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Antihyperglycemic and antilipidemic activity of *Anthocephalus cadamba* (roxb.) Miq. roots

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ABSTRACT

Methanolic root extract of Anthocephalus cadamba (Family: Rubiaceae) was evaluated for its antihyperglycemic and antilipidemic activity in alloxan induced diabetic rats. The extract was given orally in two different doses (200 and 400 mg/kg) for 28 days. Glibenclamide (2.5 mg/kg) was used as a standard drug for activity comparison. Various parameters studied were blood glucose concentration, serum lipids, glycosylated haemoglobin and liver glycogen. The extract showed significant antihyperglycemic activity in dose dependent manner. Further, the extract was favourably and significantly corrected the alterations in the values of the lipid parameters, organ weights, liver glycogen and glycosylated haemoglobin content in diabetic rats. Therefore, it may be suggested that the methanolic root extract of A. cadamba has potential ability to prevent the secondary complications of diabetes mellitus like atherosclerosis.

Keywords: *Anthocephalus cadamba*, Glibenclamide, Antidiabetic, Antilipidemic.

INTRODUCTION

The increasing prevalence of Type 2 diabetes mellitus (DM) worldwide is an issue of major socio economic concern. DM is a complex and multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins [1]. The World Health Organization estimates that approximately 150 million people have DM worldwide, and that this number may well double by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles [2]. Hyperglycemia in the diabetics is associated with alteration of glucose and lipid metabolism and modification in liver enzymes level [3, 4]. Liver is an important insulin dependent tissue which plays a pivotal role in glucose and lipid metabolism and is severely affected during diabetes [5]. Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. In DM, lipid abnormalities are almost the rule. Typical finding are elevation of total and VLDL cholesterol, triglyceride concentration, lowering of HDL cholesterol and a predominance of small, dense LDL particles [6]. Lipid abnormalities in patients with diabetes are likely to play important role in the development of atherogenesis [7]. Medicinal plants have always played an important role in the management of DM especially in developing countries since time immemorial. From the beginning of last century, evidence of lipid lowering properties of medicinal plants

has also been documented [8]. In recent years, many traditionally used medicinal plants have been tested for their antidiabetic potential in experimental animals [9]. Few of the traditional plant treatments for diabetes has received scientific scrutiny and the WHO has recommended that this area warrants immediate attention and there is still an unmet need for medicinal plants and phytopharmaceuticals with scientifically proven antidiabetic activity.

Anthocephalus cadamba (Roxb.) Miq. Syn. *Neolamarckia cadamba* var *A. chinensis* (Family: Rubiaceae), commonly known as Kadam (Hindi), distributed all over India, is a large tree up to 37.5 m high and 2.4 m in girth with straight cylindrical bole [10-12]. In folk medicine, it is used in the treatment of fever, uterine complaints, blood diseases [13, 14], skin diseases [15], eye inflammation, diarrhea [16], anaemia, leprosy, dysentery and stomatitis [17]. The tribes of Ganjam district of Odisha grind the fresh root and drink its suspension in water daily to reduce blood sugar in the patients with diabetes mellitus since time immemorial and they claim for its promising activity. Few biological activities viz. anti-hepatotoxic [18], antimalarial [19], analgesic, anti-inflammatory, antipyretic [20], diuretic and laxative [21] have been reported by various authors in the literature. Presence of few indole alkaloids viz. cadambine, 3α-dihydrocadambine, cadamine, isocadamine and isodihydrocadambin has been reported in the barks [22-24]. Cholorogenic acid has been identified in the leaves [18]. In the present paper, we report the antidiabetic and antihyperlipidemic activities of the methanol extract of the roots of *A. cadamba.*

MATERIALS AND METHODS

Preparation of Plant Extract

Fresh roots were collected from the forests of Ganjam district of Odisha during June 2012 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah, India. After authentication, fresh roots were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The powdered material (500 g) was defatted with petroleum ether and extracted with methanol for 48 h in a soxhlet extractor. The liquid extract was concentrated under vacuum to yield dried extractive. The percentage yield was calculated with respect to the dried plant material (yield: 6.4 %w/w). Preliminary phytochemical screening of the extract was performed using standard methods [25, 26].

Animals

Male Wistar rats (150-200 g) were acclimatized to the laboratory conditions for a period of 10 days at room temperature. The animals were kept in standard polypropylene cage and maintained under standard environmental conditions with free access to food and water *ad libitum.* All the procedures were performed in accordance to Institutional Animal Ethics Committee.

Screening for antidiabetic activity [27-29]

The acclimatized animals were kept fasting for 24 hours with water *ad libitum* and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided standard laboratory diet *ad libitum*. The fasting blood glucose was estimated after 48 h of alloxan administration to confirm the diabetic state. Rats showing fasting blood glucose more than 225 mg/dl were considered diabetic and divided into different groups each comprising of six animals. Glibenclamide (2.5 mg/kg) was used as reference standard for activity comparison. The test samples were suspended in 0.5% sodium carboxy methyl cellulose in distilled water. All the test samples were fed to the animals through oral route. Group I comprised of normal rats and Group II as the diabetic rats (controls) which received only vehicle (2ml/kg). Animals of Group III and IV (diabetic rats) received the methanol extract of at 200 and 400 mg/kg. Group V (diabetic rats) served as positive control and received glibenclamide (2.5 mg/kg). Treatment with the test samples (twice daily) were carried out for 28 days. Blood collection was done by tail vein method of each rat by collecting a drop of blood from the tip of the tail. The blood glucose level was measured with a pre-standardized blood glucose monitoring system using haemogluco strips (Senso card blood glucose meter supplied by M/s Avecon health care Pvt. Ltd., Himachal Pradesh). Glucose concentration in the blood of each rat from every group was estimated on 0, 7, 14, 21 and 28 day of the study (Table 1).

Biochemical studies

On the 29th day, the animals were sacrificed by cervical dislocation [30]. The entire blood was collected through cardiac puncture, centrifuged at 4000 rpm for 15 min and the serum was separated for biochemical study. The major organs like the liver, kidney and pancreas were taken out and weighed (Table 2).

Serum lipid profile (Total cholesterol, Triglycerides, HDL-cholesterol, LDL cholesterol and VLDL-cholesterol) was measured by enzymatic colorimetric methods (Table 3), using commercial kits supplied by Span Diagnostics, Mumbai, India. For the estimation of liver glycogen content, the liver was homogenized in 5% w/v trichloroacetic acid and its glycogen content was determined by the method of Caroll *et al* [31]. Glycosylated haemoglobin content was estimated by the method of Gabbay *et al* [32]. The results are depicted in Table 4.

Statistical analysis

Data from the experiments were analyzed using one way- Analysis of Variance (ANOVA) followed by Dennett's Multiple Comparison test. Values were expresses as mean ± SEM. $p<0.05$ was considered as the minimal level of statistical significance.

RESULTS AND DISCUSSION

Preliminary phytochemical tests of the methanol extract revealed presence of alkaloids, terpenoids, flavonoids and saponins.

Screening for antidiabetic activity

Single dose of administration of alloxan showed rise of blood sugar level more than two times from the normal level within 48 h. The results of effect of methanol extract of *A. cadamba* are presented in Table 1. As shown in the table, the extract showed significant decrease in blood glucose level in a dose dependant manner. In diabetic rats, the blood glucose level was reduced from 289.85 to 194.32 mg% and from 278.7 to 185.2 mg% with 200 and 400mg/kg doses of the extract respectively on the 28th day. Glibenclamide (2.5 mg/kg) caused a reduction of blood glucose level from 285.67 to 114.75 mg%.

Alloxan is known to cause direct and selective cytoxicity to the pancreatic β-cells by causing cell membrance disruption after its intracellular accumulation [33], resulting in a decrease in endogenous insulin secretion and release, which leads to decreased glucose utilization by the tissues [34]. In the present study, the dose of alloxan (150 mg/kg, i.p.) was selected in order to partially destroy the pancreatic β-cells. In these conditions, insulin was secreted but not sufficiently to regulate the blood glucose [35], thus leading to the significant increase of fasting blood glucose level in alloxan induced diabetic rats. In our present study, we have observed that the methanol extract of *A. cadamba* could reverse the hyperglycaemic condition in diabetic rats and brought about hypoglycaemic action because blood glucose once lowered by the extracts did not increase again throughout experiment as compared to untreated alloxanized control, where the blood glucose level was always remaining above the initials. The possible mechanism of action of the test extracts may be due to by promoting the insulin release from the undestroyed β-cells or its action may be insulin like [36].

Organ weights

Table 2 reveals the organ weights of experimental animals. It was observed that the weights of the isolated organs (liver, kidney and pancreas) of the sample treated groups were higher than the diabetic control group of animals. Induction of diabetes with alloxan is associated with a characteristic loss of tissue proteins [37]. The treatment with *A. cadamba* resulted in an improvement in the body weight as compared to the diabetic rats which may be due to their protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis.

Biochemical studies

As shown in Table 3, the diabetic rats showed elevated levels of serum cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol. A decreased value of HDL-cholesterol was also noticed. Oral treatment of the extract at tested doses caused significant alterations of the above lipid parameters and the effect appeared to be comparable to that of glibenclamide. Diabetes mellitus is known to cause hyperlipidemia through various metabolic derangements amongst which insulin deficiency has been known to stimulate lipolysis in the adipose tissues and give rise to hyperlipidemia and fatty liver. Thus in diabetes hyperlipidemia and hypertriglyceridaemia often occur [29]. The subsequent hyperlipidemia shown by diabetic rats can be used as an index for hyperglycemia.

Table 4 reveals the effect of the methanol extract on liver glycogen and glycosylated haemoglobin. It is observed that the extract significantly improved the liver glycogen and glycosylated haemoglobin contents at the tested doses and the activity were found to be comparable to the reference standard.

Table 1: Effect of methanol extract of *A. cadamba* on blood glucose concentration in alloxan induced diabetic rats

Experimental groups	Fasting Blood Glucose (mg/dl)				
	0 Day	7 Day	14Day	21Day	28Day
Control	78.35±9.9	77.17±11.34	79.15±13.06	80.17±12.37	79.62±10.65
Diabetic control	289.45±16.9	291.17±17.94	293.62±20.08	297.25±21.69	297.37±23.42
Diabetic + 200mg extract	289.85±19.09	278.97±19.86	258.55±19.07	240.5±23.61	194.32±25.9**
Diabetic + 400mg extract	278.7±16.77	262.95±19.31	237.4±21.19	209.3±22.79*	185.2±25.29**
Diabetic + Glibenclamide	285.67±12.69	248.7±16.12	210.55±19.17*	149.27±12.73**	114.75±12.12**

*Data expressed as mean ±SEM. Evaluation by One Way-Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control.*

Table 2: Effect of methanol extract of *A. cadamba* on organ weights in experimental rats

Experimental groups	Organ weight (g)		
	Liver	Kidney	Pancreas
Control	7.44±0.24	1.21±0.1	0.6±0.06
Diabetic control	4.38±0.16	0.82±0.1	0.44±0.06
Diabetic + 200mg extract	5.99±0.12**	0.9±0.09	0.52±0.02
Diabetic + 400mg extract	6.22±0.11**	1.17±0.08*	0.47±0.05
Diabetic + Glibenclamide	7.02±0.09**	1.18±0.05*	0.5±0.04

*n=6; Data expressed as mean ±SEM. Evaluation by One Way-Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control.*

Table 3: Effect of methanol extract of *A. cadamba* on lipid profile in experimental rats

Experimental groups	T-CH	TG	HDL-CH	LDL-CH	VLDL-CH
Control	76.65±7.8	44.5±5.24	25.45±3.34	42.3±4.64	9.56±0.6
Diabetic control	198.4±6.11	142.35±6.23	17.9±2.7	160.8±15.12	31.6±2.47
Diabetic + 200mg extract	167.17±9.15**	123.75±5.16**	23.05±2	113.77±12.19*	20.6±2.46**
Diabetic + 400mg extract	115.4±13.81**	84.62±10.8**	30.27±2.59*	76.87±11.19**	18.02±3.04**
Diabetic + Glibenclamide	96.97±9.78**	78.25±5.68**	35.3±3.76**	59.5±9.02**	17.67±2.6**

T-CH: Total cholesterol; TG: Triglycerides; HDL-CH: HDL cholesterol; LDL-CH: LDL cholesterol; VLDL-CH: VLDL cholesterol
*n=6; Data expressed as mean ±SEM. Evaluation by One Way-Analysis of Variance (ANOVA) Followed by Dunnett's Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control.*

Table 4: Effect of methanol extract of *A. cadamba* on liver glycogen and glycosylated hemoglobin in experimental rats

Experimental groups	Liver glycogen (g/100g)	Glycosylated hemoglobin (%)
Control	3.22±0.56	6.02±0.07
Diabetic control	0.91±0.06	10.05±0.18
Diabetic + 200mg extract	2.22±0.13*	8.41±0.19**
Diabetic + 400mg extract	2.91±0.08**	7.33±0.12**
Diabetic + Glibenclamide	3.25±0.14**	6.82±0.17**

*n=6; Data expressed as mean ±SEM. Evaluation by One Way-Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control.*

CONCLUSION

In the present study, antidiabetic activity of methanol root extract of *A. cadamba* was evaluated in Wister rats using alloxan induced diabetes model. The study revealed significant improvements in different biochemical parameters we have studied. Rats treated with the extract showed improvement in liver glycogen, HDL cholesterol and has shown its ability to enhance the glycogenesis process in the liver of the diabetic rats. Further, the extract significantly reduced the levels of LDL cholesterol and increased that of HDL cholesterol. The facilitation of atherogenesis by LDL cholesterol is due to its role in depositing cholesterol in the vascular bed. HDL cholesterol however carries out the reverse transport of excess cholesterol from cells of tissues to the liver. Thus along with antidiabetic activity, the methanol root extract of *A. cadamba* has the potential to prevent formation of atherosclerosis and coronary heart disease which are the secondary complications of diabetes mellitus.

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