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Research Article
Modulation of Blood Glucose Concentration, Lipid Profile and Haematological Parameters in Alloxan Induced Diabetic Rats Using Methanol Extract of *Nauclea latifolia* Root Bark

[1]N. Ogugua Victor, [1]Agu Obiora, [2]S.I. Egba and [2]Uroko Robert Ikechukwu

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria
[2]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Abstract

Objective: The study investigated the potentials of methanol extract of *Nauclea latifolia* root bark in modulating blood glucose concentration, lipid profile and haematological parameters in alloxan induced diabetic rats. **Materials and Methods:** The acute toxicity study was carried out with 18 mice while the study on lipid profile and haematological parameters were carried out with 30 male Wister Albino rats using standard reagents and assay protocols. The rats were randomly distributed into six groups of five rats each with group 1 as the normal control that was free from diabetes and received no treatment. Group 2 and 3 were diabetic rats treated with 1.5 mL normal saline and 2.5 mg kg^{-1} b.wt., of glibenclamide, respectively while group 4-6 were diabetic rats treated with 100, 200 and 400 mg kg^{-1} b.wt., of the extract, respectively for 14 days. **Results:** The result of the acute toxicity study revealed that the methanol extract was relatively safe within the maximum dose tested as no adverse reactions nor death was recorded. The extract exhibited hypoglycaemic activity in the diabetic rats comparable with the standard drug (glibenclamide) as the diabetic rats treated with graded doses of the extract showed significant ($p<0.05$) decrease in the blood glucose concentrations relative to the diabetic untreated rats with low dose having the highest hypoglycaemic activity. The diabetic rats had significant ($p<0.05$) decrease in packed cell volume count and haemoglobin concentrations, however, administration of the extract significantly ($p<0.05$) increased packed cell volume count and haemoglobin concentrations, respectively. Significant ($p<0.05$) increase in WBC count and no significant increase in red blood cell count ($p>0.05$) observed in the diabetic control relative to the normal control were significantly reversed when treated with the extract. The extract showed significant antihyperlipidaemic activity, reducing total cholesterol and low density lipoprotein concentrations significantly with no significant increase in high density lipoprotein and decrease in triacylglycerol concentration, respectively. **Conclusion:** The findings of this study have validated the use of the extract in the management of diabetes as it positively modulated blood glucose concentration, lipid profile and haematological parameters.

Key words: Hypoglycaemic activity, *Nauclea latifolia*, diabetic albino rats, total cholesterol, triacylglycerol, high density lipoprotein, low density lipoprotein, glibenclamide, haematological parameters

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Corresponding Author: Uroko Robert Ikechukwu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria Tel: +2348065914471

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

It has been established that many medicinal plants possess bioactive and therapeutic properties useful to man for prevention, treatment and management of various diseases, however, little or no information on the mechanism of actions of their components and associated toxicity[1].The growing interest in herbal medicine demands information on the various herbal preparations and scientific evaluation of medicinal plants could play an important role to the discovery of novel drugs and elucidation of toxic risks associated with the use of herbal preparations and other conventional drugs of plant origin in the treatment of diseases[2]. Herbal drugs or phytomedicines are currently recognized as the most common form of alternative medicine used by about 60% of the world population both in the developing and in the developed countries where modern medicines are predominantly in use[3]. The use of herbal remedies especially in the form of teas or extracts for the treatment of various diseases is gaining increasing popularity, making them the mainstay of health care system, especially among the rural populace in the developing countries[4]. Their increasing popularity are mostly attributed to their potency and their availability as cheap source of medical treatments[4]. Medicinal plants have served as valuable starting materials for drug development in both developed and developing countries most especially in Africa where it acts as a first line of treatment of various diseases for more than 80% of her population[4].

Nauclea latifolia is a valuable medicinal plant that is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa where it is commonly known as African peach and used in traditional medicinal[5]. Various solvent extracts of *Nauclea latifolia* parts are used for the therapeutic management of malaria, hypertension, prolonged menstrual flow, cough, gonorrhoea, stomach disorders, dysentery, ulcers and liver ailments[6]. The use of this plant in the management of these conditions has not been fully scientifically investigated and validated though the cardiovascular, spasmolytic, anti-plasmodial, sedative activities and anti-parasitic effects have been reported in studies that used various laboratory models[7-9]. Previous studies had suggested that the leaf of *Nauclea latifolia* possesses an anti-hypertensive effect[10,11] while Egbung *et al.*[12] reported that various parts of the plant are commonly prescribed as a treatment for diabetes mellitus. This study was designed to investigate the effect of the methanol extract of *Nauclea latifolia* root bark on the blood glucose levels, haematological parameters and lipid profile of alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material: The fresh root bark of *Nauclea latifolia* was collected from a natural habitat at Opi, Nsukka local government, Enugu state, Nigeria and identified at the Taxonomy Unit, Department of Botany, University of Nigeria, Nsukka.

Acclimatization of animal: Thirty male Wister Albino rats weighing 129-350 g and 18 male mice weighing 18-28 g were obtained from the Animal House of University of Nigeria formal Teaching Hospital, Enugu and were divided into six groups of five rats per group. The animals were housed in a well-ventilated animal house and 12 h day light and darkness cycle with free access to feed and water for 7 days before commencement of the study to allow the animals adapt to the new environmental condition. Feeding of the animals were done *ad libitum* with growers' mash (vital feed) and drinking water.

Preparation of plant extract: The plant material was dried under shade at room temperature and afterwards blended to powder using electronic blender. A quantity (500 g) of the pulverised sample was then extracted with 80% methanol for 48 h. The solution was filtered with Whatman filter paper to obtain the filtrate which was then concentrated by evaporating in a water bath. The percentage yield of the extract was then calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of exract after concentration}}{\text{Initial weight of extract before concentration}} \times 100$$

Experimental design: The mice were divided into six groups of three mice each and three groups were used for the phase I of the acute toxicity study and the remaining three groups were used for the phase II study, respectively. Thirty male Wister Albino rats were distributed randomly into six groups of five rats each. Group 1 rats served as normal control (no diabetes induced and no treatment given) while the group 2 animals served as negative control (diabetic rats that received only 1.5 mL of normal saline). Group 3 rats (diabetic rats treated with 2.5 mg kg^{-1} b.wt., of the standard antidiabetic drug) while groups 4, 5 and 6 were diabetic rats

treated with 100, 200 and 400 mg kg^{-1} b.wt., of the extract, respectively. Treatment lasted for 14 days and after overnight fast, the animals were sacrificed on day 15 under mild anaesthesia (10% formalsaline). The blood glucose concentration was determined every 2 days' intervals using glucometer. The administration of methanol extract of *Nauclea latifolia* root bark and the standard drug (Glibenclamide) were done using intra-gastric syringe. Blood samples were collected for haematological analysis using EDTA test tubes while other biochemical parameters were determined using serum obtained from blood samples in the plain bottles.

Induction of diabetes: Diabetic condition was successful induced after the rats were fasted overnight by intraperitoneal injection of the rats with 150 mg kg^{-1} b.wt., of alloxan-monohydrate dissolved in normal saline. The blood glucose concentration was monitored daily and rats with blood glucose concentrations more than 200 mg dL^{-1} were considered to be diabetic.

Determination of blood glucose concentrations: The blood glucose concentrations were determined using ACCU-CHEK active glucometer by roche diagnostic.

Principle: The method is based on the reaction of glucose with oxygen in the presence of glucose oxidase to yield gluconic acid and hydrogen peroxide. The hydrogen peroxide formed subsequently reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. In other words, it oxidizes the dye in a reaction mediated by peroxidase to produce a blue coloured complex. The intensity of the colour which is proportional to the glucose concentration in the sample could be read from the ONE-TOUCH glucometer which is a reflectance meter.

Determination of acute toxicity: The acute toxicity study was carried out according to the method of Lorke[13].

Determination of Packed Cell Volume (PCV): This was done using standard method as described by Ochei and Kolhatkar[14].

Determination of haemoglobin (Hb) concentration: Estimation of Hb concentration was carried out using the standard method as described by Ochei and Kolhatkar[14].

Determination of Red Blood Cell (RBC) count: This was done using standard haematological procedure as described by Ochei and Kolhatkar[14].

Determination of Met-haemoglobin (Met-Hb) concentration: This was done using standard haematological procedure as described by Ochei and Kolhatkar[14].

Determination of Total White Blood Cell (WBC) count: This was carried out following standard haematological procedure as described by Ochei and Kolhatkar[14].

Determination of serum total cholesterol concentration: Determination of total serum cholesterol was done using the method of Roeschlau *et al.*[15] as described in Randox kit.

Principle: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Determination of high density lipoprotein-cholesterol (HDL) concentration: Serum high density lipoproteins-cholesterol concentration was determined using the method of Albers *et al.*[16] as described in Randox kit.

Principle: Low density lipoproteins, very low density lipoproteins and chylomicron fractions were precipitated quantitatively by the addition of a phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the high density lipoprotein fraction, which remains in the supernatant is determined.

Determination of low density lipoprotein-cholesterol concentration: The serum low density lipoprotein-cholesterol concentration was determined using the method of Assmann *et al.*[17] as described in BioSystems cholesterol kits.

Principle: Low density lipoproteins in the sample precipitate with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation.

Determination of triacylglycerol (TAG) concentration: The serum triacylglycerol concentration was determined using method of Fossati and Prencipe[18] as described in Randox kit.

Principle: The Triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chloropenol under the catalytic influence of peroxidase.

RESULTS

Percentage yield of the methanol extract of *Nauclea latifolia* root back: The percentage yield of the extraction of 500 g finely ground sample of *Nauclea latifolia* root back extracted with 80% methanol gave a percentage yield of 10.8% equivalent to 54 g.

Acute toxicity of methanol extract of *Nauclea latifolia* root bark: The results of the acute toxicity study showed that the extract is not lethal even at higher doses (Table 1). The acute toxicity study (LD_{50}) recorded 100% survival beyond 24 h for all the animals that received 100-5000 mg kg^{-1} b.wt., of the extract orally. Since, no mortality was recorded even at 5000 mg kg^{-1}, 2, 4 and 8% of 5000 mg kg^{-1} of the methanol extract was adopted as low, medium and high effective doses for the study.

The baseline glucose concentrations indicated that all animals in the six groups were having normal glucose concentration range (70-90 mg dL^{-1}) and thus were non-diabetic before the induction (Fig. 1). Following induction of diabetes, it was observed from the results that all groups except the normal control (group 1) were diabetic on day 1. The normal control (group 1) that was not induced diabetes showed significantly ($p<0.05$) lower glucose concentrations throughout the treatment duration compared to the diabetic control (group 2). On day 3, a significantly ($p<0.05$) lower glucose concentrations were observed in groups 3 and 4 compared to the diabetic control (group 2). However, on days 5, 7, 9, 11 and 13, a significantly ($p<0.05$) lower glucose concentrations were observed in all treatment groups compared to diabetic control (group 2).

In the results of haematological parameters are presented in the Table 2, Packed Cell Volume (PCV) count and haemoglobin (Hb) concentrations showed significantly ($p<0.05$) lower values in the diabetic control (group 2) compared to the normal control (group 1). A significantly ($p<0.05$) higher PCV count and Hb concentrations were observed in the standard control (group 3) compared to the diabetic control (group 2) while non-significantly ($p>0.05$) increase in PCV count and Hb concentrations were observed in group 4, group 5 and group 6 compared to the diabetic control (group 2). The concentration of Met-Hb was significantly ($p<0.05$) higher in the diabetic control (group 2) when compared to the normal control (group 1). Following treatments, a significantly ($p<0.05$) lower concentration of Met-Hb was observed in the standard control (group 3) and non-significantly ($p>0.05$) lower concentrations of Met-Hb was observed in groups 4, 5, and 6 compared to the diabetic control (group 2). The WBC result showed a significantly ($p<0.05$) higher concentration in the diabetic control (group 2) compared to the normal control (group 1). The standard control (group 3) and group 4 showed a significantly ($p<0.05$) lower WBC count when compared to both normal control (group 1) and diabetic control (group 2). Groups 5 and 6 also showed a significantly ($p<0.05$) lower WBC count when compared with the diabetic control (group 2). A non-significantly ($p>0.05$) lower RBC count was observed in the diabetic control (group 1) compared to the normal control

Table 1: Acute toxicity result of methanol extract of *Nauclea latifolia* root bark

Phases	Dosage (mg kg^{-1} b.wt.)	Mortality rate
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

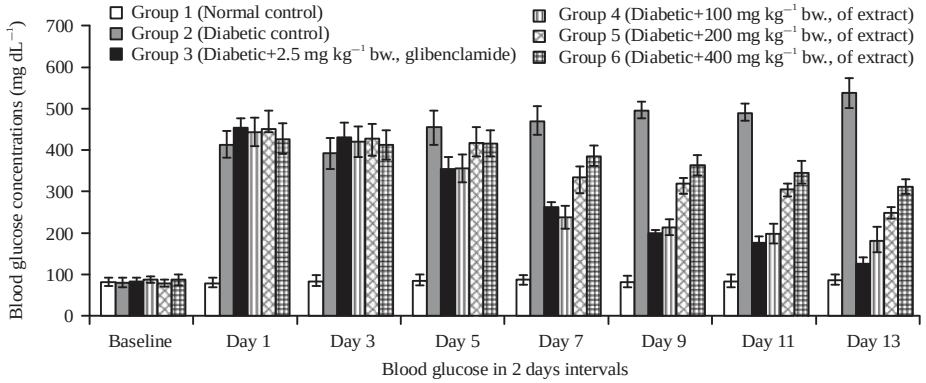

Fig. 1: Effect of methanol extract of *Naucalea latifolia* root bark on blood glucose concentrations of alloxan-induced diabetic rats

Table 2: Effects of methanol extract of *Nauclea latifolia* root bark on concentrations of some haematological parameters of alloxan-induced diabetic rats

Groups	PCV (%)	Hb (g dL^{-1})	Met-Hb (g dL^{-1})	WBC (mm^3)	RBC ($\times 10^9$ L^{-1})
Group 1	46.33±1.53[b]	16.03±0.57[b]	1.47±0.46[b]	10600.00±721.11[b,c]	286.67±2.89
Group 2	40.67±3.06[a,c]	13.83±2.06[a,c]	5.07±1.53[b,c]	12866.67±170.98[a,c]	260.00±20.00[c]
Group 3	47.00±2.65[b]	16.07±0.91[b]	2.50±1.60[a]	8466.67±901.85[a,b]	323.33±25.17[b]
Group 4	45.67±1.53	15.70±1.14	3.83±2.04[b]	8666.67±808.29[a,b]	295.00±13.23
Group 5	45.00±4.58	14.57±0.91	4.23±0.57[b]	9466.67±100.65[b]	310.00±18.03[b]
Group 6	44.67±2.52	14.83±0.73	4.17±0.84[b]	9666.67±808.29[b]	310.00±36.06[b]

Values are expressed as Mean±SD (n = 3), mean values with superscript(s) across the rows are considered significant (p<0.05) compared to normal, diabetic and standard control, respectively

Table 3: Effects of methanol extract of *Nauclea latifolia* root bark on the concentrations of lipid profile of alloxan-induced diabetic rats

Groups	Total cholesterol (mmol L^{-1})	LDL (mmol L^{-1})	HDL (mmol L^{-1})	TAG (mmol L^{-1})
Group 1	3.51±0.31[b]	2.27±0.65	1.24±0.13[b]	1.48±0.17
Group 2	4.38±0.50[a,c]	3.06±0.57[c]	0.45±0.19[a,c]	1.72±0.22
Group 3	3.60±0.13[b]	2.15±0.19[b]	1.15±0.38[b]	1.60±0.14
Group 4	4.23±0.27[a,c]	2.85±0.51	0.57±0.26[a,c]	1.67±0.07
Group 5	3.72±0.12[b]	1.63±0.58[b]	0.65±0.19[a]	1.60±0.13
Group 6	4.05±0.20[a]	2.33±0.26	0.82±0.47	1.67±0.14

Values are expressed as Mean±Standard Deviation (n = 3), mean values with superscript (s) across the rows are considered significant (p<0.05) compared to normal, diabetic and standard control, respectively

(group 1). In the treatment groups, a significantly (p<0.05) higher RBC counts were observed in groups 3, 5, 6 and a non-significantly (p>0.05) higher RBC counts were observed in group 4 compared to the diabetic control (group 2). For alhaematological parameters, there were no significant (p>0.05) differences in groups 4, 5 and 6 treated with different doses of the extract and the standard control (group 3) treated with glibenclamide. It was generally observed that positive effects were exerted on these haematological parameters of diabetic rats by the standard drug as well as different doses of the extract. However, the effects of the extract cannot be said to be dose-dependent.

The lipid profile results are presented in Table 3. A significantly (p<0.05) higher total cholesterol and non-significantly (p>0.05) increase in low density lipoprotein concentrations were observed in the diabetic control (group 2) compared to the normal control (group 1). In the treatment groups, both total cholesterol and low density lipoprotein concentrations were significantly (p<0.05) lower in groups 3 and 5 compared to the diabetic control (group 2). Also, a significantly (p<0.05) higher total cholesterol concentration was observed in groups 4 and 6 compared to the normal control (group 1). For total cholesterol, a significantly (p<0.05) higher concentration was observed in group 4 compared to standard control (group 3) but non-significant differences were observed in low density lipoprotein concentrations of groups 4, 5 and 6 treated with different doses of extract and the standard control (group 3) treated with glibenclamide, a standard anti-diabetic drug. The high density lipoprotein concentration of the diabetic control (group 2) showed significantly (p<0.05) lower value compared to the normal control (group 1). Treatments resulted in significantly (p<0.05) increase in high density lipoprotein concentration in the standard control (group 3) compared to the diabetic control (group 2). For groups 4, 5 and 6, non-significantly (p>0.05) increase in high density lipoprotein concentrations were observed compared to the diabetic control (group 2). A significantly (p<0.05) decrease in high density lipoprotein concentration was observed in groups 4 and 5 compared to the normal control (group 3) indicating that highest dose of the extract (400 mg kg^{-1} b.wt.) produced more desired effect in group 6 and compared better with the standard control (group 3). For triacylglycerol, non-significantly (p>0.05) higher concentrations was observed in the diabetic control (group 2) and all treatment groups compared to the normal control (group 1). Treated group showed non-significantly (p>0.05) lower triacylglycerol concentration compared to the diabetic control (group 2).

DISCUSSION

This study was undertaken to investigate the effects of methanol extract of *Nauclea latifolia* root bark on lipid profile, haematological parameters and its anti-diabetic properties. The median lethal dose (LD_{50}) revealed that the extract was safe up to 5000 mg kg^{-1} b.wt., as no adverse effects or death were recorded in the groups of mice that received various doses of the methanol extract of *Nauclea latifolia* root bark and this may be attributed to low or absence of toxic constituents in the methanol extract. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in

insulin secretion, insulin action or both. It is characterized by defective regulation of carbohydrate, fat and protein[19]. Therefore, in diabetic condition, the blood glucose level is usually elevated due to a decrease in the sensitivity of target tissues to the action of insulin. The finding of this study was in line with the above assertion. The baseline glucose concentrations indicated that all animals in the six groups were having normal glucose concentration range (70-90 mg dL^{-1}) due to their functional beta cells pancreatic islet and normal insulin response, so the rats were non-diabetic prior to alloxan induction. Following induction of diabetes, it was observed from the results that all animal groups except the normal control (group 1) were diabetic on day 1. Treatment of the diabetic rats with methanol extract of *Nauclea latifolia* root bark commenced on day 3 resulting in maximal reduction of blood glucose concentration from 444.60±44.28 to 183.60±30.39 mg dL^{-1} with 100 mg dL^{-1} dosage after 13 days of administering the extract suggesting that 100 mg kg^{-1} b.wt., of was the most effective dose of the extract. The hypoglycaemic effect of the methanol extract was comparable to the hypoglycaemic activity of the standard drug (glibenclamide) which resulted in the reduction of blood glucose concentration from 455.40±52.22 to 126.00±16.69 mg dL^{-1} in the standard control (group 3). The extract can effectively serve as alternative to glibenclamide in the management of diabetes most especially if it can be incorporated into the diets of diabetic patients. This result is in agreement with the findings of Anita and Okokon[20] and Abubakar *et al.*[21] who had obtained similar results from their independent investigations on the abilities of some plants extracts in reducing glucose concentrations in diabetic rats.

The haematological parameters such as total RBC, PCV counts and Hb concentrations have been reported to be lower in diabetic patients compared to normal control group, indicating the presence of anaemia in the diabetic animals[22,23]. The findings of this work revealed that PCV counts and Hb concentration showed a significantly ($p<0.05$) lower values in the diabetic control (group 2) compared to the normal control (group 1). The increase in the levels of haematological parameters recorded in this study was indicative that both the standard drug and the extract had the potential to stimulate erythropoietin release in the kidney known to enhance RBC production (erythropoiesis)[24,25]. Similar observation has been made on a number of plants[26,27].

The significant (p 0.05) increase in Met-Hb concentration of the diabetic control compared to the normal control could be well correlated with the corresponding reduction observed in Hb concentration of the diabetic control. The standard control performed better than the extract-treated groups 4, 5 and 6 as it showed a significant ($p<0.05$) reduction of Met-Hb concentration when compared to the diabetic control (group 2). Similar result was reported by Ajagbonna *et al.*[28] and Akah and Alemji[29]. The White Blood Cells (WBC) serve as scavengers that destroy micro-organisms at infection sites, removing foreign substances and debris that result from dead or injured cells[30]. Consequently, the level is known to rise as body defence in response to toxic environment[31]. The result of the total WBC obtained in this study was in consonance with the above assertion as the WBC concentration of the diabetic control (group 2) was significantly ($p<0.05$) higher compared to the normal control (group 1). A significant ($p<0.05$) decrease in WBC concentration were also observed in all treatment groups compared to the diabetic control (group 2). The decrease suggested that the extract did not exhibit toxic effect even at high dose (400 mg kg^{-1} b.wt.). The ability of the extract to maintain non-significant ($p>0.05$) differences in groups 4, 5 and 6 compared to the standard control (group 3) for all haematological parameters could be attributed to similarity in their mechanism of action.

The dyslipidaemia in type 2 diabetes is characterized by a combination of increased serum total cholesterol, LDL and decreased HDL-cholesterol which are similar to the results of the lipid profile observed in this study. The decrease in the total cholesterol and LDL concentrations observed in the diabetic rats treated with varied doses of the methanol extract demonstrated the hypolipidaemic effect of the extract which is comparable with glibenclamide, a standard anti-diabetic drug. The decrease in High Density Lipoprotein (HDL) concentration of the diabetic control relative to the normal control and increase in HDL concentration in the standard control compared to the diabetic control showed that the diabetic rats had lower concentration of good cholesterol (HDL) while the standard drug increased the good cholesterol levels in the standard control. There were no significant ($p>0.05$) increase in TAG concentrations observed in the diabetic control (group 2) and all treatment groups compared to the normal control (group 1) prior to treatments. Treatments with the standard drug for the standard control and methanol extract of *Nauclea latifolia* root bark for groups 4, 5 and 6, respectively resulted in a non-significant ($p>0.05$) decrease in TAG concentration when compared to the diabetic control (group 2). It is evidenced in this results that the methanol possesses anti-lipidemic and anti-hypercholesterolemia activity which could prevent excess deposition of LDL along the arterial wall that may lead to atherosclerosis and its associated cardiovascular diseases and also possibly activation inflammatory reactions. Partly, the

lipid lowering effect may be due to the inhibition of hepatic cholesterol biosynthesis and increased faecal bile acid secretion as reported by Kaur *et al.*[32]. These results are in consonance with the findings of Effiong and Essien[33] as well as the study by Asanga *et al.*[34].

CONCLUSION

The findings obtained from this study have shown that the methanol extract of *Nauclea latifolia* root bark administered to alloxan-induced diabetic rats positively modulated affected the haematological, hypoglycaemic and hypolipidaemic properties. The outcome, of this study gives scientific support on the medicinal values of *Nauclea latifolia* root bark and justifies its use in the management of diabetes and other ailments as claimed by traditional medicinal practitioners.

SIGNIFICANT STATEMENTS

The findings of this study validated the traditional use of the various extracts of *Nauclea latifolia* root bark in the management of diabetes as it demonstrated:

- That the extract is relatively safe for consumption
- Hypoglycaemic activity in diabetic rats
- Anti-hyperlipidaemic activity in diabetic rats
- Haematological stimulatory potentials in diabetic rats More research on this plant extract could yield potent drug/herbal products for efficient management of blood glucose level in diabetic patients

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