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Antihyperglycemic and antihyperlipidemic properties of aqueous root extract of *Icacina senegalensis* in alloxan induced diabetic rats

Akuodor GC[1*], Udia PM[1], Bassey A[2], Chilaka KC[3], Okezie OA[4]

[1]*Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria*
[2]*Department of Pharmacology, College of Health Sciences, University of Uyo, Nigeria*
[3]*Department of Pharmacology and Therapeutic, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[4]*Department of Chemical Pathology, Federal Teaching Hospital, Abakaliki, Nigeria*

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ABSTRACT

Objective: To evaluate the antidiabetic and hypolipidemic activity of aqueous root extract of *Icacina senegalensis* (*I. senegalensis*) in alloxan-induced diabetic rats. **Method:** Blood glucose levels of alloxan-induced diabetic rats were monitored after the administration of *I. senegalensis* extract (100, 200 and 400 mg/kg) to diabetic rats for 14 d. Different biochemical parameters, serum cholesterol, serum triglyceride, high density lipoprotein, low density lipoprotein and very low density lipoprotein were also examined. **Results:** Treatment of alloxan diabetic rats with the extract showed significant ($P<0.05$) activity. The activity of the extract was comparable to that of the standard drug, glinbeclamide. **Conclusions:** The results suggest that the root extract of *I. senegalensis* possesses antidiabetic and hypolipidemic properties, which might be a potential source for isolation of new orally active agent in the treatment of diabetes and its associated complications.

1. Introduction

Daibetes mellitus is a group of syndromes characterised by hyperglycermia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases. Defects in carbohydrate metabolism machinery and consistent effort of the physiological system to correct the imbalance in the carbohydrate metabolism place an overexertion on the endocrine system. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia[1,2]. Diabetes Mellitus with its fatal complications is a major public health problem in developed as well as developing countries[3].Though different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes. There is an increasing demand by patients to use natural products with blood glucose lowering activity[4,5]. The use of ethnobotanicals has a long folkloric history for the treatment of blood glucose abnormalities[6]. Therefore, the search for medicinal plants with hypoglycaemic properties are increasingly been sought for in the treatment of this debilitating condition

Icacina senegalensis (*I. senegalensis*) A. Juss (Family: Icacinaceae) is a savannah suffurex with glabrous or pubescent leafy shoots of about 2-3 feet high and a large fleshy tuber with creeping roots. The plant is indigenous to west and central Africa[7,8]. It grows wild on light sandy soils in the savannah areas of Senegal, Gambia, Ghana, Nigeria, Guinea, Central African Republic, Congo and parts of Sudan. Different parts of the plant, especially the leaves, root and stem are widely employed in traditional medicine. The plant is very extensively used in rural areas in the treatment of diarrhoea, and to cure malaria.

The present study was undertaken to evaluate the antihyperglycemic and antihyperlipidemic properties of the aqueous root extract of *I. senegalansis* in alloxan-induced

*Corresponding author: Akuodor GC, Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria.
Tel: +2348036725237.
E-mail: goddyakuodor@yahoo.com

diabetic rats.

2. Materials and methods

2.1. Collection of plant material

The roots of *I. senegalensis* were collected from a farm land in Orlu, Imo state, Nigeria. The plant material was identified and authenticated by Mr Frank. I Akpejoye of the Department of Botany, University of Calabar, Nigeria, where voucher specimen (No.620) is maintained.

2.2. Extraction

The roots were cleaned, cut into pieces and air-dried at room temperature for 7 d and ground to powder using mortar and pestle. Two hundred grams of the grounded root powder was then soaked in distilled water for 24 h and filtered. The filtrate was dried on a water bath at reduced temperature to recover the extract and the yield calculated to be 12.5% w/w. The root extract was subsequently reconstituted in normal saline at appropriate concentration for the experiment.

2.3. Phytochemical screening

Phytochemical analysis of the aqueous root extract was carried out employing standard procedures to determine the following compounds; Flavonoids, tannins, saponins, terpenoids, alkaloids, cardiac glycosides, steroids and anthraquinones[9,10].

2.4. Animals

Swiss albino rats (180-220 g) of both sexes obtained from Animal House, Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria, were used for the study. The animals were housed in cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had free access to water.

2.5. Acute toxicity study of the extract

The LD_{50} of the leaf extract was tested to determine the safety of the agent according to the guidelines set by OECD (Organization for Economic Cooperation and development) No. 423[11]. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats per group and administered 100, 600 and 1 000 mg/kg of the extract orally. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. The results of this phase informed the choice of doses for the second phase, in which 2 000, 3 000 and 5 000 mg/kg were administered to another set of three rats per group. The mice were also observed for signs of toxicity and mortality for 72 h.

2.6. Induction of diabetes

Thirty albino rats of both sexes weighing (180-220 g) were used for the study. The animals were kept in standard cages at room temperature and 12 h light/dark condition in animal house of the Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria. The animals were fed on standard feed and had water ad libitum. Diabetes was induced by intraperitoneal injection of 150 mg/kg of alloxan monohydrate (Sigma, St. Louis, MO, USA), freshly prepared in normal saline and administered to overnight fasted rats. 72 h after, rats with blood glucose levels greater than 200 mg/kg were considered diabetic and selected for the study.

2.7. Experimental design

The alloxan-induced diabetic albino rats were randomly grouped into five groups of six each (n=6). Group 1 which served as negative control received normal saline (10 mL/kg), groups 2, 3 and 4 received (100, 200 and 400 mg/kg) of the root extract, while group 5 which served as positive control received Glibenclamide (10 mg/kg). All administered orally.

2.8. Determination of blood glucose levels

All blood samples were collected by tail tipping method. The blood glucose levels and body weight were measured on day 1, 7 and 14 of the study. Determination of the blood glucose concentrations was done using Accu check glucose kit.

2.9. Hypolipidemic activity and Biochemical analysis

On day 14, the animals were anaesthetized with chloroform and the blood collected through cardiac puncture into sample bottles devoid of anticoagulant (Zayo-Sigma clot activator). The samples were centrifuged at 1 000 rpm for 5 min to obtain serum. All lipid profile parameters were determined. Total cholesterol, triglyceride and high density lipoprotein (HDL) were measured using Agappe diagnostic kit. All samples were analysed with (SM23A, MICROFIELD,

ENGLAND) spectrophotometer. The concentrations of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were determined using Sophia and Manoharan[12] method. The concentration of potassium and sodium in serum were estimated using Spectrum diagnostic kit. Serum chloride, serum urea and serum creatinine were also determined using Spectrum diagnostic kit.

2.10. Statistical analysis

The results obtained were expressed as mean±SEM. The data were analysed using one-way ANOVA. $P<0.05$ was considered as significant.

3. Results

3.1. Phytochemical sreening

Phytochemical analysis of the aqueous root extract of *I. senegalensis* revealed the presence of the following compounds; alkaloids, flavonoids, saponons, tannins, terpenoids steroids and cardiac glycosides and anthraquinones. These classes of compounds are reported to show important biological activities[13,14].

3.2. Acute toxicity

The acute oral toxicity test showed normal behaviour of the treated rats. There was no mortality observed at a high dose of 5 000 mg/kg. Hence the experimental doses used (100, 200 and 400 mg/kg p.o.) were within safe margin.

4. Discussion

This study has shown the potential antidiabetic and hypolipidemic effects of *I. senegalensis* on alloxan-induced diabetic rats. Phytochemical compounds such as tannins, saponins, alkaloids, flavonoids steroids and terpenoids present in this extract have been implicated in antidiabetic and hypolipidemic activity[15-17]. These constituents may in part be responsible for the observed significant activity of this root extract either singly or in synergy with one another. Additionally, regeneration of B-cells may not be ignored as the probable mechanism by which *I. senegalensis* root extract produced a significant reduction in blood glucose in the treated rats. This is because improved blood glucose in the treated animals suggests either increased insulin release or improved insulin activity both of which could be attributed to improvement in the integrity of

Table 1
Effect of aqueous root extract of *I. senegalensis* on body weight of alloxan induced diabetic rats (g).

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14
Control	0.2 mL	162.17±2.66	172.50±1.61	190.67±2.72
I. senegalensis	100	159.50±1.68	153.00±1.57	160.00±1.03*
	200	162.20±0.79	159.83±1.54	171.50±0.99*
	400	162.20±0.79	155.00±1.71	160.33±1.41*
Glibenclamide	10	160.33±1.08	156.00±2.09	162.00±1.41*

Data are expressed as mean±SEM, *significant at $P<0.05$ when compared to control ($n=6$).

Table 2
Effect of aqueous root extract of *I. senegalensis* on glucose level of alloxan induced diabetic rats (mg/dL).

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14
Control	0.2 mL	91.67±1.76	83.17±0.48	80.33±0.21*
I. senegalensis	100	283.50±1.94	201.00±0.86*	173.50±1.80*
	200	281.33±1.41	167.50±2.04*	122.17±0.98*
	400	282.50±1.80	141.33±1.14*	108.00±0.86*
Glibenclamide	10	282.83±1.19	138.50±0.81*	105.00±1.00*

Data are expressed as mean±SEM, *significant at $P<0.05$ when compared to control ($n=6$).

Table 3
Effect of aqueous root extract of *I. senegalensis* on lipid profile of alloxan induced diabetic rats (mg/dL).

Treatment	Dose (mg/kg)	TC	TG	HDL	LDL	VLDL
Control	0.2 mL	199.83±2.59	142.33±1.56	47.17±1.01	59.50±0.72	41.83±0.48
I. senegalensis	100	172.33±3.55*	130.17±0.54*	44.00±1.46*	56.00±0.93*	36.00±1.15*
	200	166.00±2.38*	118.83±2.66*	41.50±0.92*	53.17±1.33*	32.33±0.49*
	400	158.50±2.84*	93.17±2.60*	38.00±1.34*	50.67±0.33*	28.67±0.49*
Glibenclamide	10	153.17±2.33*	74.50±2.04*	36.83±1.22*	49.17±0.83*	25.83±0.79*

Data are expressed as mean ±SEM *significant at $P<0.05$ when compared to control ($n=6$)

Table 4
Effect of aqueous root extract of *I. senegalensis* on some kidney function parameters of alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Na^+(mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	Urea(mmol/L)	Creatinine(mmol/L)
Control	0.2 mL	143.20±0.34	5.33±0.20	106.67±0.49	7.00±0.63	88.67±2.11
I. senegalensis	100	139.33±0.49*	4.00±0.26*	99.50±2.06*	4.50±0.22*	74.50±1.82*
	200	137.17±0.60*	3.83±0.31*	91.00±3.02*	3.83±0.31*	65.67±3.17*
	400	135.33±0.71*	3.67±0.21*	87.83±1.76*	3.50±0.22*	61.17±3.73*
Glibenclamide	10	136.67±1.52*	3.83±0.30*	89.67±1.89*	4.00±0.26*	60.33±2.76*

Data are expressed as mean±SEM, *Significant at $P<0.05$ when compared to control ($n=6$).

B-endocrinocytes[18-20]. The fundamental mechanism in diabetes mellitus involves excessive hepatic glycogenolysis and gluconeogenesis and the decreased utilization of glucose by the tissues[21]. Alloxan induces diabetes in experimental animals through beta cells destruction[22]. It has been shown that cell apoptosis is related to alloxan-induced inhibition of pancreatic glucokinase function and there is selective beta cell loss[23], leading to insulinopaenic diabetes[24-28]. The antidiabetic activity of aqueous root extract of *I. senegalensis* was significant ($P<0.05$) in decreasing blood glucose level at doses of 100, 200 and 400 mg/kg p.o. In diabetes, hyperglycemia is associated with dyslipidemia[29,30], representing risk factor for coronary heart diseases[31,32]. The abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots due to the actions of insulin. In normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. Moreover, in diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia[33], and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities. The dyslipidemia is characterized by increase in TC, TG, LDL, VLDL and fall in HDL. This altered serum lipid profile was reversed towards normal after treatment with aqueous root extract of *I. senegalensis*. The underlying mechanism of lipid lowering effect of the root extract could be by inhibition of lipid absorption due to the presence of saponins and tannins[34] or inhibition of cholesterol esterase, activation of fatty acid synthase, and production of triglyceride precursors such as acetyl-CoA and glycerol phosphate[35].

Serum level of potassium, sodium, chloride, urea and creatinine were observed to significantly decrease in diabetic rats following the administration of *I. senegalensis* root extract. The kidney function is compromised in uncontrolled diabetes mellitus. This result showed that the aqueous root extract has a kidney protective effect.

In conclusion, the results of the present study indicate that indeed the aqueous root extract of *I. senegalensis* possesses antihyperglycemic and antihyperlidemic properties and alleviate kidney damage from diabetes. Further studies on its isolation, identification and characterization of the active principles are in progress in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

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