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Modulating Roles of Ethanolic Roots Extract of *Crossopteryx febrifuga* on Blood Glucose, Lipid Profile, Glycosylated Haemoglobin and Cytoarchitectural Changes on Pancreatic Beta Cells in Alloxan-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study, carried out the laboratory work and performed the statistical analysis. He equally wrote the first draft of the manuscript and undertook the final editing of the paper. Authors OSO, OTO and AMA wrote the protocol, took part in the laboratory work, part of the draft and undertook in the initial editing of the paper. Authors BJD, OAO, LAE and WSN carried out most of the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The study investigated the modulating roles of ethanolic roots extract of *Crossopteryx febrifuga* (CF) for its antihyperglycemic, antihyperlipidemic, glycosylated hemoglobin effects and cytoarchitectural changes on pancreatic beta cells in alloxan-induced diabetic rats

Study Design: Experimental diabetes using animal models.

Methodology: Twenty- Five (25) male albino rats were randomly divided into five (5) experimental groups: control, diabetic, standard drug (glibenclamide 10 mg/kg body wt) 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, Diabetic rats 5 per group received graded doses (375 and 500 mg/kg bwt) of the extracts and glibenclamide 10 $mgkg^{-1}$ for 15days. Blood was collected on days 0, 5, 10 and 15 for glucose estimation. Lipid profile was measured using DiaSys Kits from Germany which utilized the colorimetric method. Insulin Assay was measured using Monobind Insulin Microplate Elisa test while HbA_1C was analyzed by Biosystem Kits (Barcelona Kits, Spain) using chromatographic method.

Twenty (20) male albino rats were randomly distributed to four groups; I, II, III and IV with each consisting of five animals received 20% (w/v) glucoseorally at a dose of 0.5ml /100 g bwt. After 30 min, the 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. Blood glucose levels were then monitored at 30, 60, and 120 min. intervals and reported as the average glucose level of each group.

Results: A significant reduction in postprandial sugar level was observed after 60min in all treatments.

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 blood glucose, TG and LDL-cholesterol levels and a significant decrease ($p<0.05$) in HDL-cholesterol level compared to diabetic untreated rats. There were significant reductions ($p<0.05$) in low density lipoprotein (LDL)-cholesterol levels and significant increase ($p<0.05$) in the treated diabetic group compared to the negative control.

Apart from these, cytoarchitectural changes also revealed the protective nature of the ethanolic roots extract of *Crossopteryx febrifuga* against alloxan induced necrotic damage of pancreatic tissues.

Conclusion: The ethanolic roots extract of *Crossopteryx febrifuga* modulated hyperglycemic by potentiating insulin release from the beta cells of pancreas and ameliorated dyslipidaemia.

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Keywords: Crossopteryx febrifuga; postprandial test; hyperglycemia; beta cells; lipid profile; glycosylated haemoglobin.

1. INTRODUCTION

Diabetes is a chronic metabolic disorder, associated with very high morbidity and mortality rate [1].

Diabetes mellitus is now an epidemic with a worldwide prevalence from 171 million in 2000 to 366 million in 2030 [2].

The number of people living with the ailment is expected to multiply with major impact on the population of the developed and developing countries due to increased rate of industrialization [3].

Diabetes mellitus is a debilitating disease which is characterized by hyperglycemia, hyperlipidemia and raised basal metabolic rate defect in reactive oxygen species scavenging enzymes [4].

Hyperglycemia resulting tolow level of production of insulin leads to a number of complications; cardiovascular, renal, neurological and ocular [5].

Recently, hyperglycemia has been major factor in the development and initiation of diabetic complications that affects nerves and arteries [6].

In diabetes, there is an indication of severe alteration in the concentration of carbohydrate, protein and lipid. The lipid metabolism has been major aetiology of vascular disease in diabetes mellitus which is oxidative in nature [7].

In diabetic conditions, enhanced activity of this enzyme lipase increases lipolysis and release more free fatty acids into circulation [8-9].

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, bizarre behaviour, confusion, coma, obesity and seizures [10].

The increasing popularity in the use of herbal remedies could be attributed to their advantages of being efficacious and a cheap source of medical care. In addition, there is an urgent need to identify indigenous natural sources of new active substances that are less toxic, safe for human consumption and readily available to combat the disease.

Crossopteryx febrifuga belongs to family of Rubiaceae, It is a twisted tree with conspicuous tubular flowers, it 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 dry cough and 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* used in the treatment of trypanosomiasis, malaria, Staph aureus infection [13-15]. It has been reported that the extract possesses analgesic, antipyretic and anti-inflammatory activities [16]. It has also been reported that the extract has gastroprotective effect [17].

Despite the widespread use of this plant in the management of various ailments, its protective effects on the metabolic disorder have not been investigated.

The present study was performed to investigate modulating roles of ethanolic roots extract of *Crossopteryx febrifuga* on blood glucose, lipid profile, glucosylated hemoglobin and cytoarchitectural changes on beta cells of pancreas in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Collection of the Plant Material

Crossopteryx febrifuga (CF) roots were collected from cultivated farmland at Ojokoro, Ifako-Ijaiye, Lagos State, Nigeria, in the month of February. The plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) by a taxonomist, where voucher specimen has been deposited in the herbarium.

2.1.1 Preparation of the plant extract

The roots of the plant were shade-dried at room temperature for 7 days and then powdered using mortar and pestle. 900g of the root powder was soaked in 96% ethyl alcohol in three cycles using soxhlet extractor. The crude extract was filtered with filter paper (Whatman 4), and the filtrate was concentrated and dried in a rotary vacuum evaporator at 30°C to obtain 135.4 g dry residue to yield an (15% vol.) viscous brownish-coloured extract which was stored in an air tight bottle kept in a refrigerator at 4°C till used [18]

2.2 Preliminary Phytochemical Screenings

The presence of saponins, tannins, alkaloids, flavonoids, terpenoids, carbohydrates, glycosides and reducing sugars in the extract were tested by using simple and standard qualitative methods earlier described [19-21].

2.3 Experimental Design

Forty five healthy male albino rats weighing between 170-190 g were obtained from the Laboratory Animal Center of College of Medicine, Lagos State University, Ikeja, Lagos, 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°C and kept in a well-ventilated room and allowed to acclimatize to the laboratory condition for two weeks before being used. They were maintained on a standard animal pellet (CHI Feeds Plc., Nigeria) and had free access to water. 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 consist of 5 rats served as the control (normal) group.

2.4 Acute Toxicity Studies

The acute toxicity of ethanolic roots extract of *Crossopteryx febrifuga* were determined by using thirty-five (35) male and female Swiss albino mice (20-22.5g) 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 with food for12h 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 solutions 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 [22-23].

2.5 Postprandial Test

Twenty albino rats were randomly distributed to four groups; I, II, III and IV with each consisting of five animals. There were fasted for about 18hrs prior to the experiment with access to water only [24]. Glucometer (Fine test, Infopia Diagnostics) was used to estimate their initial blood sugar level. Each animal was administered orally with 20% (W/V) glucose at a dose of 0.5ml/100g bwt. The extracts suspension was respectively prepared by dispersing 3.75g of the extract dissolved in 25 ml acacia (2% W/V), solution.
After 30min, 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.

Blood glucose levels were monitored at 30, 60 and 120 min intervals and reported as the average glucose level of each group.

2.6 Experimental Design

To induce diabetes, rats were first anesthetized with inhalation of gaseous nitrous. ALX was purchased from representative of Sigma Company in Nigeria and was prepared in freshly normal saline. Diabetes was induced by intraperitoneal (ip) injection of alloxan monohydrate (150 mg/kg b wt) in a volume of 3 mL [25]. After 72 h, blood was withdrawn for blood glucose estimation monitored with a glucometer (Fine test, Infopia Diagnostics). The animals with blood glucose level ≥ 250 mg/dl were considered diabetic and included in the experiment.

The diabetic animals were randomly distributed into four groups of five animals each while the fifth (last) group, the positive control, had five normal rats.

Treatments were as follows:

Group I: Normal rats (positive control).
Group II: Diabetic untreated rats (control negative).
Group III: Diabetic rats treated with Glibenclamide 10 mg /kg bwt
Group IV: Diabetic rats treated with *Crossopteryx febrifuga* at a dose of 375 mg/kg bwt
Group V: Diabetic rats treated with *Crossopteryx febrifuga* at a dose of 500 mg/kg bwt.

2.7 Effect of Extract on Average Body Weight of Rats

On day 1 and 15 respectively, the rat weights were taken with Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland) and the difference in weight from the initial weight per group was calculated.

2.8 Sample Collection of Lipid Profile

Blood collected and centrifuged within 5 minutes of collection at 4000rpm for 10 minutes to obtain plasma, which was analyzed for total cholesterol (TC), total triglyceride (TG) and high density lipoprotein-cholesterol (HDL-Chol) levels by using DiaSys Kits from Germany which utilized the colorimetric method as described by [26]. Low density lipoprotein-cholesterol (LDL-Chol) levels were calculated using Friedwald equation [27].

2.9 Assay of Insulin and HbA1C

The insulin assay was measured using Monobind Insulin Microplate Elisa test (Diagnostic Automation Inc. USA) as described by [28]. While the HbA1C assay was analyzed by Biosystem Kits (Barcelona Kits, Spain) using chromatographic method as described by [29].

2.10 Histopathological Study

On the last day of experiment, the tail parts of the pancreas were removed from each group was fixed in Bouin's fluid.

This was done as described by Ojewale et al. [25]. Briefly, after 48h the organs were removed from Bouin's fluid and fixed in fresh Bouin's fluid for another 72h. Each pancreatic tissue was sliced into slabs of about 0.5 cm thick and dehydrated in varying degree of alcohol (70%, 90%). From 90% alcohol to 3 changes of absolute alcohol for 1 hour each, then into chloroform for about 10 h and later transferred into fresh chloroform for about 30 min. The tissues were placed in 3 changes of molten paraffin wax for 30 min each in an oven at 57°C.They were placed vertically in molten paraffin wax inside a plastic mould and left overnight to cool and solidify. They were later trimmed and mounted on wooden blocks. Serial sections were cut using a rotary microtome at 5 µm thickness. Sections were floated in a water bath and picked by albuminized slides and dried on the hot plate at 57°C. To stain, the slides were de-waxed in staining racks and placed in staining wells containing xylene and rehydrated in varying degree of alcohol (absolute, 90%, and 70%) and then to water for 5min after which they were stained with heamatoxylin for 3 min. Excess heamatoxylin was washed off with water and differentiated with 1% acid alcohol. Sections were rinsed under running tap water and then left for 5 min for blueing. Sections were counterstained with 1% eosin and washed off with water. They were dehydrated with 70%, 90% and absolute alcohol and cleared in xylene to remove all traces of water. A drop of mountant was placed on the surface of the slide and covered with a 22 by 22 cm cover slip. Light microscopy was used for the evaluations and the photomicrographs were taken.

2.11 Statistical Analysis

Data are presented as means ± SD. Student'st-test analysis was applied to test the significance of differences between the results of the treated, untreated and control groups. The difference was considered significant at the conventional level of significance (p<0.05).

3. RESULTS

3.1 Phytochemical Analyses

The phytochemical analysis revealed the presence of carbohydrates, reducing sugars,

glycosides, flavonoids, terpenoids, steroids, saponins, tannins, Anthraquinones and alkaloids as shown in Table 1.

Table 1. Phytochemical analyses of the extract of the roots of *Crossopteryx febrifuga*

Phytochemical constituents	vailability
Carbohydrates	+
Glycosides	+
Cardiac glycosides	+
Saponins	+
Flavonoids	+
Terpenoids	+
Anthracene derivative	+
Alkaloids	+

+ = Present; - = absent.

3.2 Acute Toxicity

The acute toxicity study result Table 2, showed that five out of the five animals that received 20.0, 15.0 and 10.0 g/kg bwt of the extract died within 4 h (100% death) while the animals that received 1 g/kg body weight survived beyond 24 h. The LD_{50} of the drug was therefore calculated to be 2.145 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 2. Acute toxicity of the ethanolic roots extract of *Crossopteryx febrifuga*

Groups	Dose (g/kg)	Log dose	24hr Mortality	% Mortality	Probit
I	1.0	3.00	0/5	0.0	0.0
II	2.5	3.40	2/5	40.0	4.7
III	5.0	3.70	3/5	60.0	5.2
IV	10.0	4.00	5/5	100.0	8.7
V	15.0	4.18	5/5	100.0	8.7
VI	20.0	4.30	5/5	100.0	8.7

Control group received 0.3ml each of 2% Acacia solution

3.3 Effect of the Extract on Postprandial Study

The postprandial test result Table 3 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 blood glucose level in postprandial study.

Table 3. Glucose levels (mg/dl) in the postprandial Study

Groups	0 min	30 min	60 min	120 min
I	82±5.7*	103±10.3*	96.0±4.6*	94.2±7.4*
II	94±4.5*	106±5.6*	98±4.9*	93±6.7*
III	92±7.6*	108±8.4*	99±7.2*	101±5.5*
IV	91±4.7	111±4.8	107±6.5	112±5.8

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

3.4 Effect of the Extract/Glibenclamide on Weight of the Animals

The animals Table 4 showed decrease in appetite and weight depreciation after alloxan administration. In the diabetic untreated group, there was progressive weight decrease while in the *Crossopteryx febrifuga*/glibenclamide treated, there was weight appreciation after few days of treatment as well as showed increase in appetite.

Table 4. The body weight (wt) changes

Groups	initial body wt (g)	Final body wt (g)	diff in body wt (g)
I	170.6±1.7	198.1±2.8	27.5
II	181.3±2.4	155.1±3.1	-26.2
III	176.4±1.6	198.7±2.0	22.3*
IV	172.3±2.1	179.6±2.6	15.2*
V	178.5±2.0	178.3±1.8	17.6*

**: Statistically significant when compared to control group (I) at p<0.05;*

3.5 Effect of the Extract/Glibenclamide on Blood Glucose Level

The blood glucose level in diabetic group was significantly higher ($p<0.05$) than those of the control group as shown in Table 5. On the other hand, administration of ethanolic roots extract of *Crossopteryx febrifuga* (at 375 & 500 mg/kg) exhibited a dose dependent significant antihyperglycemic activity on 5th, 10th and 15th day post-treatment. The antihyperglycemic effect of ethanolic extract (375 & 500 mg/kg) was found more effective than the reference drug, glibenclamide produced a significant reduction in blood glucose compared to diabetic control.

Table 5. Effect of ethanolic extract of *Crossopteryx febrifuga* for 15 days on plasma glucose concentration (mg/dl) in ALX-induced diabetic rats

Groups	0^{th} day	5^{th} day	10^{th} day	15^{th} day
I	78.5±7.6	76.4±8.3	84.5±10.2.	87.6±9.5
II	319.4±16.4	338.7±19.6	346.2±22.5	359.4±20.3
III	297.3+18.6*	271.2±20.4*	258.1±23.7*	244.2±22.3*
IV	286.8±15.2*	265.3±16.4*	254.6±20.4*	231.4±24.6*
V	289.5±17.4*	262.5±16.1*	243.4±15.7*	229.7±18.1*

Mean ± SD, n = 5, $p < 0.05$ vs control group: Statistically significant when compared to control group (I) at p<0.05;*

3.6 Effect of the Extract/Glibenclamide on Lipid Profile Level

Total cholesterol was significant higher when rats became diabetic. In the diabetic treated groups with the ethanolic roots extract of *Crossopteryx febrifuga* Table 6, a significant reduction ($p<0.05$) in plasma cholesterol level was observed in experimental compared to the diabetic untreated group. The higher dose of the extract of 500 mg/kg (122.3±4.4) exerted a more significant reduction than glibenclamide (158.2±1.4). There was a gradual decrease in triglyceride levels in all the treated groups as against the untreated. A significant decrease ($p<0.05$) was observed in levels of triglyceride in diabetic treated group when compared to reference drug, glibenclamide.

The study showed varied degrees of HDL-cholesterol depletion resulting from the effect of diabetes. The depletion was most severe in the untreated group. The treated groups showed significant recovery (p<0.05) over a period of time that varied with graded doses of the extract.

Increase in LDL cholesterol was observed in all the groups due to diabetic activities. The root extract treated group in relation to the untreated exhibited significant reduction (p<0.05) in plasma LDL level.

3.7 Effect of Ethanolic Roots Extract of *C. febrifuga* on Insulin Level

Table 6 showed the plasma insulin levels in diabetic untreated and treated groups. In diabetic untreated group, there was marked decrease in plasma insulin level with significant (p<0.05) increase in blood glucose level compared to normal (control) group. Administration of ethanolic roots extract of *C. febrifuga* (375 and 500 $mgkg^{-1}$bwt) showing dose dependent decrease and glibenclamide (10 $mgkg^{-1}$bwt) significantly increase (p<0.05) the plasma insulin level compared to diabetic untreated group.

3.8 Effect of Ethanolic Roots Extract of *C. febrifuga* on Blood HbA1C Level

Table 6 showed that the level of HbA1C was significantly higher in diabetic untreated group control when compared to normal. Diabetic groups treated with ethanolic roots extract of CF and glibenclamide, there was significant (p<0.05) decrease in plasma HbA1C level compared to diabetic control in which the extract treated showed dose dependent effect. The extracts exerted comparatively more effective decrease than glibenclamide

Table 6. Effect of ethanolic extract of *Crossopteryx febrifuga* for 15 days onlipid profile, insulin and glucosylated heamoglobin levels in ALX-induced diabetic rats

	Groups				
Parameters	**I**	**II**	**III**	**IV**	**V**
TCHOL mg/dL	101.6±1.2	352.6±4.5	158.2±1.4*	128.6±4.2*	122.3±4.4*
HDL mg/dL	35.3±0.4	19.8±0.6	30.6±0.7*	32.6±0.3*	31.9±1.0*
LDL mg/dL	134.6±4.4	344.2±5.8	165.4±3.2*	131.2±1.4*	122.7±2.6*
TG mg/dL	138.3±4.2	496.4±7.4	264.2±4.0*	139.4±2.1*	134.6±4.0*
Insulin µ U/ml	5.8±1.1	3.6±1.42	7.8±2.1*	8.2±1.34*	8.4±1.62*
HbA1C %	4.7±0.32	9.2±0.63	6.4±0.22*	6.1±0.34*	5.8±0.40*

*Values are mean ± SD; n = 5, *p < 0.05 compared to control (Student's t-test).*

3.9 Histopathological Findings

The photomicrograph of normal Fig 1a pancreatic tissue showed intact beta cells which appeared more numerous than the alpha cells. The pancreatic islets were demarcated from surrounding exocrine acini tissue by a thin layer of reticular fibers. In the glibenclamide treated Fig. 1b, mild pyknotic changes were observed at some peripheral cells, the diabetic untreated rats Fig. 1c showed extensive beta cells necrosis with an appearance of amorphous eosinophilia. The photomicrograph of diabetic treated group with extract Fig 1d showed no pathological changes, increased volume density of pancreatic islets and increased percentage of beta cells, in the diabetic rats that received the extracts, which may be a sign of regeneration.

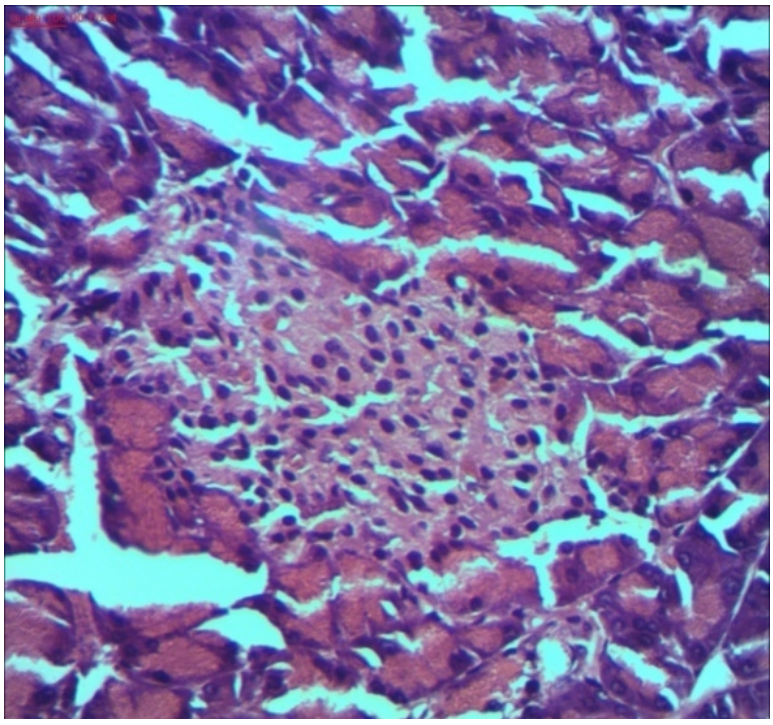

Fig. 1a. Cross section of control (+ve) group

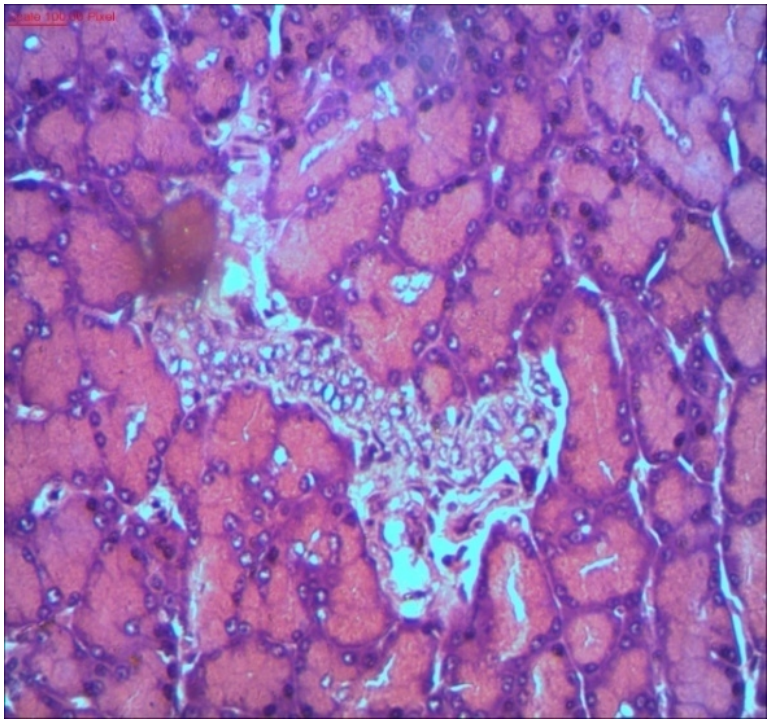

Fig 1b. Cross section of diabetic (-ve) group

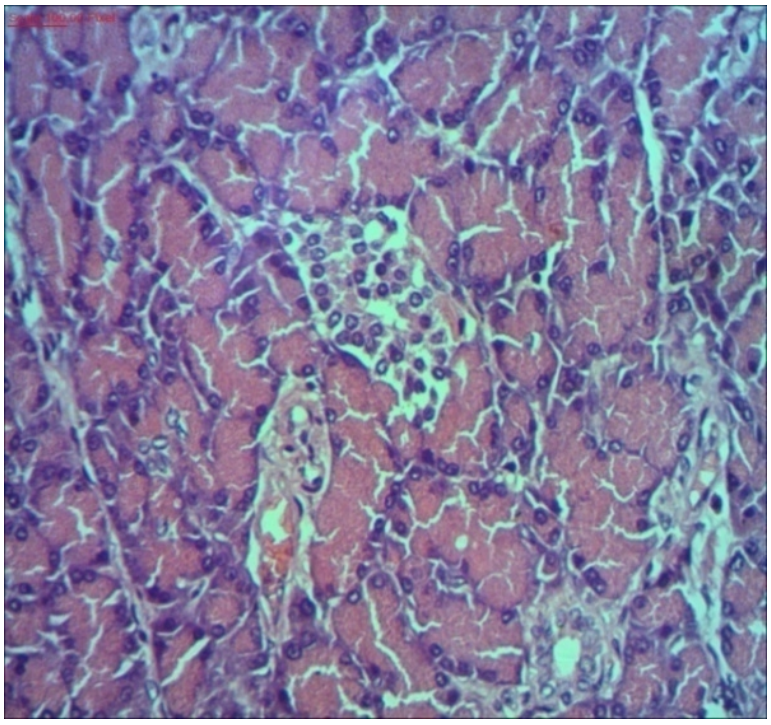

Fig. 1c. Cross section of glibenclamide group

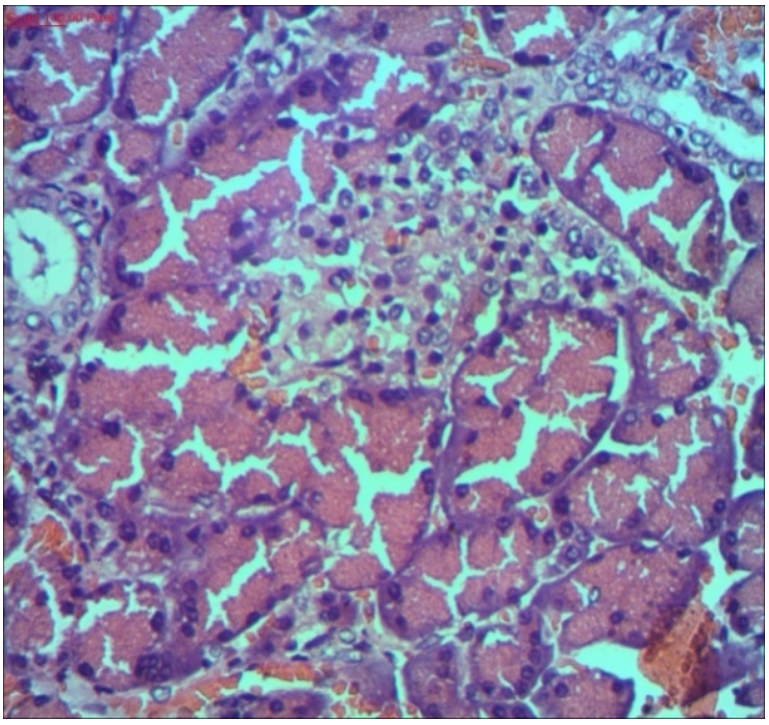

Fig. 1d. Cross section of *C. febrifuga* group

4. DISCUSSION

It was observedin this studythat *Crossopteryx febrifuga* can reverse the metabolic disorders occurring in alloxan induced diabetic rats. Our findings indicate that blood glucose, triglyceride, cholesterol, LDL-C, HDL-C and HbA1C had a significant decrease by contrast HDL-C and Insulin had a significant increase in diabetic rats treated with *Crossopteryx febrifuga* and diabetic rats treated with glibenclamide as compared with diabetic rats. Insulin level increased significantly in diabetic groups received without treatment (glibenclamide or *Crossopteryx febrifuga*) when compared with diabetic group without treatment.

Despite the advances made with biguanides, sulphonylureas therapy and thiazolidinedione therapy, plant sources for the management of DM has gained wide acceptance for a number of reasons. Investigations showed that plants are more effective in the management of diabetic complications [30]. Herbal medicine as oral anti-diabetic drug has immensely helped to eradicate the discomfort of continuous insulin infusion or subcutaneous injections in diabetic patients [31].

More importantly, plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than their synthetic counterparts [32-33].

The present study was aimed to investigate modulating roles of ethanolic roots extract of *Crossopteryx febrifuga* on blood glucose, lipid profile, glucosylated hemoglobin and cytoarchitectural changes on pancreatic beta cells in alloxan-induced diabetic rats.

The median lethal dose (LD_{50}) of the drug was calculated to be 2.145 g/kg bwt. The extract can be classified as being slightly toxic according to [23], since the LD_{50} by oral route was between1-2.5 g/kg which was much slightly closer to and higher than 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 extract, there was significant weight loss by the diabetic animals without treatment. The extracts exhibited good postprandial lowering effect on plasma glucose level after 60 min of glucose load indicating that the ethanolic roots extract of *Crossopteryx febrifuga* possess α-glucosidase inhibitory activities.

Alloxan is known for its selective pancreatic β cells cytotoxicity which has been extensively used to induce diabetes mellitus in animals. Alloxan is one of the chemicals used for the induction of diabetes mellitus apart from streptozotocin. [34-36].

The anti-hyperglycaemic effect of the extract may be due to a number of factors. CF root is rich in ingredients that have been reported to possess anti-hyperglycaemic activities like saponins known to be bioactive against diabetes [37]. Terpenoids, flavonoids, glycosides and alkaloids frequently implicated with this activity [38] were also part of its active component. Therefore, the presence of antioxidant compounds such as flavonoids, terpenoids, saponins and minerals in this plant provides further evidence for the beneficial effects of ethanolic roots extract of *Crossopteryx febrifuga* on the ALX-induced diabetic rat. It is possible to suggest that this extract might play a vital role in improving the diabetic status in terms of blood sugar by restoring the structural and functional properties of β-cells of pancreas, a primary target organ for ALX. The activities of these substances may have triggered the beta cells to increase insulin production thus leading to glucose uptake and utilization by other tissues.

Insulin plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lypolysis [11]. It inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids [39].

This investigation showed that alloxan induced diabetes led to different lipid abnormalities. Previous studies documented the activities of alloxan in lipid profile derangement [40-41]. In lipids studies, an elevated level of total cholesterol was observed when the rats became diabetic. Hypercholesterolemia, a common feature in dyslipidemia has been reported in diabetic cases [42].

The marked increase in plasma triglyceride level may be due to depletion in insulin release. Under normal conditions, insulin activates the enzyme lipoprotein lipase and hydrolyses triglyceride [43]. It has been observed that prior to full manifestation of DM, individuals often exhibit an atherogenic pattern of risk factors that include lower level of HDL-cholesterol [44]. Reports indicate that LDL concentrations are predictive of coronary events that are independent of other coronary disease risk factors [45].

The level of HbAIC in the diabetic untreated group significant increased indicating glycosylation of Hb shows reduced affinity to oxygen in the presence of hyperglycaemia HbA1C [46]. In the ethanolic roots extract of *Crossopteryx febrifuga*/glibenclamide treated, showed decrease in HbAIC concentration was observed when compared to that of diabetic rats without treatment indicating decrease in blood glucose level and recovery to Hb. The extract however exhibited more effective decrease.

It could be summarized that the possible mechanisms by which ethanolic roots extract of *Crossopteryx febrifuga* brings about its antihyperglycemic action may be through potentiation of pancreatic secretion of insulin release from the β-cells of pancreas (which was clearly justified by the increased level of insulin in diabetic rats treated with ethanolic roots extract of *Crossopteryx febrifuga* and glibenclamide).

5. CONCLUSION

The ethanolic roots extract of *Crossopteryx febrifuga* (CF) exhibited good postprandial activity, modulated hyperglycemic by potentiating insulin release from the beta cells of pancreas and ameliorated dislipidaemia. On the other hand**,** the ethanolic roots extract of *Crossopteryx febrifuga* (CF) could be considered as slightly toxic and safe for consumption.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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