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Comparison of the antiglycemic and hypolipidaemic effects of n-hexane and methanol leaf extracts of *Nauclea latifolia* in alloxan-induced diabetic rats

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ABSTRACT

Diabetes Mellitus has been known to be associated with lipid disorders and cardiovascular complications. The aim of this study was to evaluate and compare the hypoglycemic and hypolipidaemic effects of n-hexane and methanol leaf fractions of *Nauclea latifolia* in diabetic rats. The induced diabetic rats were administered 150mg/kg and 300mg/kg body weight respectively of the two fractions of *Nauclea latifolia* or 5mg/kg body weight of glibenclamide for fourteen days. Fasting blood glucose levels were measured and the lipid profile was analyzed. There were significant reductions ($p < 0.05$) in the fasting blood glucose levels in all the groups treated with the leaf fractions when compared with the diabetic control. There were also significant ($p < 0.05$) decreases on the lipid profile as shown in the level of total cholesterol and low density lipoprotein cholesterol. Results of this study show that leaf fractions of *Nauclea latifolia* possessed antidiabetic properties with the methanol fraction exerting a more potent effect.

Keywords: Diabetes mellitus, hypoglycaemic, hypolipidaemic, *Nauclea latifolia*.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It is specifically characterized by abnormality of lipid profile such as cholesterol, low and high density lipoprotein and triglyceride leading to series of secondary complications (Khursheed *et al.*, 2011).

Lipid profile is a panel of blood tests that serves as an initial broad medical screening tool for lipid abnormalities, such as cholesterol and triglyceride. It helps determine the risk for coronary heart disease. The measurement of the lipid profile of diabetic patients is highly essential in order to investigate how diabetes affects their lipid metabolism (Siva Prabodh *et al.*, 2012).

Diabetes mellitus being a devastating health problem in major populations worldwide; affecting people in both developed and developing countries (Oyedemi *et al.*,

2011), motivated research on hypoglycaemic agents of plant origin through the encouragement of the World Health Organisation (WHO). This lead to the search for alternative remedies that may produce similar degree of efficacy like most hypoglycaemic drugs required by most diabetic patients; as these drugs are not without short comings and side effects (Abubakar *et al.*, 2009).

Nauclea latifolia Smith (Syn. *Sarcocephalus latifolius*), a member of the family Rubiaceae is commonly known as Pin Cushion tree, African peach, Bishop's head, locally known in Nigeria as Mbom-ubong in Akwa Ibom and Cross River States, Tabashiya in most parts of the north, Marga in Hausa, Egbesi in Yoruba and Uburu Inu/Nbitinu in Igbo (Onike, 2010).

Recently, Effiong *et al.*, 2013 reported the antidiabetic property of the ethanol (crude) extract of the leaves of *Nauclea latifolia* plant in streptozotocin- induced diabetic rats. In this study different doses of methanol

and N-Hexane fractions of the leaves of the plant were orally administered to Alloxane-induced diabetic rats for 14 days to study and compare their antidiabetic property in these fractions. The study was necessitated by the high costs of orthodox oral hypoglycaemic medications and the claims by traditional medical practitioners (TMPs) about the potency and efficacy of herbs including *Nauclea latifolia* in the treatment of diabetes mellitus.

MATERIALS AND METHODOLOGY

Collection and Identification of Plant Materials

Fresh but matured *Nauclea latifolia* leaves were collected at the Department of Pharmacognosy and Natural Medicine Farm of the University of Uyo, Nigeria. They were authenticated by Dr. (Mrs.) Eshiet; a botanist with the Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo and a Voucher specimen was deposited in the Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo, Nigeria with herbarium number 679. The leaves were rinsed severally with clean tap water to remove dust particles and debris and thereafter allowed to completely drain. The leaves were then cut and chopped into pieces and air-dried until ready for extraction.

Preparation of Plant Extracts

The gradient method of extraction was employed for the extraction process. Five hundred grammes (500g) of pulverised *Nauclea latifolia* leaves were macerated in 2.5 litres (2500ml) of N-hexane (Analar) in a 5L capacity glass ware and was made air-tight and left to stand for 72 hours with intermittent shaking. The mixture was filtered and concentrated at room temperature yielding 8.3g of brown oily substance of *Nauclea latifolia*. The filtrate was stored in a refrigerator until use.

The residue was spread out on a clean white cardboard paper and allowed to air-dry until n-hexane was completely removed. The dried residue was then macerated in 2.5 litres of Methanol (Analar) in a 5L capacity glass ware and was made air-tight and left to stand for 72 hours with intermittent shaking. The mixture was filtered and concentrated in a water bath at 60°C yielding 44.35g of *Nauclea latifolia*. The filtrate was stored in a refrigerator until use.

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

fed 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.

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 $\geq 200\text{mg/dl}$ on the third day (72 hours) after alloxan injection.

Experimental Design

Forty-two rats were divided into seven groups of 6 rats each consisting of a diabetic control (DC) and normal control (NC) groups, 150mg/kg N-hexane, 300mg/kg N-hexane, 150mg/kg methanol, 300mg/kg methanol and lastly the group of 5mg/kg glibenclamide as the standard drug. Extracts were reconstituted in distilled water (vehicle) before use and were administered orally via gastric intubations, twice daily (7.00 am and 7.00 pm) while glibenclamide was administered once a day.

Biochemical Investigations

Blood Glucose Level (BGL) was measured with Accu-check Active™ glucose strips in Accu-check Active™ 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".

Collection of samples for analysis

Blood glucose level was measured every 3 days throughout the 14 days treatment period. At the end of treatment, the animals were fasted for 12h, then anaesthetized under chloroform vapour and dissected. Whole blood was obtained by cardiac puncture into sterile plain tubes and was allowed to clot for about 2h and thereafter centrifuged (3,000g for 10min) to remove

Table1. Effects of n-hexane and methanol leaf fractions of *Nauclea latifolia* on BGL of alloxan-induced diabetic rats.

Treatment	Dose in (mg/kg)	Blood glucose level (mg/dl) in days				
		Day 0	Day 4	Day 7	Day 10	Day 14
Diabetic control	0.2	281.00± 16.09	296.00 ± 16.62	274.30 ± 8.98	322.60 ± 14.67	233.00 ± 18.68
Normal control	0.2	132.50 ± 7.58	132.50 ± 7.58 ^a	124.25 ± 1.93 ^a	122.75 ± 3.90	84.00 ± 3.97 ^a
N-hexane fraction	150.0	281.00 ± 16.09	139.75 ± 17.27 ^a	137.00 ± 10.08 ^a	149.75 ± 15.01	116.75 ± 4.92 ^a
N-hexane fraction	300.0	385.25 ± 19.72	191.25 ± 18.53	175.00 ± 5.75 ^a	166.00 ± 8.84	138.00 ± 14.01 ^a
Methanol fraction	150.0	315.75 ± 18.36	77.50 ± 4.87 ^a	71.75 ± 9.48 ^a	140.75 ± 14.16	88.75 ± 19.54 ^a
Methanol fraction	300.0	334.75 ± 34.10	94.75 ± 9.96 ^a	99.09 ± 16.74 ^a	144.30 ± 13.17	135.30 ± 8.41 ^a
Glibenclamide	5.0	350.60 ± 10.74	290.33 ± 17.35	147.00 ± 13.33 ^a	106.00 ± 17.61	91.00 ± 4.61 ^a

Data are expressed as mean ± SEM. Significant at ^ap < 0.05. When compared to diabetic control (n=6).

Table2. Effects of n-hexane and methanol leaf fractions of *Nauclea latifolia* on Serum TC, TG, HDL-cholesterol of alloxan- induced diabetic rats.

Drug	Dose (mg/kg)	Average Serum lipids profile (mg/dl)				
		TC	TG	HDL	LDL	VLDL
DiabeticControl	0.2	383.76±79.67 ^{b,c}	122.78 ± 1.85	41.72±0.60 ^c	299.46±79.23 ^c	42.58±10.35
Normal control	0.2	97.96±2.77 ^{a,c}	181.42±7.16 ^c	35.08±5.92 ^c	45.61±3.42 ^a	36.28 ± 1.43 ^a
N-hexane fraction	150.0	73.16 ± 18.62 ^{a,c}	110.36±3.47	17.87±3.35 ^{a,b,c}	33.24± 16.19 ^a	22.04 ± 0.68 ^a
N-hexane fraction	300.0	69.78 ± 8.41 ^{a,c}	148.59±6.53 ^a	33.02±6.80 ^c	9.29 ± 5.62 ^{a,c}	29.21± 1.00 ^a
Methanol fraction	150.0	124.40 ± 14.90 ^a	175.60±13.91 ^a	31.05±2.97 ^c	50.52 ± 13.98 ^{a,b}	35.04 ± 2.85 ^a
Methanol fraction	300.0	108.25±12.77 ^a	166.90 ± 15.44 ^a	32.78± 5.23 ^c	42.09 ± 9.93 ^a	30.42 ± 3.92 ^a
Glibenclamide	5.0	129.36± 1.45 ^a	89.24± 14.30 ^a	66.45±0.21 ^a	32.06 ± 13.23 ^a	17.85 ± 2.86 ^{a,b}

Data are expressed as mean ± SEM. Significant at ^ap < 0.05 = compared to diabetic control, ^bp < 0.05 = compared to normal control, ^cp < 0.05. When compared to standard drug (n=6).

cells and recover serum which was used for the lipid profile assays.

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

RESULTS

Effects of n-hexane and methanol leaf fractions of *Nauclea latifolia* on blood glucose levels in alloxan-induced diabetic rats

Table 1 shows the changes in blood glucose following a 14-day treatment period. On day 4, there were significant decreases (p<0.05) of BGL in animals treated with 150mg/kg n-hexane (139.75±17.27mg/dl), 150mg/kg methanol (77.50±4.87mg/dl) and 300mg/kg methanol (94.75±9.96mg/dl) when compared with diabetic control (296.00±16.62) while it compared well with the normal control (132.50±7.58 mg/dl). Day 7 showed a significant decrease of BGL in 150mg/kg (137.00 ± 10.08 mg/dl) and

300mg/kg (175.00±5.75 mg/dl) of n-hexane fraction, 150mg/kg (71.75±9.48mg/dl), 300mg/kg (99.09±16.74) of methanol fraction compared with diabetic control (274.30±8.98 mg/dl). Day 14 showed significant decrease (p<0.05) of BGL in 150mg/kg (116.75±4.92 mg/dl), 300mg/kg (138.00±14.01 mg/dl) of n-hexane, 150mg/kg (88.75±19.54 mg/dl), 300mg/kg (135.30±8.41mg/dl) of methanol and standard drug (91.00±4.61 mg/dl) in comparison with diabetic control (233.00±18.68).

Effect of n-hexane and methanol leaf fractions of *Nauclea latifolia* on serum lipid profile in alloxan-induced diabetic rats

On Table 2, serum lipids showed significant difference (P<0.05) in the experimental rats compared to controls. TC decreased in all experimental groups (150mg/kg (73.16±18.62mg/dl), 300mg/kg (69.78±8.41mg/dl) of n-hexane, 150mg/kg (124.40±14.90mg/dl), 300 mg/kg (108.25±12.77mg/dl) of methanol and standard drug (129.36±1.45mg/dl) in comparison with diabetic control (383.76±79.67mg/dl). LDL cholesterol levels were also reduced in all experimental groups; 150mg/kg (33.24±16.19mg/dl), 300mg/kg (9.29±5.62mg/dl) of n-hexane, 150mg/kg (50.52±13.98mg/dl), 300 mg/kg (42.09±9.93mg/dl) of methanol and standard drug (32.06±13.23mg/dl) in comparison with diabetic control

(299.46±79.23mg/dl).

DISCUSSION

Generally, abnormal lipid profiles and lipoprotein oxidation especially, LDL-C is more common in diabetics and is aggravated with poor glycaemia control (Siva Prabodh et al, 2012). This complies with the result obtained in this research work with the untreated diabetic rats.

In a diabetic condition; the blood glucose level is usually elevated 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. Administration of n-hexane and methanol leaf fractions of *Nauclea latifolia* to the diabetic rats for 14 days caused significant reductions in the BGL of the diabetic rats when compared with DC and sustained from day 4 to day 14. However, these reductions were dose-dependent as 150mg/kg of both the methanol and n-hexane fractions produced greater reductions in BGL than 300mg/kg of the respective fractions. Thus, a maximal reduction from 315.75±18.36mg/dl to 88.75±19.54mg/dl was observed in the 150mg/kg methanol fraction after 14-days of administering the extract. This was in close consonance with the reduction observed in the standard drug group (350.60±10.74mg/dl to 91.00±4.61mg/dl). This result agreed with the research of Antia and Okokon (2014) and Abubakar et al (2005) on the abilities of some plants extract in reducing blood glucose levels in diabetic rats.

Some phytochemical compounds such as alkaloids (Karawya and Wahab, 1984), polysaccharides (Tomoda et al., 1985), steroids (Ivorra et al., 1989), terpenes and tannins (Reher et al., 1991) have been implicated in the antidiabetic activities of plants. It has been reported that *N. latifolia* contains alkaloids (Hotellier et al., 1991; Abreu and Pereira, 2001), terpenes (Morah, 1995) and some inorganic elements (Asubiojo et al., 1982). These constituents may in part be responsible for the observed significant activity of this extract either singly or in synergy with one another. In this study, continuous treatment with the leaf fractions of *Nauclea latifolia* for a period of 2 weeks caused significant decrease in BGL of treated diabetic rats compared to untreated diabetic rats. Some plants' extracts are reported to exert hypoglycaemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin (Pari and Amarnath, 2004). While others act through extra pancreatic mechanisms by inhibition of hepatic glucose production (Edduoks et al., 2003) or corrections of insulin resistance (Hu et al., 2003). This leaf fractions may have acted through one of the above mechanisms.

The dyslipidaemia in Type 2 diabetes is characterized by a combination of increased serum T),

TC, LDL and decreased HDL-cholesterol which was similar with the lipid profile results of the diabetic control rats in this research. Coleman (2011) reported that most diabetics have a high blood TG level, a high LDL level and a low HDL level. This was also in consonance with the results of the untreated diabetic rats in this work.

The results from this study showed that the serum TC and LDL levels of the leaf fractions (n-hexane and methanol) treated diabetic rats were significantly reduced dose-dependently; as the more potent effects were realised at higher doses (300mg/kg) of the respective fractions; 300mg/kg n-hexane leaf fraction produced the most potent effect while its TG levels were significantly ($p<0.05$) increased, after 14 days of treatment as against that in the untreated diabetic group. This result agreed with the findings of Edet et al (2013). The methanol and n-hexane leaf fractions of *Nauclea latifolia* caused considerable decreases in the levels of HDL of the diabetic rats. The only significant decrease was caused by n-hexane fraction (150mg/kg), while the HDL levels of the standard drug glibenclamide-treated diabetic rats was increased significantly. VLDL levels were high in the treated diabetic rats but were significant only in the group treated with 150mg/kg methanol fraction. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose (Krishnakumar et al., 2000). The action of the plant in reducing plasma cholesterol concentration could be due to the ability of one or more of the phytochemicals in the plant to activate the functioning enzymes of the rat responsible for cholesterol absorption (Malinon, 1997); or the regression of the diabetic state due to the administration of the fractions may have increased the utilization of glucose, thereby depressing the mobilization of fat. Phytochemical compounds like phenols, tannins, alkaloids, steroids, cardiac glycosides and terpenes present in this extract have been reported to exert antilipidaemic activity (Tandon, 2005). They may in part be responsible for the hypolipidaemic activity of this leaf fractions.

In conclusion, the results of this study show that leaf fractions of *Nauclea latifolia* possessed hypoglycaemic and hypolipidaemic properties with the methanol fraction exerting a more potent effect. This suggests that the active principle may be localised in the methanol fraction of *Nauclea latifolia*.

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Abbreviations

Blood Glucose Level =	BGL
Total Cholesterol =	TC
High Density Lipoprotein=	HDL
Very Low Density Lipoprotein =	V LDL
World Health Organisation =	WHO
Normal Control =	NC
Diabetic Control =	DC
Triglyceride =	TG