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HYPO-GLYCEMIC EVALUATION OF LEAF EXTRACT OF *ADIANTUM INCISUM* IN STZ INDUCED DIABETIC RATS

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ABSTRACT

Objective: Leaf of *Adiantum incisum* (Pteridaceae) has been used in traditional health systems to treat diabetes and many more disorders in southern Asia including India. However hypoglycemic potential of leaf of this valuable plant is not scientifically validated till date. The aim of present study is to evaluate hypoglycemic effect of ethanol extracts of *Adiantum incisum* leaf. **Methods**: The ethanol extracts in different dose concentration 100, 200 mg/kg of *Adiantum incisum* leaf were evaluated for hypo-glycemic potential for 21 days in streptozotocin induced diabetic rats. **Results**: The outcome of present study indicates, crude extract significantly reduce blood glucose level in diabetic rats**.** The acute oral toxicity study of *A. incisum* leaf extract did not show mortality in the animals at the limit dose of 2g/kg. In STZ induced diabetic rat, maximum BGL lowering effect produced by LE_2 followed by LE_1 after 21 days of treatment. **Conclusions:** The observed result may be due to active principles present in extract and fractions. The result showed the potential effects of *A. incisum* leaf extract as hypoglycemic in dose dependant manner compare to glibenclemide as standard drug. The claimed traditional use as anti-diabetic has scientific back ground.

KEYWORDS: *Adiantum incisum*, hypoglycemic, leaf, streptozotocin, phyto-chemical.

1. INTRODUCTION

Globally diabetes mellitus is one of the major causes of mortality and morbidity in human being.[1] According to an estimate by the International Diabetes Federation, near about 463 million persons are suffering from this disease and this is continuously increasing day by day, in India about 75-77 million people suffering from this disorder, which is expected to increase to about 90-95 million in future.[2-3]

Diabetes mellitus is caused by absolute or relative deficiency of insulin and/or reduced insulin activity or inherited and/or acquired deficiency in production of insulin. It represents serious, chronic heterogeneous group of metabolic disorder which finally produce hyperglycemia and irregularity in sugar, lipid and protein metabolism.[4-6] Chronic hyperglycemia is associated with dysfunction of heart, eyes, blood vessels, kidneys, nerves etc and it is characterized by damaged of pancreatic β cells, oxidative stress and cardiovascular complications.[2,7,8] Globally type 2 diabetes is common form which considered as one of the most recurrent lifestyle diseases. Prevalence of Type 2 is also more than type 1. Type 2 diabetes mellitus (T2DM) is a non-communicable disorder which is major causes of death worldwide because it is associated with long term side effects like retinopathy, nephropathy, neuropathy, skin complications etc. For the treatment of DM there are various classes of oral hypoglycemic agents existing along with variety of insulin, but due to so many side effects and long duration treatment there is an increasing demand by the patients to use natural products for control hyperglycemia.[9-12]

Despite considerable progress within the management of Type 2 DM by synthetic drugs, the design for natural anti-diabetic plant products for controlling diabetes is goes on. There are many hypoglycemic plants known through the folklore but their introduction into the modern therapy system awaits the invention of animal test system that closely parallel to the pathological course of diabetes in human beings.[13-16] Some medicinal herbs with proven anti-diabetic and related beneficial effects utilized in treatment of diabetes are *Tinospora cordifolia, Gymnema sylvestre, Casearia esculenta, Syzygium cumini, Commiphora wightii, Gmelina arborea, Asparagus racemosus, Boerhavia diffusa, Sphaeranthus indicus, Pterocarpus marsupium, Tribulus terrestris, Phyllanthus amarus, Swertia chirata, Glycyrrhiza glabra, Gossypium herbaceum, Berberis aristata, Piper nigrum.*[10,17,18]

A traditional medicinal maidenhair fern is evergreen fern widely distributed in mostly hilly areas, commonly called trailing maidenhair. There are countless varieties

of maidenhair ferns and they therefore offer the perfect match for every need and taste of a plant lover. Originally native to tropical rain regions, it now feels comfortable in the balcony, boxes or pots and in flower bed, although not all varieties are winter proof.[19-22] The maidenhair fern is very undemanding, but there still is a certain need for care in order for it to grow healthy and powerful for many years. This maidenhair fern also known as *Adiantum incisum* as its botanical name .It is known as Nilakantha-shikha, Mayurshikha, Vahrishikha in Ayurveda.[23-28]

The fern contains triterpenoids (including adiantone, isoadiantone) and flavonoids (including rutin and isoquercetin), hentriacontane, 16-hentriacontanone, adiantone, isoadiantone, β-sitosterol and fernene.[28-30]

There are large numbers of traditional benefits of maidenhair fern, since ancient times. It is used in the treatment of chest affections, cough, diabetes, fever and skin diseases. The leaves are used in diabetes and as a cure for coughs and fevers. It gives relief in internal heat or fever due to cooling action.[31-32]

2. MATERIALS AND METHODS

2.1 Reagent and Chemicals: Streptozotocin, glibenclamide were procured from Himedia Laboratories, Mumbai, India. Chemical kits for estimation of blood glucose were purchased from Primal Health care Limited, Lab Diagnostic Division, Mumbai, India. All other reagents of analytical grade were obtained from Merck Chemicals.

2.2 Plant Material

2.2.1 Plant collection, identification and authentication: Adiantum incisum leaves were obtained from the Jammu near new plot Army cantt downhill area in the month of July & August. The selected herb i.e. Voucher no. 16552 was identified and authenticated by the expert taxonomist. The crude drug were cleaned, dried in shade for 48-72 h and coarsely powdered. The powdered drug used for further studies.

2.2.2 Extraction of Plant material: The extraction of fern leaves with ethanol was carried out by using hot continuous percolation method in soxhlet apparatus. 90% ethanol was used as a solvent. After completion of extraction the extract was collected directly from round bottomed flask and solvent was evaporated using vacuum evaporator.

2.3 Preliminary Phytochemical screening of extract and solvent fractions

Standard Preliminary phyto-chemical qualitative test of the extract and fractions was carried out for detection of phyto-constituents. The crude extract was screened for the presence or absence of secondary metabolites such as Reducing sugars, Alkaloids, Steroidal compounds, Phenolic com- pounds, Cardiac glycosides, Flavonoids, Saponins, Tannins and Anthraquinones using standard procedures.[33-35]

2.4 Animals

The ethical clearance obtained via the institutional Animal Ethics Committee (CPCSEA registration number-1659/PO/a/ CPCSEA.) before the experiment. For acute toxicity study and for pharmacological activity evaluation Albino rats, weighing 150-200gm, were used for study, the animal were fasted whole night before the experiment starts for various extracts. Animal were kept in a constant humidity (55%), temp at (22± 2^0C), and exposed to dark and light {12hr) every day the bedding materials of the cages were changed.[36]

2.5 Acute oral toxicity studies

OECD 420 guidelines (organization for economic co-operation and development) were used to conduct acute toxicity study for determination of safe dose.[37] Rats were divided in five groups of three in each and kept fasting for overnight providing only water. 1st control group get DW. Leaf extract in different conc. 200-2000 mg/kg b.w. given to remaining group. Initially animals were observed at regular time intervals during the first 4 hrs, and then once daily for seven days. Behavioral changes and toxicity symptoms also observed closely.

2.6 Preparation of diabetic rats

STZ dissolved in Citrate buffer (0.1M) was injected in overnight fasted animal i.p. at dose of 60 mg/kg body weight. After a fortnight, rats with marked hyperglycemia were selected and used for the study.[38]

2.7 Experimental design

Animal will be randomly divided into 4 groups of 6 each and assigned as below.

Group 1:- STZ (60mg/kg, i.p., for one day) + Vehicle (for 21 days).

Group 2:- STZ (60 mg/kg, i.p., for one day) + Standard drug Glibenclamide (5mg/kg/day, p.o., for 21 days).

Group 3:- STZ (60mg/kg, i.p., for one day) + hydro-ethanolic extract of *Adianum incisum* extract (100 mg/kg/day, p.o., for 21 days- LE1).

Group 4:- STZ (60mg/kg, i.p., for one day) + hydro-ethanolic extract of *Adianum incisum* extract (200 mg/kg, p.o., for 21 days- LE-2).

2.8 Hypoglycemic potential of leaf on Streptozotocin - induced diabetes model

Rats will be treated with *Adiantum incisum leaf* suspension of ethanol (100, 200 mg/kg/day, p.o., for 21 days) and glibenclemide (5mg/kg/day) after 24h of administration, blood sample will be collected for 21 days at weekly intervals. Blood will be collected for determination of blood glucose level using Glucometer.

2.9 Statistical Analysis

Values are expressed as mean ± standard error of mean (SEM) and analyzed by using statistical package for social sciences (SPSS) version 7.5 using one way analysis of variance (ANOVA) followed by student 't' test. Data were considered statistically Significant at P<0.001 and P<0.01.

3. RESULTS

3.1 Successive Percentage yield of extract in (%)

Ethanol	-	9.27
Chloroform	-	3.03
Acetone	-	4.60
Pet. Ether	-	4.49
Distilled water	-	14.07

3.2 Preliminary Phytochemical analysis of extract

Table 1 Phytochemical analysis of leaf crude extract.

Sr. No.	Secondary metabolites	Phytochemical test	Ethanol extract
1	Alkaloids	Dragandroff's test	-
2.	Carbohydrate	Benedict's test	-
3.	Glycosides	Born tragger test	-
4.	Saponins	Foam test	+
5.	Phytosterols	Libermann burchards test	+
6.	Phenols	Ferric Chloride test	+
7.	Tannins	Gelatin test	+
8.	Flavonoids	Lead acetate test	+

3.3 Acute oral Toxicity study

For the determination of safe dose, toxicity study result shows that leaf extract caused no mortality in limit dose of 2000mg/kg b.w. in first 24 hrs as well as for the 7 days follow-up. There are also no behavioral changes and toxicity symptoms during whole period. This indicates that Median Lethal Dose (LD50) of the extract is greater than 2 g/kg.

3.4 Effect of Adiantum incisum extracts on serum glucose levels in Oral Glucose Tolerance Test model in rats:

The ethanol extract were evaluated *in-vivo* at two different concentrations under specified conditions for glucose tolerance test in rats fed with glucose. Among these concentrations both 100, 200mg/kg displayed potent anti-hyperglycemic activity and significantly prevented a sudden rise in blood glucose levels. Result concludes that the ethanol extract inhibited the enzymes responsible for the hydrolysis of carbohydrates into absorbable sugar in rat model. The results are shown in Table 2.

Table 2: Oral sucrose tolerance test in rats.

S.No.	Drug/Control	Blood glucose level (mg/dl)					
		0 min	30 min	60 min	90 min	2Hr	3Hr
1.	Group I Glucose Control	81.17 ±4.07	148.83 ±7.33	127.83 ±7.24	112.17 ±7.63	83.17 ±3.16	82.17 ±3.12
2.	Group II Glucose +Glibenclamide	84.00 ±2.05	121.00 ±8.47	98.67 ±5.38	90.83 ±3.30	80.83 ±3.22	76.17 ±0.60
3.	Group III Glucose +*A.incisum*(100mg/kg)	79.33 ±3.57	97.50 ±7.85	122.67 ±8.43	91.50 ±4.96	80.17 ±3.95	77.50 ±3.99
4.	Group IV Glucose +*A.incisum*(200mg/kg)	99.00 ±1.5	100.00 ±2.5	120.00 ±1.5	96.00 ±3.0	88.5 ±1.5	79.98 ±3.87

3.5 Anti-diabetic activity in Streptozotocin induced diabetic rat model

After development of diabetes, experimental animals showed remarkable variation in blood glucose levels when compared to normal rats (p<0.001), but no major variation in baseline fasting BGL between all groups of diabetic rats. Table 3.

Study between groups revealed that both LE_1 100 and LE_2 200 remarkably decrease the BGL on the 21st days, compared to the baseline blood glucose level. Similarly, the standard drug glibenclamide decrease the BGL significantly (P<0.01) on the 14th and 21st days respectively, compared to the baseline BGL. The maximum reduction in BGL was obtained at the 21st days 37.3%, 42.6%, and 47.1%, respectively, for LE_1 100, LE_1 200, and GLC5.

Table 3: Hypoglycemic Activity of the leaf Extract in Rats.

Group	Blood Glucose Level (mg/dl)				
	Day 0 (Baseline)	Day 7	Day 14	Day 21	% Decrease in BGL 21st Day
Vehicle	140.2±2.72	148.8 ±10.2	149 ±6.6	144 ±3.2	-2.85
STZ (D)	342.12±4.1	367 ±8.5	334.6 ±6.7	325.6 ±4.7	4.95
GLC5 (D)	314.8±3.5	282 ±2.8	230.8 ±2.5	166.6 ±3.8	47.1
LE_1(D)	308.5±5.2	299.1 ±1.7	248.6 ±3.4	193.2 ±2.5	37.3
LE_2 (D)	318.42±2.8	303.8 ±4.7	258.8 ±7.7	182.5 ±3.2	42.6

*Each value represents mean + SEM; n = 6 rats for each treatment

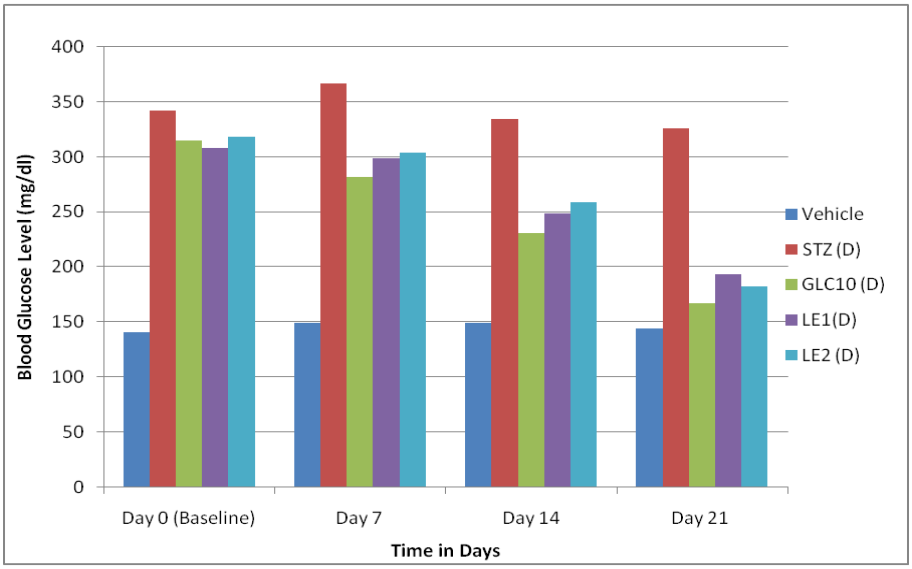

Fig. 1: Anti-diabetic potential of the leaf Extract on STZ induced diabetic Rats.

4. DISCUSSION

This work is accomplished to detect natural remedy which can be utilized in the prevention or treatment of diabetes. Phyto-constituents from plants have traditional history to treat diabetes.[39] Numerous compounds extracted from plants exhibit promising hypo-glycemic and hypo-lipidemic potential and sometimes they are more potent than oral hypoglycemic agents.[40-41] It is proven that effect of plant extract as anti-diabetic is linked with the presence of some secondary metabolites as flavonoids, phenols and tannins phyto-chemicals with antioxidant potential.[42-43] Presence of above phyo-constituents and anti-oxidant potential of said plant is already validated. So the present work analyzed the anti-diabetic potential of the ethanol extract of *Adiantum incisum* due to presence of the phenolic and flavonoids compounds.

The preliminary phyto chemical analysis of the ethanol crude extract indicates the presence of Flavonoids, Saponins, Phenols and Tannins (Table 1), which are responsible for anti-oxidant and hypoglycemic potential.

Hypo-glycemic analysis of crude extract in rats indicates that treatment with GLC5, LE_1, and LE_2 decrease blood glucose level significantly. The considerable percent (%) decreased in blood glucose levels were observed at 21st day after treatment. Table 3

5. CONCLUSION

The result of this research revealed that leaf extract contain poly-phenols as flavonoids and tannin which may be responsible for the hypo-glycemic effect. The outcome of study indicates significant blood glucose level lowering effect on diabetic rats. The outcome produces scientific support for the use of the plant as folk remedy for the management of diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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