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Research Article

Hepatoprotective and Healthy Kidney Promoting Potentials of Methanol Extract of *Nauclea latifolia* in Alloxan Induced Diabetic Male Wistar Albino Rats

¹Ogugua Victor Nwadiogbu, ²Uroko Robert Ikechukwu, ²Egba Simeon Ikechukwu and ¹Agu Obiora

¹Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria

²Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Abstract

Background and Objective: *Nauclea latifolia* root-bark extracts are used traditionally to treat various diseases. The study investigated phytochemical compositions and effects of methanol extract of *Nauclea latifolia* root-bark on liver and kidney function parameters of alloxan induced diabetic male wistar rats to understand possible effect it could have on those who use the extract to manage diabetes.

Materials and Methods: The acute toxicity study was carried out using 18 male mice while 30 male Wistar albino rats were used to study the effects of the extract on the liver and kidney function parameters using standard analytical methods. The rats were divided into six groups of five rats each with group 1 as normal control, group 2 as the negative that was alloxan induced but not treated, group 3 rats were alloxan induced but treated with 2.5 mg kg⁻¹ body weight glibenclamide. Group 4-6 rats were alloxan induced but treated with 100, 200 and 400 mg kg⁻¹ body weight of the methanol extract respectively for 14 days. The data obtained were statistically analyzed using one way analysis of variance at 95% confidence level. **Results:** Phytochemical analysis of the extract showed high concentrations of pharmacological active phytochemicals like flavonoids, saponins, proteins, carbohydrate and alkaloids. The acute toxicity study showed that the extract was relatively safe as no death or adverse reactions were observed after extract was administered to the mice. The induction of diabetic condition in rats with alloxan resulted in significant ($p < 0.05$) increase in activities of liver marker enzymes (AST, ALT and ALP) concentrations of total bilirubin, urea, creatinine and serum electrolytes. Treatments with glibenclamide and the methanol extract, respectively showed significant ($p < 0.05$) decreases in the activities of these liver marker enzymes and concentrations of total bilirubin, urea, creatinine and serum electrolytes when compared to the negative control. The effects of the methanol extract were inversely proportional to the dose of the extract administered. **Conclusion:** The findings of this study suggest that the methanol extract possesses hepatoprotective and pharmacological activities that could promote liver and kidney function and thus, could protect these organs from both endogenous and exogenous stressors.

Key words: Diabetic rats, liver marker enzymes, serum electrolyte, hepatoprotection, *Nauclea latifolia*

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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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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Nauclea latifolia is a valuable medicinal plant commonly found in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa where it is best known as African peach and used in traditional medicinal¹. Extracts of various parts *Nauclea latifolia* have been used therapeutically to treat malaria, hypertension, prolonged menstrual flow, cough, gonorrhoea, stomach disorders, dysentery, ulcers and liver ailments²⁻⁴. In Nigeria, *Nauclea latifolia* smith is commonly known as Ubulinu, ovoro ilu (Igbo i.e., South-Eastern Nigeria), Tafashiya, tafiyaigia (Hausa i.e., Northern Nigeria), Ebeyesi, egbesi (Yoruba i.e., South-Western Nigeria)⁵.

Plants play significant roles in human lives not only by functioning as a primary producer in the food chain but also by providing pharmacologically active phytochemicals. The phytochemicals produced by plants could be harnessed for the production of vital pharmaceutical drugs and herbal medicines for the treatment of common illness and some infectious diseases confronting man every day. Crude plant extracts rich in pharmacologically active phytochemicals can be effectively for the prevention, treatment and management of some diseases⁶. Every plant contains phytochemicals at varying degree depending on plant species and plant regions which could be affected by various processing methods, geographical locations and age of the plants. Many phytochemicals possess a wide range of pharmacological activities which include: antioxidant, antimicrobial, antiviral, antidiabetic, anti-inflammatory, immunological properties, a decrease of platelet aggregation and modulation of hormone metabolism and anticancer properties⁷⁻⁹. Diabetes mellitus, a medical condition associated with a defect in carbohydrate metabolism as a result of lack/deficiency of insulin secretion or a varying degree of insulin resistance could be managed successfully with dietary restrictions and herbal formulations¹⁰. At present, diabetes mellitus is incurable with about 2.8% of the world population affected and Type 1 diabetes mellitus accounts for approximately 5-10% of all diagnosed cases of diabetes in adults¹¹⁻¹³. Genetic abnormalities decreased physical activity, obesity, stress and changes in food consumption have been shown to increase the prevalence of diabetes with World Health Organization projecting that it would be one of the major health challenges in the next 14 years¹⁴.

Herbal drugs currently are the most acceptable alternative medicine for the majority of the world population both in the developing and in the developed countries¹⁵. There is an increase in the use of herbal remedies for the treatment of various diseases especially among the rural

populace in the developing countries, which are mostly attributed to their potency and their availability as a cheap source of medical treatments¹⁶. There exists cumulating evidence that medicinal plants serve as valuable starting materials for drug development in both developed and developing countries most especially in Africa where it acts as the first line of treatment of various diseases for more than 80% of her population and various parts of *Nauclea latifolia* have also been used⁹.

There is insufficient toxicological evidence to support the claim that *Nauclea latifolia* possesses medicinal properties to treat various ailments and diseases linked to it and that it does not cause significant harms to those individuals that consume it. This study was designed to evaluate the phytochemical composition and toxicological effects of methanol extract of *Nauclea latifolia* root bark on liver and kidney function parameters of alloxan induced diabetic male Wistar albino rats.

MATERIALS AND METHODS

The study was conducted at the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria Nsukka, Nigeria between April 27, 2015 and March 11, 2016.

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

Experimental animals: Thirty male Wistar albino rats weighing 129-350 g and eighteen male mice weighing 18-28 g were obtained from the Animal House of the University of Nigeria formal Teaching Hospital, Enugu, Enugu State, Nigeria. The rats were randomly divided into six groups of five rats/group and the mice were also divided into six groups of 3 mice each respectively. The animals were housed in a well-ventilated animal house under 12 h daylight 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 was 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 a powder using a blender. A quantity, 500 g of the pulverized

sample was then extracted with 80% methanol for 48 h (i.e., cold extraction). The solution was filtered through Whatman filter paper to obtain the filtrate which was concentrated by evaporating in a water bath at 55°C and percentage (%) yield of the extract calculated.

Experimental design: Three groups of the mice made up of 3 mice each was used for the phase I of the acute toxicity study and the second three groups used for the phase II study respectively. The 30 male Wistar albino rats divided into six groups of five rats each was used for the study. Group 1 rats served as normal control (no diabetes was induced and no treatment was 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 body weight of the standard antidiabetic drug) while groups 4, 5 and 6 were diabetic rats treated with 100, 200 and 400 mg kg⁻¹ body weight of the extract, respectively. Treatment lasted for 14 days and after an overnight fast, the animals were sacrificed on day 15 under mild anesthesia (10% formosaline). The administration of methanol extract of *Nauclea latifolia* root-bark and the standard drug (Glibenclamide) was done using an intra-gastric syringe. Blood samples were collected in the plain bottle for the analyses of the effect of the methanol extract on the liver and kidney function parameters biochemical parameters.

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 body weight of alloxan-monohydrate dissolved in normal saline. The blood glucose concentration was monitored daily and rats with blood glucose more than 200 mg dL⁻¹ were considered diabetic.

Phytochemical analysis of the root-bark methanol extract of *Nauclea latifolia*: The preliminary phytochemical screening of the methanol extract root bark of *Nauclea latifolia* root-bark was carried out in order to ascertain the presence of some important phytochemicals and those detected were quantified. Both qualitative and quantitative analyses were done using standard methods described by Harborne¹⁷ and Trease and Evans¹⁸.

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 and 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 an indicator. In other words, it oxidizes the dye in a reaction mediated by peroxidase to produce a blue coloured complex. The intensity of the color 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 Lorke¹⁹.

Alanine aminotransferase (ALT) activity assay: Determination of serum ALT activity was done using the method of Reitman and Frankel²⁰.

Principle: The ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-Dinitrophenylhydrazine.

Aspartate aminotransferase (AST) activity assay: Determination of serum AST activity was done using the method of Reitman and Frankel²⁰.

Principle: Aspartate aminotransferase (AST) activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-Dinitrophenylhydrazine.

Alkaline phosphatase (ALP) activity assay: Determination of serum ALP activity was done using the method by Reitman and Frankel²⁰.

Principle: Serum alkaline phosphatase hydrolyzes a colorless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which, at alkaline pH values turns into a pink color that can be photometrically determined.

Determination of total serum bilirubin (T. bil) concentration: The total serum bilirubin concentration was determined using the method of Jendrassik and Grof²¹.

Principle: Total Bilirubin is determined by reaction with diazotized sulfanilic acid, in the presence of caffeine, with the final production of an azo-pigment. The same reaction, but in the absence of caffeine is used to measure Direct Bilirubin.

Determination of urea concentration: Serum urea concentration was determined by the method of Varley and Alan²².

Principle: Urea in Serum is hydrolyzed to ammonia in the presence of urease which was then measured photometrically by Berthelot's reaction.

Determination of creatinine concentration: Determination of serum creatinine concentration was done by Jaffe's reaction as described by Peters²³.

Principle: Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Determination of sodium ion (Na⁺) concentration: The serum sodium ion concentration was determined using the method of Terri and Sesin²⁴.

Principle: Sodium was precipitated as the triple salt of sodium magnesium uranyl acetate with the excess uranium, which reacts with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Determination of chloride ion (Cl⁻) concentration: Serum chloride ion concentration was determined using the method of Skeggs and Hochstrasser²⁵.

Principle: Chloride ions form soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a color complex that absorbs light at 480 nm. The intensity of the color produced is directly proportional to the chloride concentration.

Determination of potassium ion (K⁺) concentration: Serum potassium ion concentration was done using the method of Terri and Sesin²⁴.

Principle: The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension, the turbidity of which is proportional to potassium ion concentration in the range of 2-7 mEq L⁻¹.

Statistical analysis: The results were analyzed statistically using one-way analysis of variance (ANOVA) to get the

grouped mean which was used to determine the significant difference between the group means at 95% level of confidence (p<0.05), using the statistical products and service solutions (IBM SPSS Statistics 20)²⁶.

RESULTS

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

Qualitative phytochemical compositions of methanol extract of *N. latifolia* root-bark: The results of the phytochemical analysis of the root-bark extract of *N. latifolia* are shown in Table 1. The results show that flavonoids, saponins, protein and carbohydrate are the major phytochemical constituents present in the methanol extract of *Nauclea latifolia* root bark while resin was not detected.

Quantitative phytochemical compositions of the methanol extract of *N. latifolia* root-bark: The results of the quantitative phytochemical analysis of methanol extract of *N. latifolia* root-bark are shown in Table 2. Total phenolics and carbohydrates were found to have the highest concentrations. Alkaloids, flavonoids, saponins and proteins were found to be moderately high in concentrations. Others were found in low concentrations. The results obtained correlated with the qualitative results shown in Table 1.

Acute toxicity of methanol extract of *Nauclea latifolia* root-bark: The data in Table 3 showed that the extract is not acutely lethal up to the highest dose (5000 mg kg⁻¹ body weight) administered to the experimental animals as no death or adverse reactions were observed in mice within or after

Table 1: Qualitative composition of *Nauclea latifolia*

Phytochemicals	Bioavailability
Alkaloids	++
Flavonoids	+++
Tannins	+
Protein	+++
Saponins	+++
Reducing sugar	++
Glycoside	++
Resin	ND
Cardiac glycoside	+
Carbohydrate	+++
Cyanide	+
Acidic compounds	+

+++ : High presence, ++ : Moderate presence, + : Low presence, ND: Not detected

24 h of the administration of the methanol extract of *N. latifolia* root-bark.

Effects of *Nauclea latifolia* root-bark on the liver function indices of alloxan induced diabetic rats: The data in Table 4 showed significant ($p < 0.05$) increase in the liver marker enzymes (ALT, AST and ALP) activity of the alloxan induced diabetic rats when compared with the normal control rats. Treatment of the diabetic rats with methanol extract of *Nauclea latifolia* root-bark produced significant ($p < 0.05$) decrease in liver marker enzyme activity of group 4, 5 and 6 that received 100, 200 and 400 mg kg⁻¹ body weight of the extract respectively. The group 3 rats treated with the standard drug showed a non-significant increase in liver marker enzymes activity when compared to the normal control that was not alloxan induced. The effects of the extract on the liver marker enzymes activity were to be inversely proportional to the dose of the extract administered per body weight. The same trend was observed in the effects of the

extract on the total bilirubin concentration where the alloxan induced diabetic rats showed significant ($p < 0.05$) increase in the total bilirubin concentration in the alloxan-induced untreated rats when compared to the normal control rats. Treatment with the standard drug and extract, respectively caused significant ($p < 0.05$) decrease in the total bilirubin concentration of the alloxan induced diabetic rats. The rats treated with the standard drug had a lower total bilirubin when compared to the rats treated with extract. It was also observed that the extract exhibited more hepatoprotective effects at lower concentration which gradually diminished with increasing concentrations.

Effects of methanol extract of *Nauclea latifolia* root-bark on urea and creatinine concentrations of alloxan-induced diabetic rats:

The values in Table 5 show the effects of methanol extract of *Nauclea latifolia* root-bark on urea and creatinine concentrations of alloxan-induced diabetic rats. It was observed that there were significant ($p < 0.05$) increase in the concentrations of urea creatinine in the diabetic control (group 2) when compared to the normal control (group 1). However, a non-significant decrease in urea and creatinine concentrations observed in all treatment groups when compared to the diabetic control (group 2) while no significant difference was observed between the standard control (group 3) and groups 4, 5 and 6 that were treated with different doses of the extract.

Effects of methanol extract of *Nauclea latifolia* root-bark on the serum electrolytes indices of alloxan-induced diabetic rats:

The data in Table 6 showed a non-significant increase in the serum electrolyte concentrations in the diabetic control for sodium and chloride ions and a significant ($p < 0.05$) increase in the concentration of potassium ion when compared to the normal control. Treatment with the extract produced non-significant difference in the serum electrolyte concentrations in group 3 rats treated with standard drug and groups 4, 5 and 6 which were treated with different doses of the extract when compared with the diabetic control (group 2).

Table 2: Phytochemical compositions of the methanol extract of *N. latifolia* root-bark

Phytochemical constituents	Concentration (mg/100g)
Alkaloids	13.54±0.02
Flavonoids	17.33±0.03
Total phenols	166.45±0.03
Tannins	0.05±0.01
Saponins	5.60±0.04
Reducing sugar	2.93±0.05
Glycoside	1.28±0.05
Carbohydrate	158.70±0.03
Cyanide	0.03±0.02
Protein	5.87±0.04

Values are expressed as Means ± Standard deviation (n = 3)

Table 3: Lethal doses (LD₅₀) of the methanol extract of *Nauclea latifolia* root-bark extract in male Wistar albino mice

Phases I	Dosage (mg kg ⁻¹ b.wt.)	Mortality rate
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

Table 4: Liver functional indices of *Nauclea latifolia* root-bark extract treated alloxan induced diabetic rats

Groups	AST (IU L ⁻¹)	ALT (IU L ⁻¹)	ALP (IU L ⁻¹)	Total Bil. (mmol L ⁻¹)
I	74.67 ± 17.93 ^b	66.67 ± 17.93 ^b	49.33 ± 4.04 ^b	0.38 ± 0.12 ^b
II	133.33 ± 9.45 ^a	92.67 ± 12.22 ^{a,c}	65.67 ± 2.08 ^{a,c}	0.93 ± 0.21 ^{a,c}
III	108.67 ± 13.01 ^b	68.00 ± 9.17 ^b	56.00 ± 4.58 ^b	0.55 ± 0.58 ^b
IV	112.00 ± 21.07 ^a	80.00 ± 26.00 ^a	59.67 ± 7.51 ^a	0.58 ± 0.67 ^b
V	112.00 ± 27.79 ^a	86.67 ± 13.01 ^a	60.00 ± 3.46 ^a	0.77 ± 0.87 ^a
VI	118.67 ± 21.94 ^a	89.33 ± 42.72 ^a	64.33 ± 5.51 ^a	0.78 ± 0.12 ^{a,c}

Value are expressed as the mean ± standard deviation (n = 3). Mean values with alphabets a, b, or c as superscript (s) across the rows are considered significant ($p < 0.05$) compared with normal, diabetic and standard control, respectively

Table 5: Urea and creatinine concentrations of alloxan-induced diabetic rats treated with methanol extract of *Nauclea latifolia* root-bark

Groups	Urea (mmol L ⁻¹)	Creatinine (mmol L ⁻¹)
1	68.00±14.80 ^a	2.33±0.12 ^a
2	87.33±8.33 ^b	2.87±0.42 ^b
3	72.67±5.03	2.40±0.10
4	79.67±14.47	2.77±0.50
5	64.00±4.00	2.57±0.21
6	75.00±6.25	2.77±0.21

Value are expressed as the mean ± standard deviation (n = 3). Mean values with alphabets a, or b as superscript (s) across the rows are considered significant (p<0.05) compared with normal, diabetic and standard control, respectively

Table 6: Serum electrolyte indices of alloxan-induced diabetic rats treated with methanol extract of *Nauclea latifolia* root-bark

Groups	Na ⁺ (mEq L ⁻¹)	Cl ⁻ (mEq L ⁻¹)	K ⁺ (mEq L ⁻¹)
1	141.00±6.56	79.33±5.13	4.13±0.61 ^a
2	152.33±43.00	78.33±5.67	5.27±0.31 ^b
3	129.00±14.11	84.00±6.93	4.47±0.95
4	145.67±16.65	82.33±15.50	4.47±0.55
5	146.00±33.05	77.33±2.31	4.47±0.15
6	141.33±37.55	77.33±6.03	4.60±0.62

Value are expressed as the mean ± standard deviation (n = 3). Mean values with alphabets a, or b as superscript (s) across the rows are considered significant (p<0.05) compared with normal, diabetic and standard control, respectively

DISCUSSION

The study investigated phytochemical compositions and effects of methanol root bark extract of *Nauclea latifolia* on liver and kidney function parameters of alloxan induced diabetic male Wistar albino rats. The median lethal dose (LD₅₀) revealed that the extract is safe up to 5000 mg kg⁻¹ body weight 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 which may be attributed to the low or absence of toxic constituents in the methanol extract.

The higher concentrations of pharmacological important phytochemicals such as total phenols, flavonoids, alkaloids and saponins recorded from both the qualitative and quantitative phytochemical analyses of methanol extract of root bark *Nauclea latifolia* show that it possesses bioactive compounds capable of conferring health benefits. Consumption of flavonoids rich foods in right proportions has been reported by Ikechukwu *et al.*⁹, to reduce the risk of diseases and conditions associated with oxidative stress due to the antioxidant activities exhibited by flavonoids⁵. Apart from the antioxidant properties of flavonoids, alkaloids and saponins, have also been reported to possess other useful biological activities²⁶. The result of phytochemical recorded in this study is in-line with the findings of other researcher who ethanol stem-bark and leaf extract of *Nauclea latifolia* respectively and reported similar phytochemical composition²⁷⁻²⁸.

The liver and kidneys play a critical role in various metabolic processes which make them prone to the toxic

effects of many exogenous compounds²⁹. The concentration of liver marker enzymes such as AST, ALT and ALP are frequently used to measure the status of liver function, however, only ALT activity is remarkably specific for liver function³⁰. In this study, there were significant (p<0.05) increase observed in these liver marker enzymes (AST, ALT and ALP) activity in the diabetic control which was alloxan induced but not treated suggesting that the alloxan might have compromised the liver integrity and function possibly through the mechanism of free radical generation by the alloxan. These might have damage liver membrane and resulted to the increased permeability of the membrane leading to the leakage of the liver enzymes to the extrahepatic tissues. The significant decrease (p<0.05) observed in these liver marker enzymes of the extract treated groups relative to the diabetic untreated group could be attributed to phytochemical constituents such as flavonoids, phenols and alkaloids which might have exhibited antioxidant activity against free radicals generated by alloxan which could have caused liver damage and thus, minimized the damage caused by alloxan to liver architecture and function. This is in line with the previous report that medicinal herbs can exhibit hepatoprotective effects through additive and synergistic actions of antioxidant activities of their phytochemicals constituents like phenol and flavonoids³¹. The extracts could have restored the membrane permeability thereby preventing leakage of the liver enzymes to the extrahepatic tissue and indicated that the extract could be said to have exhibited hepatoprotective activity. It also showed that the extract exhibited more hepatoprotective activity at low doses as the hepatoprotective activity decrease with increasing doses which could be an indication

that the extract may contain other non hepatoprotective components that could have interfered with its hepatoprotective activity. Identification, isolation and purification of the active hepatoprotective components in this extract will play a vital role in the development of a hepatoprotective drug or herbal formulation and reduction in the consumption of inactive components that may have adverse health effects.

Serum bilirubin levels could be expressed as total bilirubin comprising of conjugated and non-conjugated or as direct bilirubin comprising only of the conjugated bilirubin. An increase in bilirubin level could be attributed to three major causes such as hemolysis, biliary obstruction and liver cell necrosis³². The reductions in bilirubin concentrations in all treatment groups particularly group 4 that was significant when compared to the diabetic control was an indication that the extract may not have had a deleterious effect on the liver. Renal function depends on the integrity of absorption, reabsorption and excretion of these markers (urea, creatinine, sodium ion, chloride ion and potassium ion, among others. The observed lower concentrations of urea and creatinine in all the treated groups when compared to the normal control indicated that the extract possesses renal protective properties comparable to the standard drug that could have improved glomerular filtration rate of urea and creatinine concentrations in the diabetic rats. The general non-significant differences observed in serum concentrations of urea, creatinine, sodium, chloride and potassium ions of groups 4, 5 and 6 treated with different doses of the extract compared to the standard control (group 3) tend to support the efficacy of the extract to protect the nephrons from damage, which is exacerbated in diabetes mellitus. Similar results have been reported by several other researchers³³⁻³⁵.

CONCLUSION

The findings of this study suggest that methanol extract of *Nauclea latifolia* root possesses hepatoprotective property capable of maintaining liver integrity and functions through stabilization of membrane as observed in the decreased amount of liver marker enzymes and also promote proper kidney functions. The extract was most effective in the prevention of liver and kidney damage at a dose of 100 mg kg⁻¹ body weight. Thus, identification of the active components in this extract, responsible for its medicinal potentials will help in the formulation of more effective hepatoprotective drugs that will improve human health.

SIGNIFICANCE STATEMENT

The study discovers the possible synergistic effect of bioactive flavonoids, total phenolics, saponins, alkaloids and protein present in the methanol extract of *Nauclea latifolia* root-bark that can be beneficial in maintaining liver and kidney integrity and functions in alloxan induced-diabetes. This study will help the researcher to uncover the critical area of liver and kidney damage associated with uncontrolled diabetes. Thus, a new approach in the use of plant extracts and possibly diet in the management of diabetic condition may be advanced to improve the lives of diabetic patients.

REFERENCES

1. Traore-Keita, F., M. Gasquet, C. Di Giorgio, E. Ollivier and F. Delmas *et al.*, 2000. Antimalarial activity of four plants used in traditional medicine in Mali. *Phytother. Res.*, 14: 45-47.
2. Dalziel, J.K., 1957. *The Useful Plants of West Tropical Africa*. 2nd Edn., Crown Agents, London, pp: 241-248.
3. Di Giorgio, C., M. Lamidi, F. Delmas, G. Balansard and E. Ollivier, 2006. Antileishmanial activity of quinovic acid glycosides and cadambine acid isolated from *Nauclea diderrichii*. *Planta Med.*, 72: 1396-1402.
4. Nworgu, Z.A.M., D.N. Onwukaeme, A.J. Afolayan, F.C. Ameachina and B.A. Ayinde, 2008. Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. *Afr. J. Pharm. Pharmacol.*, 2: 37-41.
5. Anowi, C.F., N.C. Cardinal, C.O. Ezugwu and U.A. Utoh-Nedosa, 2012. Antimicrobial properties of the chloroform extract of the stem bark of *Nauclea latifolia*. *Int. J. Pharm. Pharm. Sci.*, 4: 744-750.
6. Shafaei, A., E. Farsi, B.M.K. Ahamed, M.J.A. Siddiqui, I.H. Attitalla, I. Zhari and M.Z. Asmawi, 2011. Evaluation of toxicological and standardization parameters and phytochemical investigation of *Ficus deltoidea* leaves. *Am. J. Biochem. Mol. Biol.*, 1: 237-243.
7. Rao, B.N., 2003. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pac. J. Clin. Nutr.*, 12: 9-22.
8. Saxena, M., J. Saxena, R. Nema, D. Singh and A Gupta, 2013. Phytochemistry of medicinal plants. *J. Pharmacogn. Phytochem.*, 1: 168-182.
9. Ikechukwu, U.R., S.R.S. Adeyi, M.K. Hadiza and A.C. Lilian, 2015. Effect of methanol extract of *Abrus precatorius* leaves on male wistar albino rats induced liver damage using carbon tetrachloride (CCl₄). *J. Biol. Sci.*, 15: 116-123.
10. Reddy, Y.S., M. Giridhar, A. Mohan, V. Mohan and N.R. Sharma, 2015. Bioinformatics based study on *Curcumin* pharmacophore and its suitability as natural remedy for diabetes. *Res. J. Pharm. Biol. Chem. Sci.*, 6: 1088-1096.

11. Bobb, A., D. Gale, S. Manmohan, A. Mohammed, F. Seetahal, P. Small and K. Mungrue, 2008. The impact of the Chronic Disease Assistance Plan (CDAP) on the control of type 2 diabetes in Trinidad. *Diabetes Res. Clin. Pract.*, 80: 360-364.
12. Etuk, E.U., 2010. Animals models for studying diabetes mellitus. *Agric. Biol. J. North Am.*, 1: 130-134.
13. International Expert Committee, 2009. International expert committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care*, 32: 1327-1334.
14. Edwin, E., E. Sheeja, V.B. Gupta and D.C. Jain, 2006. Fight diabetes the herbal way. *Express Pharm. Rev.*, 1: 41-42.
15. Ogbonnia, S.O., F.E. Nkemehule and E.N. Anyika, 2009. Evaluation of acute and subchronic toxicity of *Stachytarpheta angustifolia* (Mill) Vahl (Fam. Verbanaceae) extract in animals. *J. Biotechnol.*, 8: 1793-1799.
16. Ogbonnia, S.O., G.O. Mbaka, F.E. Nkemehule, J.E. Emordi, N.C. Okpagu and D.A. Ota, 2014. Acute and subchronic evaluation of aqueous extracts of *Newbouldia laevis* (Bignoniaceae) and *Nauclea latifolia* (Rubiaceae) roots used singly or in combination in Nigerian traditional medicines. *Br. J. Pharmacol. Toxicol.*, 5: 55-62.
17. Harborne, J.B., 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edn., Chapman and Hall, London, UK, ISBN-13: 9780412572609, pp: 88-185.
18. Trease, G.E. and M.C. Evans, 1989. *Trease and Evans' Pharmacognosy*. 13th Edn., Bailliere Tindall, London, UK, ISBN-13: 9780702013577, pp: 144-148.
19. Lorke, D., 1983. A new approach to practical acute toxicity testing. *Arch. Toxicol.*, 54: 275-287.
20. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
21. Jendrassik, J. and P. Grof, 1938. *In-vitro* determination of total and direct bilirubin in serum or plasma. *Biochemistry*, 6: 269-275.
22. Varley, H. and H.G. Alan, 1984. *Tests in Renal Disease*. In: *Practical Clinical Biochemistry*, Varley, H. (Ed.). William Heinemann Medical Book Ltd., London, UK, pp: 10.
23. Peters, J.H., 1942. The determination of creatinine and creatine in blood and urine with the photoelectric colorimeter. *J. Biol. Chem.*, 146: 179-186.
24. Terri, A. and P. Sesin, 1958. Determination of serum potassium by using sodium tetraphenylborate method. *Am. J. Clin. Pathol.*, 29: 86-90.
25. Skeggs, L.T. and H. Hochstrasser, 1964. Multiple automatic sequential analysis. *Clin. Chem.*, 10: 918-936.
26. IBM., 2011. *IBM SPSS Statistics for Windows, Version 20.0*. IBM Corporation, Armonk, New York, USA.
27. Udobre, A.S., C.O. Usifoh, O.A. Eseyin, A.E. Udoh and O.A. Awofisayo *et al.*, 2012. The wound healing activity of methanol extract of the stem bark of *Nauclea latifolia*. *Int. J. Pharm. Biomed. Sci.*, 3: 136-139.
28. Egbung, G.E., I.J. Atangwho, I.A. Iwara, M.O. Odey and P.E. Ebong, 2013. Chemical composition of root and stem bark extracts of *Nauclea latifolia*. *Arch. Applied Sci. Res.*, 5: 193-196.
29. Prochazkova, D., I. Bousova and N. Wilhelmova, 2011. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 82: 513-523.
30. Bidhe, R.M. and S. Ghosh, 2004. Acute and subchronic (28-day) oral toxicity study in rats fed with novel surfactants. *AAPS PharmSci*, 6: 7-16.
31. Horton, H.R., L.A. Moran, R.S. Ochs, J.D. Rawn and K.G. Scrimgeour, 1996. *Proteins: Three-Dimensional Structure and Function*. In: *Principles of Biochemistry*, Lehninger, A.L., D.L. Nelson and M.M. Cox (Eds.). 2nd Edn., Prentice Hall, USA, pp: 79-119.
32. Wang, X., N. Wang, F. Cheung, L. Lao, C. Li and Y. Feng, 2015. Chinese medicines for prevention and treatment of human hepatocellular carcinoma: Current progress on pharmacological actions and mechanisms. *J. Integr. Med.*, 13: 142-164.
33. Tilkian, S.M., C.B.M. Sarko and A.G. Tilkian, 1979. *Clinical Implications of Laboratory Tests*. 2nd Edn., C.V. Mosby Company, St. Louis, MI., USA.
34. Uko, E.K., O. Erhabor, I.Z. Isaac, Y. Abdulrahman and T.C. Adias *et al.*, 2013. Some haematological parameters in patients with type-1 diabetes in Sokoto, North Western Nigeria. *J. Blood Lymph*, Vol. 3. 10.4172/2165-7831.1000110.
35. Shang, Q., J. Xiang, H. Zhang, Q. Li and Y. Tang, 2013. The effect of polyhydroxylated alkaloids on maltase-glucoamylase. *PLoS ONE*, Vol. 8. 10.1371/journal.pone.0070841.