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FREE RADICAL SCAVENGING ACTIVITY AND HISTOLOGICAL PROPERTIES OF ETHANOL EXTRACT OF *CROSSOPTERYX FEBRIFUGA* STEM BARK IN ALLOXAN-INDUCED DIABETIC RATS

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Key words: *Crossopteryx febrifuga***,** phytochemical, antihyperglycemic, free radical, antioxidant, hepatoprotective.

Abstract

Crossopteryx febrifuga is known for its ethno-medicinal value for the treatment of diabetes mellitus in Nigeria. This study investigates the antioxidant property and antihyperglycemic effect of the ethanol extract of this plant's stem bark on alloxan-induced diabetic albino rats. The effect on the liver and pancreatic cells of the diabetic rats was also studied. Thirty rats were used in the antidiabetic study. The rats were divided into six groups (I-VI) of five rats each. A single interpreitoneal (i.p) injection of 150mg/kg alloxan monohydrate constituted in normal saline induced diabetes in Groups **II-VI** rats. Group **I** was however not induced and used as positive control. Group **II** served as negative control/diabetic control while Groups **III**, **IV** and **V** were treated with *C. febrifuga* extract in the doses of 500mg/kg, 1000mg/kg and 1500mg/kg body weight respectively, Group **VI** served as standard group and was treated with 5mg/kg glibenclamide (a standard antidiabetic drug). Treatment of the diabetic rats was done for seven days through orogastric procedure. The extract showed a significant ($p < 0.05$) hypoglycemic effects in all the doses compared to the diabetic control and standard antidiabetic drug, glibecamide. Qualitative **p**hytochemical screening of the stem bark extract revealed the presence of flavonoids, steroids, alkaloids, saponins, tannins, glycosides, carbohydrates, phenols and sterols. Quantitative, phytochemical screening revealed alkaloids (4.13±0.23%), flavonoids (6.27±2.05%), saponnins (22.67±2.31%), Phenols (3.50±0.32 mg/g) and tannins (0.35±1.02 mg/100g). The extracts showed good antioxidant activity which may be attributed to its rich phytochemicals. Histological examination of the liver and pancreatic tissues of the extract treated diabetic rats showed signs of protection, recovery and regeneration of cells when compared to the diabetic non treated rats. Above all, this investigation suggests that the ethanol stem bark of *C. febrifuga* possesses antioxidant, antidiabetic and hepatoprotective effect.

Introduction

Diabetes mellitus is a multifactorial and heterogeneous disorder characterized by high blood sugar level (hyperglycemia) and glycosuria (Bunza and Dallatu, 2017). Known causes are usually either lack of insulin or the insulin's inability to do its biological functions due to resistance by the cells (Wesam et al, 2016). This leads to high concentration of glucose and lipids in the blood which coupled with oxidative stress exposes the cells to severe devastating effects such as long-term damage, dysfunction and failure of various organs, especially eyes, kidneys, nerves, heart and blood vessels (Szkudelski, 2001).

The use of plants in the traditional health care system is well documented (WHO, 1999; Mahomoodally, 2013). In developing countries alone, well over 80% of the population relied on traditional medicine practices to treat many diseases due to diverse biochemical constituents of plants with pharmacological properties (Piero *et al*., 2012).

Plants are well known in traditional herbal medicine for their hypoglycemic activities and available literatures indicated that there are more than 800 plant species with hypoglycemic activity (Alarcon-Aguilara *et al*., 1998). The use of plant products with anti-diabetic activity may be attributed to its low cost, accessibility and less side effects.

Crossopteryx febrifuga, a plant of the family Rubiaceace 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 Irkwar gbande by 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, malaria and diabetes (Audu, 1989; Odugbemi, 2008; Salawu *et al*., 2008).

A lot of scientific evidences has emerged to support the tradomedicinal use of *C. febrifuga* as herbal remedies. The methanol crude extract of the plant roots was reported to contain bioactive substances with potential values in the treatment of trypanosomiasis and malaria (Hostettman *et al*., 2000; Yusuf *et al*., 2005). The root bark extract was also reported to have analgesic, antiinflammatory, antipyretic, antimalaria, antidiabetic and antilipidemic activities. The hypoglycemic and hypolipidemic effect of ethanol root and stem bark extracts of the plant were also reported (Idris and Nenge, 2019; Ojewale et al., 2013). Even though other parts of this plant had been explored for various therapeutic purposes, there is no scientific report so far on the antioxidant and histological activity of its stem bark. The present work investigates the antioxidant property of the stem bark extract and its effect on histology of pancreas and liver of alloxan-induced diabetic rats.

MATERIALS

Deionised water, Centrifuge (Denley B5400, England) Micropipette, Weighing balance, Jenway 6310 Spectrophotometer, Rotary evaporator (Bauchi Vacuum Controller V-850: Rotavapor, R-210, Germany), Accu- check Active Glucometer (Roche Diabetes Care GmbH Standhofer Strasse 11668305 Mannheim, Germany), test tubes, refrigerator, measuring cylinder, conical flasks, wash bottles razor blades, needles and syringes.

Chemicals used

Normal saline (0.9%), Glibenclamide -5mg/Kg, absolute ethanol 99.9% (JBH), 1, 1-diphenyl-2-picrylhydrazyl radical, DPPH and Trichloroacetic acid were obtained from Sigma-Aldrich, anhydrous ferric chloride, potassium ferricyanide, anhydrous sodium carbonate, potassium persulphate, $K_2S_2O_8$ and Ascorbic acid were obtained from BDH Chemical Laboratory, England, UK. All the chemicals were of analytical grade.

Plant Collection

The stem barks of *Crossopteryx febrifuga* were collected from wild in Mbarumun-Nanev, Kwande Local Government Area of Benue State, Nigeria. The plant was authenticated by Joseph Waya of Botany Department, Benue State University Makurdi and the specimen's Voucher No: 232 were deposited at the Herbarium unit.

METHODS

Preparation and Extraction of Plant Extract

The stem barks of *C. febrifuga* were chopped and naturally air dried for one month. The dry sample was crushed to powder using a mortar and pestle. The powdered sample (600g) 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^{o}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 VOM, Plateau State. The animals were kept in standard cages at room temperature and 12hrs daylight cycle for two weeks (to acclimatize) in the animal house at the Pharmacology Department, Bayero University Kano. These animals were fed freely on commercial feed (Vital Feed) and water.

Phytochemical Analysis

Qualitative and quantitative phytochemical analysis of the stem bark extract were done using standard procedures as described by Amita and Shalini, (2014) and Chukwuma and Chigozie, (2016) respectively.

Experimental design

This research work was organized into 4 phases: I, II, III and IV. Phase I was done according to the method of Lorke (1983) to determine the Lethal Dose LD_{50} (acute toxicity) of the extract. Phase II dealt with antioxidant study, Phase III constituted the antidiabetes studies and phase IV covered histological studies.

Acute Toxicity

Modified 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.

The LD50 was calculated via the formula $LD_{50} = (Da \times Db)^{1/2}$ where Da and Db represents group with highest dose with no mortality and lowest dose with mortality respectively.

Antioxidant study

The determination of free radical scavenging activity of crude ethanol stem bark extract was carried out using the DPPH (1,1-diphenyl-2-piccrylhydrazyl) quenching assay and ABTS (2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid) cation decolorization assay as described by Aliyu *et al*, 2010 and Re *et al*, 1999 respectively.

DPPH Radical Scavenging Assay

The extract solutions (25 µg, 50 µg, 75 µg, 150 µg and 300 µg) in ethanol (1 ml) were transferred into 1ml of DPPH solution (0.2 mM in ethanol) and allowed to stand at room temperature for 30 min. The absorbance of the solution was measured at 517 nm. The ability of the extract to scavenge the DPPH radical was calculated using the equation:

DPPH scavenging activity (%) $= {A_0 - A_1}/{A_0} \times 100$. Where A_o is the absorbance of the blank (control) and A_1 is the absorbance of the sample. The blank contained ethanol (1 ml) and sample solution (2 ml). Ascorbic acid was used as standard.

ABTS Radical Scavenging Assay

ABTS cation radical was produced by mixing 14mM ABTS solution (5 ml) and 4.9mM potassium persulphate, $K_2S_2O_8$(5 ml), stored in the dark at room temperature for 16 hrs. The plant extract at various concentrations (25µg, 50µg, 75µg, 150µg and 300µg) in 1ml of ABTS solution were homogenized and their absorbance recorded at 734nm. Ethanol blanks were run at each concentration and all measurements were recorded after 7min. Similarly, the reaction mixture of the standard solution was obtained by mixing ABTS solution (950 µl) and ascorbic acid (50 µl). The ABTS scavenging ability was expressed as IC50 µg/ml. The inhibition percentage of ABTS radical was calculated using the formula: ABTS scavenging activity (%) $= {A_0 - A_1}/{A_0} \times 100$. Where A_o is the absorbance of the blank (control) and A_1 is the absorbance of the sample..

Antidiabetes Study

Thirty diabetic albino rats were assigned into six groups of Five (5) rats each and treated according to the schedule below:

Group	Description	Treatment/Drug	Dose(mg/kg)
A	Normal/positive control	Normal saline	-
B	Diabetic/negative control	Normal saline	-
C	Diabetic treated	*C. febrifuga* extract	500
D	Diabetic treated	C.febrifuga extract	1000

E	Diabetic treated	C.febrifuga extract	1500
F	Diabetic treated	Standard drug (Glibenclamide)	5

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.

Induction of Diabetes mellitus

The rats were subjected to 12hrs fast. Diabetes mellitus was then induced by interpretoneal injection (ip) of 150 mg/kg body weight of alloxan monohydrate 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 Diabetic Rats and Determination of Blood Glucose Level, (BGL)

The diabetic 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).

Histology study

The liver and pancreatic tissue were dissected out and washed on ice cold saline immediately. A portion of the tissue was fixed in 10% neutral normal- saline fixation solution for histology studies. The procedure employed in the study was that described by *Strate et al*., (2005). The slides were viewed at magnification of x 400 and the photo micrograms taken.

Statistical Analysis

Data obtained were expressed as Mean ± SD 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.

Results

Qualitative phytochemical analysis of the ethanol stem bark extract of *C. febrifuga* indicated the presence of flavonoids, steroids, alkaloids, saponins, tannins, glycosides, carbohydrates, phenols and sterols. The content of some of these classes of natural products are shown in table 2. The extract has high quantity of saponnins (22.67±2.31%) and phenols (3.50±0.32mg/g).

Table 1: Phytochemical Content of Ethanol Crude Extracts of *C. febrifuga* Stem Bark

Class of Phytochemicals	Content
Alkaloids,%	4.13±0.23
Flavonoids, %	6.27±2.05
Saponnins %	22.67±2.31
Phenols,mg/g	3.50±0.32
Tannins, mg/100g	0.32±0.20

Values expressed as mean ± SD, n=3

In the phytotoxicity study, the rats were administered with the extract to as high as 5000mg/kg body weight doses. However, no sign of toxicity was noticed in the test rats throughout the 24 hours of administration of the extract. Thus, *C.febrifuga* extract at the dose of 5000mg/kg is physiologically safe.

Antioxidant properties of *C. febrifuga* ethanol stem bark crude extract are presented in Tables **2a** and 2**b** below. The extract showed antioxidants activity (%) which is demonstrated in *in vitro* ABTS and DPPH methods of free radical scavenging assay. The activity was however concentration dependant and increased proportionally with increase in concentration of the extract similar to the standard antioxidant compound, vitamin C.

In ABTS assay, the % inhibition activity of the extract was significantly lower ($p < 0.05$) at every concentration compared to the standard except, at 300µg/ml. At 300µg/ml, the inhibition activity of the extract was 87.19±0.03% and that of ascorbic acid was 95.44±00%. The IC_{50} of the extract was 149 µg/ml and that of ascorbic acid was 18µg/ml. This showed that *C. febrifuga* extract has poor scavenging ability for ABTS radical compared to ascorbic acid.

In DPPH assay, a similar situation was observed with the extract % inhibition being significantly lower (**p** < 0.05) throughout the entire concentrations compared to the Vitamin C. The Highest inhibition (81.65±0.00%) was however recorded at 300µg/ml as against 92.08±0.00% of the vitamin C with the IC_{50} of 68µg/ml and 25µg/ml respectively**.**

Table 2a ABTS Free Radical Scavenging Assay

Concetration, ug/ml	*C. febrifuga* (%)	Vitamin C (%)
25	7.95±0.01*	33.16±0.02
50	20.89±0.01*	55.39±0.14
75	33.03±0.01*	80.90±0.05
150	52.59±0.04*	88.10±0.05
300	87.19±0.03**	95.44±00
	IC_{50} =149 µg/ml	IC_{50} =18 µg/ml

Values expressed as mean ± SD. *= Results were significant (at p<0.05) and **= result not significant

Table 2b DPPH Free Radical Scavenging Assay

Concetration, ug/ml	*C. febrifuga* (%)	Vitamin C (%)
25	2.16±0.01*	29.29±0.01
50	5.87±0.01*	62.43±0.02
75	38.42±0.01*	80.27±0.00
150	71.56±0.01*	86.66±0.01
300	81.65±0.00*	92.08±0.00
	IC_{50} =68 µg/ml	IC_{50} =25 µg/ml

Values expressed as mean ± SD and *= Results were significant (at p<0.05)

Results of the antidiabetic study of the crude extract of *C. febrifuga* are presented in **figure 1** below. The result showed blood glucose level of various groups from day0 through day7.

The blood glucose level in the diabetic group was significantly higher ($p < 0.05$) than those of the normal control group, indicative of the diabetic state of the rats. On the other hand, administration of ethanol extract of *Crossopteryx febrifuga* stem bark (at 500, 1000 & 1500 mg/kg) exhibited a dose dependent antihyperglycemic activity on blood glucose level (BGL) when compared with the negative control group. The 500mg/kg dose showed a significant reduction ($p < 0.05$) in BGL as from day5 through day7 compared to the diabetic control. The difference is significant ($p < 0.05$) at a dose of 1000mg/kg as from day6 to day 7.

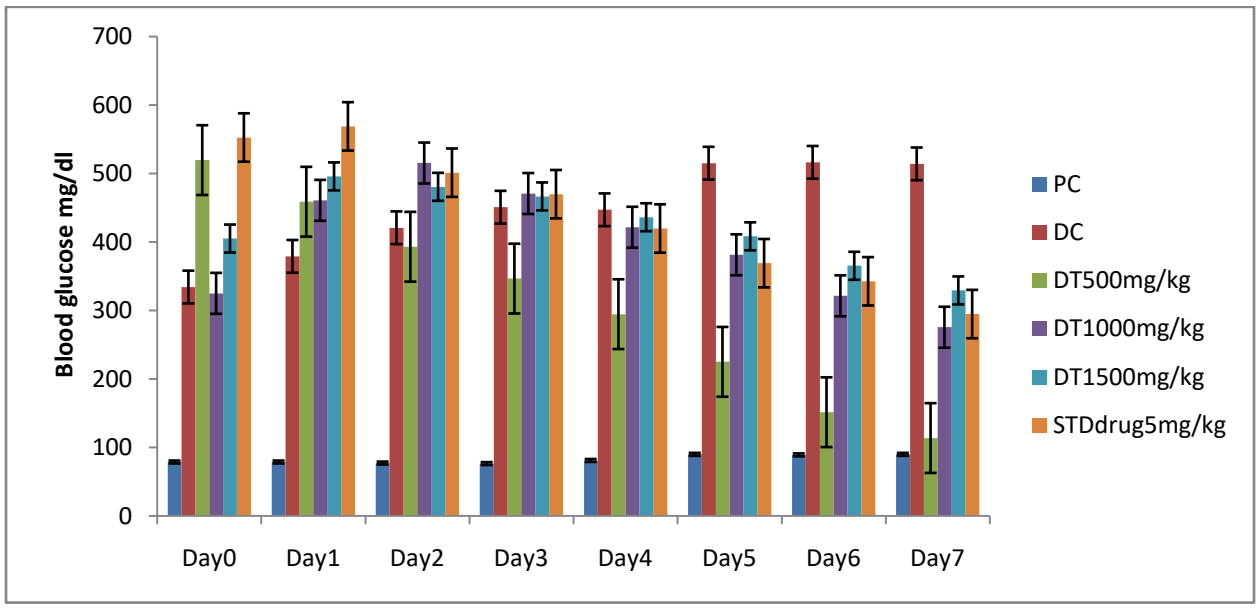
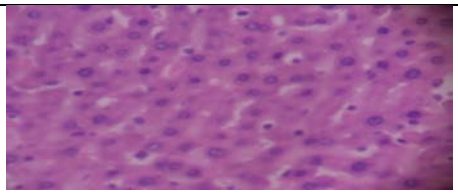
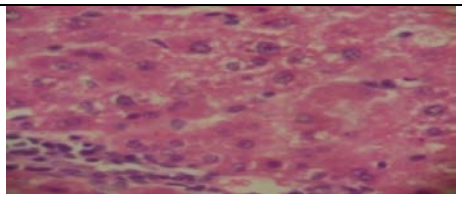
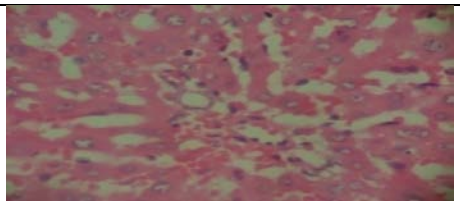
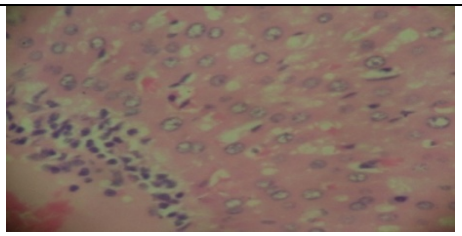


Figure1 Effect of Ethanol crude Extract of *C. febrifuga* Stem Bark on Alloxan-Induced Diabetic Rats.

Values were expressed as mean (n=5) ± standard deviation at 95% confidence level

Keys: PC = Positive control group, DC = Diabetic control group (alloxan-induced non-treated), STD = Standard control group, DT = Diabetes treated (500mg/kg), DT = Diabetes treated (1000mg/kg), DT = Diabetes treated (1500mg/kg).

Group	Photomicrograph	Result
Normal/positive control		The hepatocytes appear normal with intact cytoplasm and nuclei.
Diabetic control/Negative control		There is perivascular inflammation of the cell, mostly around the blood vessels. Mild vacuolation was evident followed by dissolution of cellular components which make cell demarcation difficult.
Diabetic treated (500mg/kg)		There is mild perivascular inflammation and mild atrophy with increase in sinusoidal spaces. Cell nuclei remain intact and surrounded by intact cytoplasmic material.
Diabetic treated(1000mg/kg)		There is massive intercellular inflammation as shown by the presence of inflammatory cells within the interstitial spaces, haemorrhage shown by the presence of red blood cells outside the blood vessels and necrosis

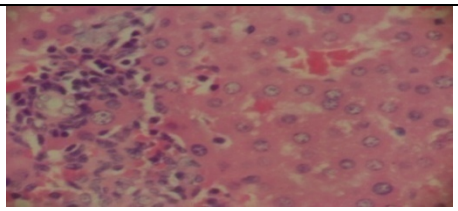
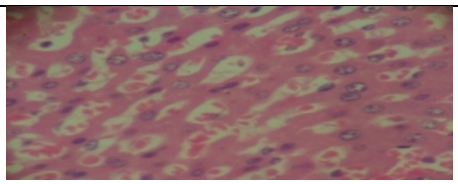
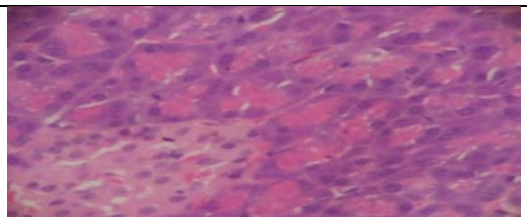
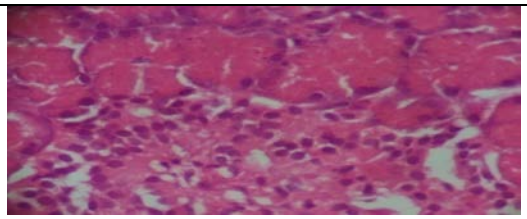
		evident by loss of cellular materials.
Diabetic treated(1500mg/kg)		There is severe hepatic cord atrophy with increase sinusoidal spaces. Heamorrhage is evident by the presence of red blood cells outside the blood vessels and within the tissues. Mild inflammation is also observed.
Diabetic treated(glibenclamide 5mg/kg)		Photomicrograph section showing liver of rat of the standard control group. There is mild hepatic atrophy and mild haemorrhage, but tissue morphology is intact. H&E stained X400

Figure 2: Histogram Showing the Effects of Ethanol Extract of *Crossopteryx febrifuga* Stem Bark and Standard Drug (glibenclamide) on Hepatic Cells of Alloxan-induced Diabetic Albino Rats.

Group	Photomicrograph	Result
Normal/positive control		Pancreatic tissues appear normal, no area of necrosis and no inflammation observed. H&E stained X400
Diabetic control/Negative control		There is increase in sinusoidal spaces and necrosis is observed with inflammation of cells H&E stained X400

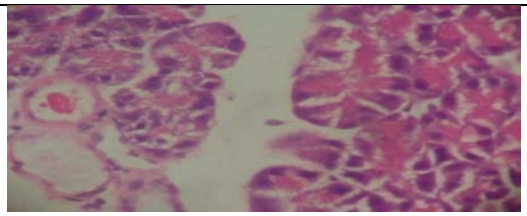
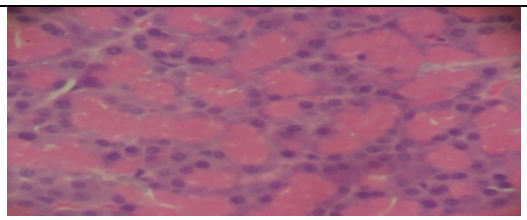
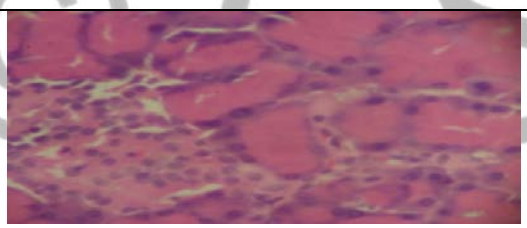
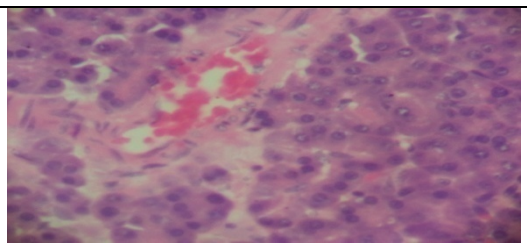
Diabetic treated (500mg/kg)		There is mild inflammation evident by the presence of inflammatory cells, increase in interlobular connective tissue septa. H&E stained X400. There is evidence of recovery although the cells have not reverted to normal.
Diabetic treated(1000mg/kg)		There is cellular infiltration by inflammatory cells and cellular hypertrophy evident by the decrease in intercellular spaces. H&E stained X400
Diabetic treated(1500mg/kg)		There is mild inflammation and increased connective tissue fiber. There is mild hypertrophy evident by the reduction in the intercellular spaces. H&E stained X400
Diabetic treated(glibenclamide 5mg/kg)		The cells and their components appear normal. No signs of inflammation or area of necrosis. There is evidence of recovery, although the cells have not reverted to normal. H&E stained X400

Figure 3; Histogram Showing the Effects of Ethanol Extract of *Crossopteryx febrifuga* Stem Bark and Standard Drug (glibenclamide) on Pancreatic Cells of Alloxan-induced Diabetic Albino Rats.

Histological results of the liver and pancreatic cells were as presented in Figure 2 and 3 above.

Discussion

Diabetes is a chronic disorder in the metabolism of carbohydrates, lipids and protein. It results from either insulin deficiency or malfunction (Wesam et al. 2016). Today, there is increase in the number of diabetic patients worldwide. This dramatic increase could be attributed to lifestyle changes, environmental and hereditary factors and damage of the insulin secreting organ, the pancreatic cells (Govindappa, 2015). When the pancreatic cells are impaired, insulin becomes insufficient or not produced at all or resisted by the relevant biomolecules like carbohydrates, protein and lipids etc. On the other hand, the inability of the cells to metabolise carbohydrates (glucose) leads to abnormally high concentration of glucose in the blood. A condition referred to as 'hyperglycemia' that is predisposed to diabetes mellitus.

Alloxan induces diabetes by damaging the insulin secreting beta cells of pancreas, resulting in decreased endogenous insulin release (Szkudelski, 2001). Consequently, there is hyperglycemia due to hepatic glucose overproduction. Interpretoneal administration of alloxan (150mg/kg) effectively induced diabetes in normal rats and those with BGL above 229mg/kg were used for the study. This study therefore, investigates the hypoglycemic and antioxidant property of the ethanol stem bark crude extract of *C. febrifuga* in alloxan-induced diabetic rats. Phytochemical analysis of the crude extract indicates the presence of flavonoids, steroids, alkaloids, saponins, tannins, glycosides, carbohydrates, phenols and sterols. The content of some of these classes of natural products were shown in table 1. The extract has high quantity of saponnins (22.67±2.31%) and phenols (3.50±0.32). Evidence supports the premise that a pro-oxidant condition exists in diabetic patients, a result of an overabundance in the production of reactive oxygen forms combined with a multilevel deficiency in metabolic sources of antioxidants (Sruthi *et al*. 2017). The presence of flavoniods, alkaloids and phenols with good antioxidants property therefore, suggests that extracts of *C. febrifuga* could be used as a source of natural antioxidants to limit free radical damage occurring in patients by acting in a synergistic manner and inhibit the destruction of cells.

Treatment of diabetic rats with *C. febrifuga* stem bark extract showed a significant reduction (at $p < 0.05$) in blood glucose (BGL) compared to diabetic untreated group as shown in figure 1. The 500mg/kg dose showed a gradual reduction in BGL throughout the period of treatment but the result was however significant (at $p < 0.05$) as from day 5 through day 7. On the other hand, 1000mg/kg dose result was significant at day 6 and day 7 while 1500mg/kg showed significant reduction at day 7 compared to diabetic untreated group (diabetic control). The reduction in the glucose levels was however dose dependent but that notwithstanding, the extract had proven to be a potential antihyperglycemic agent as used in folklore. This may be due to the synergy in the action of the rich phytochemical constituents of the plant which had already been established to have reputable effect in reviving and stimulating pancreas and beta cells to produce insulin (Switi *et al*. 2014).

Complications arising from prolonged diabetic condition are attributed to oxidative stress (Sruthi *et al*. 2017) initiated by reactive oxygen and nitrogen species. This reactive oxygen species or free radicals can produce the normal cellular metabolism and react with biomolecules like proteins, DNA and lipids to cause cellular damage and is responsible for degenerative changes (Manisha *et al*. 2017).

Hyperglycemia induces free radical production and impairs the endogenous antioxidants defense system such as enzymes. This could be seen

in the inflammation of the liver and pancreatic cells of the diabetic rats (Figures 2 and 3). However, upon treatment with plant extract, there was sign of recovery/remedy as indicated by the disappearance of the inflammation and necrosis (Figure 2 & 3). This may be possible due to the active antioxidants compounds of the plant extract. These antioxidants lower the oxidants by donating electron to stabilize free radical so that its harmful effect is minimized. This invariably was responsible for the regeneration of the beta cells of the diabetic rats to produce insulin to stimulate the enzymes that metabolize the glucose, thus reducing its level in the blood.

Although the extract showed antihyperglycaemic properties (Figure 1), it has deteriorating effect on the pancreatic and liver cells of the rats especially at higher doses (1000mg/kg and 1500mg/kg) as shown in Figure 2 and 3 respectively. Perhaps, acute toxicity studies suggested that the extract was relatively safe up to 5000mg/kg. This could only be possible when such a high dose is administered once. But for prolonged treatments; lower doses may be required to avoid damage to body organs.

Conclusion

C. febrifuga has been reported to have therapeutic uses in managing various ailments in traditional health care system. Various parts of this plant have been investigated that sustained these claims owing to the rich classes of phytochemical (alkaloids, flavoniods, polyphenols etc) with reputed history of biological activities.

However, the present work investigated the antioxidant and histological properties (in diabetic rats) of extract of C. febrifuga stem bark.

The results of the investigation have indicated that *C. febrifuga* stem bark possesses antioxidant, antihyperglycaemic and histoprotective properties and therefore could be used in the management of such disease conditions. Furthermore, research is on going to isolating the antioxidants and antidiabetes constituents of the plant.

References

E.C. Agishi. Tiv, Idoma, Etulo, Igede, Akweya, Hausa, English and Scientific Names of Plants. pp. 23- 26, 2010.

P. Amita and T. Shalin. Concept of Standardization, Extraction and PrePhytochemical Screening Strategies for Herbal Drug. Journal of Pharmacology and Phytochemistry, vol. 2, no.5, pp. 115-119, 2014 available at http://www.phytojournal.com.

J. Audu. Medicinal Plants and Their Use in Bauchi. The Nigeria Field, vol.54, pp.157-168, 1989.

A. B. Aliyu1, H. Ibrahim, A. M. Musa, M. A. Ibrahim, A. O. Oyewale and J. O. Amupitan. *In vitro* evaluation of antioxidant activity of *Anisopus mannii* N.E. Br. *African Journal of Biotechnology*, vol. 9, No**.** 16, pp: 2437-2441, Apr. 2010. http://www.academicjournals.org/AJB

F.U. Bunza and M.K. Dallatu. Hematological Indices in Alloxan-Induced Diabetic Rats: Effect of Supplementation with the Antioxidant Dimethyl Sulfoxide. *Asian Journal of Medicine and Health*, vol. 8, no. 1, pp. 1-7, Nov. 2017.doi.org/10.9734/AJMH/2017/36038.

S.E. Chukwuma and M.E. Chigozie. Qualitative and quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science*, ept.2016: 1/7-8/7. doi.org/10.1155/20161327.

M. Govindappa. A Review on Role of Plant(s) Extracts and its Phytochemicals for the Management of Diabetes. *Journal of Diabetes Metabolism*, vol. 6, pp. 565, Jun. 2015, doi:10.4172/2155-6156.1000565.

K. Hostettmann, A. Marston, K. Ndjoko and J. Wolfender. The Pontential of African Plants as a Source of Drugs. *Current Organic Chemistry*, vol. 4, no. 1, pp. 973-1010, 2000. Doi :10.2174/1385272003375923.

D. Lork,e. A New Approach to Practical Acute Toxicity Testing. Archive of Toxicology, Vol. 54, no. 4, pp. 275-289, Dec. 1983.

A. Maiga, K.E. Maiterud, D. Diallo, B.S. Paulsen. Antioxidant and 15-lipoxygenase inhibitory Activities of the Malian Medicinal Plants Diospyros abyssinaica (Hiern) F. White (Ebenaceae), Lonnea velutina A. Rich (Anacardiaceae) and Crossoptyerx febrifuga (Afzel) Benth(Rubiaceae). *Journal of Ethnopharmacology*, vol. 104, no. 1-2, pp. 132-137, Mar. 2006. Doi: 10.1016/j.jep.2005.08.063

W. H. Manisha, R. Richa, and J. Deepali. Oxidative Stress and Antioxidants: An Overview. *International Journal of Advanced Research and Review*, vol. 2, no. 9, pp. 110-119, Sept. 2017.

T. Odugbemi. Medicinal plant by species names: Outlines and pictures of medicinal plants from Nigeria. Lagos, University press. pp. 596, 2006.

A.O. Ojewale, A.O. Adekoya, O.A. Odukanmi, O.T.Olaniyan, O.S. Ogunmodede and B.J. Dare. Protective effect of ethanolic roots extract of Pseudocedrela kotschyi on some hematological and biochemical parameters in alloxan-induced diabetic rats. *World Journal of Pharmacy and Pharmaceutical Sciences.* Vol. 2, no**.** 3, pp. 852-866, May 2013.

C.C. Rhenny and K.K. Kirk. Performance of Three Blood Glucose Meter. Annals of Parmacotherapy. Vol. 34, no. 3, pp. 317-321, Mar. 2000.

R. Re, N. Pelligrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. Antioxidant Activity Applying an Improved ABTS Radical decolorization Assay. *Free Radical Biology and Medicine*, vol. 26, no. 9-10 pp. 1231-1237, May 1999.doi.org/10.1016/50891-5849(98)00315-3

O.A. Salawu, B.A. Chindo, A.Y. Tijani and B. Adzu. Analgesic, Anti-inflammatory, Anti-pyretic and Antiplasmodial Effects of the Methanol Etract of Crossoptyerx febrifuga: *Journal of Medical Plants Research*, vol. 2, no. 8, pp. 213- 218, Sept. 2008.Available at www.academicjournals.org/JMPR

G. Sruthi, H.J. Haritha, V.J. Nisha Ullas and E. Abraham. Role of Antioxidants in the Management of Diabetes mellitus. *International Journal of Phamarceutical Sciences and Nanotechnology,* vol.10 no. 4, pp. 3763-3767, July-August 2017.

M. P. Stanley and M. P. Venugopal. Antioxidant Action of Tinospara cordifolia Roots Extract in Alloxan-Induced Diabetic Rats. *Phytotherapy Research*, vol. 15, no. 3, pp. 213-218, May 2001, d0.1002/ptr.707.

T. Strate, O. Mann, H. Kleighans, S. Rusani, C. Schneider, E. Yekebas, M. Freitag, T. Standl, J.R. Izbick, and C. Bloechle. Micro circulatory function and tissue damage is improved after the therapeutic injection of bovine hemoglobin in severe acute rodent pancreatitis. *Pancreas Aprroach*, vol. 30 no. 3, pp. 254-259, Apr. 2005.

B. G. Switi, G. K. Mohan and M. Sandhya Rani. Phytochemicals for Diabetes Management. *Pharmaceutical Crops,* vol. *5,* (Suppl 1: M2), pp. 11-28, 2014.

T. Szkudelski. The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas.
Physiological Research, vol. 50, pp. 537-546, 2001.

M. N. Piero, J.M. NAJI, C. M. Kibiti, J. J.N. Ngeranwa, E. N. M. Njagi and P. M. Miriti. The Role of Vitamins and Minerals Elements in Management of Type 2 Diabetes Mellitus: A Review South. *Asian*. *Journal of Biological Sciences*, vol, 2, supp.1, pp. 107-115, Jan 2012.

K. Wesam, M. Farokhipour, Z. Asadzadeh, D. Ashtary-Larkey and M. Asadi-Samani. The role of Medicinal Plants in the Treatment of Diabetes: A Systematic Review. *Electron Physician*, vol. 8, no. 1, pp. 1832-1842, Jan. 2016, doi 10.19082/1832.

Yusuf, A., Iliyasu, B., Abubakar, A., Onyekwelu, E.L., Bot, D.Y.(2004). Preliminary Evaluation for Anti trypanosomial Activity of Aqueous Stem Bark Extract of C. febrifuga in T. congolensis infection in Rats. *Journl of Pharmacy and Bioresources*, vol, 2 no. 2, pp. 111-115 Oct.2005. Doi:10.4314/jpb.v2i2.32072.

M. M Idris and H, P. Nenge. Antihyperglycaemic and Antilipidemic Properties of Ethanol Stem Bark Extract of *Crossopteryx febrifuga* in Alloxan-Induced Diabetic Rats. *Chemsearch Journal,* vol 10, no. 2, pp. 130-137, Dec.2019.www.ajol.info/index.php/csj

M.F. Mahomoodally. Traditional Medicine in Africa: An Appraisal of Ten Potent African Medicinal Plants. *Evidence-Base Complementary and Alternative Medicine*, vol. 2013, pp.1-14, Dec. 2013. Doi.org/10.1155/2013/617459.

WHO. The Use of Traditional Medicine in Health Care System. 17th Meeting of Ministers of Health of Countries of South-Easten Asia Region(SEAR) Yangon, Myanmar, pp. 1-10, October 1999.

F.J. Alarcon-Aguilara, R. Roman Ramos, S. Perez-Gutierrez, A. Aguilar-Contreras, C.C. Contreras-Weber and J.L. Flores-Saeuz. Study of the Antihyperglycemic Effect of Plants Used as Antidiabetics. *Journal of Ethnopharmacology*, vol. 61, no. 2, pp.101-110, Jun. 1998. Doi:10.1016/s0378-8741(98)00020-8