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Comparative effect of *Nauclea latifolia* leaf fractions on blood glucose and lipid profile parameters of alloxan induced-diabetic rats

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The antidiabetic effects of methanol, N-hexane and ethyl acetate leaf fractions of *Nauclea latifolia* were investigated in diabetic model rats. 150 and 300mg/kg fractions of *N. latifolia* were administered orally to the experimental animals at two weeks interval, while their blood glucose levels were taken daily. At the end of the experiment, their lipid profiles were assayed using standard methods. The research data indicated significant decreases (P=0.05) of the blood glucose in all the fractions at a dose-dependent manner (ethyl acetate (150 mg/kg = 47.83%; 300 mg/kg = 64.17%), N-hexane (150 mg/kg = 58.45%; 300 mg/kg = 64.18%), methanol (150 mg/kg = 44.5%), except for the 300 mg/kg dose methanol fraction (0.82%) as compared to the increased level in the negative control (33.33%). In the lipid profile assay, there were also a dose dependent significant decrease (P=0.05) of serum Total Cholesterol (TC) and Low Density Lipoprotein (LDL) cholesterol in all the fraction groups (ethylacetate (150 mg/kg- TC = 69.88 ± 8.52 mg/dl, LDL-cholesterol = 8.23±7.76 mg/dl; 300 mg/kg-TC = 51.08±9.93 mg/dl, LDL-cholesterol = 6.44±7.66 mg/dl), N-hexane (150 mg/kg-TC = 73.16 ± 18.62mg/dl, LDL-cholesterol = 33.24±16.19 mg/dl; 300 mg/kg-TC = 69.78±8.41 mg/dl, LDL-cholesterol = 9.29±5.62 mg/dl and methanol (150 mg/kg; TC = 116.86 ± 13.34 mg/dl, LDL-cholesterol = 50.68±14.13 mg; 300 mg/kg-TC = 108.66 ± 12.77 mg/dl, LDL-cholesterol = 42.09±9.93mg/dl) as compared to the high concentration of the negative control group (TC = 383.76 ± 79.68 mg/dl, LDL-cholesterol = 299.46 ± 79.23 mg/dl). Ethylacetate and N-hexane fractions showed significant reductions (P=0.05) of TC and LDL-cholesterol as compared to the positive control (TC=116.36±14.69 mg/dl, LDL-cholesterol = 32.06±13.23mg/dl) also at a dose-dependent manner; thus, portraying a more efficient hypolipidaemic activity than the standard drug. This antidiabetic research on *N. latifolia* suggest that ethylacetate fraction produces the best effect, followed by N-hexane and lastly by methanol fraction.

Key words: *Nauclea latifolia,* ethyl acetate, n-hexane, methanol, leaf fractions, glibenclamide, antidiabetic, blood glucose, lipid profile.

INTRODUCTION

Diabetes mellitus can be defined as an increase in blood glucose without the presence or non-enough pancreatic insulin secretion which could involve the concurrent impairment of insulin action (Martha, 2009). It is an endocrine disorder characterized by aberration in carbohydrate, protein, blood relating functions and fat

metabolism as a result of complete or relative insufficiency of insulin secretion and action (American Diabetes, 2006; Canadian Diabetes Associates, 2008).

Herbal medicine entails the usage of herbs and plant parts (roots, stems, barks, or even fruits) in enhancing an improved health (Bussmann and Sharon, 2006). The recommendation by WHO on diabetes mellitus has shown the importance of research on hypoglycemic agents from medicinal plants. The photochemistry and their pharmacological values shows that about 800 plants could be applied for the treatment of diabetes traditionally throughout the world (Kavishankar et al., 2011).

Nauclea latifolia Smith, (Rubiaceae) is known commonly as Pin cushion tree and a strangling shrub that is native to tropical Africa and Asia. *N. latifolia* is largely used in the traditional folklore (Akpanabiatu et al., 2005). The pharmacological usage of *N. latfolia* leaves reported include hypoglycemic, hypolipidemic and hypo-cholesterolemic property (Gidado et al., 2008; Asanga et al., 2013), antihypertensive property (Nworgu et al., 2008).

Blood sugar level could be measured using a glucose meter either in mg/dl or mmol/L of blood. The normal value of glucose level for an average person is 4.5 to 7.0 mmol/L (81 to 126 mg/dl) (Briscoe, 2006). Values greater than 13 to 15 mmol/L (230 to 270 mg/dl) are considered high; usually referred to as hyperglycaemic needs to be closely monitored to return to normalcy. The patient required an urgent medical attention if after 2-3 tests blood sugar levels continue to rise. Values of 3.8 mmol/L (< 70 mg/dl) are referred to as a hypoglycaemic attack (low blood sugar).

The main lipid found in the blood, bile, and brain tissues is cholesterol and it is one of the most important steroids which is a precursor of many steroid hormones of the body (Siedel et al., 1981). Esterified cholesterol is present in two thirds of the blood. The metabolized cholesterol in the liver is transported by lipoproteins in the blood stream (Flegg et al., 1973). The reported abnormal lipid disorders in diabetics are hyperlipidemia, atherosclerosis, etc. (Friedewald et al., 1972; Nelson et al., 2008). The important diagnostic of serum lipids include very low density lipoprotein, triacylglycerol, high density lipoprotein, total cholesterol etc.; thus, one of the reasons for premature atherosclerosis in persons with diabetes mellitus is an abnormal lipid metabolism (Khanna et al., 1996).

The medical community is still facing the challenge of managing diabetes without any side effect. Presently there are several drugs available to treat diabetes mellitus which include thiazolidinediones, biguanides and sulphonylurea (De-Fronzo et al. 1997). These drugs usage are restricted by their secondary failure rates, pharmacokinetic properties and accompanying side effects (Donath et al., 2006). Hence, there is an essential need for the search for a new class of compounds to overcome diabetes problems; ultimately it leads to a continuous search for an alternative medicine (Hansotia et al. 2005). *N. latifolia* for example may provide the useful dietary adjunct to existing therapies or as source for the development of pharmaceutical entities (Pepato et al., 2005). This research will reveal the efficacy of the fractions of *N. latifolia* on the lipid profile and blood sugar of diabetic rats and also establish the fraction with the highest hypoglycaemic and hypolipidemic property.

MATERIALS AND METHODS

Plant material's collection and identification

The fresh leaves of *N. latifolia* were collected at the Pharmacy farm, University of Uyo, Akwa Ibom state, Nigeria, in February, 2016. The identification and authentication of the plant were made by Dr. (Mrs.) Eshiet a Botanist in the Faculty of Pharmacy, University of Uyo, Nigeria. The voucher number deposited at the herbarium was 679. Dust particles and debris were removed from the leaves by rinsing them severally with clean tap water and then allowed to drain completely. Then, the leaves were cut, chopped into pieces, air-dried and extracted.

Plant extract's preparation

The preparation of the ethanol extract was done using the wet method of extraction: A kilogramme of the fresh leaves was cut and chopped into pieces on a chopping board using a knife and an electric blender was used to blend it in 1.5 L of 96% ethanol. It was transferred into an amber colored bottle and kept cool at 4°C in a dark compartment for 72 h. Thereafter, it was filtered with a cheese material and then Whatman No 1 filter paper was used to obtain a homogenous filtrate. A rotary evaporator was used at 37- 40°C to concentrate *in vacuo* this filtrate to about one tenth the original volume. The concentrates were dried in a water bath at 40°C, while in an open container to yield 78.95 g of brown oily substances of *N. latifolia*. A dessicator filled with self-indicating silica gel was used to completely dry it and then refrigerated at 2-8°C until needed. The residue was spread out on a clean white cardboard paper and allowed to air-dry until ethanol was completely removed.

Preparation of the fractions

Fractionation was done using the gradient method. Air-dried crude extract residue (500 g) of the *N. latifolia* were macerated using 2.5 L of N-hexane in a 5 L capacity glass ware. It was made air-tight, intermittently shaken while standing for 72 h. Whatman No.1 filter paper was used to filter the mixture and concentrated *in vacuo* using a rotary evaporator at 37-40°C to give one tenth the original volume. The water bath at 40°C was used to dry the concentrate which was left open to yield 8.3 g of an oily brown N-hexane leaf fraction of *N. latifolia*.

The residue was air-dried to completely remove n-hexane and then macerated in 2.5L of ethyl acetate for 72 h with occasional agitation. The filtrate of the filtered marc was concentrated *in vacuo* with a rotary evaporator at 37-40°C also to one tenth its original volume. The water bath at 40°C was used to dry the concentrates while open; yielding 7.95g ethylacetate leaf fraction of *N. latifolia*. The residue obtained was

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left to air dry, after which it was macerated in 2.5 L methanol in a glass ware capacity of 5 L, made air-tight, intermittently shaken and left standing for 72 h. The rotary evaporator at 37-40°C was used to concentrate *in vacuo* the filtered mixture to about one tenth its original volume. Finally, the water bath at 40°C was used to dry the concentrates while allowing open yielding 44.35 g methanol leaf fraction of *N. latifolia*.

The desicator filled with a self-indicating silica gel was used to completely dry the different concentrated fractions of the plant. They were transferred into a beaker, corked using aluminum foil and then kept at 2-8°C in a refrigerator until they are required for usage.

Experimental animals

Sixty six Albino Wistar rats; two-three weeks of age, weighing (140-150g) were purchased from the Department of Biochemistry, University of Calabar, Nigeria. The animals were kept inside a stainless steel wire cages, in well-ventilated Faculty of Pharmacy animal house, University of Uyo, Nigeria strictly under a standard environmental and adequate nutritional conditions (12 h light : 12 h dark cycle; 25 to 30°C; 35-60% humidity) throughout the period of experiment. Feeding with commercial feed and drinking water of the animals were done *ad libitum*. The animals were left to acclimatize for fourteen days before commencement of the study. The ethical guide for care and use of laboratory animals of the University of Uyo, Nigeria were strictly adhered to throughout the experiments.

Diabetes induction

Sixty overnight fasted rats were injected intraperitoneally with 150 mg /kg alloxan (Sigma, St. Louis, Mo, USA) for diabetes induction. Hypoglycemia was prevented in the induced animals by placing them on a 10% glucose solution in the next 24 h of induction (Jarald et al., 2008). Hyperglycemia were recorded for 48 rats with fasting blood glucose (FBG) > 200 mg/dl) on the fourth day of diabetes induction and thus inculcated into the diabetic group of the study.

Experimental design

Eight groups of diabetic animals and a group of normal non-diabetic animals were used as shown in Table 1. The fractions were orally administered twice daily for 14 days, while glibenclamide (a reference drug) was administered once daily, and also orally. Blood glucose of individual rat was monitored at every 24 h interval. After the end of 14-days treatment, the rats were fasted overnight and anaesthetized in the morning with chloroform. Blood was collected through cardiac puncture and kept for 2 hours to clot. The whole blood was centrifuged at 6000 rpm for 20 min to separate out the serum. Lipid profile assay was done using the sera.

Biochemical analysis

The level of blood glucose (BGL) was obtained using Accucheck ActiveTM on strips of glucose in Accu-check ActiveTM test meter with the blood obtained via the tail vein of fasted rats. Enzyme method of Asanga et al. (2013) was used to assay the level of total cholesterol (TC), Heber et al. (2013) enzymatic colorimetric method was used to determine the triglyceride (TG), while the phosphotungstate method of Tripathi et al. (2012) was applied in the assay of high density lipoprotein (HDL-C); all kits were of Agappe laboratory. Very low density lipoprotein concentration (VLDL-C) and low density lipoprotein concentration (LDL-C) were extrapolated using the values obtained for TC, TG and HDL; thus, VLDL-C = TG/5, while LDL-C = TC - (HDL-C + VLDL-C) (Friedewald et al., 1972).

Analysis of data

Data are reported as mean ± standard error of mean (S.E.M). One-way analysis of variance (ANOVA) and the Duncan's post hoc test were used in analyzing the results. Statistically, significant was considered at P = 0.05.

RESULTS AND DISCUSSION

Treatment effect on concentrations of blood glucose

Significant differences (P=0.05) were observed in the concentrations of blood glucose in the negative control (394.66±84.47mg/dl) in comparison with that of the positive control (49.33±16.2 mg/dl) and normal control (84.00±3.98 mg/dl). All the plant fractions indicated a significant difference (P=0.05) in levels of blood glucose (150 mg/kg N-hexane =150.50±31.44 mg/dl, 300 mg/kg N-hexane =134.25±28.62 mg/dl, 150 mg/kg ethyl acetate =163.75±93.65 mg/dl, 300 mg/kg ethyl acetate = 368.67±79.82 mg/dl, 150 mg/kg of methanol =116.75 ± 4.92 mg/dl, 300 mg/kg of methanol 138.00 ± 14.01 mg/dl) when compared with all the controls as shown in Table 2.

Treatment effects on lipid profile

All the lipid profiles had a significant differences (P=0.05) in the negative control (TG=122.79±21.65 mg/dl, TC=383.76±79.68 mg/dl, HDL-C=41.72±0.61 mg/dl, VLDL-C=42.58±10.35 mg/dl, LDL-C=299.46±79.23 mg/dl) when compared with the positive (TG=89.24±14.30 mg/dl, TC=116.36±14.69 mg/dl, HDL-C=66.45±0.21 mg/dl, VLDL-C=17.85±2.86 mg/dl, LDL-C=32.06±13.23 mg/dl) and normal controls (TG=181.43±7.17 mg/dl, TC=97.97±12.78 mg/dl, HDL-C=35.09±5.93 mg/dl, VLDL-C=36.51±1.49 mg/dl, LDL-C=24.64±12.70 mg/dl), respectively. Also, all the fractions: 150 mg/kg-ethyl acetate (TC=69.88±8.52 mg/dl, LDL-C=8.23±7.76 mg/dl), 300 mg/kg ethyl acetate (TC=51.08±9.93 mg/dl, LDL-C=6.44±7.66 mg/dl), 150 mg/kg N-hexane (TC=69.88±8.52 mg/dl, LDL-C=8.23±7.76 mg/dl), 300 mg/kgN-hexane (TC=51.08±9.93mg/dl, LDL-C=6.44±7.66mg/dl) 150 mg/kg-methanol (TC=116.86±13.34 mg/dl, LDL-C=50.68±14.13 mg/dl), and 300 mg/kg-Methanol (TC=108.66±12.77 mg/dl, 42.09±79.93 mg/dl) had a significant differences (P=0.05) on LDL-C and TC in comparison with the controls. except the 150 mg/dl methanol TC which was similar to the positive control. For VLDL-C, the fractions: 150 mg/kg-ethyl acetate=34.94±1.84 mg/dl), 300 mg/kg ethyl acetate (=22.24±2.77 mg/dl), 150 mg/kg-methanol =35.13±2.78 mg/dl), and 300 mg/kg-methanol=33.76±3.17 mg/dl) indicated a significant differences (P=0.05) when compared with the positive and negative controls, while

Table 1. The designed experimental study.

Group	Quantity of rats	Pre-treatment	Treatment
1 = Normal control	6	Non-alloxan treated rats (normal rats)	Distilled water
2 = Negative control	6	Alloxan treated	Distilled water
3 = Positive control	6	Alloxan treated	5 mg/kg glibenclamide
4=N-hexane fraction	6	Alloxan treated	150 mg/kg N-hexane fraction
5=N-hexane fraction	6	Alloxan treated	300 mg/kg N-hexane fraction
6=Ethylacetate fraction	6	Alloxan treated	150 mg/kg Ethyl acetate fraction
7=Ethylacetate fraction	6	Alloxan treated	300 mg/kg ethyl acetate fraction
8=Methanol fraction	6	Alloxan treated	150 mg/kg methanol fraction
9=Methanol fraction	6	Alloxan treated	300 mg/kg methanol fraction

Table 2. Treatment effect of blood glucose concentration.

S/N	Group	Treatment	Blood glucose concentrations		
			Starting value (mg/dl)	Ending value (mg/dl)	Percentage change (%)
1	Normal control	Distilled water	132.50±7.59	84.00±3.98*[b]	36.60*,[b]
2	Negative control	Distilled water	296.00±16.62	394.66±84.47[a,b]	33.33,[a,b]
3	Positive control	5 mg/kg Gliberclamide	589.00±10.02	49.33±16.2*,[a]	91.62*,[a]
4	N-hexane fraction	150 mg/kg N-hexane fraction	288.50±18.37	150.50±31.44*,[a,b]	47.83*,[a,b]
5	N-hexane fraction	300 mg/kg N-hexane fraction	374.75±14.27	138.00±14.01*,[a,b]	64.17*,[a,b]
6	Ethylacetate fraction	150 mg/kg ethyl acetate fraction	310.75±20.45	163.75±93.65*,[a,b]	44.5*,[a,b]
7	Ethylacetate fraction	300 mg/kg ethyl acetate fraction	365.66±20.35	134.25±28.62*,[a,b]	0.82.*,[a,b]
8	Methanol fraction	150 mg/kg of methanol fraction	281.00 ±16.09	116.75 ± 4.92*,[a,b]	58.45*,[a,b]
9	Methanol fraction	300 mg/kg of methanol fraction	385.25 ±19.72	368.67±79.82*,[a,b]	64.18*,[a,b]

Data were expressed as mean ± SEM, n = 6. *P = 0.05: compared with Negative Control; [a]P = 0.05: compared with normal control; [b]P = 0.05: compared with positive control, that is, Glibenclamide.

they were similar to that of the normal control. There was a significant difference (P=0.05) in the positive control in the levels of HDL in comparison with the normal and negative controls (Table 3).

DISCUSSION

Induction of diabetes with alloxan presented significantly raised blood glucose by 3-5 times its normal values in non-diabetic rats. Research carried out by Kahn et al. (2005) and Henry et al. (2005) on the fractions of *Ocimum sanctum* and *N. latifolia,* respectively in diabetic and non-diabetic rats produced similar result as seen in this work. Contrary, the 14-day treatment with 150 mg/kg N-hexane, 300 mg/kg N-hexane, 150 mg/kg ethyl acetate, 300 mg/kg ethyl acetate, 150 mg/kg methanol fractions and glibenclamide presented significant decrease (P=0.05) in blood glucose of diabetic animals by 58.45%, 64.18%, 47.83%, 64.17%, 44.50% and 91.62% of their initial values, respectively as shown on Table 2. Some researchers (Asanga et al., 2013; Effiong and Essien, 2014) also gave similar report on the ability of fractions of *N. latifolia* in reducing blood glucose concentrations. Research carried out by Kahn et al. (2005) on the fractions of *O. sanctum* on normal and diabetic rats also produced similar result as in this research.

The hypoglycemic effects of ethyl acetate and N-hexane fractions decreases with dose increase as their 300 mg/kg dose gave a greater decrease as compared to their 150 mg/kg dose. On the other hand, the blood glucose concentration was significantly increased (P=0.05) when treated with 300 mg/kg-methanol fraction as it gave a 0.82% decrease from the initial value in comparison with the negative control group. Animals of the negative control group were still hyperglycemic (368±79.82 mg/dl). They may have developed diabetic ketoacidosis, a diabetic emergency, which is usually characterized by extreme hyperglycemia. This occurs due to the absence of insulin, possibly caused by severe pancreatic beta cell damage by the alloxan.

The significant hypoglycemic activity of N-hexane, ethyl acetate and methanol (150 mg/kg) fractions could be an

Table 3. Treatment effect of lipid profile.

S/N	Group	Treatment	Lipid profile (mg/dl)				
			TG	TC	HDL-C	VLDL-C	LDL-C
1	Normal control	Distilled water	181.43±7.17*,b	97.97±12.78*	35.09±5.93	36.51±1.49b	24.64±12.70*
2	Negative Control	Distilled water	122.79±21.65a	383.76±79.68a,b	17.87±3.35a,b	42.58±10.35a,b	299.46±79.23a,b
3	Positive Control	5mg/kg Gliberclamide	89.24±14.30a	116.36±14.69*	66.45±0.21±,a	17.85±2.85*,a	32.06±13.23*
4	N-hexane fraction	150mg/kg N-hexane fraction	174.73±9.19*,b	69.88±8.52*	26.70±1.17*,b	34.94±1.84b	8.23±7.76*,a,b
5	N-hexane fraction	300mg/kg N-hexane fraction	111.17±13.87a	51.08±9.93*	22.41±3.75*,a,b	22.24±2.77*,a	6.44±7.66*,a,b
6	Ethylacetate fraction	150mg/kg Ethyl acetate fraction	175.62±13.91*,b	116.86±13.34*	31.06±2.67b	35.13±2.78b	50.68±14.13*,a
7	Ethylacetate fraction	300mg/kg Ethyl acetate fraction	166.91±15.44*,b	108.66±12.77*	32.78±5.24b	33.76±3.17*,b	42.09±9.93*
8	Methanol fraction	150mg/kg of Methanol fraction	110.36±3.47	73.16±18.62*,b	41.72±0.61*a,b	22.04±0.6*	33.24±16.19*
9	Methanol fraction	300mg/kg of Methanol fraction	148.59±6.53*	69.78±8.41*,b	33.08±6.80b	29.21±1.00*	9.29±5.62*,b

Data were expressed as mean ± SEM, n = 6. *P = 0.05: compared with negative control; aP = 0.05 : compared with normal control; bP = 0.05: compared with positive control, that is, Glibenclamide. TC = total cholesterol, TG = Triacylglyceride, HDL-C = high density lipoprotein-cholesterol, VLDL-C = very low density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol.

effect of flavonoids, triterpenes and saponins present in *N. latifolia* leaf (Gidado et al., 2008; Asanga et al., 2013). The plant extracts may also contain some biomolecules that could sensitize the insulin receptor to insulin or stimulate the islets of Langerhans beta cells to release insulin; thereby, enhancing the carbohydrate metabolizing enzymes to re-establish an improved blood glucose level (Umar et al., 2010; Asanga et al., 2013).

Increased levels of VLDL, LDL-C, TG and TC are seen in the negative control rats accompanied by insulin resistance, heart disease, diabetes mellitus because the peripheral fat deposits mobilized more fatty acids from it (Bopanna et al., 1997; Nelson et al. 2008); these were decreased after treatment with *N. latifolia* fractions which could be their ability to arrest some diabetes mellitus symptoms related to lipid and its suggestion for the disease management. However, there were significant reductions (P = 0.05) of LDL-C concentrations in most fractions and glibenclamide in comparison with the negative control, and which is similar to an earlier report by Nikkila (1984) and Asanga et al. (2013). LDL-C concentration in the blood has positive correlation with incidence of cardiovascular diseases. Also, there were significant reduction (P = 0.05) in the concentrations of VLDL-C in the glibenclamide treated group, 300 mg/kg of ethyl acetate fraction compared with the normal and negative controls. Similar result were earlier reported by Dhandapani et al. (2002) and Akah et al. (2009).

Flavonoids, phenols, saponins and sterols which are present in *N. latifolia* have been reported by Katsumata et al. (1999) to be accompanied with hypocholesterolemia and hypolipidemia. This suggest the reason for the obtained result in N-hexane and ethylacetate particularly, the 300 mg/kg ethyl acetate fraction which had the greatest effect in TG, TC, VLDL-C and LDL-C concentrations in comparison with the negative control as seen in Table 3. Furthermore, an improved result was noted in all the fractions as compared to the glibenclamide treated group which is a reference drug for diabetes in lowering total cholesterol and LDL-C, as N-hexane showed a significant difference, while the difference in ethylacetate was not significant (P ≠ 0.05).

Conclusion

Decreased total cholesterol, low density lipoprotein-cholesterol and fasting blood glucose concentrations with improved level of high density lipoprotein by the fractions indicates that, the fractions have hypoglycaemic and hypolipidemic activity. These effects observed in the fractions were dose dependent as the higher doses produced a more significant effect as seen in Tables 2 and 3. From the study, ethyl acetate fraction may be the best agent in arresting hyperglycemia and hyperlipidemia arising from diabetes mellitus followed by N-hexane and then

methanol. And thus, affirms the rationale for their usage to treat diabetic.

CONFLICTS OF INTERESTS

The authors have declared no conflict of interests.

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