Antihyperglycaemic and Antilipidaemic Properties of Ethanol Stem Bark Extract of Crossopteryx Febrifuga in Alloxan-Induced Diabetic Rats

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ABSTRACT

This study investigated Crossopteryx febrifuga stem bark extract for antihyperglycemic and antihyperlipidemic effects in alloxan–induced diabetic rats. Thirty diabetic albino rats were assigned into six groups of five rats each and treated with extract of varying doses (500mg/kg, 1000mg/kg and 1500mg/kg) and standard antidiabetic drug - glibenclamide. Treatment of the diabetic rats was done in seven days through orogastric procedure. The extract showed a significant (p < 0.05) hypoglycemic and hypolipidemic effect in all the doses compared to the diabetic control and standard antidiabetic drug, glibenclamide. The high density lipoprotein (HDL) increased significantly (p < 0.05) and the low density lipoprotein (LDL) decreased in all the groups, as against the diabetic control. On percent scale, the HDL increased by 55.18% and the LDL reduced significantly (p < 0.05) by 73.52% at the dose of 500mg/kg as against the standard drug which showed just 20.69% reduction. Acute toxicity study was done prior to the diabetic study. The method adopted was that of Lorke and plant extract was found to be safe up to the dose of 5000mg/kg. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, flavonoids phytosterols and polyhenolics. The presence of these rich phytoconstituents was the reason for the antihyperglycemic and hypolipidemic property of this plant thus, confirming its use in folklore.

Keywords: Antihyperglycemic, Crossopteryx febrifuga, Diabetes mellitus, Phytochemical, Toxicity

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defect in insulin secretion and insulin inaction (Kangralkar et al. 2010; Adoum et.al. 2012). It is a disease with worldwide significance and increasing prevalence because of its impact on health, quality of life and life expectancy of patients as well as health care system (Amos et al. 1997; Subbiah et al. 2006). This disease affects millions of people worldwide and the number of patients increasing by the day with a population projected to be about 592 million or more by the year 2035 (Guariguata et al. 2013).

Besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogeneses (Seikat et al. 2008). The elevated reactive oxygen species and the simultaneous decline in antioxidative defense mechanisms promote the development of late complications such as ulcer, blindness, neuropathy and renal failure (Eden, 2009). Diabetes produces disturbance in lipid metabolism that is evident as hyperlipidemia and cholesterolemia - a risk factor for atherosclerosis (Schwartz, 2006; Li, 2007).

In different regions of the world particularly Africa and Asia, plant materials have played an important role in the traditional management of diabetes mellitus and herbal remedies continue to be more accessible and affordable than conventional drugs and represent the first line of treatment available to diabetic patients especially in the rural communities (Eze et al. 2012). Today many researchers across the globe have demonstrated the role of medicinal plants in the control of hyperglycemia and hyperlipidemia (Subbiah et al. 2006; Rajagopal and Sasikaka, 2008).

Crossopteryx febrifuga, a plant of the family Rubiaceae is widely reported for its medicinal value (Maiga et al. 2006; Salawu et al. 2008). The plant is a deciduous savanna tree, 1.8-15 meter tall, with a rounded crown and pendulum branches. The bark is pale grey to dark brown. It is widely distributed throughout West and tropical Africa. C. febrifuga is known as Ikwargh gbande by the Tiv tribe of North Central Nigeria. It is called Ayeye among the Yorubas of Southwest and Kasifiya by the Hausas of the Northern Nigeria (Agishi, 2010). The plant is widely used traditionally for the treatment of dry cough, respiratory disease, fever, dysentery, pains and malaria (Audu, 1989; Odugbemi, 2008; Salawu et al. 2008).
A lot of scientific evidences have emerged to support the tradomedical use of *C. febrifuga* as herbal remedies. Thus, the methanol crude extract of the root of the plant was reported to contain bioactive substances with potential values in the treatment of trypanosomiasis, malaria and staphylococcus infection (Hostettman *et al.* 2000; Yusuf *et al.* 2004). The root bark and leaf extract was also reported to have analgesic, anti-inflammatory, antipyretic, antimalaria, antidiabetic and antilipidemic activities (Salawu *et al.* 2008; Ojewale *et al.* 2014; Ajayi *et al.* 2016). Antipyretic activity of aqueous stem bark extract of this plant was reported by Elisee *et al.* (2018). Even though other parts of this plant had been explored for its antihyperglycemic and antilipidemic activity, there is no scientific report so far on the hypoglycemic and hypolipidemic activity of its stem bark. And in view of the fact that different parts of plant may contain different classes of natural products, this research work was carried out to evaluate the phytochemical constituents of ethanol stem bark crude extract of *C. febrifuga* as well as its hypoglycemic and hypolipidemic effects on alloxan-induced diabetic rats.

**MATERIALS AND METHODS**

**Materials**
Deionised water, Centrifuge (Denley B5400 centrifuge, England), Micropipette, Weighing balance, Jenway 6310 Spectrophotometer, Rotary evaporator (Buchi Vacuum Control V-850; Rotavapor, R-210, Germany), Normal saline and Glibenclamid, Accu-check Active Glucometer (Roche Diabetes Care GmbH, Standhofer Strasse 11668305 Mannheim, Germany), Absolute ethanol 99.9% (JBH). All chemical were of analytical grade.

**Plant Collection**
The stem bark of *Crossopterix febrifuga* was collected from wild in Mbalumun-Nanev, Kwande Local Government Area of Benue State, Nigeria. The plant was authenticated by Joseph Waya of Botany Department, Benue State University Makurdi where the specimen’s voucher no: 232 was deposited in the Herbarium unit.

**METHODS and Extraction of Plant Extract**
The stem bark of *C. febrifuga* was chopped and air dried under shade in the natural way for one month. The dry sample was crushed to powder using a mortar and pestle. 600g of the powdered sample was macerated in two liters (2l) of ethanol with occasional shaking for 3 days (72hrs). The mixture was decanted and filtered using Whatmann number one filter paper through vacuum filtration procedure. The filtrate was concentrated using computerized rotary evaporator at 40°C. It was finally evaporated to dryness under standard conditions of temperature and pressure and the dried extract kept in the refrigerator for use.

**Experimental Animals**
Wistar strain albino rats of both sexes (120-200) were purchased from the animal holding unit Central Diagnostic division of the National Veterinary Research Institute of Nigeria VON, Plateau State. The animals were kept in standard cages at room temperature and 12hrs daylight cycle for two weeks (to aclimatize) in the animal house at the Pharmacology Department, Bayero University Kano. These animals were fed freely on commercial feed (Vital Feed) and water.

**Phytochemical Screening**
Qualitative phytochemical screening of the stem bark extract was done using standard procedure (Amita and Shalini, 2014; Chukwuma and Chigozie, 2016).

**Experimental design**
This research work was organized into 3 phases: I, II and III. Phase I was done according to the method of Lorke (1983) to determine the Lethal Dose LD₅₀ of the extract. Phase II constituted the antidiabetes studies. Thirty diabetic albino rats were assigned into six groups of five (5) rats each and treated according to the schedule below (Table 1). Treatment was done once daily by orogastric intubation for seven (7) days. The third III phase was the collection and preparation of serum sample for lipid profile.

**Table 1** Experimental Design and Treatment Schedule for Anti-diabetic Study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Treatment/Drug</th>
<th>Dose(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal/positive control</td>
<td>Normal saline</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic/negative control</td>
<td>Normal saline</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Diabetic treated</td>
<td><em>C. febrifuga</em> extract</td>
<td>500</td>
</tr>
<tr>
<td>D</td>
<td>Diabetic treated</td>
<td><em>C. febrifuga</em> extract</td>
<td>1000</td>
</tr>
<tr>
<td>E</td>
<td>Diabetic treated</td>
<td><em>C. febrifuga</em> extract</td>
<td>1500</td>
</tr>
<tr>
<td>F</td>
<td>Diabetic treated</td>
<td>Standard drug (Glibenclamide)</td>
<td>5</td>
</tr>
</tbody>
</table>

**Acute Toxicity**
Modify method of lorke (1983) was used. Nine (9) albino rats of either sex were divided into 3 groups of 3 rats each. In the first phase, they were administered orally with extract at a single dose of 500mg/kg, 1000 mg/kg and 1500 mg/kg respectively and observed for 24 hrs for signs of toxicity and death. In the second phase, another 3
groups of 1 rat each were given a single dose of 2500 mg/kg, 3500 mg/kg and 5000 mg/kg respectively and were observed for 24hrs for signs of toxicity and death.

**Induction of Diabetes mellitus**

The rats were subjected to 12hrs fast. Diabetes mellitus was then induced by intraperitoneal injection (ip) of 150 mg/kg body weight of alloxan reconstituted in normal saline (0.9%) after fasting blood sugar (78-100mg/dl) were taken. The rats were left on 5% glucose to prevent hypoglycemia (Stanley and Venugopal, 2001). Forty eight hours later, diabetes was confirmed in the rats that had blood glucose level equal to or greater than 230mg/dl.

**Treatment of Diabetes Rats and Determination of Blood Glucose Level, (BGL)**

The diabetes rats were sorted out and treated as outlined in the experimental design above. Treatment was done orogastrically for Seven days with a single dose given per day. Blood sample for the sugar level determination was collected from the tail tip by aid of lancet at 24hrs interval of each treatment made. The blood sugar levels were measured by the glucose- oxidase principle (Beach and turner, 1958) using one Touch Basic Accu-check (active) Glucometer test strips. Results were reported as mg/dl (Rheney and kirk, 2000).

**Collection and Preparation of Serum Sample for Lipid Profile Analysis**

Blood samples were collected from overnight fasted rats via cardiac puncture with sterile syringe into plain tubes and were allowed to clot. Thereafter, the serums were separated by centrifugation using Denley B5400 centrifuge (England) at 3000rpm for 10 minutes. The serums (supernatants) were collected and then assayed for lipid profile.

**Lipid Profile Assay**

This was done spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Northern Ireland) as follows:

(a) Serum Total Cholesterol (TC): The serum total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by the method of Stein (1987).

(b) Serum Triglyceride (TG): This was determined after enzymatic hydrolysis of the sample with lipase as described by the method of Tietz (1990).

(c) Serum High density Lipoprotein Cholesterol (HDL-C): This was measured by the method of Wacnic and Albert (1972).

(d) Serum Low Density Lipoprotein Cholesterol, LDL-C: This was determined according to protocol of Friedewald et al. (1972). All measurements were made in mg/dl.

**Statistical Analysis of Data**

Data obtained were expressed as Mean ± SEM and analyzed using the Analysis of Variance ‘ANOVA’ via the Statistical Package for Social Scientists, SPSS Version 21. Values at p < 0.05 were regarded as significant compared with appropriate controls.

<table>
<thead>
<tr>
<th>Table 2 Phytochemical constituents of the crude ethanol stem bark Extract of <em>Crossopteryx febrifuga</em>.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytochemicals</strong></td>
</tr>
<tr>
<td>Flavonoids</td>
</tr>
<tr>
<td>Tannins</td>
</tr>
<tr>
<td>Saponnins</td>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>Cardiac glycosides</td>
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<tr>
<td>Phenols/polyphenols</td>
</tr>
<tr>
<td>Phytosterols</td>
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<tr>
<td>Anthraquinones</td>
</tr>
</tbody>
</table>

Key: (+) means detected
### Table 3 Effect of Ethanol Stem Bark Extract of *C. febrifuga* on Blood Glucose Level (mg/dl) of Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Day0</th>
<th>Day1</th>
<th>Day2</th>
<th>Day3</th>
<th>Day4</th>
<th>Day5</th>
<th>Day6</th>
<th>Day7</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>78.75±4.89</td>
<td>78.75±4.85</td>
<td>77.25±4.66</td>
<td>76.50±4.03</td>
<td>81.00±2.12</td>
<td>90.00±3.72</td>
<td>89.21±2.05</td>
<td>90.01±3.75</td>
</tr>
<tr>
<td>B</td>
<td>334.25±17.55</td>
<td>379.00±9.42</td>
<td>420.75±20.13</td>
<td>450.75±17.67</td>
<td>447.00±19.66</td>
<td>515.00±8.53</td>
<td>516.23±10.50</td>
<td>514.00±7.53</td>
</tr>
<tr>
<td>C</td>
<td>519.50±9.08</td>
<td>458.75±18.55</td>
<td>393.00±24.70</td>
<td>346.50±16.88</td>
<td>294.60±12.35</td>
<td>225.01±9.50</td>
<td>151.50±18.26</td>
<td>113.75±7.25</td>
</tr>
<tr>
<td>D</td>
<td>325.00±39.68</td>
<td>460.75±34.01</td>
<td>515.25±12.02</td>
<td>470.75±23.71</td>
<td>421.50±24.95</td>
<td>381.25±19.08</td>
<td>321.40±14.53</td>
<td>275.52±11.51</td>
</tr>
<tr>
<td>E</td>
<td>405.00±55.22</td>
<td>495.75±41.00</td>
<td>480.50±28.44</td>
<td>466.50±21.55</td>
<td>436.11±19.63</td>
<td>408.25±12.54</td>
<td>365.23±14.45</td>
<td>329.25±15.33</td>
</tr>
<tr>
<td>F</td>
<td>552.50±9.25</td>
<td>568.75±7.57</td>
<td>501.12±13.65</td>
<td>469.75±19.88</td>
<td>419.67±15.60</td>
<td>369.00±10.68</td>
<td>342.58±30.05</td>
<td>294.75±4.23</td>
</tr>
</tbody>
</table>

Result is significant at p< 0.05 and expressed as mean ± SEM; n=5, a = significant compared to diabetic control; b = significant compared to standard drug. A= Normal control/Positive control; B = Diabetic control; C= Diabetic treated (500mg/kg); D = Diabetic treated (1000mg/kg); E= Diabetic treated (1500mg/kg); F= Standard drug (glibenclamide, 5mg/kg) (student t-test)
Phytochemical analysis of ethanol stem bark extract of *Crossopteryx febrifuga* revealed the present of flavonoids, tannins saponins, alkaloids, cardiac glycosides, phenols, phyoesters and anthraquinones as shown in Table 2. This result is in line with the work reported by Salawu et al. (2008) on the methanol leaf extract and Ojewale et al. (2014) on methanol root extract of the plant respectively. These classes of phytochemicals have been implicated for antihyperglycemic properties (Switi et al. 20014). Previous studies have shown that glycosides for instance have inhibitory effect on alpha-amylase, aldose reductase, alpha-glucosidase and could increase the level of serum insulin. In addition, flavonoids, beside their antioxidant properties, reduce glucose transporter (Cazarolli et al. 2008; Switi et al. 2014). Alkaloids do have a stimulatory action on insulin secretion by activation of imidazole I binding sites in the pancreatic β-cell (Ujah et al. 2015). Thus, these phytochemicals in synergy or unilaterally, could have contributed to improving insulin secretion and action on the glycemic level. Hence the rationale for *C. febrifuga* stem bark extract exerting hypoglycemic effect (Table 3) in the alloxan-induced diabetic rats and its use in the management of diabetes mellitus by folklore.

Phytotoxicity study showed that there was no sign of toxicity in the test rats throughout the 24 hours of administration of the extract up to a dose of 5000mg/Kg. This indicated that ethanol extract of the stem bark of *C. febrifuga* was physiologically safe to the test rats; suggestive that it will reduce the risk posed by synthetic drugs to humans.

Hyperglycemia is an important pathophysiological characteristic of diabetes mellitus. It is a condition that is characterized by persistent high level of unmetabolised sugar in the blood that could eventually lead to diabetic condition (Goldberg, 2018).

Alloxan, a β- cytoxin induces diabetes mellitus by damaging the insulin secreting β- cells of the pancreas resulting in decreased endogenous insulin release (Rajagopal and Sasikala, 2008).

Consequently, intraperitoneal administration of alloxan (150mg/kg) in the test rats effectively induced them and this was used to explore the scientific basis of the ethanol stem bark extract of *C. febrifuga* for correction of hyperglycemia in diabetes mellitus.

Oral treatment of diabetic rats with ethanol stem bark extract of *C. febrifuga* gave a promising results as shown in Table 3. The result showed that the extract significantly (p<0.05) reduced the blood glucose level in all the treated groups (Groups C, D and E) compared to the rats in the diabetic control group (Group B). The 1000mg/kg dose showed a significant reduction of the blood glucose level after day 5 of treatment (with a total reduction of 46.53%). A significant reduction in the blood glucose level was also observed in the diabetic rats treated with 500mg/kg and 1500mg/kg extract starting from day 2 and day 6 respectively. These results were significantly different compared to the diabetic control group which shows a continuous rise in the glucose level. The effect of the extract at all of these doses is also comparable to the standard antidiabetic drug (glibenclamide) which showed significant hypoglycemic effect as from day 5 (Table 3). The most interestingly was the result obtained from treatment at 500mg/kg extract, with significant hypoglycemic effect from day 2. This dose gave the best result and is favourably compared to the standard drug, with a marked reduction in at day 5 by 56.31% as against 28.35% respectively.

Oral hypoglycemic agents (or pharmaceutical agents) such as sulphonylureas, meglitimide, insulin, thiazolidinedione, sodium glucose cotransporter inhibitors among others are used to manage diabetes mellitus. However, these are without some serious risk factors to be considered such as efficacy, cost, side effect, weight gain, cornorbidities and hypoglycemia. For instance, sulphonylures lead to hypoglycemia, insulin injection causes hypokalemia and ketoacidosis is cause by the use of sodium glucose cotransporter inhibitors (Chaudaury et al. 2017).

However, the use of medicinal plants in the management of diabetes mellitus has been encouraged (Shokeen et al. 2008). These medicinal plants contain rich phytochemicals (that improve pancreatic cells performance) and are relatively devoid of numerous disadvantages associated with pharmaceutical agents (Dey et al. 2002; Switi et al. 2014).

### RESULTS AND DISCUSSION

- **Phytochemical analysis** of the ethanol stem bark extract of *C. febrifuga* revealed the presence of flavonoids, tannins saponins, alkaloids, cardiac glycosides, phenols, phytoesters and anthraquinones as shown in Table 2. This result is in line with the work reported by Salawu et al. (2008) on the methanol leaf extract and Ojewale et al. (2014) on methanol root extract of the plant respectively. These classes of phytochemicals have been implicated for antihyperglycemic properties (Switi et al. 20014). Previous studies have shown that glycosides for instance have inhibitory effect on alpha-amylase, aldose reductase, alpha-glucosidase and could increase the level of serum insulin. In addition, flavonoids, beside their antioxidant properties, reduce glucose transporter (Cazarolli et al. 2008; Switi et al. 2014). Alkaloids do have a stimulatory action on insulin secretion by activation of imidazole I binding sites in the pancreatic β-cell (Ujah et al. 2015). Thus, these phytochemicals in synergy or unilaterally, could have contributed to improving insulin secretion and action on the glycemic level. Hence the rationale for *C. febrifuga* stem bark extract exerting hypoglycemic effect (Table 3) in the alloxan-induced diabetic rats and its use in the management of diabetes mellitus by folklore.

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Hyperlipidemia is another important consequence of diabetes mellitus. The condition is characterized by prolonged high concentration of lipid in the blood and is the leading cause of atherosclerosis in diabetic patients (Goldberg, 2018).

Result of the effect of ethanol stem bark extract of C. febrifuga on rats is presented in Table 4. The result showed a slight increase in total triglyceride level, low density lipoprotein (LDL) cholesterol; a significant increase (p<0.05) in total cholesterol level and a slight decrease in the level of high density lipoprotein (HDL) of the diabetic rats compared to the normal control (non-diabetic rats). However, these parameters were reversed after treatment. Total Cholesterol and Triglyceride slightly decreased while LDL concentration decreased significantly (p<0.05) when compared to that of the diabetic control group (Group B). HDL increased in all the doses however, the increase was not significant as against the diabetic control. On percent scale, the HDL increased by 55.18% (at 1000mg/kg) and the LDL reduced significantly by 73.52% at the dose of 500mg/kg as against the standard drug which showed just 20.69% reduction.

The significant increase in serum lipid profile of the diabetic rats is a consequence of the uninhibited actions of lipolytic hormones on the peripheral fat depots due to insulin defect. This condition led to high concentration of free fatty acids in the plasma which are subsequently converted to phospholipids and cholesterol by the liver. The two compounds with excess triglycerides are discharged into lipoprotein in the blood thus resulting to hyperlipidemia (Rajagopal and Sasikala, 2008). Administration of ethanol extract of C. febrifuga stem bark reversed the above changes and improved the HDL levels. This result is tandem with that reported by Ojewale et al. (2014) on the root bark extract of the plant.

CONCLUSION

The results of the present investigation indicates that ethanol C. febrifuga stem bark extract have hypoglycemic effect on alloxan-induced diabetic rats. It was also found to be effective in managing hyperlipidemia. Therefore, C. febrifuga stem bark has a therapeutic property in the management of diabetes mellitus, and this justifies it used by folklore. Finally, work is on going towards investigating the antioxidant activity and isolating the bioactive compound(s) which is/are responsible for these effects of plant extract.

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