ejpmr, 2024, 11(7), 314-319

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF HYPOGLYCEMIC POTENTIAL OF *ADIANTUM CAUDATUM* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Article Received on 01/05/2024 **Article Revised on 21/05/2024** **Article Accepted on 10/06/2024**

ABSTRACT

▪ **Objective:** Leaves, Root and fruit of *Adiantum caudatum* (Pteridaceae) has been used in traditional health systems to treat diabetes and many more disorders in various country. However hypoglycemic potential of leaves of this valuable plant is not scientifically validated till date. The aim of present study is to evaluate hypoglycemic effect of ethanol extracts and solvent fractions of *A. caudatum* leaves. **Methods:** The ethanol extracts and solvent fractions in different dose concentration 150, 300, 600 mg/kg of *A. caudatum* leaves were evaluated for hypo-glycemic effect in single dose and multiple dose study for 21 days in streptozotocin induced diabetic rats. **Results:** The acute toxicity study of *A. caudatum* extract and fractions did not show mortality in the animals at the limit dose of 2000mg/kg during the observation period. The outcome of present study indicates that extract significantly decreases elevated level of blood glucose in dose dependant manner when compared to untreated diabetic rats. **Conclusion:** The observed result may be due to active principles present in extract and fractions. ethanolic extracts and chloroform fractions of *Adiantum caudatum* (150, 300 & 600 mg/kg b.w) were tested, in which 300 and 600 mg/kg dose have shown significant ($P<0.01$) anti-diabetic activity compared to standard drug. The claimed traditional use as anti-diabetic has scientific back ground.

KEYWORDS: *Adiantum caudatum*, hypo-glycemic, streptozotocin, ethanol.

INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by elevated blood glucose with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Without the enough insulin, the cells of the body cannot absorb sufficient glucose from the blood, hence blood glucose levels increase, which is termed as hyperglycemia.[1,2] If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death. Type 2 diabetes (Non-insulin-dependent diabetes mellitus) is a chronic metabolic disease that results from defects in insulin secretion and insulin receptor kinase.[3,4]

Diet, exercise, modern drugs including insulin and oral administration of hypoglycaemic drugs such as sulfonylureas and biguanides manage the pathogenesis of diabetes mellitus. Insulin plays a key role in glucose homeostasis along the side of a counter regulatory hormone, glucagon, which raises serum glucose.[5]

Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost. A list of medicinal plants with proven antidiabetic and related beneficial effects and of herbal drugs used in treatment of diabetes is compiled. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an antidiabetic compound with antioxidant properties would be more beneficial. Therefore information on antioxidant effects of these medicinal plants is also included.[6,7]

This maidenhair fern also known as *Adiantum caudatum* as its botanical name. It is known as Nilakantha-shikha, Mayurshikha, Vahrishikha in Ayurveda. It has astringent, tonic and febrifuge properties. Medicinally, the leaves are used as a cure for cough, fever and chest affection. They are also employed for diabetes and externally the leaf paste is used to cure skin diseases.[8-10]

MATERIAL AND METHODS

In the present study, the leaves of *Adiantum caudatum* was collected from local area of Alwar district,

Rajasthan, with the help of field botanist. Streptozotocin was procured from Sigma Chemical Co., USA. Pure drug Glibenclamide was obtained from Aventis Pharma Ltd., Goa.

Methods: Preparation of extract: Dried course powder of the leaves was extracted with alcohol (90%) and water by using soxhlet apparatus separately until the extraction solvents becomes colorless.

Preliminary Phytochemical Screening

Extract of *Adiantum Caudatum* leaves will be subjected to preliminary quantitative phytochemical investigation for the detection of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, gums and mucilages, fats & fixed oils using the following standard methods.[11-13]

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± 20C), and exposed to dark and light {12hr) every day the bedding materials of the cages were changed.[14]

Anti Diabetic activity: Acute oral toxicity: The acute oral toxicity studies of extracts were carried out as per the guidelines of Organization for Economic Co-operation Development (OECD) guidelines.[15]

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.[16]

Streptozotocin induced Diabetes in rats

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

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

Group 2:- Streptozotocin (60 mg/kg, i.p., for one day) + Standard drug Glibenclamide (10mg/kg/day, p.o., for 21 days).

Group 3:- Streptozotocin (60mg/kg, i.p., for one day) + hydroethanolic extract of *Adianum caudatum extract* (100 mg/kg/day, p.o., for 21 days).

Group 4:- Streptozotocin (60mg/kg, i.p., for one day) + hydroethanolic extract of *Adianum caudatum extract* (200 mg/kg, p.o., for 21 days).

Induction of diabetes: Diabetes will be induced in overnight fasting animals by single intraperitoneal injection of Streptozotocin (60mg/kg) prepared in citrate buffer (0.1M, at 4.5 pH). After 72 hr of STZ injection, blood sample will be drawn by tail vein and glucose estimation is done using Glucometer.

RESULTS AND DISCUSSION

Organoleptic characters of crude drug

Table 1: Organoleptic characters of *Adiantum caudatum*.

Crude drugs	Organoleptic characters			
	Nature	**Colour**	**Odour**	**Taste**
A. caudatum	Coarse powder	Light Green/	Odourless	Characteristic
Leaves Feature	Scaly at the base epicalyx 5	Bulbiferous apex	Veins: Distinct above and below	

Ethanolic extraction of *A. caudatum* leaves

The percentage yield of petroleum ether and ethanol extract of *A. caudatum* leaves was found to be 1.28%, 9.68%, where as chloroform soluble extract, acetone soluble fraction and acetone insoluble fraction of ethanolic extract were found 28.44%, 21.46%, and 42.20% respectively. These extracts and fractions were stored in airtight container for further studies.

Table 2: Percentage yield, consistency and color of extracts of A. caudatum.

S. No.	Extraction /fraction	Colour	Consistency	(% w/w) Yield
1	Petroleum ether extract	Light brown	Sticky mass	1.28%
2	Ethanol extract	Dark brown	Sticky mass	9.68%
3	Chloroform soluble extract	Dark brown	Sticky mass	28.44 % of ethanolic extract
4	Acetone soluble fraction	Dark brown	Sticky mass	21.46 % of ethanolic extract
5	Acetone insoluble fraction	Dark brown	Sticky mass	42.20 % of ethanolic extract

Qualitative phytochemical investigation of *A. caudatum*

Table 3: Qualitative chemical tests of different extracts of *A. caudatum*.

Sr. No.	Secondary metabolities	Phytochemical test	Ethanolic extract of *Adiantum incisum*
1.	Alkaloids	Mayer's test Wagner's test Dragandroff's test Picric acid 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	++
9.	Proteins & Amino acid	Ninhydrine test	-
10.	Fixed oil & fats	------	-
11.	Steroid	------	+
12.	Terpenoids	-------	+
13.	Quinones	-------	-

RESULTS AND DISCUSSION

Ethanolic extract of *Adiantum incisum* shows high amount of Phytosterols, flavonoids and Saponins compound.

Anti Diabetic activity: Acute oral toxicity

Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hrs. In all the cases no death was observed within first 24 hrs. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern. Attention was also given to observation of tremors and convulsions.

The therapeutic dose was calculated for the purpose of anti-diabetic and antioxidant investigations. The LD_{50} value determined by the method as per guidelines of Organization for Economic Co- operation Development was found to more than 2000 mg/kg b.w. by oral route.

Streptozotocin induced diabetic model

After fourteen days of STZ injection, the confirmed diabetic rats (i.e., random blood glucose level 250mg/dl) were selected for the experiment. Blood samples of diabetic rats were collected before and 2, 4, 6 and 8 hr after treatment. Blood glucose levels were determined. The results were compared with that of the standard drug.

Statistical Analysis[44]

The results of the study were subjected to one way analysis of variance followed by student t-test for multiple comparisons. Values with $P < 0.05$ were considered significant.

Table 4: Anti-diabetic potential of leaves in Diabetic-Rats (Single Dose study).

Group	BGLs(mg/dl)				
	0 Hrs	1 Hrs	2 Hrs	4 Hrs	8 Hrs
DW10 (NC)	78.12±3.44	76.66±3.62	77.16±3.74	77.83±4.44	80.13±3.44
Diabetic Control	372.25±8.04	375.66±8.61	386.33±9.84	380.16±9.83	375.83±9.48
GLC10	382.00±9.83	248.33±6.80**	216.16±7.47**	162.50±6.76**	130.00±11.23**
EEAC 300	395.09±4.30	385.87±5.03	352±6.93	292.82±7.33	282.86±4.63
CEAC 300	386.00±8.41	346.00±8.15	271.16±7.47*	212.16±9.74**	200.83±8.32**
AEAC 300	398.02±4.33	386.76±4.68	362.75±6.53	312.06±6.33*	296.61±5.39**

n=6, *p<0.05- significant, **p<0.01-more significant v/s diabetic control, SEM= standard error mean, SD=standard deviation, n= number of animal

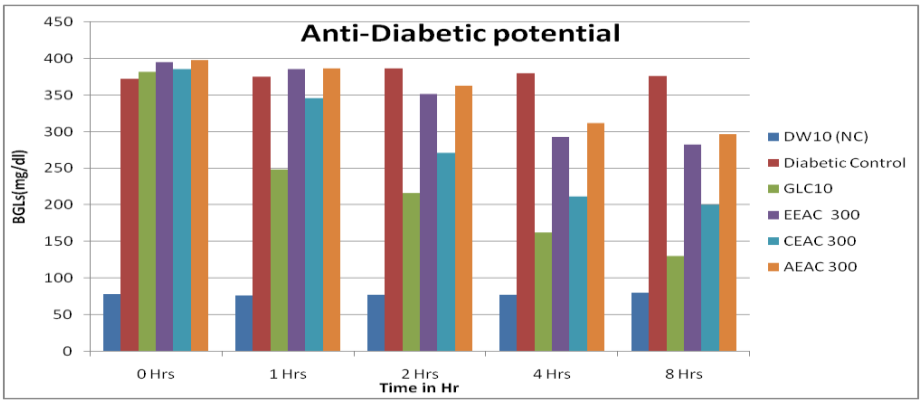

Figure 1: Anti-diabetic potential of leaves in Diabetic-Rats.

RESULT AND DISCUSSION

The anti-diabetic activity of *A. caudatum* of various extracts were studied against standard drug Glibenclamide. It was observed that there was marked activity was found in chloroform fraction of ethanolic extract as compare to acetone soluble fraction of ethanolic extract and crude ethanol extract did not show any appreciable activity. This suggests that chloroform fraction of ethanolic extract is major contributor of anti-diabetic activity and may be investigated further for isolating active compound.

The reference drug glibenclamide induce a remarkable decrease in blood glucose levels at the 2nd, 4th and 8th hr compared to NC. In similar manner extract, their fractions treated groups indicates a remarkable decrease in BGL, for CEAC 300 at 2nd Hrs, at 4th and 8th Hrs, AEAC 300 ($p < 0.05$) at 8th Hrs. Reference compound also cause remarkable decrease in BGL at 2nd, 4th & 8th hrs on baseline comparison.[17-19]

Evaluation of Anti-diabetic potential of chloroform fraction of leaves extract in Diabetic-Rats (Sub Acute Study).

Hypoglycemic potential of repeated daily doses up to 21st days is summarized in Table 5.14. After development of diabetes in rats, BGLs were recorded every week up to 3 weeks in normal and diabetic rats treated with DW, CEAC -150, CEAC -300, CEAC -600 and GLC10.

Table 5: Effect of *A. caudatum* on blood glucose level in streptozotocin induced diabetic male wister rats (sub acute study)

Groups	Treatment	Blood glucose level mg/dl				
		0 Day	7 Days	14 Days	21 Days	% Decrease in BGL 21st Day
I	NC	83.6±3.44	80.16±3.74	84.1±4.44	82.5±3.44	1.88%
II	DC	370.42±8.04	388.66±11.52	382.16±10.87	375.2±10.30	-2.2%
III	GLC	386.5±8.63	193.66±8.95**	172.83±6.94**	143.00±5.65**	62.95%
IV	CEAC -150	398.50±9.33	340.33±10.51*	312.00±7.22*	282.33±7.45**	29.1%
V	CEAC -300	382.6±10.68	297.00±7.94*	257.50±8.08*	212.16±5.67**	44.5%
VI	CEAC -600	392.2±11.16	282.50±5.96**	242.33±5.39**	202.66±5.31**	48.46%

n=6, *p<0.05- significant, **p<0.01-more significant v/s diabetic control, SEM= standard error mean, SD = standard deviation n= number of animals

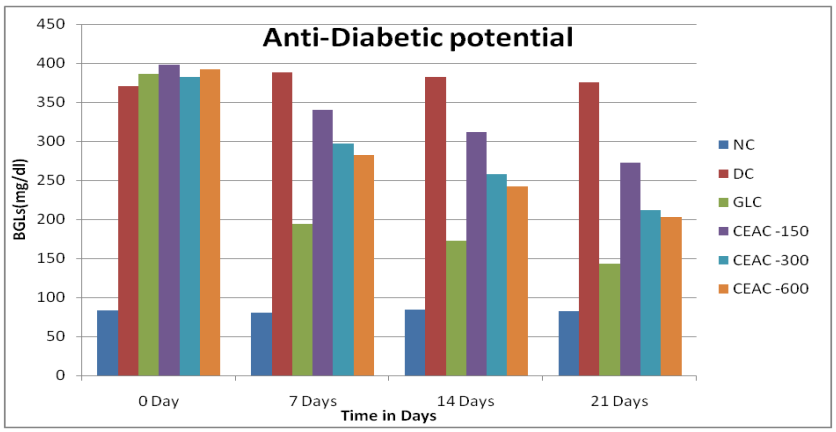

Figure 2: Effect of *A. caudatum* on blood glucose level in streptozotocin induced diabetic rat (sub acute study).

RESULT AND DISCUSSION

After development of diabetes, glucose levels in experimental animals showed remarkable variation with normal rats. Result of study indicates that CEAC -150mg/kg (29.1% Reduction) has very less effect on blood glucose level compared to standard drug glibenclamide.

During study, group analysis indicates that on treatment with CEAC -300 and CEAC -600 significantly decrease the BGL on 14th and21st when compared to DC. In the similar manner, GLC10 treated group showed significantly decrease in BGL on the 7th day, 14th and 21st days.

Maximum decrease in BGLs was obtained on 21st days 44.52%, 48.46%, and 62.95%, respectively, for CEAC -300, CEAC -600 and GLC10 (Table 14, Fig 5.4).

Study between groups also revealed that both CEAC -300 and CEAC -600 remarkably decrease BGLs, while other fraction treated group did not show considerable changes in BGL. As study indicates that there is no remarkable difference between result of CEAC -300 and CEAC -600 so 300mg/kg body wt dose is suitable group for treatment.[17-19]

CONCLUSION

- Color, consistency and the % yield of extracts were determined. Among all the extracts, the % yield of the chloroform fraction of ethanolic extract was found to be the highest. Various extracts were subjected to phytochemical investigation and revealed presence of glycosides, saponins, phytosterols, flavonoids, tannins, phenolic compounds and proteins. TLC profiles confirmed the presence as reported in literature.
- The chloroform fraction of ethanolic extract of *A. caudatum* was investigated for anti-diabetic activity.
- In streptozotocin induced model, administration of streptozotocin, led to elevation of fasting blood sugar (FBS), which was maintained over a period of study in diabetic control group. The results were comparable with reference standard glibenclamide.
- Various doses of ethanolic extracts and chloroform fractions of *Adiantum caudatum* (150, 300 & 600 mg/kg b.w) were tested, in which 300 and 600 mg/kg dose have shown significant ($P<0.01$) anti-diabetic activity compared to standard drug.

Conflict of interest

No conflicts of interest are mentioned by the researchers.

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