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ANTI-DIABETIC ACTIVITY OF *LYGODIUM FLEXUOSUM* (L) EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Objective: To evaluate the anti-diabetic activity of ethanolic extract of Lygodium flexuosum (L) on alloxan induced diabetic rats. Methods: Diabetes was induced in Wistar rats by intraperitoneal injection of alloxan monohydrate (100mg/kg b.w/i.p). Ethanolic extract of Lygodium flexuosum (L) (100, 200mg/kg b.w/p.o) was prepared freshly, administered to alloxan induced diabetic rats for 14 days. Blood glucose levels monitored at 1, 3, 5, 7 and 14 days, serum lipid profile and Histopathological changes in pancreas were examined after 14 days. OGTT was performed by administration of 100 and 200 mg/kg b.w/p.o of ethanolic extract of Lygodium flexuosum (L) 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, elevated cholesterol, triglyceride, VLDL, LDL levels and also increase in the levels of HDL were observed in diabetic rats treatment after 14 days of extract. Improved in regeneration of β-cells of langerhans of pancreas in rats by histopathological studies. Oral glucose tolerance test, blood glucose levels significantly lower at all time points (In extract and standard Wistar rats) that blood was sampled after oral glucose load. Conclusion: The results were suggested that the whole plant extract of Lygodium flexuosum (L) having potent Antidiabetic activity on alloxan-induced diabetic rats and this justifies its use in ethanomedicine and can be exploited in the management of diabetes.

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INTRODUCTION

Diabetes is a condition in which the body either does not produce enough, or does not properly respond to, insulin a hormone produced in the pancreas [1]. Diabetes is the world's largest endocrine disease with deranged carbohydrate, fats and protein metabolism. As per WHO report, approximately 150 million people have diabetes mellitus worldwide, and this number may well double by the year 2025. Statistical projection suggests that the number of diabetics will rise from 15 million in the year 1995 to 57 million in 2025, making India apart the country with the highest number of diabetics in the world [2]. Allthough many drugs and interventions are available to manage diabetics, these are expensive for a developing country like India apart from their inherent adverse effects. Therefore, it is necessary to look for new avenues to manage this major health problem. As part of the pathogenesis of Type II diabetes mellitus, skeletal muscle, liver and adipose tissues become resistance to the hormonal effect of insulin, which in turn leads to decreased insulin-mediated glucose disposal, hepatic glucose over production and a marked increase in lipolysis. The plants kingdom has become a target for the search by multinational drugs and biological active compound. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [3].

Herbal medicines are used to treat various diseases and now they had become an item of global importance, with both medicinal and economic implications. The demand of herbal medicine is being increasing day by day due to their safety and efficacy. Now herbals had taken over the allopathic system due to their less side effect and efficient working mechanism. Herbals are playing a pivotal role in increasing the economy of the country and had taken the nation on to the new path to achieve the goal of development [4]. Based on the chemical constituents medicinal value, herb *Lygodium flexuosum* (L) has selected for this study.

The *Lygodium flexuosum* (L) Sw.(family,Schizaeaceae), common name Maiden hair creeper, a rhizomatous perennial terrestrial fern, common in Southeast Asia and Australia), have been recorded as increasing memory power. Traditionally,the whole plant of *Lygodium flexuosum* (L) is used for hepato-fibrosys, cough, rheumatism,sprains, scabies, eczema, jaundice including wounds and skin diseases [5]. Hence, the present study was under-taken to explore Anti-diabetic activity of *Lygodium flexuosum* (L) extract on alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material

The plant of *Lygodium flexuosum* (L) were collected in the month of February 15th 2014 and authentication was done by botanist Dr. K. Madhava chetty, Assistant Professor, Department of Botany in S.V. University, Titupathi, and A voucher specimen number (3021).

Preparation of plant extract

The whole plant were examined for integrity and absence of dust and insect contamination then the plant of *Lygodium flexuosum* (L) were shade dried for 48 h to avoid the evaporation of volatile active constituents before extraction. The dried material was grinded into course powder and kept in the Soxhlet apparatus for 24 h for obtaining ethanolic extract. The ethanolic extracts obtained were concentrated in rotary flash evaporator under vacuum and their percent yield was determined [6,7].

Phytochemical screening

Phytochemical screening of the crude extract was carried out employing standard procedures [8,9] like percolation method, to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, Saponins, Anthraquinones, cardiac glycosides, carbohydrates and others.

Animals

Wistar rats (150-220g) of male were used for this experiment. They were obtained from Smt. Sarojini Ramulamma College of Pharmacy, (Reg.No 51/01/CPCSEA), Mahabubnagar, India and Maintained under standard environmental 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 *Lygodium flexuosum* (L) 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 min after dosing, periodically during the first 24 hours (with special attention during the first 4 hours), and daily thereafter for a period of 14 days [10].

Oral glucose tolerance test (OGTT)

The oral glucose test was performed in overnight fasted (18h) rats. The rats were divided into four group of six rats each. Group I (normal control) received orally 0.9 % saline. Group II received reference drug Glibenclamide at dose of 10mg/kg b.w/p.o Group III and IV received 100 and 200mg/kg b.w/p.o of ethanolic extract of *Lygodium flexuosum* (L) dissolved in 0.5% tween 80 respectively. After 30 min of treatment, all the groups were orally loaded with 2g/kg b.w/p.o of glucose. Blood samples were collected just prior to glucose administration and at 30, 60, 120 and 150 min after glucose loading. Blood glucose levels were measured using GOD-POD method [11].

Induction of diabetes

Alloxan monohydrate was first weighed individually for each animal according to its weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 100 mg/kg b.w/i.p. After 1 hr of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation, and after 48 hr, blood glucose was measured. One group served as a control which received vehicle alone. The diabetic rats (glucose level > 150 mg/dl) were considered diabetic and selected for experiment [12-14].

Evaluation of Parameters:

Evaluation of antidiabetic activity of extract

The animals were randomly divided into five groups with 6 rats in each group and treated as follows:

- Group I: Normal control group rats were administered 0.9 % w/v Normal saline (0.5 ml/kg.b.w/p.o) orally for 14 days.
- Group II: Diabetic control group rats were administered 0.9 % w/v Normal saline (0.5 ml/kg.b.w/p.o) orally for 14 days.
- Group III: Standard control group rats were administered standard (Glibenclamide 10mg/kg b.w) orally for 14 days.
- Group IV: Test-1 group rats were administered ethanolic extract of *Lygodium Flexuosum* (L) (100 mg/kg b.w/day) orally for 14 days.
- Group V: Test-2 Group rats were administered ethanolic extract of *Lygodium Flexuosum* (L) (200 mg/kg b.w/day) orally for 14 days.

The change in the fasting BGLs of all the rats were recorded at regular intervals during the experimental period. In alloxan induced diabetic rats the BGLs were monitored at the end of 1, 3, 5, 7, and 14 days after administration of extract for prolonged treatments. The BGLs were monitored in the blood of the diabetic rats by retro-orbital plexus puncture method. Blood glucose levels were determined by GOD-POD method [12-14].

Measurement of Serum lipid profile

Different groups of rats were anesthetized with di ethyl ether using desiccator and the blood samples were collected in tubes after retro orbital puncture. Plasma was obtained by centrifugation (1000rpm, 15 min, 4 °C) and stored at −20 °C until its analysis. The total Cholesterol, Triglycerides, HDL were estimated in serum by the kits specific for the test using Autoanalyzer [15,16]. The VLDL and LDL was estimated using standard formulas.

Histopathological studies

The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and Sections of 5μ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination [17,18].

Statistical analysis

All the values of the experimental results were expressed as mean±SEM and analyzed by one way ANOVA followed by "Dunnett's Test".

RESULTS

Phytochemical screening

The *Lygodium flexuosum* (L) whole plant leaves extract was conformed to contain carbohydrates, flavonoids, alkaloids, terpenoids, tannins, steroids and glycosides.

Toxicity Study

In toxicity study (limit test) the ethanolic extract *Lygodium Flexuosum* (L) was shown no signs and symptoms, morbidity and mortality on *Wistar* rats.

Hypoglycemic activity in normal rats (OGTT) and alloxan induced diabetic rats

A dose- dependent reduction in BGLs was observed in alloxan-induced diabetic rats treated with ethanolic extract of *Lygodium flexuosum* (L).

In the Oral glucose tolerance test (OGTT) in normal rats (Table 1) showed the blood glucose levels of the control, Glibenclamide (10mg/kg b.w/p.o) and ethanolic extract of *Lygodium Flexuosum* (L) (100 mg and 200 mg/kg b.w/p.o) at different time points (0, 30, 60, 120, 150 min) after administration of glucose (2 g/kg b.w/p.o) there was a peak increase in the blood glucose at 30 min in all the groups. In Glibenclamide and Extract treated groups, there was a decrease in blood glucose level at 150 min when compared to control group.

During prolonged study (14 days), the extract produced a sustained significant reduction in BGLs in the diabetic rats compared to control (Table 2). At the end of the study the extract at dose of 100 mg/kg b.w/p.o showed a significant reduction in the blood glucose from 3 day and 200 mg/kg b.w/p.o extract showed from 1 day onwards. The effects of the highest dose of the extract were more than that of the standard drug glibenclamide,10 mg/kg b.w/p.o on day 15.

Table 1. Oral glucose tolerance test (OGTT).

Group	Blood glucose level (mg/dL) 0 Min Mean±SEM	30 Min Mean±SEM	60 Min Mean±SEM	120 Min Mean±SEM	150 Min Mean±SEM
I Normal Control (0.9% w/v normal Saline 0.5ml/kg b.w/p.o)	82.8±1.35	125.0±1.95***	142.0±1.91***	114.0±1.64***	105.0±1.11***
II Standard Glibenclamide (10mg/kg/b.w./p.o)	83.8±1.350	125.0±1.200***	134.0±1.540***	112.0±1.650***	82.0±0.856
III Extract Treated (100mg/kg/b.w./p.o)	83.0±1.830	105.0±2.160***	123.0±1.670***	97.7±0.882***	86.5±0.764
IV Extract Treated (200mg/kg/b.w./p.o)	80.7±1.310	122.0±1.620***	135.0±0.989***	107.0±1.480***	81.7±1.120**

Values are Mean±SEM; n=6. *P value <0.01, **p <0.05, ***p < 0.001 Vs. 0 min.

Table 2. Effect of *Lygodium flexuosum* (L) extract on alloxan induced diabetic rats.

Group	Blood glucose level (mg/dl) 0 day Mean±SEM	1 day Mean±SEM	3 day Mean±SEM	5 day Mean±SEM	7 day Mean±SEM	14 day Mean±SEM
I (Normal control)	88.00±2.408	86.83±2.344	86.50±2.349	87.00±2.366	87.17±2.088	86.67±2.319
II (Diabetic control)	241.8±2.638	246.3±2.431	256.8±2.638	270.3±2.404	283.0±2.817	313.8±4.301
III (Standard)	217.80±2.561	194.30±2.692***	170.20±2.822***	142.30±2.813***	119.80±1.815***	86.67±4.104***
IV (Test-I)	230.0±4.367	217.6±4.151	204.7±4.631**	183.7±4.773***	163.3±4.566***	134.8±4.377***
V (Test II)	232.20±3.270	218.2±3.198*	192.2±3.420***	159.3±5.162***	122.7±2.616***	89.67±3.844***

Values are Mean±SEM; N=6. *P value< 0.01, **p <0.02, ***p < 0.001Vs. Diabetic Control.

Serum lipid profile:

In serum profile the elevated cholesterol, triglyceride, VLDL, LDL levels and decreased HDL levels were reported in diabetic rats. In this study administration of extract of *Lygodium flexuosum* (L) significantly reduced the elevated cholesterol, triglyceride, VLDL and LDL levels in diabetic rats. Also increased the levels of HDL were observed in diabetic rats (Table 3). Therefore this plant extract may be helps in preventing the diabetic associated complications.

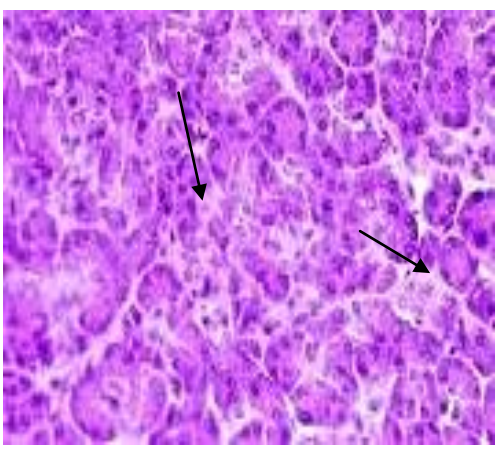
Table 3. Mean data of Serum profile in Alloxan induced diabetic rats.

Group	Serum lipid profile (mg/dl) Total cholesterol Mean±SEM	Triglycerides Mean±SEM	HDL Mean±SEM	LDL Mean±SEM	VLDL Mean±SEM
I (Normal control)	63.2±2.280	60.4±1.47	33.0±1.040	18.1±2.500	12.1±0.293
II (Diabetic control)	158.0±1.900	144.0±4.87	14.3±0.282	114.0±1.820	28.8±0.973
III (Standard)	73.6±1.130***	63.9±2.19***	26.2±1.500***	34.6±1.230***	12.8±0.438***
IV (Test-I)	92.5±2.030***	95.7±1.42***	20.8±0.801**	52.6±2.520***	19.1±0.283***
V (Test II)	75.1±1.590***	65.3±1.86***	25.8±1.380***	36.2±2.240***	13.1±0.373***

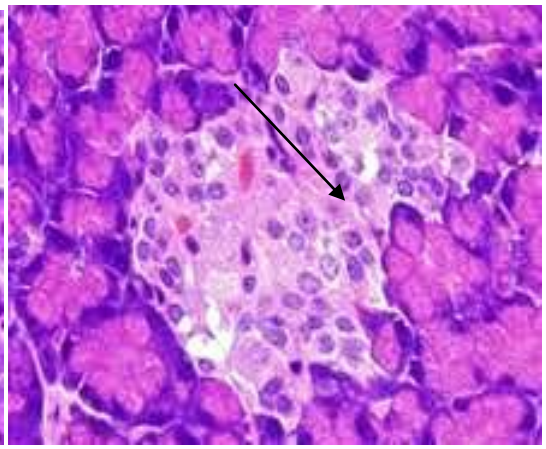
Values are Mean±SEM; N=6. *P value< 0.01, **p <0.02, ***p < 0.001Vs. Diabetic Control.

Histopathological changes in rat pancreas:

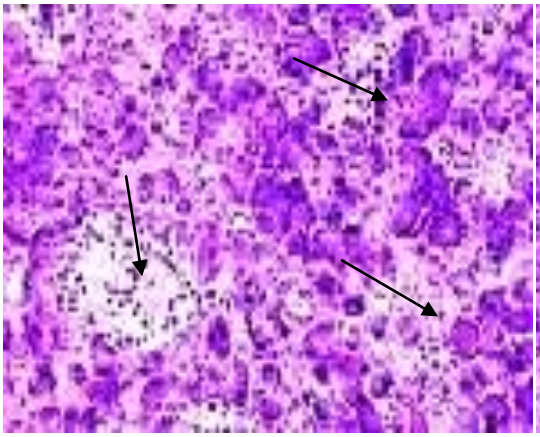
There is no destruction of β cells in normal control group, complete damage of β cells was observed in diabetic control group, and regeneration beta cells was observed in all test groups and standard group but better regeneration was observed in test-II when compared to diabetic control group i.e. equal to that of standard drug treated group. The results showed that the better regeneration of β cells is observed in test-II (200mg/kg b.w./p.o) group and standard drug treated group when compared to diabetic control group.



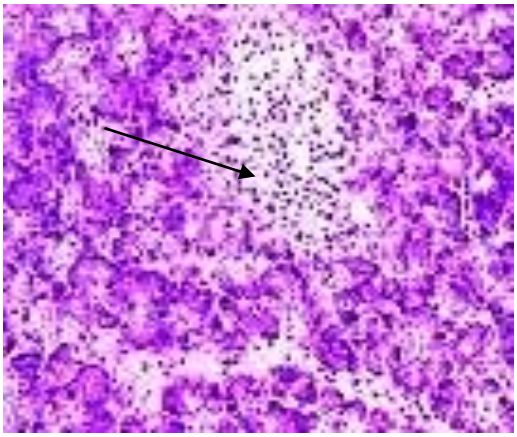
Normal control



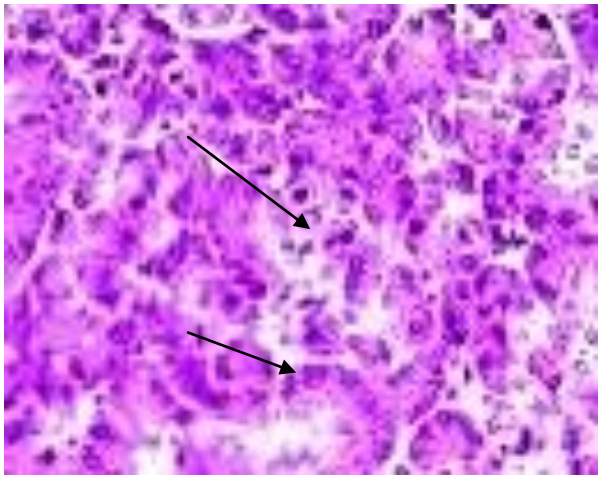
Diabetic control



Standard



Test -I



Test-II

DISCUSSION

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyurea (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).[1]

Evaluation of antidiabetic activity of *Lygodium flexuosum* (L) was carried in alloxan induced diabetic rats. The whole plant *of Lygodium Flexuosum* (L) is a rhizomatous perennial fern, contains carbohydrates, flavonoids, alkaloids, terpenoids, tannins, steroids and glycosides. Some of these classes of compounds have been implicated in the antidiabetic activity of the plants. Ex: Flavonoids and tannins. The ethanolic extract of *Lygodium Flexuosum* (L) was shown significant antidiabetic activity when compared to standard drug glibenclamide 10mg/kg b.w/p.o in alloxan induced diabetic *Wistar* albino rats.

In toxicity study (limit test) the ethanolic extract *Lygodium Flexuosum* (L) was shown no signs and symptoms, morbidity and mortality on Wistar rats.

The hypoglycemic effect of the ethanolic extract of *Lygodium Flexuosum* (L) on normal rats by oral glucose tolerance test (OGTT) there was a peak increase in the blood glucose at 30 min in all the groups. In Glibenclamide and Extract treated groups, there was a decrease in blood glucose level at 150 min when compared to control group. In sub-acute study the fasting blood sugar levels of diabetic rats, the test-I and test-II started significant reduction in blood glucose level from 3rd and 1st day onwards. At the end of the study the extract at dose of 100 and 200 mg/kg b.w/p.o showed a significant ($p<0.001$) reduction in the blood glucose level comparable with that of glibenclamide (10 mg/kg b.w/p.o) treated group.

In serum profile the elevated cholesterol, triglyceride, VLDL, LDL levels and decreased HDL levels were reported in diabetic rats. In this study administration of extract of *Lygodium flexuosum* (L) significantly reduced the elevated cholesterol, triglyceride levels, VLDL and LDL in diabetic rats. Also increased the levels of HDL were observed in diabetic rats. Therefore this plant extract may be helps in preventing the diabetic associated complications.

From the histopathological studies it was suggested that the regeneration of β cells following destruction by alloxan might be the primary cause for the antidiabetic activity of the extracts.

The *Lygodium Flexuosum* (L) extract (200mg/kg b.w/p.o) was shown a better with significant antidiabetic activity. In the subacute study the extract at dose of 200 mg/kg b.w/p.o showed a significant blood glucose reduction from first day of treatment. The damage of pancreas in alloxan-treated diabetic control rats and regeneration of β-cells by standard rats was observed.

The comparable regeneration was also shown by ethanolic extracts of *Lygodium Flexuosum* (L). Hence the above discussion revels that ethanolic extract at high dose (200 mg/kg b.w/p.o) is effective and shows similar curative effect as standard (glibenclamide 10mg/kg b.w/p.o).

CONCLUSION

It can be concluded that the ethanolic extract of *Lygodium flexuosum* (L) exhibited significant antidiabetic activity via chemical constituents(Flavonoids, Alkaloids, Beta Sitosterol), antioxidant property of the extract. Histopathological study, β-cells regeneration in alloxan-induced diabetic rats, further studies is needed to identify the active principles responsible for the antidiabetic effect, and to evaluate its mechanism of action in different model.

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