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RESEARCH ARTICLE

HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITIES OF ETHANOLIC ROOTS EXTRACT OF *CROSSOPTERYX FEBRIFUGA* IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The study investigated the activities of antihypoglycemic and antihypolipidemic of ethanolic roots extract of *Crossopteryx febrifuga* (CF) in alloxan-induced diabetic rats. **Methods:** Twenty male albino rats were randomly distributed to four groups; I, II, III and IV with each consisting of five animals received extracts as follows: Group I, *C .febrifuga* (500 mg/kg bwt); Group II, *C.febrifuga* (250 mg/kg bwt); Group III, *C. febrifuga* (100 mg/kg bwt); Group IV, 0.5 ml (2% w/v) acacia solution and served as control. After 30 min, the animals were each administered orally with 20% (w/v) glucose at a dose of 0.5ml /100 g bwt. Blood glucose levels were then monitored at 30, 60, and 120 min. intervals and reported as the average glucose level of each group. Twenty- Five (25) Albino rats were randomly divided into five (5) experimental groups: control, diabetic, standard drug (glibenclamide) and *C. febrifuga* (375 and 500 mg/kg bwt) treated diabetic groups. The animals in four out of five groups were fasted for 18 h and were made diabetic by injecting with a single dose of alloxan (ALX) 150 mg/kg. Blood was collected on days 0, 5, 10 and 15 for glucose estimation and day 15 for lipid profile assay. **Results:** A significant reduction in postprandial sugar level was observed after 60 min in all treatments. Alloxan-induced diabetic rats without treatment showed significant increases ($p < 0.05$) in the levels of blood glucose, triglycerides, total cholesterol, low density lipoprotein LDL-cholesterol while the high density lipoprotein HDL-cholesterol level were significantly decreased ($p< 0.05$) compared to normal rats. In addition, the diabetic rats treated with the CF and glibenclamide showed significant decrease ($p < 0.05$) in both blood glucose, TG and LDL-cholesterol levels and a significant decrease ($p < 0.05$) in HDL-cholesterol level compared to diabetic untreated rats. **Conclusion**: The ethanolic roots extract of *Crossopteryx febrifuga* (CF) possesses potential hypoglycemic and hypolipidemic activities in alloxan-induced diabetic rats.

Keywords: *Crossopteryx febrifuga*, glibenclamide, alloxan, hypoglycemic, hypolipidemic, postprandial.

INTRODUCTION

Diabetes has become one of the major devastating diseases afflicting health of many people, in recent times, and has accounted for a high proportion of health problems worldwide [1]. It affects at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [2].It is also associated with the long term complications, including retinopathy, nephropathy, neuropathy and angiopathy [3]. Managing blood sugar levels and keeping it closer to normal values is able to reduce the risk of diabetes and death by these complications. Impaired carbohydrate and protein utilization in the diabetic also leads to accelerated lipolysis, which results in elevated plasma triglycerides levels [4].

To reduce the risk of vascular complications in diabetes mellitus, control not only of blood glucose levels but also lipid levels and weight are necessary [5].

Although treatment of diabetes with insulin and many oral hypoglycaemic agents has recorded huge successes, they were however, associated with some serious side effects like recurrent cases of hypoglycemic coma and obesity [6].

Moreover, these orthodox medications seem to be insufficient to prevent diabetic complications. These limitations to a large extent accounted for drift towards alternative therapies that include herbal formulations [7]. Plant products have been long used in the treatment of diabetic patients through improving the diabetic status in terms of blood sugar [8, 9, 10]. For these vital reasons, therefore, there is an urgent need for a search for cheap and safe blood sugar lowering oral hypoglycaemic agents that would be effective in the treatment of diabetes and lack of serious side effects of the currently used oral hypoglycemic agents.

Crossopteryx febrifuga belongs to Rubiaceae Family, It is a twisted tree with conspicuous tubular flowers, which is widely distributed throughout the Savannah region of West and Tropical Africa. It is commonly found in all part of Nigeria and known as Ayeye among yorubas in Nigeria. It is used traditionally for symptomatic relief of dry cough and for treatment of respiratory infections, fever, dysentery and pain [11]. In northern Nigeria, the plant has been used for treatment of pain and malaria [12]. It has been reported that the crude methanolic extract of *C. febrifuga* contains biologically active substances with potential values in the treatment of trypanosomiasis, malaria, Staph aureus infection [13, 14]. [15] have reported that the extract possesses analgesic, antipyretic and anti-inflammatory activities. [16] has also reported the gastroprotective effect of the plant. There are claims by some herbal practitioners that aqueous roots extract of *Crossopteryx febrifuga* is effective in the management of DM and diabetic complications. The scientific evidence to back this claim is lacking. In view of this, the study was aimed at the evaluation of the hypoglycemic and hypolipidemic effects of ethanolic roots extract of *Crossopteryx febrifuga* in alloxan-induced diabetic rats.

MATERIALS AND METHOD

PLANT MATERIAL

Collection of Plant material

Crossopteryx febrifuga (CF) was collected from cultivated farmland at Ojokoro, Ifako-Ijaiye, Lagos State, Nigeria, in the month of November, 2012. The plant was identified and authenticated by a botanist, Prof. J. Dele Olowokudejo of Botany department of University of Lagos, Nigeria, where voucher specimen has been deposited in the herbarium.

Preparation of plant extract

The roots of the plant were shade-dried at room temperature for 7 days and then powdered using mortar and pestle. The root powder 1150 g was soaked with 96% aqueous alcohol in three cycles using soxhlet extractor. The crude extract was filtered with Whitman filter paper 4, and the filtrate was concentrated and dried in a rotary vacuum evaporator under reduced pressure in vacuole 30^0C to obtain 108.2g dry residue to yield an (9.41% vol.) viscous brownish-coloured extract which was stored in an air tight bottle kept in a refrigerator at 4^0C till used.

Experimental Animals

Forty five healthy male albino rats weighing between 170-200 g were obtained from the Laboratory Animal Center of College of Medicine, University of Ibadan, Ibadan, Nigeria. The rats were housed in clean cages with the filter tops under controlled conditions of 12 h light/dark cycle, 50% humidity at 26 ± 2^0C and kept in a well-ventilated room and allowed to acclimatize to the laboratory condition for two week before being used. They were maintained on a standard animal pellet (CHI Feeds Plc., Nigeria) and had free access to water *ad libitum*. The animals were distributed randomly into four groups of five animals each for postprandial study and into four groups of five rats each for the alloxan-induced diabetic experiment and the fifth group consists of 5 rats served as the control (normal) group.

Acute Toxicity Studies

The acute toxicity of ethanolic roots extract of *Crossopteryx febrifuga* were determined by using thirty-five (35) male Swiss albino mice weighing between 20-22.5 g, which were maintained under the standard conditions. The animals were randomly distributed into a control group and six treated groups, containing five animals per group. After depriving them food with 12 h prior to the experiment with access to water only, the control group was administered with single dose of ethanolic roots extract of *Crossopteryx febrifuga* with at a dose of 0.3ml of 2% Acacia solution orally while each treated group was administered with single dose of ethanolic roots extract of *Crossopteryx febrifuga* orally with at a doses of 1.0, 2.5, 5.0, 10, 15 and 20.0g/kg body weight respectively of 2% acacia solution. They were closely observed in the first 4 hours and then hourly for the next 12 hours followed by hourly intervals for the next 56 hours and continued for the next 2weeks after the drug administration to observe any death or changes in behaviour, economical, neurological profiles and other physiological activities [17, 18].

Postprandial Test

Twenty albino rats were randomly distributed to four groups; A, B, C and D with each consisting of five animals. There were only fasted for about 18hrs prior to the experiment with access to water only [19]. Glucometer (Accu-Chek, Roche Diagnostics) was used to estimate their initial blood sugar level. The extracts suspension was respectively prepared by dispersing 3.75 g of the extract dissolved in 25 ml acacia (2% W/V), solution.

The animals were treated as follows:

Group I received *Crossopteryx febrifuga* (500 mg/kg b wt)

Group II received *Crossopteryx febrifuga* (250 mg/kg b wt)

Group III received *Crossopteryx febrifuga* (100 mg/kg b wt)

Group IV received 0.5ml (2% W/V) acacia solution and served as control.

After 30min, each animal was administered orally with 20% (W/V) glucose at a dose of 0.5ml/100g bwt. Blood glucose levels were monitored at 30, 60 and 120 min intervals and reported as the average glucose level of each group intervals after post glucose challenge.

Experimental Design

Diabetes was induced by intraperitoneal (ip) injection of alloxan (150 mg/kg body weight) dissolved in 3ml of normal saline [20]. After 72 h blood was taken from the lateral veins of the tail and the blood sugar levels were monitored with a glucometer (ACCUCHEK, Roche Diagnostics) for estimation blood sugar level. The animals with blood sugar level more than 200 mg/dl [21] were considered diabetic and included in the experiment.

The diabetic animals were randomly distributed into four groups of five animals each while the last group, the positive control, consists of five normal rats and were treated as follows:

Group I: Normal rats (positive control).

Group II: ALX- induced diabetic animals but untreated to serve as negative control.

Group III: ALX- induced diabetic treated with Glibenclamide (10 mg/kg b wt)

Group IV: ALX- induced diabetic treated with *Crossopteryx febrifuga* extract (375 mg/kg b wt)

Group V: ALX- induced diabetic treated with *Crossopteryx febrifuga* extract (500 mg/kg b wt)

Evaluation of Hypoglycaemic and Hypolipidaemic Effects

The animals were treated daily for fifteen days. On the 15th day after treatment they were starved overnight, and on the 16th day, they were anaesthetized with diethyl ether and blood obtained via cardiac puncture into heparinized container. The blood was centrifuged within 5 min of collection at 5000rpm to obtain plasma which was analyzed for glucose level, total cholesterol, total triglyceride, HDL-cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics [22]. LDL-cholesterol levels were calculated using Friedwald equation [23].

Statistical Analysis

Data is reported as Mean ± SD. Statistical comparisons were determined by analysis of variance (ANOVA) and means were separated using Duncan's multiple range test ($p < 0.05$).

RESULTS

Acute Toxicity

The acute toxicity study result (Table 1), showed that five out of the five animals that received 20.0 g/kg body weight of the extract died within 4 h (100 % death) while one out of the five animals that received 1 g/kg body weight of the extract died within 24 h (20%). The oral route of LD_{50} of the drug was found to be from 1.0- 2.5 g/kg b wt. The median lethal dose is therefore calculated from the graph to be 1.85 g/kg bwt. The LD_{50} of the extract was determined by plotting a graph of probit on the Y-axis against the log dose on the X-axis.

Table 1: Acute Toxicity Study of Ethanolic Roots Extract of *Crossopteryx Febrifuga* on Mices

Groups	Dose g/kg	Log dose	24hr Motility	% Motility	Probit	Probit Approx
I	1.0	3.000	1/5	20	4.158	4.2
II	2.5	3.397	2/5	40	4.746	4.7
III	5.0	3.699	4/5	80	5.841	5.8
IV	10.0	4.000	5/5	100	8.719	8.7
V	15.0	4.176	5/5	100	8.719	8.7
VI	20.0	4.301	5/5	100	8.719	8.7

Control group received 0.3ml each of 2% Acacia solution.

Effect on Body Weights

The animals (Table 2) showed decrease in appetite and weight loss after alloxan induction. In the diabetic untreated group, exhibited progressive weight decrease occurred while in the ethanolic roots extract of *Crossopteryx febrifuga* /glibenclamide treated, there was slight weight appreciation after few days of treatment with the ethanolic roots extract of *Crossopteryx febrifuga* /glibenclamide as well as showed increased in appetite.

Table 2: Weight Variations

Groups	Day 1	Day 5	Day 10	Day 15
I	165.6±0.8	170.4±1.0	172.2±1.2	178.7±1.5
II	172.8±1.5	164.6±1.3	157.2±1.7	142.9±1.0
III	175.2±2.2	178.2±2.3	184.5±2.8	198.5±1.7
IV	172.4±1.1	173.6±0.9	175.2±1.2	176.1±1.3
V	175.4±1.4	176.8±1.5	178.6±1.1	180.4±1.6

Mean ± SD, n = 5, *P< 0.05 vs control group

Effect of the Extract on Postprandial Study

The postprandial test result (Table III) showed a significant decrease ($p < 0.05$) in blood glucose levels in all treated groups after 60min of oral glucose administration compared to the control. The extract was observed to be active in lowering the postprandial the blood glucose level (Table 3).

Table 3: Postprandial Test

Groups	0 min	30 min	60 min	120 min
I	89±6.8*	105±10.6*	93.0±4.1*	94.2±6.3*
II	90±4.3*	108±5.9*	96±4.7*	97±7.1*
III	88±9.7*	109±8.2*	99±8.3*	100±6.5*
IV	89±5.5	108±4.8	115±6.5	118±6.0

Values are Mean ± SD, n = 5, *p < 0.05 compared to control group.

Effect of the Extract/Glibenclamide on Blood Glucose

Table 4 showed the results of the extract and glibenclamide effects on the blood glucose. The plasma glucose levels of the diabetic rats treated with the extract and glibenclamide were significantly reduced ($p < 0.05$) compared to the diabetic control. The drug proved to have a better plasma glucose lowering effect than glibenclamide.

Table 4: Effect of ethanolic roots extract of *crossopteryx febrifuga* and glibenclamide on glucose levels.

Groups	Day 0	Day 5	Day 10	Day 15
I	91.4±3.8	88.5±5.8	75.8±4.5	87.3±5.6
II	324.2±22.7	337.6±23.5	354.4±25.2	365.7±28.5
III	197.3±8.5*	160.2±10.3*	122.3±8.5*	87.6±7.8*
IV	189.6±5.7*	147.7±6.2*	105.2±6.8*	62.4±6.4*
V	181.7±3.5*	136.8±5.6*	91.7±5.4*	54.8±4.7*

Values are Mean ± SD, n = 5, *p < 0.05 compared to control.

Effect of the Extract/Glibenclamide on Lipid Profile

Table 5 showed a significant decrease ($p < 0.05$) in TCHOL, TG and LDL-cholesterol levels while significant increase in HDL-cholesterol level was observed in all diabetic animals treated with the drug or glibenclamide. In contrast, the untreated diabetic animals showed significant increase in both TG and LDL-cholesterol levels and a significant decrease in HDL-cholesterol level.

Table 5: Effect of ethanolic roots extract of *crossopteryx febrifuga* on lipids levels in alloxan-induced diabetic rats.

Groups	Total Chol	HDL	LDL	TG
I	64.5±1.2	35.7±1.0	10.8±3.6	47.1±1.4
II	66.6±3.7	19.2±0.7	58.2±6.5	125.8±1.2
III	42.5±1.3*	30.7±0.9*	28.6±2.7*	57.5±2.5*
IV	36.4±0.4*	30.5±1.2*	21.3±2.0*	32.5±0.8*
V	29.5±2.1*	32.5±0.4*	18.8±4.1*	22.6±1.1*

Values are Mean ± SD, n = 5, *p < 0.05 compared to control.

DISCUSSION

Diabetes is now recognized as one of the major threat diseases and a leading cause of mortality and morbidity, claiming many lives worldwide over [24]. Oral hypoglycemic agents such as sulphonylureas and biguanides have been commonly used in the management of diabetes especially type II but are with serious side effects. It has been observed that hypoglycaemic activities occur from the stimulation of pancreatic beta cells to release insulin [25, 26]. In addition, attention has been focused on the use of plants and herbal remedies believed to be safer and lack of serious side effects as alternatives in the management of DM and diabetic complications.

The median acute toxicity value (LD_{50}) of the drug was determined to be 1.85 g/kg bwt. According to 27, 28, the extract can be classified as being slight toxic, since the LD_{50} by oral route was found to be 1-2.5 g/kg which was much slight closer to WHO toxicity index of 2 g/kg.

Although increase in appetite and water consumption was observed in the diabetic and normal animals treated with the ethanolic roots extract of *crossopteryx febrifuga*, there was significant weight loss by the diabetic animals without treatment.

The significant slight weight gain observed in the diabetic animals treated with the drug clearly suggested that the ethanolic roots extract of *crossopteryx febrifuga* might not have the obesity forming tendency, which is one of the undesirable side effects normally encountered when treating diabetics with sulphonylureas. There were also no changes observed in the macroscopic examinations of the organs of the diabetic animals treated with the ethanolic roots extract of *crossopteryx febrifuga* or glibenclamide while the organs of diabetic untreated animals showed some changes compared to the normal control.

The ethanolic roots extract of *crossopteryx febrifuga* showed to be effective in decreasing plasma glucose levels on the diabetic rats and proved to have a better plasma glucose lowering effect than glibenclamide.The effective lowering of blood sugar level demonstrated by this drug supports its local use as a hypoglycaemic agent.

Lipid inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids [29]. In diabetic conditions, enhanced activity of this enzyme (lipase) increases lipolysis and release more free fatty acids into circulation [30]. The lowering of plasma TC, TG and LDL-cholesterol levels and significant increase in HDL-cholesterol level in the treated animals clearly demonstrated the presence hypolipidaemic agents in the ethanolic roots extract of *Crossopteryx febrifuga*.

CONCLUSION

The study observed that the *Crossopteryx febrifuga* have good postprandial sugar lowering effect and showed that the *Crossopteryx febrifuga* possesses some hypoglycaemic and hypolipidaemic activities.

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