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STUDIES ON GLUCOSE LOWERING EFFICACY OF THE ANTHOCEPHALUS CADAMBA (ROXB.) MIQ. ROOTS.

S. ACHARYYA[1*], G. K. DASH [1], S. MONDAL [1] AND S.K. DASH [2].

[1]Matushree V. B. Manvar College of Pharmacy, Dumiyani, Rajkot dist., Gujarat -360 440.
[2]P.G. Department of Biosciences, College of Pharmaceutical Sciences, Mohuda, Berhampur Ganjam dist., Orissa-760002

***Corresponding Author** sumanacharyya83@yahoo.com

ABSTRACT

Diabetes, the most prevailing metabolic disorder is attracting present research attention towards it. The methanol and aqueous extracts of the roots of *Anthocephalus cadamba* (Roxb.) Miq. (Family-Rubiaceae) was tested for hypoglycaemic activity in normoglycaemic and alloxan induced hyperglycaemic rats at dose levels of 100, 200 and 400 mg/kg, p.o. respectively. The extract was further subjected to oral glucose tolerance test in normal rats. The hypoglycaemic activity of the root was compared with the reference standard glibenclamide (2.5 mg/kg, p.o.). The study revealed that the roots extract caused significant reduction in the blood glucose level in both normoglycaemic and alloxan induced diabetic rats at the tested dose levels in a dose dependant manner. In glucose-loaded animals, the extract also reduced the elevated blood glucose concentration. The study established the scientific basis for the utility of this plant in the treatment of diabetes and justifies the use of the roots of the plant for treating diabetes as suggested in folklore remedies.

KEYWORDS

Anthocephalus cadamba, Alloxan, Glibenclamide, Hyperglycaemic, Normoglycaemic, Oral glucose tolerance Test (OGTT).

INTRODUCTION

The diverse culture of our country is a rich source of traditional medicines, many of which are of plant origin. Scientific data on such plant derivatives could be of clinical use[1]. *Anthocephalus cadamba* (Roxb.) Miq. Syn. *Neolamarckia cadamba* var *A. chinensis* (Family: Rubiaceae) commonly known as Kadam is a large tree up to 37.5 m high and 2.4 m in girth with straight cylindrical bole. The bark is gray, smooth in young trees, rough and longitudinally fissured in old trees. Leaves opposite, simple, elliptic-oblong; Flowers in solitary globose head, orange or yellow; Fruits pseudo carps, found all over India[2, 3]. In folk medicine it is used in the treatment of fever, uterine complaints, blood diseases [4,5], skin diseases[6], eye inflammation, diarrhoea [7], anaemia, leprosy, dysentery and stomatitis [8]. The reported uses of this are anti-hepatotoxic[9], antimalarial[10], antimicrobial, wound healing, antioxidant[11], anthelmintic[12], analgesic, anti-inflammatory, antipyretic[13], diuretic and laxative [14]. The major constituents of bark are

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triterpenes, tripernoid glycosides, saponins, indole alkaloids cadambine, 3α-dihydrocadambine, cadamine, isocadamine and isodihydrocadambin[15-17]. Cholorogenic acid isolated from the leaves[9]. The tribes of Ganjam district of Orissa drink the root paste duly suspended in water in reducing blood sugar in the patients with diabetes mellitus. Studies substantiating its use in diabetes are lacking. In the present study was undertaken to evaluate the hypoglycemic properties of the root in experimental animal models to provide a scientific support to the folklore claims.

MATERIALS AND METHODS

Plant Material

The plant material (root) was collected from the forests of Ganjam district of Orissa during June 2007 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah. A voucher specimen [Sp. No: CNH/ I-I / (17)/2009/Tech.II/28] has been kept in our research laboratory for further reference. After authentication, fresh root were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of Extract

The powdered roots (500 g) after defatting with petroleum ether (60-80^0 C) for 48 h was successively extracted with methanol and water for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. The above extracts were used for further studies such as colour, consistency and extractive values. Fluorescence characteristics of liquid extracts were observed under daylight and ultraviolet light separately at short and long wavelengths [18]. Standard methods [19-23] were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them. The results are depicted in the tables (Table 1 to 3).

Table 1.
Data showing the colour, consistency and extractive values of methanol and aqueous extracts of A. cadamba roots.

Sl. No.	Solvent extract	Colour	Consistency	% w/w of extract.
I.	Methanol	Dark Brown	Sticky with brown stain	9.2
II.	Aqueous	Brown	Sticky	15.6

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Table 2.

Fluorescence characteristics of liquid extracts of A. cadamba roots under daylight and ultraviolet light.

Sl. No.	Reagents	Colour		
		Day light	Short uv	Long uv
I.	Methanol	Light brown	Violet	Yellowish green
II.	Aqueous	Brown	Dark Brown	Brown fluorescence

Table 3.

Preliminary Phytochemical Test for methanol and aqueous extracts of A. cadamba roots.

Extract	Phytoconstituents present
Methanol extract	Alkaloids, Flavonoids, Tannins, Saponins, Sugars
Aqueous Extract	Flavonoids, Tannins, Saponins, Sugars

Animals

Swiss albino mice (20–25 g) of either sex were used for acute toxicity study and adult Wistar albino rats (150-200 g) of either sex were used for the antidiabetic evaluation. The animals were kept in standard polypropylene cages at room temperature of 34 ± 2^0 C and at 60-65 % relative humidity during the experimental work. The institutional Animal Ethics Committee approved all the experimental protocols (registration number: 1050/ac/07/CPCSEA).

Acute toxicity study

The test was carried out as suggested by *Ganapaty et al*., 2002 [24]. Selected animals were divided into different groups of six in each. The control group received 1% Tween-80 in normal saline (2 ml/kg, p.o.). The other groups separately received 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg/kg of the test extracts respectively in a similar manner. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any.

Antidiabetic evaluation

Determination of the blood glucose levels

Blood glucose concentrations (mg/dl) were determined using a Medsource osazone biomedicals Pvt. Ltd commercial test (Batch No. GLU-1012 E), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns under mild anaesthesia.

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Using normoglycaemic rats

The method of *Mondal et al.*, 2009 was followed [25]. The animals were fasted for 18 h but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h). The normal rats were then divided into eight groups of six animals each. Group-I served as solvent control and received only vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III to VIII received different extracts at doses of 100, 200 and 400 mg/kg in a similar manner. Blood glucose levels were measured after 1, 2, 4 and 8 h of administration of single dose of test samples. The results are depicted in Table 4.

Table 4.

Effect of methanol and aqueous extracts of the roots of A. cadamba on the blood glucose level in normal rats

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (normoglycaemic study)			
				Time (h) after treatment			
				1	2	4	8
I	Control	2 ml/kg	100.16±3.04	100.83±3.34	100.33±2.51	100.16±2.66	100±1.98
II	Glibenclamide	2.5 mg/kg	96.5±2.95	60.83±.98** (36.96%)	51±2.12** (47.15%)	44.5±4.85** (53.88%)	42.66±4.85** (55.79%)
III	Methanol extract	100	109.33±2.88	102.16±93.83 (6.55%)	93.83±4.28 (14.17%)	73.83±3.76* (32.47%)	69.33±4.35** (36.58%)
IV		200	109.83±3.37	92.33±5.95 (15.93%)	77.5±4.83* (29.43%)	66.16±7.03** (39.76%)	59.83±4.6** (45.52%)
V		400	106.83±2.56	78.33±3.57* (26.67%)	61.16±6.95** (42.75%)	50.5±4.26** (52.72%)	48.16±2.88** (54.91%)
VI	Aqueous extract	100	108.5±3.16	105±4.35 (3.22%)	98±8.36 (9.67%)	75.5±7.16* (30.41%)	73±5.5* (32.71%)
VII		200	110±3.79	102.83±7.42 (6.51%)	84.66±8.12 (23.03%)	75.16±7.92* (31.67%)	70.5±7.43* (35.9%)
VIII		400	109.33±3.32	94.66±8.02 (13.41%)	75.83±9.75* (30.64%)	70.5±7.93* (35.5%)	63.66±8.57** (41.77%)

*Results expressed as Mean ±SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (*One way, *ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.*

Oral glucose tolerance test (OGTT) in rats

The method of *Dash et al.*, 2008 was followed [26]. Fasted rats were divided into eight groups of six rats each. Group I served as a control and received only vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III to VIII received the test extract at doses of 100, 200 and 400 mg/kg respectively in a similar manner. After 30 min of treatment, rats of all groups were loaded

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orally with glucose (2 g/kg, p.o). The Blood glucose concentrations were determined 30, 60, 150 and 180 min after the glucose loading (Table 5).

Table 5.

Effect of methanol and aqueous extracts of the roots of A. cadamba on oral glucose tolerance in normal rats.

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (oral glucose tolerance study)			
				Post treatment			
				30 min.	60 min.	150 min.	180 min.
I	Control	2 ml/kg	93.66±2.69	153.33±13.63	159.83±13.26	167.66±12.44	161.5±13.71
II	Glibenclamide	2.5 mg/kg	96.83±2.84	128.16±7.32	105.16±9.38* (17.94%)	91±10.8** (28.99%)	77.66±10** (39.4%)
III	Methanol extract	100	92.83±9.63	135.5±9.97	122.83±11.48 (7.13%)	117±11.21* (13.65%)	110.83±12.11* (18.2%)
IV		200	94.5±3.75	135.66±12.32	120±10.68* (12.54%)	113.33±10* (16.46%)	106.33±11.32* (21.62%)
V		400	98.83±10.01	135.5±11.92	112.66±8.16* (16.85%)	98.83±7.3** (27.06%)	83.33±6.96** (38.5%)
VI	Aqueous extract	100	91.33±3.79	135.5±13.54	130.16±13.35 (3.94%)	127.66±14.8 (5.78%)	113.83±14.46* (15.99%)
VII		200	90.16±8.83	135.83±10.95	127.5±12.83 (6.13%)	122.66±12.45* (9.69%)	109.16±14.09* (19.63%)
VIII		400	94.16±8.24	136.33±13.5	118.83±11.17 (12.83%)	115.16±10.1* (15.52%)	98.33±9.81** (27.87%)

*Results expressed as Mean ± SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (*One way, *ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.*

Alloxan Induced hyperglycaemic rats

The method of *Mondal et al.*, 2009 was followed [25]. The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided standard laboratory diet *ad libitum*. Two days after the injection, the blood glucose levels were measured and the animals with blood glucose levels higher than 225mg/dl were considered to be diabetic. The animals were segregated into eight groups of six animals in each. Group-I served as negative control and received vehicle (2 ml/kg p.o.) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III to VIII received the different extracts at doses of 100, 200 and 400 mg/kg in a similar manner. Blood glucose levels were estimated at 0, 1, 2, 4 and 8 h respectively after administration of single dose of test samples (Table 6).

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Table 6.

Effect of methanol and aqueous extracts of the roots of A. cadamba on the blood glucose level in alloxan induce diabetic rats.

Group	Treatment	Dose (mg/kg)	Fasting	Blood glucose concentration (mg / dl) (Hypoglycemic study)			
				Time (h) after treatment			
				1	2	4	8
I	Control	2 ml/kg	239.33±2.2	249.83±1.86	255.66±1.9	258.83±2.12	262.16±5.02
II	Glibenclamide	2.5 mg/kg	240.16±10.2	187.5±10.68* (21.92%)	155±14.88** (35.45%)	112.66±9.23** (53.08%)	98.33±9.93** (59.05%)
III	Methanol extract	100	236.83±14.84	210.83±16.24* (10.97%)	204.66±15.09* (13.58%)	190.33±16.2* (19.63%)	177.5±22.89* (25.05%)
IV		200	234.83±10.16	201±10.11* (14.4%)	186.66±10.15* (20.51%)	158.66±13** (32.43%)	128.16±10.2** (45.42%)
V		400	235.5±14.73	194.66±14.75* (17.34)	156.33±14.05** (33.61%)	128.5±6.58** (45.43%)	102.83±8.92** (56.33%)
VI	Aqueous extract	100	239.83±11.29	233.33±11.35 (2.84%)	219±13.25 (8.68%)	206.83±11.39* (13.75%)	199.83±14.17* (16.67%)
VII		200	237.5±13.59	228.66±13.21 (3.72%)	202.66±15.02* (14.66%)	199±20.26* (16.21%)	192.83±14.8* (18.8%)
VIII		400	235±13.69	218.66±14.46 (6.95%)	183.5±13.86* (21.91%)	175.83±17.8* (25.17%)	148±22.88** (37.2%)

*Results expressed as Mean ± SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (*One way, *ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.*

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's-t test. A P-value<0.05 were considered to be significant. All the values were expressed as mean ± SEM.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening of methanol and aqueous extracts revealed presence of alkaloids, saponins, flavonoids, tannins and sugars in the test extracts. In acute toxicity study, it was found that the methanol and aqueous extract induced sedation, diuresis and purgation at all tested doses. However, there was no mortality in any of the extracts at tested doses till the end of 14 days of observation. Reports of the normoglycaemic study (Table 4) reveals that the test extracts exhibited significant reduction in blood glucose concentration in

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a dose dependant manner as compare to control. It was observed that methanol and aqueous extracts reduced 54.91% and 41.77% blood glucose levels at 400 mg/kg, p.o. respectively where as glibenclamide (2.5 mg/kg, p.o) showed 55.79%in rats after 8h treatment.

The effect of test extracts on glucose tolerance test in normal rats is shown in Table 5. At 30 min after glucose administration the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. All the tested extracts (100, 200 and 400 mg/kg, p.o.) exhibited significant hypoglycaemic effect but glibenclamide and methanol (200 and 400 mg/kg) extract significantly depressed the peak of blood glucose level at 60 min after glucose loading.

In antihyperglycaemic study (Table 6), the rise in the blood glucose level was observed after 24 h of alloxanization to the animals. Single administration (100, 200 and 400 mg/kg, p.o.) of the methanol and aqueous extracts of root of *A. cadamba* in diabetic rats showed significant reduction in blood glucose level, where as methanol extract (400 mg/kg) was found maximum reduction in blood glucose level (56.33%) at the end of 8 h. The results of the methanol extract are comparable to that of the reference standard glibenclamide.

Traditional medicinal plants with various active principals and properties have been used from ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, cancer and coronary heart diseases. Beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring the integrity and function of beta-cells, releasing insulin activity, improving glucose uptake and utilization, and the antioxidant properties present in medicinal plants, offer an exciting opportunity to develop them into novel therapeutics [27]. The antihyperglycaemic activity of ***A.*** *cadamba* extract may be due to the presence of several bioactive antidiabetic principles.

Administration of alloxan caused rapid destruction of pancreatic beta-cells in rats, which led to impaired glucose-stimulated insulin release and insulin resistance, both of which are marked feature of type II diabetes. The hypoglycaemic effect of plant extract is generally dependent upon the degree of pancreatic beta-cell destruction and useful in moderate alloxan induced diabetes. The lesser the degree of pancreatic beta-cell destruction, the more useful the herb is in treating diabetes in animals.

The active ingredient in the extract that reduces the blood sugar is not known at present. There is ongoing research to isolate and characterize the bioactive compound(s) responsible for the antidiabetic activity of *A. cadamba.*

CONCLUSION

From the present study it is apparent that the roots of *A. cadamba* possess hypoglycaemic activity and it justify the use of the roots of the plant for treating diabetes as suggested in the folklore remedies.

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