Full Length Research Paper

The effect of *Nauclea latifolia* leaf extract on some biochemical parameters in streptozotocin diabetic rat models

*[1]Effiong, Grace Sylvester and [2]Akpan, Henry Dan.

[1]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo. P.M.B. 1017, Uyo, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo. P.M.B. 1017, Uyo, Nigeria

*Corresponding author's e-mail: graceffiong2007@yahoo.com

Abstract

The effect of *Nauclea latifolia*, a promising vegetable used in the traditional management of metabolic disorders on some biochemical assay was investigated in diabetic rat. The study design consisted of twenty four rats divided into four groups of six rats each. Whereas groups 1 and 2, non diabetic and diabetic controls received placebo treatment, groups 3 received 200mg/kg b.w. of *Nauclea latifolia* twice a day while the 4th group received subcutaneous insulin, 5IU/kg b.w. per day, for 21 days. Thereafter, the animals were sacrificed, and their serum was used to assay lipid components and liver function enzymes, using standard analytical kits. Measured blood glucose in diabetic animals decreased significantly from initial by 61.51% upon treatment with *Nauclea latifolia*. Whereas diabetes induction caused significant increases (p<0.05) in total cholesterol by 54.42% and low density lipoprotein by 55.0% compared to the normal control (NC), treatment with extract of NL significantly decreased (p<0.05) these by 24.79% and 33.38% respectively. Also the amino transferases (ALT and AST) activities which increased by 66.83% and 72.87% in the diabetic control rats indicating hepatotoxicity secondary to hyperglycemia became reduced upon treatment with NL. Thus, *Nauclea latifolia* extract may provides a high efficacy in protection against atherosclerosis and hepatotoxicity in diabetes.

Keywords: Aminotransferases, blood glucose, diabetes mellitus, lipid profile, *Nauclea latifolia*

Abbreviations

Nauclea latifolia: NL; Blood Glucose Level: BGL; Total Cholesterol: TC; High Density Lipoprotein: HDL; Very Low Density Lipoprotein: VLDL; World Health Organisation: WHO; Normal Control: NC; Diabetic Control: DC; Triglyceride: TG.

INTRODUCTION

Diabetes mellitus is a multifactorial disease, which is characterized by hyperglycemia and glucosuria (Atangwho, 2008) among others. Diabetes mellitus is also a non-communicable disease, which is considered as one of the five leading cause of death in the world (Zimmet, 1999).

Serum enzymes in this case viz. alanine aminotransferase, (ALT), Aspartate aminotransferase (AST), are present in the hepatic and biliary cells (Jensen et al., 2004). These enzymes are usually released from the hepatocytes and leak into circulation causing increase in their serum levels under hepatocellular injury or inflammation of the biliary tract cells. Serum levels of these enzymes are particularly high in acute hepatocellular damage caused by drug toxicity and xenobiotics (Norman, 1998). The extent of the enzymes changes is related to the nature, closes to toxic agent and duration of toxicity (Brukner et al., 1984; Shi et al., 2003; Song et al., 2003).

Many investigations have shown that diet treatment or drug therapy to regulate cholesterol can decrease subsequent cardiovascular disease (CVD) associated mortality and morbidity (Kwiterovich, 1997). On the basis of this, great effort have been made to reduce the risk of CVD through the regulation of cholesterol, thus the therapeutic benefits of plant foods have been focused on many extensive dietary studies (Yoko-Zawa et al., 2006 and Zhang et al., 2007).

Nauclea latifolia (NL) (Rubiaceae) commonly known as Pin cushion tree is a strangling shrub or small tree native to tropical Africa and Asia. It grows in Akwa Ibom and Cross River states of Nigeria and is called "Mbom-mbong" whilst in the Northern Nigeria; it is called "Tabasiya. Parts of the plant are commonly prescribed traditionally as a remedy for diabetes mellitus and hypertension (Akpanabiatu *et al.,* 2005; Nworgu *et al.*, 2008 and Okwori *et al.*, 2008).

However, there are not much empirical data or scientific reports to support the antidiabetic effect of the plant. The working hypothesis is 'if *Nauclea latifolia* extract may provide high efficacy in the protection against atherosclerosis and hepatotoxicity in diabetes or not.' This present study was designed to test the antidiabetic effect of ethanolic extract of *Nauclea latifolia* in normoglycemic and STZ-induced diabetic rats.

MATERIALS AND METHODS

Plant material

Fresh *Nauclea latifolia* leaves were collected by the fence of Industrial Training Fund office in Calabar, Cross River State of Nigeria. The sample was authenticated by Dr. E. G. Amanke, a Botanist in the Department of Botany, University of Calabar, Nigeria and voucher specimen deposited at the Department of Botany herbarium, University of Calabar.

Preparation of ethanol extract of plants

The ethanol extract was prepared using the wet method of extraction. One kilogram of the fresh leaves of the plant were cut into pieces, blended in1.5litres of ethanol (96%) with an electric blender and transferred into amber coloured bottle and kept in cool (4°C) dark compartment for 72hours. The blend was filtered using a cheese cloth and thereafter with Whatman No 1 filter paper. The extract was concentrated *in vacuo* using a rotary evaporator at 37-40°C and dried completely in a desiccator containing a self-indicating silica gel.

Animals

Albino Wistar rats of (150-250g) of both sexes obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy of

the University of Uyo, Nigeria were used with each cage containing the same sex of animals to avoid mating and pregnancy. They were kept in clean cages (wooden bottom and wire mesh top), maintained under standard laboratory conditions (Temperature 25± 5°C, Relative humidity 50-60%, and a 12/12h light/dark cycle) and were allowed free access to standard diet (Vital Feed from Grand Cereals and Oil Mills Limited, Jos, Plateau State of Nigeria) and water *ad libitum*. Animals were acclimatized for 14 days in the animal house of the Department of Biochemistry, University of Calabar, Nigeria before each of the experiments. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of the Faculty of Pharmacy, University of Uyo, Nigeria.

Acute toxicity study

Animals

Swiss albino mice (20-25g) of both sexes obtained from the Department of Pharmacology and Toxicology, University of Uyo, Nigeria were used for the experiment after a14 day acclimatization. The animals were kept in clean plastic cages under standard conditions. Studies were carried out in accordance with the principles of good laboratory practice and animal handling.

Methods

Lethal dose (LD$_{50}$) determination was conducted using the method of Nofal et al., (2009). The evaluation was done in two phases. In phase one, four groups of six mice each, were treated with 2000, 4000, 6000 and 8000 mg extract b.w orally respectively. The mice were observed for clinical signs , symptoms of toxicity and death within 24 h. Based on the results of phase one, another four groups of six (6) fresh mice per group were each treated with 1000, 2000, 3000 and 4000 mg extract/kg b.w intraperitoneally (ip) respectively in the second phase. Clinical signs and symptoms of toxic effects and mortality were then observed for 24h. The LD$_{50}$ was then calculated according to Nofal et al., (2009) using the formulae;

$$LD_{50} = DM - \frac{\sum(Z \times d)}{n}$$

Where:
Dm = the largest dose which kills all animals.
z = Mean of dead animals between 2 successive groups
d = the constant factor between 2 successive doses.
n = Number of animals in each group.
$\sum$= the sum of (z × d)

Experimental Induction of Diabetes

The diabetes was induced in the overnight fasted animals by a single intraperitoneal injection of freshly prepared

solution of streptozotocin (Sigma, USA) 50mgkg^{-1}b.w. in 0.1M cold sodium citrate buffer pH 4.5 (Ghoraishian, 2006 and Rao and Naidu, 2010). The animals were considered as being diabetic if the blood glucose value were >200mg dl^{-1} on the third day after streptozotocin injection and were used in the experiment. This was estimated using One Touch Glucometer (Lifescan, inc 1995 Milpas, Califonia, USA) with blood obtained from the tail vein of the overnight fasted rats.

Experimental Protocol

Albino rats weighing 150-250g were used in this study. Twenty (24) rats included in the study were divided into four (4) groups of six (6) animals each (three diabetic and one non diabetic). The experimental groupings were as follows: NL = Diabetic rats treated with 200mg/kg.b.w of *Nauclea latifolia* twice daily, Insulin = diabetic rats treated with 5 units/kg.b.w of insulin, DC = diabetic control; administered distilled water as placebo orally via gastric intubations and NC = non-diabetic rats; also given placebo treatment orally via gastric intubations. Extracts were reconstituted in distilled water (vehicle) before use and were administered twice daily (7.00 am and 7.00 pm) while Streptozotocin was administered once a day.

Blood was collected on every 3 days through the rat's tail vein for glucose estimation. At the end of the experimental period, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapor and sacrificed. Immediately, overnight fasting blood samples were collected for sera preparation by cardiac puncture into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3,000rpm for 10 minutes using bench top centrifuge (MSE Minor, England) and stored frozen until needed for analysis. All analysis was completed within 24 hours of sample collection.

Biochemical Assays

Assay kits used for the biochemical assays were obtained from Randox Laboratories Ltd., Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom, Qt 94QY: Lipid profile- Triglycerides (TG), Total cholesterol (TC), High density lipoprotein (HDL), , Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were determined using Reitman and Frankel method, 1956., Glucose concentration were determined by the use of One Touch Glucometer (Lifescan, Inc., 1995 Milpitas, Califonia 95035, USA). The concentration of Very Low Density Lipoprotein (VLDL) was extrapolated by dividing the respective concentration of TG by 5 while Low Density Lipoprotein (LDL) was estimated using the method by Friedewald (1972) that is; "LDL = TC - HDL - VLDL".

Statistical Analysis

The results were analyzed by one-way ANOVA, using SPSS statistical package. All data were expressed as Mean ± SE and difference between groups considered significant at P=.05.

RESULTS

Acute toxicity studies

The LD$_{50}$ was calculated to be >5000mg/kg b.w with oral route of administration while intraperitoneally, the LD$_{50}$ was calculated to be 1500mg/kg.

Effect of treatment on Blood and Serum Glucose levels

Every three days changes in blood glucose monitored for 21 days following daily treatment with extract and insulin in diabetic and non-diabetic rats is shown on Table 1. Whereas diabetic induction (initial=292.00±21.05 mmol/L, final=214.67±14.42 mmol/L) causes a significant increase (P<0.05) in blood glucose level of the test animals, there was a significantly decreased (P<0.05) levels in NL (initial=406.67±27.68 mmol/L, final=194.67±14.42mmol/L) and insulin (initial=578.00±6.34mmol/L, final=77.33±10.36 mmol/L) treated rats. The serum glucose level in the untreated diabetic rats (DC) (11.92±0.80mmol/L) was significantly P<0.05) higher compared to NC (4.64± 0.20 mmol/L), while NL treated animals showed a rather significant decrease in the serum glucose (8.50±1.28 mmol/L) compared with the DC (Table 1).

Effect of treatment on serum lipid profile

Table 2 shows the changes in serum lipid concentration following a 21-day treatment period. Serum total cholesterol (TC) and low density lipoprotein (LDL) concentration which increased significantly (p<0.05) in diabetic control (DC) rats compared to non-diabetic control (NC) was decreased in the diabetic group treated with NL and that of insulin. These decrease were however non significant, compared to NC but were significant in comparison with DC. Also, NL treated diabetic rats showed a significant decrease when compared to the insulin group. Triacylglycerol (TG) in the serum of DC and NL indicated a non significant decrease compared to NC Serum, VLDL concentration showed a non significant decrease in NL treated group compared with NC.

Table 1. Effect of treatment on Blood and Serum Glucose levels of Diabetic and Non-Diabetic Rats

	FASTING BLOOD GLUCOSE LEVELS			
GROUP	**INITIAL (mg/dl)**	**FINAL (mg/dl)**	**%CHANGE**	**Serum glucose (mmol/L)**
DC	292.00±21.05	214.67±14.42* [b]	26.43* [b]	11.92±0.80*[b]
NC	73.59±2.21	73.16±2.06 [a]	0.58 [a b]	4.64±0.20 [a b]
NL	406.67±27.68	194.67±14.42*[a b]	61.51*[a b]	8.50±1.28*[ab]
INSULIN	578.00±27.68	77.33±10.36* [a]	86.62*[a]	3.97±0.66* [a]

*P<0.05 vs NC; a = P<0.05 vs DC; b = P<0.05 vs Insulin; Mean ± SE, n = 6,
DC = diabetic control, NC = non-diabetic control

Table 2. Effect of treatment on serum lipid profile

GROUP	TG (mg/dl)	TC(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
DC	82.86±9.91	448.59±36.04 [a, b]	8.26±1.04 [a, b]	398.80±38.17 [a, b]	16.57±1.98
NC	76.41±9.65	115.95±14.59 [a, b]	64.22±2.33[a]	92.40±15.06 [a, b]	15.28±1.93
NL	61.07±4.60	191.34±21.70*,[a, b]	66.83±0.14*, [a]	136.72±20.79* [a, b]	12.21±0.92
INSULIN	75.23±14.56	254.40±29.84*	66.36±0.15*	205.23±31.01*, [a]	15.05±2.91

*P<0.05 vs NC; a = P<0.05 vs DC; b = P<0.05 vs Insulin; Mean ± SE, n = 6, DC = diabetic control, NC = non-diabetic control

Table 3. Effect of Treatment on some Serum Enzymes in Diabetic and non- Diabetic Rats

GROUP	AST (U/L)	ALT (U/L)	Alpha-Amylase (U/L)
DC	70.67±0.42*	69.33±18.09*	254.14±45.41*
NC	19.17±2.52 [a]	83.00±4.02 [a]	166.59±26.65 [a]
NL	50.33±12.23*, [a, b]	14.33±1.38*, [a, b]	216.37±5.74[a, b]
INSULIN	73.33±2.11	46.67±1.72*, [a]	347.78±26.83*

*P<0.05 vs NC; a = P<0.05 vs DC; b = P<0.05 vs Insulin; Mean ± SE, n = 6, DC =
diabetic control, NC = non-diabetic control

Effect of Treatment on some Serum Enzymes

The effect of extracts treatments on biochemical indices of liver and pancreas functions in serum viz: alanine amino transferase (ALT), aspartate aminotransferase (AST) levels and alpha amylase are expressed in Table 3. Serum AST and ALT were raised significantly (P<0.05) by 3.19 and 3.01 fold respectively in the diabetic control (DC) relative to non-diabetic control (NC). Treatment with extract of NL and insulin for 21 days caused a significant reduction in AST and ALT in all diabetic treated groups relative to diabetic control. Alpha-amylase in the DC group (254.14±45.41U/L) increased significantly compared to NC (166.59±26.65U/L). Intervention with the

NL extract caused a significant decrease in the level of the enzyme (171.37±9.97U/L) compared to insulin treated rats (347.78±26.83U/L), indicating potentials of the plant, in protecting the pancreas. It was observed that the enzyme level in the treatment with NL (216.37±5.74U/L) was rather significantly increased (p<0.05) in comparison with NC (Table 3).

DISCUSSION

The acute toxicity test has been investigated to establish the adverse effects of the administration of the ethanol leaf extract of *Nauclea latifolia* on some behavioural and

clinical parameters. When administered orally, in mice, the ethanolic extract of *NL* produced no lethality even at doses as high as 8000mg/kg. Apart from weakness, N*L* did not produce any major signs of clinical toxicity over the 24hour observation period. Intraperitoneal administration produces no noticeable neurological or behavioural effects within the six hour observation. However, 100% lethality at 2000mg/kg and 0% lethality at 1000mg/kg were recorded after 24 hours. According to the (OECD, 2001) protocol NL leaf extract may be classified as non toxic since the limited dose of an acute toxicity is generally considered to be 5.0 g/kg bw (Nofal et al., 2009; Assam et al 2010).

Glucose concentration in blood at any time of the day is determined by metabolic processes in three major organs viz; liver, adipose and muscle, which themselves are under regulation by hormones (Champ *et al.*, 2005). In diabetes mellitus, this integrated regulation deteriorates over time precipitating chronic hyperglucemia. A treatment option, if efficacious, should over time re-establish this fine control.

Administration of Streptozotocin in this study led to 1.5-fold elevation of fasting blood glucose levels which was maintained over the experimental period of 3 weeks, the result shows that induction of diabetes with STZ caused a significant increase in blood glucose levels as seen in the diabetic control (DC) rats. This was in consonance with the work of Moore et al., 2012. Three weeks of daily treatment with NL led to a fall in blood sugar levels by 25-68 percent. This seems to reach a maximum after 15 days of treatment and remained constant to the third week. The continued treatment of diabetic rats for 21 days with the plant extract caused a significant reduction of blood glucose level by 61.51% comparable to insulin which is used for the treatment of type II diabetes; this was similar to the result of the research of Al-Zuhair et al., (2010).

Nwanjo, (2005) has shown that the administration of aqueous leaf extract of *Vernonia amygdalina* produced hypoglycaemic, hypolipidaemic and antioxidant effects in rats which is similar to the observation of this finding. The result is also in line with the findings of Adaramoye et al., (2007) and Ugochukwu et al., 2003). The cholesterol lowering effects of these plant extracts could be beneficial in preventing lipid abnormalities which may arise in certain metabolic disorders (Cho et al., 2002). Gidado et al (2008) have reported that flavonoids, tannins and saponins may play some roles in the hypolipidaemic effect of some plants. The mechanism of hypocholesterolaemic action of these plant leaves may be due to inhibition of the absorption of dietary cholesterol in the intestine or its production by the liver (Ahmed-Raus et al., 2001) or stimulation of the biliary secretion of cholesterol and cholesterol excretion in faeces (Anderson et al., 1991).

In an inflammatory condition, there is a leakage of cytoplasmic enzymes into circulation, hence ALT levels increased above that of AST. Thus, when there is gross cellular necrosis, as in STZ-induced diabetes-damaged to the pancreatic cells by STZ, the level of AST may rise higher than that of ALT (Recknagel, 1987). This is because ALT levels is increased in the serum due to conditions where cells of the liver have been inflamed or undergo cell death, and is specific for the liver cells (Jensen et al., 2004) but the AST levels can be triggered in other conditions such as myocardial infarction apart from hepatocellular damage (Jensen et al., 2004).

Some medicinal plants possess hepatoprotective effects. These effects are present because they contain some bioactive compounds (Shinkim and Anderson, 1963). The presence of saponins in a variety of herbal preparations administered to humans proved to be potent against cancer and hepatic cell proliferation (Lipkin, 1995), this effect was seen in NL as it reduced the concentration of the aminotransferases. This was in line with the study by Etim *et al.*, 2008 where Gongronema latifolium ameliorated the increase in serum AST and ALT levels caused by acute CCl_4 induced hepatotoxicity *in vivo*. Reduced levels of ALT and AST in rats treated with the extract could also be attributed to the ability of the NL to prevent the metabolism of streptozotocin into more toxic metabolite and minimized the production of free radicals, it also boost the activities of the scavengers of free radicals (STZ: Safe Working Practices Information page (2009), thus minimizing hepatocellular injury produced. The present study shows that NL markedly inhibit pancreatic α-amylase which is in accordance with the report of Kwon et al (2007) and Ebong et al (2008). These results suggest that NL may be potentially useful to control postprandial hyperglycaemia in patients with type 2 diabetes through inhibition of pancreatic α-amylase.

It is evident from the result of this investigation that *Nauclea latifolia* extract may provide high efficacy in the protection against atherosclerosis and hepatotoxicity in diabetes hence the need to adopt this strategy in bio-prospecting for antidiabetic natural products.

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