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EFFECT OF ETHANOL EXTRACT AND FRACTIONS OF NAUCLEA LATIFOLIA LEAVES ON SOME BIOCHEMICAL PARAMETERS OF ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus as an endocrine disorder has over the years been treated with orthodox medicine and this have many drawbacks including undesirable side effects and high cost of long term treatment hence ethno botanicals have provided greater advantage over the earlier. The aim of this study is to determine the effect of ethanol extract and its fractions on biochemical parameters such as total protein, urea, Creatinine, AST and ALT levels of alloxan induced diabetic albino wistar rats. The respective extract and fractions at doses of 100 and 250 mg/kg were carried out using their sera. There were significant reductions ($p < 0.05$) in urea levels in groups treated with methanol fractions. All the fractions significantly reduced ($p < 0.05$) creatinine levels in the diabetic rats as compared with the diabetic control group. AST and ALT levels were significantly reduced ($p < 0.05$) in the groups treated with glibenclamide as well as methanol and butanol fractions. Analysis further showed slight increases in total protein levels in groups treated with dose dependent (100mg/kg) methanol and ethyl acetate fractions, ethanol extract (250 mg/kg) as well as n-hexane fractions. Hence butanol and methanol fractions of this plants exhibited some nephroprotective and hepatoprotective properties.

KEYWORDS: Nauclea latifolium, Biochemical parameters, diabetes

1.0 INTRODUCTION

Derangements in protein, fat and carbohydrate metabolism caused by complete or relative insufficiency of insulin secretion or action have been reported in diabetic rats (1,2). The prevalence of this disease is too alarming and in year 2000, global estimate was 150 million people, while in 2010 it was 221 million (3).

Nauclea latifolium is a tropical plant that grows commonly in Akwa Ibom and Cross River States. It is called "Mbom-Ibong" (Ibibio) while the northern Nigerian calls it "tabashiya" (Hausa). It has been known to have medicinal properties and is commonly used in the treatment of diabetes, hypertension, malaria and also as laxative (4,5). Preliminary phytochemical screening of this plant showed the presence of saponins, flavonoids, phenols cardiac glycosides, sterols/terpenoids, tannins, alkaloids and carbohydrates. This work was undertaken to evaluate the effects of the ethanol extract and its respective fractions on urea, creatinine, AST, ALT and total protein levels in alloxan induced diabetic (wistar) rats.

2.0 MATERIALS AND METHODS

2.1 Collection and Extraction of plant material

Nauclea latifolium leaves were collected fresh at endocrine farm, University of Calabar, Cross River State, Nigeria; identified in Department of Pharmacognosy, University of Uyo, Nigeria; dried under shade and blended to powder (2kg). The 2kg powder was double macerated in 20 litres of 95% ethanol, filtered and concentrated in in vacuo (40^{o}C). The ethanol extract (363.07g) was successively partitioned with n-hexane (4 x 250ml), ethyl acetate (3 x 250 ml), butanol (4 x 250ml) and methanol (1 x 250 ml) and all were concentrated to obtain their respective fractions (6)

2.2 Animals/Diabetes Induction

Eighty albino (wistar) rats weighing (100-250g) obtained from animal house of faculty of pharmacy, University of Uyo, Uyo, Nigeria, fed ad libitum with vital commercial feed and drinking water were used. For induction of diabetes, overnight fasted rats were intraperitoneally injected with alloxan (Sigma, St. Louis, Mo, USA) at a dose of 150mg/kg body weight of rats administered as 5g/100ml distilled water. After 4 days, seventy surviving rats with blood glucose levels above 250mg/dl were considered diabetic and used for the study (7,8,9). All experiments were conducted in compliance with the University of Uyo's ethical guide for care and use of laboratory animals.

2.3 Treatment groups

The following treatment groups were used for the studies:

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).

There were 5 rats per group. Each fraction was administered once orally to the rats for a period of 14 days after which the rats were fasted overnight. Blood was collected from the rats through cardiac puncture under chloroform anesthesia while their sera was used for assay. All reagent and chemicals for this work were of analytical grade. Urea, creatinine, AST, ALT and total protein levels were estimated using their respective Randox Laboratory reagent Kits (10). Their absorbance were measured using AJ 122 Chemistry analyzer (spectrophotometer).

3.0 STATISTICAL ANALYSIS

The various data were determined using student t-test and ANOVA, while the group data are presented as mean ± SEM and $p<0.05$ was regarded as significant.

4.0 RESULT AND DISCUSSION

The results are presented in Table 1. From the result, there were significant decreases in urea levels in groups administered with both 100 and 250 mg/kg dose of methanol fraction within the treatment period, this was similar to the earlier report by Akah et al (11). Suggesting a possible reduction in Serum urea levels of diabetic rats treated with Vernonia amygdalina. Also, both doses of ethanol extract and all the fractions significantly decreased ($p < 0.05$) creatinine levels in all the treatment groups within the treatment period, the result was similar to the report by Akah et al (11) on the abilities of Vernonia amygdalina to reduce serum creatinine levels in diabetic rats.

Moreover, marked significant decreases ($p < 0.05$) in ALT levels to normal range was noted in the group treated with butanol fraction (250mg/kg) and glibenclamide, this result was similar to the report by some researchers (11,12) on the possible reduction in serum ALT level of diabetic rats after treatment with Vernonia amygdalina for a period of two weeks. AST levels was significantly reduced ($p < 0.05$) in the treatment groups of butanol and methanol fractions as well as glibenclamide, there by supporting the earlier reports (11, 12).

Furthermore, the total protein levels in the groups treated with n-hexane fractions, ethanol extract (250 mg/kg), ethyl acetate (100 mg/kg) and methanol fraction (100 mg/kg) showed significant increases ($p < 0.05$) when compared with the diabetic control group, this result was similar to the reports by Manchester (13) Venkateswarlu on the ability of (6) methanolic fraction of Salacia macrosperma roots in improving serum level of total protein in diabetic rats.

In conclusion, the results of this work show that butanol and methanol fractions may contain some phytochemicals that can revert nephrotoxicity and hepatotoxicity caused by alloxan in diabetic rats to some extent.

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TABLE 1

Effect of Nauclea latifolium leaf ethanol extract and fractions on the blood glucose, serum creatinine and urea levels (mg/dL) of diabetic

S/NO	Treatment groups	Glucose	Glycaemic Change (%)	Creatinine	Urea
1	Diabetic Control (30 % tween 80)	298.2±6.05	2.5 *	41.82±0.08	204.51±2.11
2	Glibenclamide (5 mg/kg).	170.6±12.0	48.1 *	3.22±0.22*	73.83±3.82
3	Ethanol Extract (100 mg/kg). .	146.2±42.0	62.7*	2.30±0.36*	196.8±75.0
4	Ethanol extract (250 mg/kg).	126.2±19.8	64.8 *	2.10±0.17*	178.0±22.2
5	n-Hexane fraction (100 mg/ Kg).	85.6±14.7	73.2 *	2.06±0.11*	424.0±97.8**
6	n-Hexane fraction (250 mg/kg).	108.8 ±5.7	66.1 *	2.43±0.18*	347.4±22.7
7	Ethyl acetate fraction (100 mg/Kg).	106.8±18.5	64.5 *	2.60±0.26*	211.9±15.6
8	Ethyl acetate fraction (250 mg/kg).	104.0±8.7	63.3 *	2.64±0.08*	193.2±1.7
9	Butanol fraction (100 mg/kg).	167.4±30.74	50.0 *	1.69±0.17*	59.10±8.01

10	Butanol fraction (250 mg/kg).	113.4±8.87	71.2 *	1.78±0.06*	53.97±5.42
11	Methanol fraction (100 mg/kg).	127.4±23.36	54.0 *	1.85±0.36*	40.11±2.89*
12	Methanol fraction (250 mg/kg).	125.5±24.60	65.2*	1.77±0.22*	44.10±1.49*

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

* significant decrease at $p < 0.05$ when compared with diabetic control group, n=5.

TABLE 2 Effect of Nauclea latifolium leaf ethanol extract and fractions on total protein , AST and ALT levels of diabetic rats

S/no	**Treatment groups**	**Total protein** (mg/dL)	**AST** (mg/dL)	**ALT**(mg/dL)
1	Diabetic Control (30 % tween 80)	5.75± 0.21	82.54 ± 3.61	27.44 ± 0.35
2	Glibenclamide (5 mg/kg).	7.22 ±0.13	3.56 ± 0.18*	7.24 ± 0.65*
3	Ethanol Extract(100 mg/kg).	5.72 ± 1.70	100.6±8.12**	20.62 ± 1.45
4	Ethanol Extract (250 mg/kg).	8.88±0.27**	83.6 ± 32.30	31.52 ± 2.69
5	n-Hexane fraction (100 mg/Kg).	9.74±0.38**	106.0 ± 2.72	37.1 ± 3.95
6	n-Hexane fraction (250 mg/kg).	9.33±0.33**	98.4 ± 3.48**	31.94 ± 1.29
7	Ethyl acetate fraction (100 mg/kg).	8.68±0.35**	103.6±3.60**	40.74 ± 2.00
8	Ethyl acetate fraction (250 mg/kg).	7.78 ± 0.16	92.2 ± 0.96	32.18 ± 0.59
9	Butanol fraction (100 mg/kg).	7.98 ± 0.35	6.52 ± 0.62*	2.68 ± 0.16*
10	Butanol fraction (250 mg/kg).	7.67 ± 0.20	5.84 ± 0.33*	3.68 ± 0.21*
11	Methanol fraction (100 mg/kg).	8.55±0.82**	8.88 ± 0.93*	2.28 ± 0.34*

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

**significant increase at $p < 0.05$ when compared with diabetic control group, n=5.