Evaluation of hypoglycemic and hypolipidemic effect of chloroform and methanolic extract of *Nauclea latifolia* (rubiaceae) on alloxan-induced diabetic rats

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Abstract

The abnormal lipid profiles and lipoprotein oxidation especially, Low density lipoprotein cholesterol is more common in diabetics and is aggravated with poor glycaemia control. In this study, hypoglycemic and hypolipidemic properties of chloroform and methanolic fractions of Nauclea latifolia leaves were investigated. 150mg/kg and 300mg/kg chloroform and methanol fractions were administered to alloxan- induced diabetic rats twice daily for a period of fourteen days. Their blood glucose level was measured every three days while Lipid profile was assayed at the end of the experiment. The two fractions significantly reduced ($p<0.05$) the fasting blood glucose levels compared to the diabetic control except in the 300mg/kg methanol fractions. Total Cholesterol and Low Density Lipoprotein Cholesterol were both reduced significantly ($p<0.05$) by the two fractions of Nauclea latifolia dose-dependently. Total Cholesterol reduced thus: Chloroform (150mg/kg=77.50±10.15mg/dl, 300mg/kg=82.72±16.21mg/dl) and methanol (150mg/kg=116.86±13.34 mg/dl and 300mg/kg=108.66±12.77 mg/dl) when compared to the diabetic control (383.76 ±79.68 mg/dl). Low density lipoprotein cholesterol: chloroform; (150mg/kg=8.77±8.02 mg/dl, 300mg/kg=27.93±11.38 mg/dl) and methanol (150mg/kg=50.688±14.13mg/dl, 300mg/kg=42.09±79.23mg/dl) when compared to the diabetic control (299.46±79.23mg/dl). Chloroform fraction shows more reductions of the two parameters. Thus suggesting a more hypoglycemic as well as favorable lipidemic potentials /effects on type 2diabetes.

Keywords: Antidiabetic, Chloroform, Hypoglycemic, Hypolipidemic, Methanol, Nauclea latifolia.

Abbreviations

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 one of the common metabolic disorders with micro- and marovascular complications that results in significant morbidity and mortality (Vats et al., 2004). Serum lipids of diagnostic importance include Total Cholesterol (TC), Triglycerides (TG), Low density lipoprotein (LDL), High density lipoprotein (HDL) hence abnormal lipid metabolism is one of the reasons for premature atherosclerosis in patients with diabetes mellitus (Khanna et al., 1996).

Nauclea latifolia (Rubiaceae), is a shrub commonly called Pincushion tree (English), mbong- ibong (Ibibio) and Tabashiya (Hausa), Ubuluinu (Igbo) and Scile maritime (French) has been reported to posses hypoglyceamic property (Ameh et al., 2009). Preliminary

phytochemical screening by Nkafamiya, (2006) showed that it contains saponins, tannins, alkaloids and polyphenols.

Diabetes is associated with chronic hyperglycemia and lipid disorder such as hyperlipidemia, and atherosclerosis. (Friedwald *et al*.1972; Nelson and Cox (2000). This study was to ascertain the hypoglycemic effect of the chloroform and methanolic leaf fractions of Nauclea lafifolia and their effect on the lipid profile of diabetic rats as well as establishing the fraction with the highest antidiabetic properties.

MATERIALS AND METHOD

Collection and Identification of Plant Materials

The fresh leaves of Nauclea lafifolia were obtained from the University of Uyo, Facuty of Pharmacy Medicinal plants Farm and authenticated by Dr. Mrs. Eshiet, a taxonomist in the department of Botany and Ecological Studies, University of Uyo, Nigeria assigned a voucher number 679 in the Herbarium.

The fresh leaves were washed, dried under shade and reduced to coarse powder. Gradient method of extraction was employed to fractionate the plant. 500g of powder was macerated in 2.5L of chloroform for 72 hours with occasional shaking, filtered and concentrated at room temperature. The residue obtained was air-dried and macerated in 2.5L of methanol for 72 hours. The extract was filtered and the filtrate concentrated in water bath (25° c). The dried extracts obtained were stored in a beaker covered with aluminum foil and stored in a refrigerator at 4°c until when needed.

Experimental Animals

Forty two adult Albino Wistar female rats (80-155g) obtained from the animal house of the Department of Biochemistry, University of Calabar, Nigeria, were used for the experiment. They were housed in wooden cages at room temperature under standard conditions and maintained in a 12h light/dark cycle. The animals were feed on pelletized Growers Feed and water *ad libitum*. The animals were allowed to acclimatize for two weeks in the animal house of the Department of Pharmacology and Toxicology, University of Uyo, Nigeria before commencement of the experiment. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals.

Induction of Diabetes

Diabetes was induced in thirty-six rats, fasted overnight, by a single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (150mg/kg) in distilled water. The Alloxan monohydrate solution was prepared by dissolving 1.25g of Alloxan monohydrate in 25ml of distilled water. The animals were considered as being diabetic if the blood glucose values were ≥ 200mg/dl on the third day (72 hours) after alloxan injection.

Experimental Design

Forty-two rats were divided into the following treatment groups for the study: - Diabetic control group(DC), Non-Diabetic group(NC), Low dose chloroform extract group (150mg/kg), High dose chloroform extract group (300mg/kg), low dose methanol extract group(150mg/kg), High dose methanol extract group (300mg/kg) and Glibenclamide group (5mg/kg), thus there were a total of seven groups with six animals per group in all. Each fraction of *Nauclea latifolia* was administered orally twice daily while the glibenclimide group was administered once daily with the aid of syringes and needles coated with plastic tube to avoid injury to the animals. Blood glucose levels were measured every three days during the experimental period and the treatment lasted for a period of fourteen days after which the animals were fasted overnight, anaesthetized under chloroform fumes, sacrificed and their sera collected for the lipid profile assay.

Biochemical analysis

Blood Glucose Level (BGL) was measured with Accu-check Active^{TM} glucose strips in Accu-check Active^{TM} test meter using blood obtained from the tail vein of overnight fasted rats. Total Cholesterol (TC) level was determined by the enzyme method (Asanga et al. 2012), triglyceride (TG) by the enzymatic colorimetric method (Heber et al. 2013) and High Density Lipoprotein (HDL) by the phosphotungstate method (Tripathi et al. 2012) using Agappe laboratory kits. 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 analysed for statistical significance by one – way ANOVA followed by Tukey-kramer multiple comparison test. All data were expressed as Mean ± SEM and values of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Result

Effect of treatment on blood glucose level of diabetic rats

Table 1 shows the blood glucose levels of Alloxan-

Table 1. Effect of treatment on blood glucose level of diabetic rats.

GROUP	Blood Glucose CONC.(mg/dl)		
	INITIAL	FINAL	%CHANGE
Normal Control	132.50±7.59	84.00±3.98*[a]	36.6
Diabetic Control	296.00±16.62[b]	394.66±103.45	33.3
DG-1500CL	271.75±29.58[bc]	97.00±21.46*[a]	64.3
DG-300CL	343.66±16.60[bc]	201.00±48.54*[a]	41.5
DG-150ME	310.75±20.45[bc]	163.75±93.65*[a]	44.5
DG-300ME	365.66±20.35[b]	368.67±79.82[bc]	0.82
DG-GLIB	589.00±10.02[b]	49.33±16.27*	91.6

DG- 150CL= Diabetic group treated with chloroform fraction 150mg1kg body weight
DG- 300CL= Diabetic group treated with chloroform fraction 300mg/kg body weight
DG- 150ME= Diabetic group treated with methanol fraction 150mg/kg body weight
DG- 300ME= Diabetic group treated with methanolic fraction 300mg/kg body weight
DG-GLIB= Diabetic group treated with Glibenclamide (5mg/kg)
*a=<0.05 indicates a significant difference compared with diabetic control;
b= (p<0.05) indicates a significant difference compared with normal control;
c= (p<0.05) indicates a significant difference compared with standard agent, glibenclamide. n=6

Table 2. Effect of treatment on serum lipid profile in alloxan-induced diabetic rats after 14 days of treatment

Groups	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-C (mg/dl)	VLDC-C (mg/dl)	LDL-C (mg/dl)
Normal Control	**181.43± 7.71**	**97.97±12.78**	**35.09±5.93**	**36.51±1.49**	**24.64±12.70*[a]**
Diabetic Control	**122.79±21.65[b]**	**383.76±79.68[b]**	**41.72±0.61**	**42.58±10.35**	**299.46±79.23[c]**
Chloroform 150mg/kg	**150.25±16.59**	**77.50±10.15*[a]**	**38.69±4.20[c]**	**30.05±3.32[c]**	**8.77±8.02* [a]**
Chloroform (300 mg/kg)	**142.16±23.23[c]**	**82.72±16.21***	**26.36±0.40*[c]**	**28.43±4.65[c]**	**27.93±11.38*[a]**
Methanol (150 mg/kg)	**175.62±13.91*[ac]**	**116.86±13.34*[a]**	**31.06±2.67[c]**	**35.13±2.78[c]**	**50.688±14.13*[a]**
Methanol (300 mg/kg)	**166.91±15.44*[a,c]**	**108.66±12.77*[a]**	**32.78±5.24[c]**	**33.76±3.17[c]**	**42.09±79.23*[a]**
Glibenclamide (5mg/kg)	**89.24±14.30[b]**	**116.36±14.69*[a]**	**66.45±0.21*[a, b]**	**17.85±2.86*[a, b]**	**32.06±13.23*[a]**

*a=<0.05 indicates a significant difference compared with diabetic control;
*b= (p<0.05) indicates a significant difference compared with normal control;
*c= (p<0.05) indicates a significant difference compared with standard drug, glibenclamide, n=6

induced diabetic animals treated with the fractions and standard drug.

There was a significant decrease (p<0.05) in the BGL of NC group (84.00±3.98mg/dl), standard drug (49.33±16.27mg/dl), low (97.00±21.46) and high (201.00 ±48.54mg/dl) dose chloroform group, low dose methanol group (163.75±93.65mg/dl) compared to the DC group(393.66±103.45mg/dl) while there was a significant increase in the high dose methanol group(368.67±79.82mg/dl).

Effect of treatment on serum lipid profile in alloxan-induced diabetic rats

In this study, The DC group showed a significant increases (p<0.05) in both LDL-C (299.46±79.23mg/dl) and VLDL-C (42.58±10.35mg/dl) when compared to the standard drug group (LDL-C=32.06±13.23 mg/dl and VLDL-C=17.85±2.86mg/dl) and NC group (LDL-C=24.64±12.70mg/dl, VLDL-C=36.51±1.49mg/dl).

There was a significant decrease (p<0.05) in TC level of the low (77.50±1.50mg/dl) and high (82.72±16.21mg/dl) dose chloroform groups, low (116.86±13.34mg/dl) and high (108.66±12.77mg/dl) dose methanol groups when compared to the DC group (383.76±79mg/dl). LDL-C also showed a significant decrease (p<0.05) in low (8.77±8.02mg/dl), high (27.93±11.38mg/dl) chloroform groups and low (50.68±14.13mg/dl), high (42.09±13.23mg/dl) methanol groups when comparing with the DC group (299.46±79.23mg/dl) (Table 2).

DISCUSSION

Diabetes is usually associated with chronic hyperglycemia due to a decrease in the sensitivity of target tissues to the action of insulin. This was in line with this study as there were increases in blood glucose levels of the untreated diabetic animals. The study presented facts that the daily oral administration of different doses of chloroform and methanolic fractions of *Nauclea latifolia* significantly reduced the BGL of the alloxan- induced diabetic rats to near normal values. This result is in consonance with the work done by Fazil et al 2010 on the antidiabetic activity of *Vinca rosea* extract in alloxan-induced diabetic rats. Also, the research of Antia and Okokon (2014) showed the abilities of some plants extract in reducing blood glucose levels in diabetic rats which is in consonance with this research work.

Results of this study showed the abilities of both fractions of *Nauclea latifolia* to significantly reduce TC, LDL and VLDL in the treated diabetic rats. This was similar to the report by Akah *et al.* (2009), on the ability of some plants extracts in reducing TC levels in diabetic rats. Akah *et al* (2009) Reports that fraction of *Vernonia amygdalina* significantly reduced TG level in diabetic rats within two weeks of treatment but this observation was contrary to the result of this study as insignificant decrease in TG levels were observed in the treatment groups when compared to diabetic control.

Flavonoids, phenols, saponins and sterols have been reported to be associated with hypolipidemia and hypocholesterolemia as reported by (Bopanna *et al*., 1997; Katsumata, *et al*., 1999) this may contribute immensely to the obtained result. Also the lipid lowering effect may be due to inhibition of hepatic cholesterol biosynthesis and increased fecal bile acid secretion as reported by Kaur *et al.* (2006). The reduction of the parameters after treatment with the plant fraction was in consonance with the work reported by Okokon *et al* (2011) and also compared well with the work by (Asanga *et al*., 2013), thereby suggesting their ability in arresting some lipid related symptoms of diabetes mellitus and its recommendation for managing incidences of the disease.

REFERENCES

Akah PA, Alemji JA, Salawu OA, Okoye TC, Offia NV (2009). Effects of Verona amygdalina on Biochemical and Hematological parameters in Diabetic rats. *Asian J. Med. Sci*. 1(3): 108-113.

Ameh, D.A., Gidado, A., Atawodi, S.E., and Ibrahim, S. (2009). Hypoglycaemic Activity of Nauclea Latifolia SM. (Rubiaceae) in Experimental Animals. *Afr. J. Tradition Complement Alternative Medical*, 5(2): 201-208.

Asanga EE, Ebong PE, Eseyin OA, Udoh IE, Eyo RA (2013). Effect of ethanol extract and fractions of Nauclea Latifolia leaves on some biochemical parameters of alloxan-induced diabetic rats. *Int. J. Res. and Rev. in Pharm. Appl. Sci.* 3(1): 147-154

Bopanna KN, Bhagy a lakshmi N, Kanna J, Balaraman R, Rathod SP(1997). Antidiabetic and Antihyperlipidemic effects of neem seed and kernel powder on Alloxan-induced Diabetic rats. Phamacoal. Res. 46(3):251-255.

Friedwald WT, Levi RI, Fredrickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge Clinical Chemistry, 18:499-502.

Huvponen RK, Viikari JS, Saarima H (1984). Correlations of serum Lipids with Diabetic control in sulphonylurea Treated Diabetic patients. Diabetes care, 7:575-578.

Katsumata KY, Katsumata TO, Katsumata K (1999). Potentiating Effects of combined usage of three sulphonylurea Drugs on the occurrence of Alloxan-induced Diabetes in rats. Horm. Metab. Res. 25:125-126.

Kaur G, Alams MS, Jabbar Z, Javed K, Athar M (2006). Evaluation of antioxidant activity of Cassia siamea flowers. *J. Ethnopharmacol.* 108: 340-348

Nelson DL, Cox MM (2000). Lehninger Principles of Biochemistry (3rd Ed.). New York. Worth publishers, 808-841

Khanna AK, Ramesh C, Kapoor NK (1996). Terminalia ayurverdic cardiotonic regulates lipid metabolism in hyperlipidemic rats. Phytotherapy Research, 10: 663-669.

Nwanjo HV (2005). Efficacy of Aqueous leaf Extract of Vernonia amygdalina on Plasma Lipoproteins and oxidative status in Diabetic rats models. *Niger. J. Physiol. Sci.* 20(1-2):39-42.

Nkafamiya I (2006). Biochemical Evaluation of the fruit of Nauclea latifolia. *Afr. J. Biotechnol.* 6 (19) 2461-2463

Okokon JL, Paul A Nwafor, Emem E Umoh, Patience J Okokon, John A Udobang (2011). Anti diabetic and Hypolipidemic Activities of Croton Zambesicus on Alloxan- induced Diabetic Rats. *Asian J Phar Biol.*

Vats V, Yadar SP, Grover JK (2004). Ethanolic extract of Ocimum Sanctum leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolisms in rats. *J. Ethnopharmacol*. 90 (155-160)