups of rats (6 rats/group) were used. The rats were fasted for 16 hr before the test. Group I served as normal control and received normal saline *p.o.* only. Group II received glucose (3 g/kg) only. Groups III and IV received methanol extract at 200 mg/kg and 400 mg/kg, respectively. Thirty minutes after extract administration, rats in groups III and IV were given glucose (3 mg/kg, *p.o.*). Blood samples were collected from the retro-orbital plexus just prior to extract administration, and 30, 90 and 150 min after glucose load, for measurement of serum glucose levels using ACCU-CHEK® Active meter (Indianapolis, USA).

Effect of extract on alloxan-induced diabetes in rats

The rats were divided into five groups, each with six rats, and were treated as summarized below:

Group I: Normal control (saline).

Group II: Alloxan-treated control (150 mg/kg.ip).

Group III: Alloxan (150 mg/kg. *i.p.*) + *Galium tricornutum* extract (200 mg/kg, *p.o.*).

Group IV: Alloxan (150 mg/kg. *i.p.*) + *Galium tricornutum* extract (400mg/kg, *p.o.*).

Group V: Alloxan (150 mg/kg.ip) + Standard drug, glibenclamide (5mg/kg, *p.o.*).

All treatments lasted for 21 consecutive days. The extract, standard drug glibenclamide (5 mg/kg) and saline were administered via a feeding cannula.

Induction of diabetes in experimental animals

Diabetes was inducted in the experimental animals by a single intraperitoneal (*i.p.*) dose of alloxan monohydrate (150 mg/kg). Alloxan was dissolved in normal saline prior to administration. Since alloxan causes fatal hypoglycemia because of massive release of insulin, the rats were given 20 % glucose solution *i.p.* after 6 hours. In addition, 5 % glucose solution bottles were kept handy for 24 hr to prevent hypoglycemia [5]. After 48 hours of alloxan injection, rats with blood glucose levels less than 200 mg/dL were included in the study. Treatment with extract and glibenclamide was started 48 h after alloxan injection.

Collection of blood samples and blood glucose determination

Blood samples were taken from tail tip of the rats. Estimations of fasting blood glucose and body weight measurement were done onset, and on the 1st, 7th, 14th and 21st days of the study. Blood glucose levels were checked by one-touch electronic glucometer using glucose test strips. On day 21, fasting blood glucose was estimated in overnight-fasted rats, with blood taken from the retro-orbital plexus under mild ether anesthesia [6]. Serum was separated and analyzed for LDL [7] and HDL [8] cholesterol, as well as triglycerides [9], urea [10], creatinine [11], ALP [12], bilirubin [13], protein [14] and ALT/AST [15].

Statistical analysis

Data were expressed as mean ± SEM. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for inter-group comparisons using the least significant difference (LSD) test. *P* values less than 0.05 were deemed statistically significant.

RESULTS

Acute oral toxicity

The OECD guidelines AOT-425 was used for determination of acute toxicity of the extract in rats. A single oral dose of *Galium tricornutum* (5000 mg/kg) did not cause any signs of toxicity or mortality in the rats within 4 hrs. The animals were observed continuously and were found to be safe at a dose of up to 5000 mg/kg.

Oral glucose tolerance test

The effects of methanol extracts of *Galium tricornutum* (200 and 400 mg/kg) on oral glucose tolerance test are shown in Table 1. The extract improved glucose tolerance in fasted normal rats, with significant decreases in serum glucose levels at 90 minutes and at 150 minutes post-glucose load.

Effect of *G. tricornutum* on fasting blood glucose levels of alloxan-diabetic rats

The anti-diabetic effect of *G***alium tricornutum** on the fasting blood glucose levels of diabetic rats is shown in Table 2. Administration of alloxan (150 mg/kg, *i.p*.) led to increases in fasting blood glucose levels, which were maintained over a period of three weeks. However, daily administration of *G. tricornutum* extract for 3 weeks led to dose-dependent decreases in blood glucose levels. The decreases in blood glucose peaked on day 21 of treatment. Rats in normal control showed slight increases in body weight but the body weights of the diabetic rats were significantly decreased during the 21–day study period.

Effect of *G. tricornutum* extract on body weight and on some serum, biochemical indices in alloxan-diabetic rats

Alloxan caused decreases in body weight, which were reversed by the extract (400 mg/kg and 200 mg/kg) after 21 days of treatment (Table 3). It also bought about significant increases in serum cholesterol, LDL, creatinine, urea and ALP; and significant decreases in HDL levels ($p < 0.05$; Table 4).

Table 1: Changes in serum glucose in the glucose tolerance test (mg/dL)

Group	0 min	30 min	90 min	150 min
Normal	69.5±2.2	68.5±5.4	71±5.1	69.3±4.1
Glucose (3g/kg)	69.3±5.4	110.1±4.7	121.3±4.3	129.6±4.5
Glucose (3mg/kg) + 200mg/kg *G. tricornutum*	70.6±4.6	100±4.9	93.8±3.7	89.1±5.1
Glucose (3g/kg) + 400mg/kg *G. tricornutum*	70.3±5.4	95.3±4.7	89.3±2.9	84.6±2.7

Table 2: Effect of extract on fasting blood glucose levels of alloxan-diabetic rats (mg/dL)

Group	Initial	1st day	7th day	14th day	21st day
Normal	70.66±2.6	70.16±2.8	70±2.4	70±3.5	72.83±4.1
Diabetic control	222.6±3.1	222.5±7.1	240±2.4	246.16±3.1	260.16±3.7
Diabetic + 200m/k *G. tricornutum*	261.3±2.8	248.6±6.1	206.16±1.8	148.5±4.0	130.5±3.1
Diabetic + 400m/kg *G. tricornutum*	257.6±3.0	246.5±5.6	190.3±3.8	144.83±2.5	123.16±3.2
Diabetic + glibenclamide	249.8±7.1	189.16±3.8	140.3±4.0	88.5±3.1	69.66±2.6

Table 3: Effect of extract on body weight of rats (grams)

Group	Day 1	Day 7	Day 14	Day 21
Normal	170±2.5	174.6±5.0	176±2.7	178.6±3.8
Diabetic control	181.8±3.8	177.6±4.7	169.8±3.1	161±4.3
Diabetic+200mg/kg extract	170±2.4	165.3±5.2	160.3±4.7	157±5.1
Diabetic+400mg/kg extract	170±3.1	168±6.0	163±3.2	160±3.1
Diabetic + glibenclamide	175.8±5.0	174.5±2.4	175.8±2.9	173.3±2.8

Table 4: Effect of extract on serum biochemical profile of alloxan-diabetic rats after 21 days

Group	LDL (mg/dl)	HDL (mg/dl)	Cholesterol (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	ALP (mg/dl)	Bilirubin (mg/dl)	TG (mg/dl)	Protein (g/dl)	ALT (U/l)
Normal	93±4.6	37±1.8	155±5.1	25±1.0	0.71±0.01	128±4.1	0.73±0.1	95±5.4	6.7±0.4	49±1.4
Diabetic control	171.8±1.2	28.8±1.9	261.3±4.5	60.3±3.2	2.18±1.0	265.8±5.1	2.11±0.5	171.5±4.7	4.24±0.3	90.6±1.5
Diabetic + 200mg/kg extract	129±3.2	32±3.1	209.8±6.1	39.8±2.1	0.97±0.4	146.5±2.6	0.62±0.2	159.6±4.1	4.86±0.5	78.3±2.1
Diabetic + 400mg/kg extract	99.8±5.0	33.6±2.0	170±3.1	33.6±2.3	0.60±0.3	129.6±4.5	0.62±0.1	144.8±3.8	5.23±0.1	67.8±3.4
Diabetic + glibenclamide	91.8±3.7	35.5±1.7	147±2.4	30±1.2	0.57±0.2	121.8±2.5	0.59±0.2	125±3.9	6.34±0.5	58.6±2.7

Similarly, serum levels of other biochemical parameters (bilirubin, triglycerides, ALT and AST) were significantly increased, while total protein was significantly decreased by alloxan ($p < 0.05$). These alloxan-induced changes were reversed by glibenclamide and *G. tricornutum* extract.

DISCUSSION

The management of diabetes mellitus is faced with the major challenge of finding antidiabetic agents without side effects. This has led to increased interest in natural products with anti-hyperglycemic potential, which have the advantage of producing much less side effects when compared with orthodox hypoglycemic drugs. Alloxan exerts its effect by destroying insulin-producing beta cells of the pancreas [16]. *In vitro* studies have revealed that alloxan is selectively toxic to beta cells of pancreas and can induce cell necrosis [17]. The cytotoxic effect of alloxan is mediated by ROS, which increase cytosolic calcium concentrations, leading to destruction of beta cells of pancreas [18]. Many plant extracts exert anti-diabetic effects by promoting beta cells regeneration or by protecting these cells from destruction, through enhancement of unrestricted endogenous insulin action. Plant extracts may also induce release of insulin by beta cells, or activate insulin receptors [19].

Results from the present study showed that oral administration of *G. tricornutum* extract (400 mg/kg) for 21 days produced significant improvements in alloxan-induced diabetes and glucose tolerance test in rats. These effects are evident from results obtained in assays of LDL, HDL, cholesterol, urea, creatinine, ALP, bilirubin, triglyceride, protein, ALT and AST. Interestingly, the anti-diabetic effect of the extract was comparable to that of glibenclamide, a standard hypoglycemic agent. In addition, the anti-diabetic effect of the extract was higher at a dose of 400 mg/kg than at the lower dose of 200 mg/kg. Thus the observed anti-diabetic potential was dose-dependent. These results indicate that *G. tricornutum* has very good anti-diabetic properties. It has been established that the antioxidant properties of plant extracts are due to the presence of flavonoids and tannins [20]. Preliminary phytochemical screening of the *G. tricornutum* extract also revealed the presence of flavonoids and tannins. Thus these components are likely to be responsible for the observed anti-diabetic properties of the extract.

CONCLUSION

The results obtained in this study indicate that methanol extract of *G. tricornutum* possesses significant anti-diabetic effects. This finding is considered useful for the development of new and safer anti-diabetic drugs from natural sources.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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