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Lipid Profile of Alloxan-Induced Diabetic Albino Wistar Rats Treated with Ethanol Whole Extract and Fractions of Nauclea Latifolia Leaves

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ABSTRACT

The ethanol extract and its fractions were evaluated in the study to unravel their effects on the lipid profile of diabetic rats after daily administration for a period of two weeks. The analysis of the results showed that there were significant decreases ($p < 0.05$) in the HDL-cholesterol and total cholesterol levels in all the treatment groups. Insignificant decreases ($p > 0.05$) in TG levels were recorded in all the treatment groups where as VLDL-C levels significantly decreased ($p < 0.05$) in the groups treated with glibenclamide, methanol fraction (100 mg/kg) as well as butanol and ethyl acetate fractions. The result of the phytochemical screening showed heavy presence of saponins, flavonoids, phenols, terpenoids, cardiac glycosides and carbohydrates in ethyl acetate, butanol and methanol fractions which may be the reason for the hypolipidaemic property of the above fractions and suggest its potency in arresting oxidative stress in diabetic rats. Also, the n-hexane and ethyl acetate fractions as well as ethanol extract gave hope in arresting hyperlipidemia resulting from diabetes mellitus in diabetic rats.

Keywords: *Nauclea latifolium, lipid profile, diabetes mellitus, photochemistry*

1. INTRODUCTION

Diabetes mellitus has become a major public health concern mostly in the developing countries. Diabetics have been reported to show abnormal lipid disorders such as hyperlipidaemia, atherosclerosis etc[9],[14]. Serum lipids of diagnostic importance include total cholesterol, triacylglycerol, very low density lipoprotein, high density lipoprotein etc, hence abnormal lipid metabolism is one of the reasons for premature atherosclerosis in patients with diabetes mellitus[13].

Nauclea latifolia (Rubiaceae) is a shrub commonly called pincushion tree (English), mbom-ibong (Ibibio) and tabashiya (Hausa), ubuluinu (Igbo) and scille maritime (French) have been reported to posses hypoglycemic property[3]. Preliminary phytochemical screening by [2] showed that it contains alkaloids, saponins and polyphenols. This study will help to unravel the effect of the ethanol extract and its fractions on the lipid profile of diabetic rats as well as establishing the fraction with the highest hypolipidaemic property.

2. MATERIALS AND METHODS

The fresh leaves of Nauclea latifolium were obtained from endocrine farm of University of Calabar, Nigeria. They were identified by Mr. Etefia, a Technologist in Department of Pharmacognosy, University of Uyo, Nigeria, washed, dried under shade and blended to powder (2 kg). The powder was macerated twice in 20 litres of 95 % ethanol with occasional shaking, filtered and concentrated in water bath (40 ^{o}C). The concentrate (363.07 g) was successively partitioned with n-hexane (4 x 250 ml), ethyl acetate (3 x 250 ml), butanol (4 x 250 ml) and methanol (1 x 250 ml) and all were concentrated to their respective fractions [17]. Phytochemical analysis was performed according to the method described by [8] and [18] .

a. Laboratory Animals/Diabetes Induction

Eighty Albino Wistar rats weighing (100 - 250g) obtained from the animal house of Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. They were fed ad libitum with Vital commercial feed, Nigeria and safe drinking water. The animals were fasted overnight and intraperitoneally injected with alloxan monohydrate (Sigma St. Louis, M. O., USA) dissolved in 0.9 % v/v cold normal saline solution at a dose of 150 mg/kg body weight [10], [16]. The rats were then kept for the next 24 hours on 5 % glucose solution bottles in their cages to prevent hypoglycemia [7]. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals.

b. Experimental Design

The following treatment groups for the study:were used Diabetic control (30% Tween 80), glibenclamide (5 mg/kg), ethanol whole extract (100 mg/kg), ethanol whole extract (250 mg/kg), n-hexane fraction (100 mg/kg), n-hexane fraction (250 mg/kg), ethyl acetate fraction (100 mg/kg), ethyl acetate fraction (250 mg/kg), butanol fraction (100 mg/kg), butanol fraction (250 mg/kg), methanol fraction (100 mg/kg), methanol fraction (250 mg/kg). Each fraction was administered once orally Via feeding tube for a duration of two weeks, the animals were fasted overnight, anaesthesized under chloroform fumes, sacrificed and their

sera used for the lipid profile assay. There were 5 rats per group [4].

c. Chemical/Reagents

Analytical grade reagents and chemicals were used for this study. The respective Randox laboratory reagent kits, UK for Triglyceride (TG) and total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) were used. The concentration of the respective parameters was read directly using AJ 122 chemistry analyser (spectrophotometer) China, whereas the concentration of VLDL was extrapolated by dividing the respective concentration of TG by 5 while LDL-cholesterol was estimated using the method by Friedewald (1972) that LDL-C = TC -(HDL-C) – VLDL.

d. Statistical Analysis

The results in the group are presented as mean ±

SEM while 95% was considered as confidence level. The various group data were statistically compared using student t-test and one way-ANOVA.

3. RESULT

Table 1: Phytochemical evaluation of Nauclea latifolium leaf ethanol extract and fractions

1	Saponins (a) Frothing test (b) Emusion test (c) $NaHCO_3$	Ethanol Extract ++ + +	n-hexane fraction ++ ++ ++	Ethyl acetate fraction + + +	Butanol fraction + + +	Methanol fraction +++ ++ ++
2	Flavonoids (a) Shinoda Reduction test (b) Mg fillings	+ +	- +	Orange + +	Red ++ ++	Red +++ ++
3	Phenol Folin- cucalteau	++	+	++	++	+++
4	Cardiac Glycosides (a) Salkowskis test (b) Keller-killianii test (c) Libermann test	++ ++ ++	+ + +	+ + +	++ ++ ++	+++ +++ +++
5	Sterols/Terpenoids (a) Burchard & Salkowski's test (b)Liberman- Burchard test	++ ++ Brownish colour	+ +	+ +	++ ++	Red interface + +
6	Tanins (a) Ferric Chloride test	+	+	++	++	++
7	Alkaloids Drangendoff's reagent Mayer's reagent Hagger's reagent	+ + +	+ + +	+ + +	+ + +	+ + +
8	Carbohydrates Molisch's test	+++	++	+	++	+++

⇒ absent, + ⇒ little, ++ ⇒ moderate, +++ ⇒ high

Table 2: Effect of Nauclea latifolium leaf ethanol extract and fractions on the lipid profile (mg/dL) of diabetic rats

S/No.	Treatment groups	TG	VLDL	Total cholesterol			
1	Diabetic Control (30 % tween 80)	177.30± 7.56	35.46±1.51	238.12±1.51			
2	Glibenclamide (5 mg/kg).	67.58±4.62	13.52±0.92*	49.17±0.95*			
3	Ethanol Extract (100 mg/kg).	114.34±23.48	22.87±4.70	100.19±11.72*			
4	Ethanol Extract (250 mg/kg).	94.10±17.02	26.82±12.18	79.33±5.48*			
5	n-Hexane fraction (100 mg/kg).	119.55±11.93	23.91±2.39	112.69±15.40*			
6	n-Hexane fraction (250 mg/kg).	104.44±12.18	20.89±2.44	91.37±5.99*			
7	Ethyl acetate fraction (100 mg/kg).	68.76±10.38	13.75±2.08*	88.75±12.14*			
8	Ethyl acetate fraction (250 mg/kg).	78.90±15.07	15.78±3.01*	64.75±5.56*			
9	Butanol fraction(100 mg/kg).	58.74±6.52	11.75±1.30*	43.86±1.89*			
10	Butanol fraction (250 mg/kg).	57.20±1.42	11.44±0.29*	56.01±5.91*			
11	Methanol fraction (100 mg/kg).	71.79±1.56	14.36±0.31	40.11±3.89*			
12	**Methanol fraction (250 mg/kg).**	**99.87±8.95**	**19.98±1.79**	**42.70±7.38***			

*⇒ significant decrease at p<0.05when compared with diabetic control group, n=5, (Mean±SEM).
**⇒ significant increase at p<0.05when compared with diabetic control group, n=5.

Table 3: Effect of Nauclea latifolium leaf ethanol extract and fractions on the lipid profile (mg/dL) of diabetic rats

S/No.	Treatment groups	LDL-C	HDL-C
1	Diabetic Control (30 % tween 80)	184.40±3.11	18.25±4.32
2	Glibenclamide (5 mg/kg).	35.15±3.80*	30.5±2.30**
3	Ethanol Extract (100 mg/kg).	42.16±6.03*	35.16±1.91**
4	Ethanol Extract (250 mg/kg).	29.11±4.05*	23.40±2.73
5	n-Hexane fraction (100 mg/kg).	48.54±2.08*	40.24±5.03**
6	n-Hexane fraction (250 mg/kg).	41.77±3.19*	28.71±0.77**
7	Ethyl acetate fraction (100 mg/kg).	42.99±1.91*	32.01±2.11**
8	Ethyl acetate fraction (250 mg/kg).	28.25±0.15*	20.72±0.82
9	Butanol fraction(100 mg/kg).	24.09±2.20*	21.02±1.13
10	Butanol fraction (250 mg/kg).	35.93±3.02*	20.63±0.05
11	Methanol fraction (100 mg/kg).	25.50±4.20*	22.25±4.03
12	Methanol fraction (250 mg/kg).	27.82±3.04*	22.90±5.80

*⇒ significant decrease at p<0.05when compared with diabetic control group, n=5, (Mean±SEM).
**⇒ significant increase at p<0.05when compared with diabetic control group, n=5.

4. DISCUSSION

The study presented fact that the may be some hypocholesterolemic substances in all the fractions and ethanol whole extract of the plant due to their abilities in significantly reducing ($p < 0.05$) total cholesterol levels in the respective treatment groups when compared with the diabetic control group. This result was similar to the report by some researchers [1], [17] on the abilities of some plants extract in reducing total cholesterol levels in diabetic rats.

[1], [15] that fraction F_6 of Vernonia amygdalina significantly reduced ($p < 0.05$) Serum TG level in diabetic rats within two weeks of treatment but proposition was not the same with the result of this study as insignificant decreases ($p > 0.05$) in TG levels were noticed in all the treatment groups when compared with the diabetic control group.

More over, the significant decreases ($p < 0.05$) in the levels of VLDL in the groups treated with glibenclamide, methanol fraction (100 mg/kg), butanol and ethyl acetate fractions were similar to the earlier report by [1]. Hence, high levels of TG, LDL-C, VLDL-C and total cholesterol been associated with diabetes mellitus, heart disease, insulin resistance due to increased mobilization of free fatty acids from the peripheral fat depots [14], [5] were reduced after treatment with the plant fractions suggesting their abilities in arresting some lipid related symptoms of diabetes mellitus and its recommendation for managing incidences of the diseases. Flavonoids, phenols, saponins and sterols have been reported to be associated with hypolipidemia and hypocholesterolemia as reported by [11], [5] 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 [12] .

Furthermore, worthy of note was the improved result of ethanol extract, n-hexane and ethyl acetate fractions showed when compared with glibenclamide, a conventional anti-diabetic drug in arresting hyperlipidemia arising from diabetes mellitus in diabetic rats. Therefore, the significant reduction in TG, LDL-C, VLDL, total cholesterol levels as well as the significant increase ($p < 0.05$) in HDL-C levels of some of the fractions may be either through the scavenging of the reactive oxygen metabolites due to the presence of anti-oxidant compounds in the extracts or by increasing the synthesis of anti-oxidant molecules in diabetic rats [15].

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