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RESEARCH ARTICLE

Antidiabetic Activity of Methanolic Extract of *Polygonum glabrum Wild* Leaves in Diabetic Rats

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ABSTRACT

To evaluate the anti-diabetic activity of the Plant leaves of *Polygonum glabrum* in alloxan induced diabetic rats. Method: Diabetes was induced in Wistar rats using freshly prepared solution of Alloxan monohydrate (100 mg/kg b. wt.) by Intraperitoneal route of drug administration. Methanolic extract of *Polygonum glabrum* (200, 400 mg/kg bwt/p.o) was prepared freshly, administered to alloxan induced diabetic rats for 28 days. The standard drug glibenclamide (10 mg/kg of b. wt) orally. Blood glucose levels was estimated on 0, 7th, 14th, 21st and 28th days, serum glucose level, lipid profile, and histopathological changes in pancreas were examined after 28 days. OGTT was performed by administration of 200 and 400 mg/kg b.w/p.o of methanolic extract of *Polygonum glabrum* and 10 mg/kg b.w /p.o of Glibenclamide to different groups respectively in normal rats. Results: significant($p<0.001$) results were observed in the estimated parameters like reduction in blood glucose , Improved in regeneration of beta cells of langerhans of pancreas in rats by histopathological studies. Conclusion: The results were suggested that the whole plant extract of *Polygonum glabrum* having potent antidiabetic activity on alloxan induced diabetic rats.

KEYWORDS

Anti-diabetic, Polygonum glabrum, alloxan induced diabetic rats, OGTT, Glibenclamide

INTRODUCTION

Diabetes mellitus is a most common endocrine disorder, affecting more than 300 million people worldwide. Diabetes mellitus is a debilitating and life threatening disease to mankind. It is a series of metabolic conditions associated with hyperglycemia and caused by defects in insulin secretion and/or insulin action[1-4]. It is characterized by derangements in carbohydrate, protein and fat metabolism[5].

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Diabetes is a condition primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. Diabetes mellitus is a chronic disease whose global spread has given it the characteristics of a pandemic. The most frequent form is Type 2 diabetes which represents more than 85% of the cases. Other forms are Type 1

(10%), specific diabetes and gestational diabetes (5%)[6].

Therapies developed with the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. Although, oral hypoglycaemic agents and insulin is the mainstay of treatment of diabetes and are effective in controlling hyperglycaemia, they have prominent side effects and fail to significantly alter the course of diabetic complications[7].

Polygonum glabrum Linn. (Family: Capparaceae) is a weed distributed throughout the tropics of the world and the plains of India. The plant is an annual, sticky herb with a strong penetrating odour, yellow flowers and long slender pods containing seeds. It is known as Hurhur (Hindi), Hurhuria (Bengali), and Nayikkadugu (Tamil) in Indian traditional medicine[8].

MATERIAL AND METHODS

Plant Material

The plant *Polygonum glabrum* was collected during the march 2014 from Sri Venkateshwara University Tirupati, India. The plant was authenticated by Dr. Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library.

Preparation of Plant Extract

Fresh plants collected, were washed to remove adhered dirt, rinsed with distilled water, blotted and dried in shade. The shade-dried specimens were powdered in a mixer. This powder was subjected to Soxhlet extraction using methanol as solvent. This cycle was repeated many times, over hours or a few days. The extracts were concentrated under reduced pressure and preserved in refrigerator until further use. At the end of the hot extraction process each extract was filtered. The extracts were then kept in desiccators to remove remaining moisture, if present, and finally stored in air tight containers at 4ºC for further use[9].

Phytochemical Screening

Phytochemical screening of crude extract was carried out employing standard procedures to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, saponins, Tannins, glycosides, carbohydrates and others[10].

Animals

Healthy adult male wistar rats (180-200g) were used for this experiment. They were obtained from Smt. Sarojini Ramulamma College of pharmacy, mahabubnagar, India and maintained under standard environment laboratory conditions and fed with Laboratory diet and water ad libitum.

Determination of Acute Oral Toxicity Studies

The LD (50) of the extract was determined by using wistar rats. Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose (2000 mg/kg/b.w) of *Polygonum glabrum* extract. After the administration of extract food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 mins after dosing, periodically during the first 24 hours and daily therefore for a period of 14 days[11].

Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18hr) normal rats. Healthy rats were randomly selected and distributed into five groups (n=6). One of those groups was administered distilled water and the rest 4 groups were given following treatment. Glucose (2g/kg bw.), glibenclamide (10mg/kg bw.) and Methanolic extract of polygonum glabrum (200 and 400 mg/kg bw. respectively) 1 hr after the administration of extract and glibenclamide, glucose is administered orally. Blood was withdrawn from the tail vein at 0, 60, 90, 120 and 150 min of glucose administration and glucose levels were estimated using Gluco check blood glucose monitoring kit[12].

Induction of Diabetes

Diabetes was induced in each group using freshly prepared solution of Alloxan monohydrate dissolved in normal saline (0.91% w/v of NaCl) except the group "A" which served as normal control. For inducing diabetes the rats were kept on fasting for 18 hours and diabetes was induced by giving a single IP injection of Alloxan monohydrate (100 mg/kg b. wt.) following standard methodology (Intraperitoneal route of drug administration)]. To prevent fatal hypoglycemia due to massive pancreatic insulin release, the rats were provided with 20% glucose solution after six hours supplied in water bottles in their cages for next 24 hours[13-15].

Treatment

Group 'A' was fed with simple drinking water which served as normal control; group 'B' in which diabetes was induced was also fed with simple drinking water, serving as diabetic control; Group 'C' in which diabetes was induced was given 200 mg/kg b. wt. of extract orally; Group 'D' in which diabetes administered 400 mg/kg b. wt. of extract orally and; group 'E' in which diabetes was induced was given the standard drug glibenclamide (10 mg/kg of b. wt) orally. All the groups were given respective treatments daily for 28 days.

Blood was collected on the 0th day, means the day on which the dosing was started, 7th day, 14th, 21st and 28th day, through the retro orbital sinus of the rats[13-15].

Measurement of Serum Lipid Profile

The serum from the blood was separated as under:

Sample was collected (preferably in ependorff tubes)

The serum was centrifuged at 1000 rpm for 5 min. The serum was pipette out using a micropipette. The serum was labeled with the animal number and the estimations were made. The serum glucose level; the enzymes SGOT, SGPT and ALP level and the lipid profile (total cholesterol HDL, LDL, VLDL and triglyceride level) was determined enzymatically on prietest bio chemistry analyser[16,17].

Histopathological Studies

At the end of the study i.e. on 28th day the rats were sacrificed and the tissues (pancreas) were collected. The whole histopathological process was carried out in accordance with the SOPs (Standard Operating Procedures) (Tissue fixation, Processing and Embedding)[18,19].

Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean± standard Error of Mean (S.E.M) and analyzed for ANOVA and post hoc Dunnett's *t*-test.

RESULTS

Phytochemical Screening

The *Polygonum glabrum* methanolic extract was conformed to contain carbohydrates, flavonoids, alkaloids, terpenoids, tannins, saponins, and glycosides.

Toxic Study

In toxic study the methanolic extract of *Polygonum glabrum* has shown no signs and symptoms, morbidity and mortality on wistar rats.

Body Weight

The diabetic group showed decrease in the body weight but the extract treated groups and the standard treated group showed increase in the body weight and the increase in body weight was more for standard treated group followed by and Methanolic extract treated groups.

Hypoglycemic Activity in Normal Rats (OGTT) and Alloxan Induced Diabetic Rats.

The blood glucose levels of the normal rats reached a peak at 30 min after the oral administration of glucose and gradually decreased to the pre-glucose load level. Of the two different doses, viz.200, and 400 mg/kg,

Table 1: Effect of Methanolic extract of *Polygonum glabrum* on body weight of Alloxan monohydrate induced diabetic rats

Groups	**0th day**	**7th day**	**14th day**	**21st day**	**28th day**
Normal	205±1.99	207±2.35	210±1.77	212±2.43	216±2.12
Diabetic control	200±2.75	194±2.32*	189±2.48*	184±2.12	180±2.51
Glibenclamide 10mg/kg	203±1.98	211±2.48**	216±2.62*	220±1.86	226±1.92
Methanolic Extract, 200mg/kg	200±2.24	202±2.57	204±2.21	206±2.22	211±1.59
Methanolic Extract, 400mg/kg	202±3.00	211±2.41	216±2.70*	219±2.54	224±2.79

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01

Table 2: Effect of Methanolic extract of *Polygonum glabrum* on OGTT of rats

Groups	**Blood glucose level mg/dl**			
	0 min	**30 min**	**60 min**	**120 min**
Normal	80.12 ±0.89	82.11 ± 1.32	85.11 ± 0.65	88.29 ± 0.99
Control (Glucose 2gm/kg)	91.32 ± 0.95	161.31 ± 1.03*	182.87 ± 0.56**	215.87 ± 1.06*
Standard (GLB 10mg/kg)	73.49 ± 1.03	132.32 ± 1.28***	105.18 ± 0.98***	91.48 ± 1.23***
Methanolic (200mg/kg)	99.03 ± 1.39	167.98 ± 1.96	156.90 ± 0.23***	118.32 ± 0.86***
Methanolic (400 mg/kg)	84.97 ± 1.45	150.67 ± 0.84	126.88 ± 0.69***	101.45 ± 0.99***

Table 3: Effect of Methanolic extract of *Polygonum glabrum* on Blood Glucose levels of Alloxan monohydrate induced diabetic rats

Groups	0th day	7th day	14th day	21st day	28th day
Normal	95.69 ± 1.51	85.95 ± 1.37	93.83 ± 0.94	94.51 ± 0.99	95.69 ± 1.51
Diabetic control	264.84 ± 1.26	277.01 ± 1.19	269.86 ± 1.01	260.18 ± 1.12	264.84 ± 1.26
Glibenclamide 10mg/kg	275.45 ± 1.52	203.01 ± 1.39***	159.72 ± 1.05***	118.39 ± 1.04***	112.45 ± 1.52***
Methanol Extract, 200mg/kg	273.02 ± 1.43	217.67 ± 1.60***	197.94 ± 1.22***	134.64 ± 0.89***	130.02 ± 1.43***
Methanol Extract, 400mg/kg	283.03 ± 1.21	213.49 ± 1.34***	178.02 ± 1.18***	126.15 ± 1.03***	122.03 ± 1.21***

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01

Table 4: Effect of Methanolic extracts of *Polygonum glabrum* on SGOT, SGPT and ALP levels of alloxan monohydrate induced diabetic rats

Groups	SGOT	SGPT	ALP
Normal	65.3±2.8	72.4±2.9	142.4±2.9
Diabetic control	256.1±2.4*	198.3±3.3*	196.32±2.4*
Glibenclamide, 10mg/kg	76.5±2.2*	58.5±2.8*	164.5±1.4
Methanol Extract, 200mg/kg	85.68±2.8*	68.76±1.9*	184.54±1.9
Methanol Extract, 400mg/kg	82.66±2.3*	67.56±3.5*	179.22±2.8*

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01

Table 5: Mean data of serum lipid profile in alloxan induced diabetic rats

Groups	Triglycerides	Total cholesterol	HDL	LDL	VLDL
Normal	69.13±0.32	76.14±1.98	38.12±1.58	31.06±0.98	13.20±0.67
Diabetic control	161.04±0.63	142.01±0.62	13.23±0.2	92.62±0.17	41.22±0.27
Glibenclamide, 10mg/kg	68.05±0.10***	77.11±0.44***	30.47±0.37***	39.97±1.09***	14.00±0.18***
Methanol Extract, 200mg/kg	65.10±0.46***	80.11±0.52***	27.64±0.95***	42.10±0.08***	15.12±0.77***
Methanol Extract, 400mg/kg	67.12±1.02***	78.20±1.06***	29.10±0.84***	39.18±0.94***	14.65±0.47***

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01

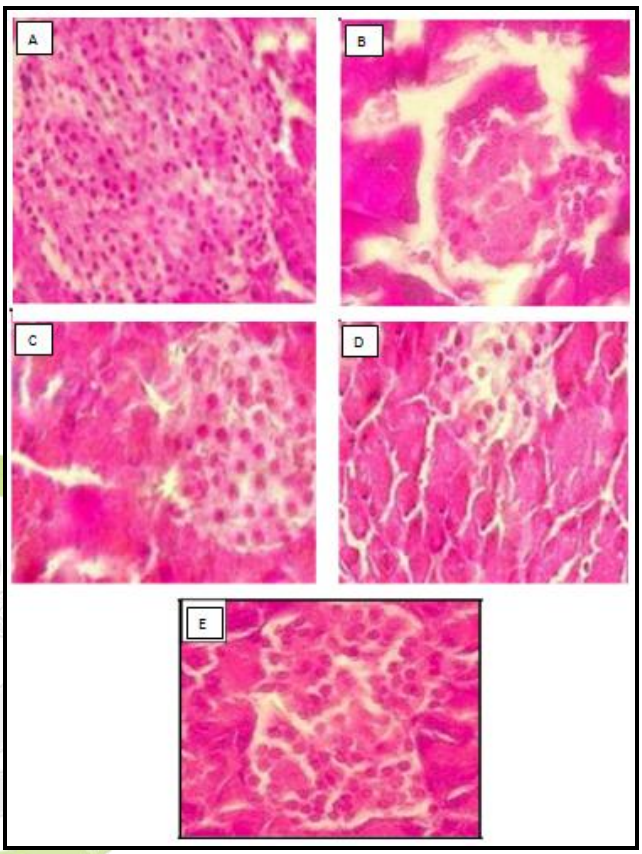
the lowest dose, i.e. 200mg/ kg caused a significant attenuation in the blood glucose at 120 min when compared to the vehicle-treated control group ($P<0.05$). Methanol extract (400 mg/kg) produced a significant decrease ($P<0.01$) in blood glucose level 60 min and 120 min after the administration of an oral glucose load. In the diabetic rats, the fasting blood glucose levels were 4–5 times higher than that of the normal rats. At a dose of 400 mg/kg produced a significant attenuation in the blood glucose ($P<0.05$) at 120 min after the oral glucose load. There was no significant attenuation in the rats administered 200 mg/kg at 120 min. Of the two doses of tested, the both doses (200 and 400mg/kg) was found to be most effective in improving glucose tolerance ($P<0.05$). Hence it was selected for the 4-week study.

Serum Lipid Profile

The serum level of triglycerides and cholesterol illustrate that the diabetic group shows significant hyperlipidemia when compared with the normal control group. The extract treated groups and the standard treated group significantly decreased the serum levels of cholesterol and triglycerides when compared with the diabetic control group ($p<0.001$). The effect of Methanolic extract on serum lipid levels was even better than that of the standard treated group, showing the hypolipidemic potential of the plant.

Histopathological Studies

In Figure, slide A and B represents islets of langerhans from normal and alloxan-induced diabetic rats, respectively. Comparison of these two slides clearly indicates the reduction in the number of β-cells in the islet of langerhans of pancreas of diabetic rats. As it is evident from slide B the islet is irregularly shaped, relatively small and atrophic. Most cells of the islets are small, degranulated and dark with scanty cytoplasm. However, compared to the untreated diabetic rats, histopathological examination of the plant *polygonum glabrum* extract-treated diabetic rats revealed an increase in the number of β-cells within the pancreatic islets, along with a reduction in the vacuolation (slides C, D and E). In other words, the plant extract treated diabetic samples histopathologically tend to approach the histopathology of the healthy pancreatic samples. The standard treated group also shows recovery and tends to approach the histopathology of the normal rat pancreas.



DISCUSSION

Crude methanol extracts of *polygonum glabrum* at a dose of 400mg/kg showed significant effect on the glucose tolerance of rats and also showed reduction in the fasting blood glucose levels of the normal rats, thus revealing the hypoglycaemic nature of the extracts. These findings indicate that the extracts might be producing hypoglycaemic effect by a mechanism independent from the insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption.

The histopathological studies of the rat pancreas showed recovery of the alloxan induced damage of the insulin secreting beta pancreatic cells, thus providing a hint for the mechanism of action of the plant. In several parts of our body, tissue parts were protected from the damage due to

toxicant. However, it appears that in the absence of major external stimuli, the beta-cell population has only a very limited potential for regeneration. This is probably due to the limited replication capacity of beta cells and to the fact that neogenesis from precursor cells is not readily reactivated. Yet, under certain conditions where major external stimuli are applied, there can be a quite vigorous regenerative expansion of the beta-cell mass. Such regenerative growth may result from activation of otherwise quiescent precursor/progenitor stem cells.

An increase in the SGPT, SGOT and ALP activities was recorded in diabetic rats in comparison with non diabetic rats, indicating an altered liver function in diabetic condition. *polygonum glabrum* extracts significantly controlled SGOT, SGPT and ALP values in the alloxan induced diabetic rats. In diabetic animals a change in the serum enzymes is directly related to changes in the metabolism in which these enzymes are involved. The increased levels of transaminases which are active in the absence of insulin because of increased availability of aminoacids in diabetes are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes. In the present study, the *polygonum glabrum* extracts significantly decreased SGOT and SGPT enzyme activities.

Hence, the improvements noticed in the levels of these enzymes are as a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of SGOT and SGPT levels after treatment also indicates a revival of insulin secretion. Elevation of ALP has been reported in diabetic rats and rabbits. This increase in ALP was significantly reversed by the extracts of *polygonum glabrum.*

Moreover, hyperglycemia in diabetic rats was associated with a high serum concentration of total cholesterol, LDL, VLDL and triglycerides and lower levels of HDL as present in the normal diabetic conditions. However, methanol extracts of *polygonum glabrum* at a dose level of 400 mg/kg and 200mg/kg reversed the diabetes-induced hyperlipidemia compared to the diabetic control group. In extract treated rats, there was a reduction in the levels of cholesterol, LDL, VLDL and triglycerides, increase in HDL showing the hypolipidemic effect of this plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis. In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after treatment with extracts of *polygonum glabrum* may be directly attributed to improvements in insulin levels.

Phytochemical analysis of the methanolic extracts of *polygonum glabrum* revealed the presence of flavonoids and alkaloids that have been shown to possess antidiabetic effect in other plants. Saponins**,** alkaloids and flavonoids which were responsible for the antidiabetic effect in other plants were also detected in the extracts of this plant. The presence of phenols in the plant could also be responsible for the antidiabetic effect as phenols have antioxidant effect and antioxidants have been shown to prevent the destruction of β-cells by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes.

CONCLUSION

It can be concluded that the phytochemical analysis of *polygonum glabrum* revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, phenolics and glycosides as the possible biologically active principles, due to which it possess antidiabetic activity. The extracts also show increase in the glucose tolerance of the rats and decrease in the fasting blood glucose level of normal rats, showing the hypoglycaemic activity. Histopathological examination of pancreas show the less damage of tissue when sections of treated groups are compared with diabetic control.

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