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Research Article

HYPOGLYCEMIC AND ANTIHYPERLIPIDEMIC EFFECTS OF *ADIANTUM CAUDATUM* IN ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT

Objective: To investigate the hypoglycemic and antihyperlipidemic effect of a successive ethanolic extract of *Adiantum caudatum* (EEAC) whole plant in alloxan induced diabetic rats.

Methods: Diabetes was induced in Wistar albino rats by the administration of alloxan (140 mg/kg b. w., i.p.). EEAC (200 mg/kg b. w., p.o.) was administered to diabetic rats for 21 days in alloxan induced diabetic rats. The effect of EEAC on blood glucose and body weight was studied in alloxan induced diabetic rats. All these effects were compared with glibenclamide (10 mg/kg b. w., p.o.) as a reference antidiabetic drug.

Results: The administration of EEAC (200 mg/kg b. w., p.o.) resulted in a significant decrease in blood glucose level and a significant increase in body weight in alloxan induced diabetic rats. Furthermore, EEAC showed antihyperlipidemic activity as evidenced by a significant decrease in serum total cholesterol and triglyceride levels in alloxan induced diabetic rats.

Conclusion: The results suggest that the EEAC possess a promising hypoglycemic effect in alloxan induced diabetic rats.

Keywords: Hypoglycemic, Antihyperlipidemic, *Adiantum caudatum*, Alloxan, Diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in the production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentration of glucose in the blood (hyperglycemia), which in turn leads to metabolic disorders and also damage many of the body's systems, in particular, the blood vessels and nerves [1]. It is also estimated that there are 30-33 million diabetic patients in India now and every fourth diabetic in the world today is an Indian. Indians are genetically more susceptible to diabetes, and the WHO predicts the number of diabetic persons in India would go up to 74 million by 2025 [2]. Apart from currently available therapeutic options, many herbal medicines have been recommended for treatment of diabetes. Furthermore after the recommendation made by WHO on DM, investigation on a hypoglycemic agent from medicinal plants has become more important [3]. The alloxan induced diabetic rat models have been regarded as one of the generally used animal model to detect the efficacy of test articles on diabetes.

The plant *Adiantum caudatum* is distinctive in appearance with dark, often black stripes and rachises, and bright green often delicately cut leaf tissue. The sori are borne submarginally and are covered by reflexed flaps of leaf tissue which resemble indusial. It generally prefers humus rich, moist, well-drained sites ranging from bottomland soils to vertical rock walls. Three triterpenoids, 8a-hydroxyfernan-25, 7b-olide, 3a-hydroxy-4a-methoxyfilicane, and 19a-hydroxyferna-7, 9 (11)-diene were isolated from the fresh fronds of *A. caudatum* [4]. Coumarin is showed to be present in *A. caudatum* [5].

The present study is aimed at investigating the antidiabetic effect of a successive ethanolic extract of *A. caudatum* (whole plant) on blood glucose levels and other biochemical parameters in normoglycemic and alloxan induced diabetic rats. The present study is carried out to validate scientifically the folklore use of *A. caudatum* as antidiabetic.

METHODS

Collection of plant material

Fresh plant of *A. caudatum* was collected in the month of June from local areas of Chhaygaon, Guwahati, Assam and authenticated by Botanical Survey of India, Shillong, Meghalaya (Ref. no. Plant identification/14/297). Voucher specimen was deposited in departmental herbarium for future reference.

Preparation of extract

The collected plant materials were shade dried and subjected to size reduction to a coarse powder using a grinder and passed through a sieve. The powdered materials were extracted successively with petroleum ether, chloroform, and ethanol (40:60), respectively, at a temperature of 30-50°C. All the extracts were further concentrated to semisolid mass using rota flash evaporator and were stored in desicator. The suspensions of ethanolic extracts were prepared by carboxymethyl cellulose (CMC) in distilled water for the experiment.

Phytochemical screening

The extract was tested to know the different constituents present in it by the standard procedures [6]. Terpenoids, phenyl propanoids, steroids, and flavonoids have been found in the plant *A. caudatum*.

Animals

Antidiabetic activity was carried out using healthy albino Wister rats of either sex weighing between 150 and 300 g. Animals were housed in polypropylene cages and allowed free access to tap water and pellet diet *ad libitum* throughout the study. After randomization into various groups and before initiation of the experiment, the rats were acclimatized for a period of 7-day under standard environmental conditions of temperature, relative humidity, and dark/light cycle. All the experiments on animals were conducted according to ethical protocols that were approved by the Institutional Animal Ethics Committee. (Reference letter no. GIPS/IAEC/2012/05).

Table 1: Effect of EEAC on blood glucose level in alloxan induced diabetic rats

Groups	0 day	7th day	14th day	21st day
Normal control	82.58±0.2789	83.28±0.08139	81.53±0.1078	82.77±0.1573
Diabetic control	316.7±0.1392	354.7±0.15[a]***	378.7±0.194[a]***	342.7±0.3032[a]***
Standard drug	315.4±0.2659	242.3±0.4751[b]***	138.1±0.3754[b]***	148.8±0.3025[b]***
EEAC	314.5±0.28	234.6±0.288[b]***	162.2±0.3769[b]***	174.3±0.7786[b]**

Each value is mean±SEM of 6 rats in each group. **p<0.01, ***p<0.001 in comparison to diabetic control, SEM: Standard error of mean, EEAC: Successive ethanolic extract of *Adiantum caudatum*, Where, [a]Normal control, [b]Diabetic control

Table 2: Effect of EEAC on body weight in alloxan induced diabetic rats

Groups	Before treatment	After treatment
Normal control	230±0.6831	232±0.3651
Diabetic control	220±0.4472	207.3±4.014[a]***
Standard drug	220±0.4472	232±0.5774[b]***
EEAC	210±0.5164	218±0.2582[b]***

Each value is mean±SEM of 6 rats in each group. ***p<0.001 in comparison to diabetic control, SEM: Standard error of mean, EEAC: Successive ethanolic extract of *Adiantum caudatum*. Where, [a]Normal control, [b]Diabetic control

Table 3: Effect of EEAC on biochemical parameters in alloxan induced diabetic rats

Groups	Biochemical parameters (mg/dl)	
	Total cholesterol	Total triglyceride
Normal control	33.72±0.2957	72.45±0.1965
Diabetic control	86.01±0.1828[a]***	175.2±0.2853[a]***
Standard drug	62.04±0.1003[b]***	84.43±0.2482[b]***
EEAC	63.52±0.1830[b]***	87.37±0.2088[b]***

Each value is mean±SEM of 6 rats in each group. ***p<0.001 in comparison to diabetic control, SEM: Standard error of mean, EEAC: Successive ethanolic extract of *Adiantum caudatum*. Where, [a]Normal control, [b]Diabetic control

Acute toxicity study

Albino mice of either sex weighing between 20 and 30 g were used. The animals were fasted overnight. Acute toxicity was performed according to OECD guidelines; method followed is according to number 420 [7]. Effective dose for *A. caudatum* extract was found to be 200 mg/kg body weight.

Standard drug

Glibenclamide tablet (Daonil tablet, Aventis Pharma Pvt. Ltd.) was used as standard. It was purchased from New Multicare Pharmacy, Chhaygaon. The tablets were suspended in distilled water using CMC as suspending agent and used for the study.

Alloxan induced diabetes

The albino rats weighing 150-300 g of either sex were allowed to fast for 12 hrs prior to experimentation and rendered diabetic by a single dose of i.p. injection of alloxan 140 mg/kg body weight as described by Joy and Kutton (1999) [8]. The antihyperglycemic activity on these animals was carried out after 5 days of alloxan injection when the stabilization of diabetes was ensured. The animals with sugar level more than 200 mg/dl were selected for the study [9-13].

Experimental design

All the animals were randomly divided into five groups.

Group 1: Healthy normal animals received only the vehicle (CMC).
Group 2: Untreated but diabetes-induced animals served as a negative control.
Group 3: Diabetes-induced animals and treated with standard drug glibenclamide 10 mg/kg body weight/day orally.
Group 4: Diabetic animals and treated with a successive ethanolic extract of *A. caudatum* (EEAC).

The extract was given orally at the dose of 200 mg/kg body weight, respectively, and the study was carried out for a period of 21-day.

On the 21st day of treatment, blood samples were collected by retro-orbital plexus puncture method under mild ether anesthesia, and serum was separated by centrifugation. The serum was analyzed for blood glucose level, total cholesterol, and total triglyceride level.

Statistical analysis

The quantitative measurements were made on six animals in each group, and the values of biochemical estimations were expressed as mean ± standard error of the mean. The data obtained were subjected to one-way ANOVA by multiple comparison tests.

RESULTS

Effect of EEAC on blood glucose levels in alloxan induced diabetic rats

The effect of EEAC on blood glucose levels of diabetic rats is given in Table 1. Alloxan induced diabetic rats showed a significant increase in blood glucose levels when compared to normal control rats. Oral administration of EEAC at 200 mg/kg b.w. showed a significant decrease ($p < 0.001$) in blood glucose levels.

Effect of EEAC on body weight in alloxan induced diabetic rats

Body weight of animals in all groups was recorded before and after treatment and change in body weight was also mentioned. The highest change in body weight during the study period was found to be in the diabetic control group which decreases. In all treatment group with 200 mg/kg of EEAC body weight was increased, $P < 0.001$ (Table 2).

Effect of EEAC on biochemical parameters in alloxan induced diabetic rats

EEAC at a dose of 200 mg/kg b. w. significantly decreases total cholesterol and total triglyceride levels ($p < 0.001$). Glibenclamide 10 mg/kg b.w. significantly decreases total cholesterol and triglyceride levels ($p < 0.001$) (Table 3).

Histopathology of pancreas in alloxan induced diabetic rats

From the histopathological examinations (Fig. 1) of the pancreas, in the normal control group, the islet boundaries were clear, and the profiles of the islet cells were clearly visible. No necrosis or fatty degeneration observed. After inducing alloxan, in diabetic control group completely destructed cells were observed, ballooning, picnosis, and Necrosis occurred. There was fatty layer degeneration, normal eco-structure had been lost, central lobes were destroyed; normal cellular integrity was completely lost along with fatty degeneration. Irregular gap junctions appeared coagulation occurred due to necrosis. In test control group (200 mg/kg of bodyweight), central Lobule is intact, cellular integrity was normal to a great extent. In standard treated Group (10 mg/kg of b.w.), cellular integrity was normal. Fatty layer degeneration had occurred. Slight ballooning occurred, but no blood clotting or necrosis had been noticed.

DISCUSSION

The successive ethanolic extract *A. caudatum* was effective in normalizing the elevated levels of blood sugar and blood lipid-like cholesterol, triglycerides. Results are comparable with that of reference

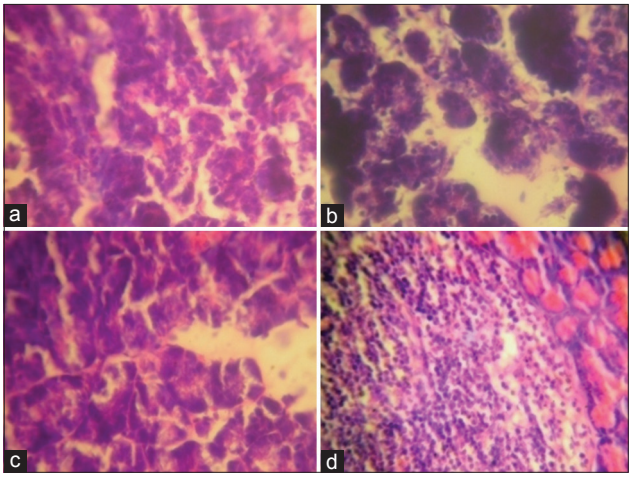

Fig. 1: Histopathology of pancreas in alloxan induced diabetic rats. (a) Normal control, (b) Diabetic control, (c) Standard drug, (d) Successive ethanolic extract of *Adiantum caudatum* (200 mg/kg b.w.)

drug glibenclamide. The phytochemical screening of the extract revealed the presence of flavonoids, alkaloids, phenolic compounds, carbohydrates, and triterpenoids.

Administration of alloxan (140 mg/kg, i.p.) let to an elevation of fasting blood glucose levels, which was maintained throughout the period of treatment for 21-day of daily treatment of EEAC led to a fall in blood sugar levels. The effect seems to reach a maximum after 14 days of treatment. Vehicle-controlled animals were found to be somewhat stable in their body weight, but diabetic rats showed a significant reduction in body weight during the entire experiment.

In light of the results, our study indicates that successive ethanolic extract of *A. caudatum* without significant change in body weight (200 mg/kg b.w.) exhibited significant antidiabetic activity in alloxan induced hyperglycemic rat. The antidiabetic effect may be due to the presence of flavonoids, phenolic compounds, alkaloids, and triterpenoids. Thus, the claim made by the traditional Indian system of medicine regarding the use of this plant in the treatment of diabetes stands confirmed.

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