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Full Length Research Paper

Hypoglycemic Effect of Ethanol Extract and Fractions of *Nauclea latifolium* leaf on normal and alloxan-induced diabetic rats

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The hypoglycemic effect of ethanol extract and fractions of *Nauclea latifolium* leaf was evaluated in this study. The result in normoglycemic rats showed maxima glycemic reduction of 26.4% within 4 hours in the group treated with butanol fraction (250 mg/kg) which proved efficacious than glibenclamide at $p < 0.05$. Also, similar advantage over glibenclamide was recorded in ethanol extract (250 mg/kg) given alloxan-induced diabetic rats with % glycemic change of 44.4% as compared to 32.2% of glibenclamide within 4 hours n-hexane fraction (100 mg/kg) and butanol fraction (250/kg) recorded maxima % glycemic changes of 73.2% and 71.2%, respectively, within a period of 14 days at $p < 0.05$ in alloxan-induced diabetic rats. These findings suggest that ethanol extract and fractions of *Nauclea latifolium* leaves possess hypoglycemic.

Key Words: Hypoglycaemic, nauclea latifolium, alloxan-induced diabetes.

INTRODUCTION

Nauclea latifolium (Rubiaceae) is an evergreen multi-stemmed shrub or tree; it is commonly called pin cushion tree (English), mbom Ibong (Ibibio), Tafashiya (Hausa), ubuluinu (Igbo) and scille maritime (French). Scientific studies on the plant reported that the aqueous extract possessed hypoglycemic effects (Ameh, 2009), antihypertensive property (Nworgu et al., 2008), anti-malaria property (Boye, 1990), antibacterial property (EI–mahmood et al., 2008). Some phytochemicals such as alkaloids, saponins and polyphenols (Akpanabiatu et al., 2005), terpenes (Morah, 1995) and some inorganic elements (Asubiojo et al., 1982) have been reported to be associated with this plant.

However, apart from Ameh (2009) who reported only the hypoglycemic effect of the aqueous extract of the plant, there is no scientific report on the use of solvents of different polarity in extracting the phytochemicals in the leaves of *Nauclea latifiolium* as well as establishing their hypogycemic property in normoglycemic and alloxan-induced diabetic rats, thereby making this study relevant and necessary.

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MATERIALS AND METHODS

Plant materials

Fresh leaves of *Nauclea latifolia* were collected from the Endocrine research farm of the University of Calabar, Nigeria. The plants were identified at the Pharmacognosy Department, University of Uyo, Nigeria, by the Chief Technologist, Mr. Etefia.

Extract Preparation

The fresh leaves were dried under shade and grounded into powder (2kg). The powder was macerated twice in 95% ethanol, filtered and concentrated to dryness in water bath at 40^{o}C. The concentrated ethanol extract (363.07g) was successively partitioned with n-hexane (4 x 250ml), ethyl acetate (3 x 250 ml), butanol (4 x 250 ml) and methanol (1 x 250 ml), the resulting extracts were concentrated to obtain their respective fractions

Laboratory Animals

One hundred and fifteen albino Wistar strain rats weighing

(100 - 250g) obtained from the animal house of the Faculty of Pharmacy, University of Uyo, Uyo were used for the study. The animals were fed *ad libitum* with commercial feed and drinking water. All experiments on rats were carried out in compliance with the University of Calabar graduate school ethical guide for care and use of laboratory animals for graduate research.

Experimental Design

The research was carried out on both normoglycemic and alloxan-induced diabetic rats, the rats were fasted overnight before each experiment and their blood samples collected from their tails. The following treatment groups were used: diabetic control group, normal control group, glibenclamide group (5 mg/kg), ethanol extract (100mg/kg), ethanol 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 (250mg/kg), methanol fraction (100 mg/kg) and methanol fraction (250 mg/kg). The study was divided into two models.

Model one A (acute studies on blood glucose levels of normglycemic rats), model one B (Acute studies on blood glucose levels of diabetic rats and model two (sub acute studies on blood glucose levels of diabetic rats). The acute studies involved the estimation of the blood glucose levels in the rats at a time intervals of 0 hour, 1 hour, 2 hours and 4 hours while the sub acute studies provided the evidence for assessing the blood glucose concentration of diabetic rats on the 1^{st}, 5^{th}, 10^{th} and the 15^{th} day before the rats were sacrificed and their respective blood collected through cardiac puncture was used for glucose estimation. The diabetic and normal control groups were administered with 30% Tween 80.

Induction of Diabetes

Eighty overnight fasted albino (Wistar) rats were intraperitoneally injected with alloxan (Sigma, St. Louis, Mo, USA) at a dose of 150 mg/kg body weight of rats. After 4 days, seventy surviving rats with blood glucose levels above 250 mg/dl were considered diabetic and used for the study.

Assessment of Hypoglycemic Activity In Non-Diabetic Rats

The respective fraction and extract doses 100 mg/kg and 250 mg/kg body weight of rats were administered orally, blood glucose concentrations were estimated at 0, 1, 2 and 4 hours after administration. Normal control group received 30% Tween 80 Similarly, glibenclamide (5 mg/kg) was given orally to another group of rats. Glucose level estimation was estimated using One-Touch® Lifescan glucometer (USA).

Acute Hypoglycaemic Activity in Alloxan-Induced Diabetic Rats

Single oral administration of the respective fractions and extract at doses 100 mg/kg and 250 mg/kg body weight of diabetic rats after an overnight fasting of the diabetic rats via BMI feeding tube (size (5 mg/kg) was given to the other group of rats.

Sub-Acute Hypoglycaemic Activity Evaluation on Alloxan Induced Diabetic Rats

Ethanol extract, plant fractions at doses of 100 and 250 mg/kg bodyweight of rats and glibenclamide (5 mg/kg) were administered once daily for 14 days. Their blood glucose concentrations were monitored on the 1^{st}, 5^{th}, 10^{th} and 15^{th} day after overnight fasting. After sacrifice, blood was collected through cardiac puncture and glucose estimation was determined based on the principle of Barhan and Trinder (1972) using glucose oxidase kits (Randox, UK).

Statistical analysis

The results are presented as mean □ SEM. The data were analysed statistically using analysis of variance (ANOVA), turkey test and Student t-test to evaluate significance at $p < 0.05$.

RESULTS AND DISCUSSIONS

In normoglycemic rats, the maximal % glycemic changes of 22.5% and 26.4% at 2^{nd} and 4^{th} hours were recorded in the groups of rats treated with butanol fraction (250 mg/kg). Comparison showed improved efficacy of ethanol extract (100 mg/kg) and butanol (250 mg/kg) over an oral hypoglycemic drug glibenclamide with significant reduction in blood glucose levels at $p < 0.05$ (Table 1). The results showed that induction of diabetic mellitus in rats using alloxan results in insulin deficiency (Ali, B. H. 1997) which leads to increase in blood glucose levels of rats (Venkaleswarlu, 1993) as seen in the diabetic control group. It also showed that significant reductions ($p < 0.05$) in blood glucose levels were recorded in groups treated with glibenclamide, 250 mg/kg of ethanol extract, n-hexane fraction (100 mg/kg), ethyl acetate fractions (250 mg/kg and 100 mg/kg), butanol fraction (100 mg/kg), methanol fraction (100 mg/kg) in the 4^{th} hours (Table 2). Further analysis and comparison with the glibenclamide showed that butanol fraction (100 mg/kg), ethyl acetate fraction (100 mg/kg), n-hexane fraction (100 mg/kg), and

Table 1. Effect of ethanol leaf extract and fractions of *Nauclea Latifolium* on the blood glucose levels (mg/dL) of normoglycemic rats.

S/No.	Treatment groups	0 Hour	1 Hour	2 Hours	4 Hours
1	Normal Control (30 % Tween 80)	62.2±5.8	55.6±5.0 (10.6%)	50.0±3.2 (19.6%)	46.6±4.3 (25.1%)
2	Glibenclamide drug (5 mg/kg).	68.4±8.4	56.0±3.0 (18.1%)	43.8±4.2 (36%)	37.2±3.8 (45.6%)
3	Ethanol Extract (100 mg/kg).	80.2±1.6	72.4±5.3 (9.7%)	68.0±3.9 (15.2%)*	66.4±3.4 (17.2%)*
4	Ethanol Extract (250 mg/kg).	75.6±6.4	83.4±3.7 (-10.3%)*	76.6±2.0 (-1.3%)*	73.4±1.9 (2.9%)*
5	n-Hexane fraction (100 mg/Kg).	77.0±9.2	72.8±2.4 (5.5%)*	68.4±2.8 (11.2%)*	56.6±2.4 (26.5%)
6	n-Hexane fraction (250 mg/kg).	71.8±7.1	66.8±6.4 (7.0%)	55.0±5.9 (23.4%)	51.8±4.5 (27.9%)
7	Ethyl acetate fraction (100 mg/kg).	87.0±7.4	78.2±5.9 (10.1%)	69.0±5.6 (20.7%)*	60.2±4.4 (30.8%)
8	Ethyl acetate fraction (250 mg/kg).	82.2±7.6	72.6±5.7 (11.7%)	66.4±4.8 (19.2%)*	55.2±4.0 (32.9%)
9	Butanol fraction (100 mg/kg).	80.2±4.9	79.6±5.1 (0.8%)*	75.0±5.2 (6.5%)*	68.6±4.5 (14.5%)
10	Butanol fraction (250 mg/kg).	81.0±7.1	70.6±5.8 (12.8%)	62.8±3.1 (22.5%)*	59.6±2.6 (26.4%)*
11	Methanol fraction (100 mg/kg).	82.6±5.0	77.8±.3.3 (5.8%)*	74.0±3.1 (10.4%)*	69.8±3.9 (15.5%)*
12	Methanol fraction (250 mg/kg).	77.0±4.4	81.6±3.0 (-6.0%)*	73.4±4.6 (4.7%)*	61.0±5.6 (20.8%)

* Significant reduction at $p < 0.05$, n = 5, Mean ± SEM, Figures in bracket are the % changes in glucose levels.

Table 2. Effect of ethanol leaf extract and fractions of Nauclea latifolium on the blood glucose levels (mg/dl) of diabetic rats.

S/No	Treatment groups	0 Hour	1 Hour	2 Hours	4 Hours
1	Diabetic Control (30 % Tween 80)	305.7±27.9	300.0±21.9 (1.9%)	300.6±25.2 (1.7%)	295.7±25.6 (3.3%)
2	Glibenclamide drug (5 mg/kg).	328.6±38.2	255.2±26.9 (22.3%)	240.9±23.0 (26.7%)	222.9±21.1 (32.2%)*
3	Ethanol Extract (100 mg/kg).	391.4±53.8	336.0±56.6 (14.2%)	274.8±64.3 (29.8%)	208.8±56.2 (46.7%)
4	Ethanol Extract (250 mg/kg).	358.4±53.2	315.6±53.2 (12.0%)	273.6±35.4 (23.7%)	199.4±19.1 (44.4%)*
5	n-Hexane fraction (100 mg/Kg).	319.8±26.7	282.4±19.6 (11.7%)	223.8±36.7 (30.0%)	182.2±20.0 (43.0%)*

Table 2. contd.

6	n-Hexane fraction (250 mg/kg).	321.0±48.6	276.8±40.6 (13.8%)	241.4±54.4 (24.8%)	196.4±41.7 (38.8%)
7	Ethyl acetate fraction (100 mg/kg).	300.6±24.4	251.8±35.6 (16.2%)	238.8±46.1 (20.6%)	179.0±41.7 (40.5%)*
8	Ethyl acetate fraction (250 mg/kg).	284.4±19.2	250.4±22.8 (11.6%)	238.2±30.8 (16.0%)	190.8±19.4 (32.7%)*
9	Butanol fraction (100 mg/kg).	334.6±42.8	314.6±43.4 (6.0%)	243.8±26.1 (27.1%)	196.2±23.8 (41.4%)*
10	Butanol fraction (250 mg/kg).	393.4±79.0	359.8±64.2 (8.5%)	321.6±47.3 (18.3%)	255.2±42.6 (35.1%)
11	Methanol fraction (100 mg/kg).	276.8±34.2	262.0±30.1 (5.4%)	238.2±28.6 (14.0%)	198.0±23.7 (28.5%)*
12	Methanol fraction (250 mg/kg).	360.2±33.2	323.2±28.5 (10.3%)	271.2±22.2 (24.7%)	215.8±25.7 (40.1%)

* Significant reduction at $p < 0.05$, n = 5, Mean ⌖ SEM, Figures in bracket are the % changes in glucose levels.

Table3. Effect of ethanol leaf extract and fractions Nauclea latifolium leaf on the blood glucose levels(mg/dl) of diabetic rats during sub-acute studies.

S/NO	Treatment groups	Glucose	Glycaemic Change (%)
1	Diabetic Control (30 % Tween 80)	298.2±6.05	48.1 *
2	Glibenclamide (5 mg/kg).	170.6±12.0	62.7 *
3	Ethanol Extract(100 mg/kg)	146.2±42.0	64.8*
4	Ethanol extract (250 mg/kg).	126.2±19.8	73.2 *
5	n-Hexane fraction (100 mg/ Kg).	85.6±14.7	66.1 *
6	n-Hexane fraction (250 mg/kg).	108.8 ±5.7	64.5 *
7	Ethyl acetate fraction (100 mg/Kg).	106.8±18.5	63.3 *
8	Ethyl acetate fraction (250 mg/kg).	104.0±8.7	50.0 *
9	Butanol fraction (100 mg/kg)	167.4±30.74	71.2 *
10	Butanol fraction (250 mg/kg)	113.4±8.87	54.0 *
11	Methanol fraction (100 mg/kg)	127.4±23.36	65.2 *
12	Methanol fraction (250 mg/kg).	125.5±24.60	2.5 *

* => Significant reduction at $p < 0.05$, n = 5, Mean ⌖ SEM, Figures in bracket are the % changes in glucose levels.

ethanol extract (250 mg/kg) were more potent than glibenclamide as seen from the % glycemic change of 44.4% ethanol extract (250 mg/kg) when compared to 32.2% for glibenclamide. These showed that the ethanol extract and fractions of *Nauclea latifolium* possessed antihyperglycemic property and confirmed the earlier

report that aqueous extract of this plants can reduce blood glucose in diabetic rats (Ameh, 2009). From the sub-acute studies, earlier report showed that blood glucose levels in diabetic rats increased gradually and produced symptoms like hyperglycemic, hyperlipidemia (Rerup, C. C. 1970). All the fractions and extract significantly reduced ($p < 0.05$) blood glucose levels (Table 3) and was in consonance with the earlier report by Kar *et al.,* (2003). The highest were noted in groups of rats treated with n-hexane fraction (100 mg/kg, 73.2%) and butanol fraction (250 mg/kg, 71.2%). The blood glucose levels of rats were reduced to physiological levels in the following groups treated with n-hexane fraction (100 and 250 mg/kg), ethyl acetate fraction (100 and 250 mg/kg) and butanol fraction (100 mg/kg). It is worthy of note that all the fractions from their % glycemic changes significantly reduced $p < 0.05$ blood glucose levels in diabetic rats better than glibenclamide.

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