RAT SERUM ELECTROLYTES, LIPID PROFILE AND CARDIOVASCULAR ACTIVITY ON *Nauclea Latifolia* LEAF EXTRACT ADMINISTRATION

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ABSTRACT

Aqueous extract of the leaf and root of *Nauclea latifolia* Sm. (Rubiaceae) is used in Nigerian folk medicine for the treatment of hypertension. This work is carried out to investigate the effect of *Nauclea latifolia* leaf extract on lipid profile and cardiovascular activity of rats. Normal and 10% coconut oil fed rats were treated with the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaf for 2 weeks. Forty-eight mature male albino rats of the Wistar strain were divided into two experiments of four groups, each group having 6 animals. Experiment I animals were treated with the water-soluble fraction of the ethanol extract whilst experiment II animals were fed 10% coconut oil meal before treatment with the water-soluble fraction of the ethanol extract. A single oral dose of *Nauclea latifolia* was 170, 340 and 510 mg/kg body wt/day of the extracts respectively for 2 wks. There was no significant change in the lipid profile of the experimental animals as compared with the controls. There was about 40% relaxation on contracted thoracic aorta that was pre-contracted with 2 μ M phenylephrine. The viability of the tissue was tested against 10 μ M of acetylcholine. There was no significant ($P > 0.05$) change in Na^+ concentration in the serum. However, the K^+ concentration in the serum of the experimental animals showed a significant increase. The study shows that ethanol extract of *Nauclea latifolia* has vasodilator action on the aorta and that lipid profiles of experimental rats were not affected. Furthermore, the increase in the K^+ may be contributing to the vasodilator effect of *Nauclea latifolia*.

KEY WORDS

Cardiovascular activity, Electrolytes, Lipid profile, *Nauclea latifolia*.

INTRODUCTION

In Africa and in most Asian countries where the use of folk medicine is prevalent, the search for herbal cures is but common practice. The user may be exposed to toxicity due to a lot of many unknown constituents of the concoction. *Nauclea latifolia* Sm (Rubiaceae) is a tropical plant that grows commonly in Akwa Ibom and Cross River States of Nigeria and also in most parts of the Northern Nigeria. In Akwa Ibom and Cross River States, it is called "Mbom-mbog" whilst in the Northern Nigeria it is called "Tabasiya". *Nauclea latifolia* has been known to have medicinal properties and is commonly used in the treatment of hypertension, malaria and also as a laxative. Scanty literature is

available on the use of this plant. However, the key phytochemical constituents are indole - quinolizidine, alkaloids, glycoalkaloids and saponins but the actual quantification of the chemical content is yet to be carried out (1). The local herbal physician combines the leaves and the roots for medication (1). It is now known that the anti-hypertensive effect of this herb is due to the leaf and not the root (1). The effects of the leaf and root extracts of *Nauclea latifolia* on purinergic neuro-transmission in the rat bladder have been reported (2).

High blood lipids are associated with hypertension and lipid peroxidation with drug toxicity (3). However in case of toxicity micronutrients such as vitamins A and C naturally present in most plants serve as antioxidants (4, 5). Changes in blood lipids have been associated with some herbs (6, 7, 8, 9, 10). Other plants such as *Elaeophorbium drupifera* have been reported to have some cardiovascular effects in experimental animals (11). Decreases in atherosclerotic (cardiovascular) diseases have been linked to elevated high-density lipoprotein concentrations (12, 13). In this investigation we intend to look at the

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possibility of having lipid changes in the blood of rats treated with extracts of *Nauclea latifolia* leaves. Serum K⁺ and Na⁺ will also be investigated since both ions play important role in cardiovascular activity (14). An attempt will be made in this work to investigate aortic tissue response to this herb since the enzyme that cause lipid changes has been found in the aorta of rabbits. It is our belief that this investigation will take us another step forward in our quest to understand the mechanism of action of *Nauclea latifolia* in hypertension.

MATERIALS AND METHODS

Preparation of Plant Material and Animal Care

Fresh leaves of *Nauclea latifolia* were obtained from the premises of the Medical School, University of Calabar, Nigeria, in the month of June 2001. The leaves were identified and authenticated by the Botanist in the botanical garden of the University. Voucher specimen No. MIA 2001 was deposited at the herbarium in the Department of Botany, University of Calabar. The leaves were dried in a Plus 11 oven at 55°C and crushed using laboratory blender (National blender). The ground leaf was stored in a glass bottle with a plastic screw cap and kept in the refrigerator (4°C).

Forty-eight mature male albino rats of the Wistar strain, weighing 200-230 g were obtained from animal stock in the Department of Biochemistry, University of Calabar, Nigeria. The animals were housed in a well-ventilated experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. Feeding of animals was done *ad libitum*, along with drinking water. The 48 animals were divided into 8 groups of 6 rats each. All experiments on rats were carried out in absolute compliance with the University of Calabar Graduate School Guideline for Graduate Research, which also consider ethical guide for care and use of laboratory animals.

Experiment I

Group 1 (normal control) : Rats were subjected to oral administration of 1.5 ml of normal saline as placebo.

Group 2, 3 and 4 : Rats were treated with 170, 340 and 510 mg/kg body weight of the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaves.

Experiment II

Group 1 (Coconut oil control) : In this group, rats were fed with 10% coconut oil diet for eight weeks before treatment with normal saline as placebo.

Group 2, 3 and 4 : In these groups, rats were fed with 10% coconut oil diet for eight weeks before treatment

with 170, 340 and 510 mg/kg body weight of the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaves.

Preparation of Coconut Oil and Coconut Oil Meal

Dry coconut fruits were purchased from Akim Market, Calabar Municipality in Cross River State, Nigeria. The nuts were broken manually and the coconut meat was removed and grated using aluminium grater. The grated meat was washed with sufficient tap water to obtain coconut milk. The milk was filtered and evaporated to dryness using aluminium pot on a gas cooker. The oil was decanted into a stainless steel cup and dried in a Plus II oven at 65°C for 2 h to remove water residues from the oil. The oil was further decanted into a bottle with a plastic screw cap and stored in the refrigerator for use. 10 g of the oil was mixed and mashed with 90 g of normal rat chow until there was homogeneity. The 10% coconut oil meal was prepared daily on demand.

Preparation of Plant Extracts

Bulk ethanol extraction of the crushed leaf of *Nauclea latifolia* was done by soxhlet extraction method using an electro-thermal heating mantle. The extract was dried in an oven at 55°C to remove all the ethanol and was stored in the refrigerator until required for use. The ethanol extract was re-extracted in cold water for use in this experiment. The residue was removed by filtration using Whatman No. 1 filter paper. The concentration of the extract was determined gravimetrically by drying a known volume and weighing the dried residue. Storing in the refrigerator throughout the period of the experiment preserved the prepared extract.

Animal Treatment and Preparation of Serum

Administration of *Nauclea latifolia* leaf extract was done by intra-gastric syringe. In experiment I, group 1 animals served as the control and received normal saline (1.5 ml). In group 2, 3 and 4, animals were treated with graded doses of 1, 2 and 3 ml of the water-soluble fraction of the ethanol extract corresponding to 170, 340 and 510 mg/kg body weight of animal respectively. The same procedure was repeated in experiment II. Administration of the extract was between the hours of 10 am and noon. Twenty-four hours after the last administration, the animals were anaesthetized under chloroform vapour. Overnight fasting blood samples for sera preparation were collected by cardiac puncture into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3000 g for 5 min using bench top centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection.

Preparation of Aortic Rings

Four normal Albino Wistar rats weighing 200-230 g were killed by cervical dislocation and the thoracic aorta immediately removed, freed from adherent tissue and placed in normal physiological salt solution (PSS) made of the following composition (mM) : NaCl : 119, KCl : 4.7, KH_2PO_4 : 1.2, MgSO_4 : 1.2, NaHCO_3 : 14.9, CaCl_2 : 1.6 and glucose : 11.5. The solution was continuously bubbled with 95% O_2 and 5% CO_2 gas mixture at 37°C. The aorta was cut into 2 mm rings and suspended between 2 stainless steel L-shaped rods in a 200 ml tissue bath containing PSS. One rod was hooked to the base of the bath and the other was connected to a force displacement transducer (Grass FT 03) which was in turn connected to a Grass model 7D polygraph for recording of isometric contractions. The rings were allowed to equilibrate for 90 min under a resting tension of 2 g during which it was stimulated with 2 μM phenylephrine until a base line contraction was recorded. With the formation of a plateau due to the phenylephrine contraction of the aorta, 0.23 and 0.68 mg/ml of the extract and acetylcholine (10 μM) were added cumulatively and their respective tissue response recorded.

Chemicals and Reagents

All reagents and chemicals that were used in this work were of analytical grade. Serum total cholesterol and high-density lipoprotein cholesterol were determined using Randox Laboratory kit reagents. Serum triacylglycerol level was estimated using Randox

Laboratory test kit and VLDL-cholesterol was calculated using the formula $\text{TG}/2.2 \text{ mmol/l}$. Low-density lipoprotein (LDL) cholesterol was determined by differential subtraction of the sum of the cholesterol fractions from the total cholesterol. Na^+ and K^+ were determined by flame photometry using Jenway P7 flame photometer. The absorbances of all the tests were determined using spectrophotometer (HAICH, DR 3000, Germany).

Statistical analysis of data was determined with the use of Standard student's 't'-test method and $P < 0.05$ were regarded as significant. The group data are expressed as mean $\pm$ SD.

RESULT

Table 1 and 2 contain the results of the lipid profiles of normal adult male albino rats of the Wistar strain that were gavaged the water-soluble fraction of the ethanol extract of *Nauclea latifolia* respectively at the doses of 170, 340 and 510 mg/kg body weight. In Table 1 the results of the lipid profiles of normal rats treated with the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaf are reported. These results also show that there was no significant ($P > 0.05$) alteration in the serum lipid profile of the experimental animals when compared with the controls.

The results in Table 2 are those of experimental animals that were fed on 10% coconut oil meal for 8 weeks before the administration of the water-soluble

Table 1. Serum electrolytes and lipid profiles of normal adult male albino Wistar rats treated with the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaves

Parameter (mmol/L)*	Serum total cholesterol	Serum HDL cholesterol	Serum Triglyceride	Serum VLDL-cholesterol	Serum LDL-cholesterol	Serum Na^+	Serum K^+
Group 1 (Control)	2.061 $\pm$ 0.29	1.124 $\pm$ 0.25	0.950 $\pm$ 0.57	0.431 $\pm$ 0.03	0.506 $\pm$ 0.12	71.75 $\pm$ 2.21	5.80 $\pm$ 1.44
Group 2	1.923 $\pm$ 0.2	1.023 $\pm$ 0.08	0.928 $\pm$ 0.08	0.422 $\pm$ 0.014	0.448 $\pm$ 0.16	76.5 $\pm$ 3.41	7.8 $\pm$ 1.38**
Group 3	1.769 $\pm$ 0.23	0.944 $\pm$ 0.12	0.785 $\pm$ 0.14	0.356 $\pm$ 0.03	0.468 $\pm$ 0.08	71.25 $\pm$ 5.37	6.4 $\pm$ 1.65
Group 4	1.858 $\pm$ 0.3	1.024 $\pm$ 0.09	0.752 $\pm$ 0.12	0.342 $\pm$ 0.1	0.534 $\pm$ 0.09	68.75 $\pm$ 3.09	3.09 $\pm$ 1.25**
* Mean $\pm$ SD of 6 determinations, ** $P < 0.05$ compared with control.							

Table 2. Serum electrolytes and lipid profiles of adult male albino Wistar rats fed on 10% coconut oil meal before treatment with the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaves

Parameter (mmol/L)*	Serum total cholesterol	Serum HDL cholesterol	Serum Triglyceride	Serum VLDL-cholesterol	Serum LDL-cholesterol	Serum Na ⁺	Serum K ⁺
Group I (Control)	2.185 ± 0.06	0.971 ± 0.03	1.277 ± 0.21	0.580 ± 0.10	0.677 ± 0.12	61.75 ± 1.50	5.850 ± 0.50
Group 2	2.243 ± 0.16	0.975 ± 0.08	1.29 ± 0.32	0.586 ± 0.14	0.681 ± 0.07	72.5 ± 1.91	6.10 ± 0.66
Group 3	1.864 ± 0.27	0.968 ± 0.04	0.979 ± 0.1	0.450 ± 0.07	0.457 ± 0.03	71.75 ± 6.55	6.30 ± 0.72
Group 4	1.764 ± 0.18	0.858 ± 0.01	1.09 ± 0.13	0.50 ± 0.054	0.460 ± 0.12	60.5 ± 1.72	4.25 ± 0.70**

* Mean ± SD of 6 determinations, ** P < 0.05 compared with control.

fraction of the ethanol extract of *Nauclea latifolia* leaf. The results in Table 2 did not show any significant change in the lipid profile ($P > 0.05$). The results of serum electrolytes (Na⁺ and K⁺) as reported in Table 1 and 2 show a slight increase in K⁺ level as compared with the controls.

Figure 1 shows the results of tissue response to the ethanol extract of *Nauclea latifolia* leaves. The phenylephrine (2 µM) induced contraction of the aorta was regarded as base line response. The administration of 0.23 mg/ml of *Nauclea latifolia* leaf extract when the response had reached plateau did not result in any significant decrease in the contraction of the thoracic aorta. However, a reduction of 2.0 mm ± 0.48 was noticed and this corresponds to 10.92 ± 2.36%. Following the administration of 0.68 mg/ml of the same extract, a further reduction of 7.50 ± 1.23% was observed. This reduction was also not statistically significant ($P > 0.05$). The administration of acetylcholine (10 µM) gave a reduction in response of 49.67 ± 6.52% which was statistically significant ($P < 0.01$).

DISCUSSION

The use of herbal medicine in the third world countries is high. *Nauclea latifolia* is widely used in Nigeria for the treatment of hypertension and other diseases due to its active components. In this study some serum indices such as HDL-cholesterol, triacylglycerol, very low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol were measured. Serum K⁺ and Na⁺ concentrations and thoracic aorta response were also measured. The serum total and HDL cholesterol of all the experimental groups did not show significant ($P > 0.05$) change. Also the TG,

Fig. 1. Effect of phenylephrine and acetylcholine on the activity of the extract, values are means of 4 experiments

VLDL- and LDL-cholesterol did not change significantly ($P > 0.05$).

Several studies have consistently shown correlation between serum level of cholesterol, TG, HDL- and VLDL-cholesterols with hypertension. The observation in this work is likely due to the fact that the mechanism involving lipid alteration may not have been disturbed by the administration of the extract of *Nauclea latifolia* leaves. Rightly, if the hepatic synthesis or degradation machinery is not stimulated, there will be no observable change in these parameters.

A study on garlic indicated that it prevents hypertension by chronic inhibition of nitric oxide synthesis without altering blood pressure in garlic fed rats. This indicates that though the base line of measured parameter remains the same, there may be a change in situation when there is hypertension (15). Also most anti-hypertensive drugs like nifedipine and bisoprolol have significantly lowered blood pressure but do not alter lipid profile (16, 17). It may, therefore be that the action of *Nauclea latifolia* leaf extracts in reducing blood pressure, may lie some where else. This is further supported by the study on lipid profile and anti-hypertensive effect in hyperlipidaemic patients : comparison of rilmenidine and captopril (18).

Nauclea latifolia did not significantly reduce the LDL-cholesterol in the experiments but there was slight reduction. The importance of this downward trend observed in treatment groups can not be underscored since a low level of LDL-cholesterol is known to correlate with a low incidence of coronary heart disease, particularly arteriosclerosis. An increase of 1% cholesterol is reported to have resulted in a 3% increase in coronary heart disease. Equally a reduction in LDL-cholesterol by 2 mg/dl can result in 1% reduction in the risk for coronary artery disease (19).

Interestingly the results of the thoracic aorta of normal rats showed that the ethanol extract of *Nauclea latifolia* at concentration of 0.68 mg/ml has vasodilatation effect of about 40% when compared with 10 μ M acetylcholine. This is a confirmation of earlier report that the leaf of *Nauclea latifolia* contains anti-hypertensive properties (1). The ability of this extract to interfere with the phenylephrine (an α -1-adrenoceptor) against induced contraction of the thoracic aorta and the potentiation of the extract activity by acetylcholine points to the fact that the extract of *Nauclea latifolia* could be mediating its vasodilator effect probably in a cholinceptor pathway. Also the serum levels of K^+ and Na^+ were measured and there was no significant difference ($P > 0.05$) in the Na^+ level but K^+ concentration changed significantly ($P < 0.05$). Sodium is associated with blood pressure and in many hypertensive patients, a reduction in sodium intake lowers blood pressure. On the other hand, potassium, which is in the intra-cellular fluid, has been reported to be among the protective electrolytes against hypertension (14). The increase in the serum K^+ is also an indication that membrane channels may possibly be affected by *Nauclea latifolia* leaf extract.

CONCLUSION

Nauclea latifolia leaf extract was investigated for effects on lipid profiles, serum electrolytes and thoracic

aorta response. There were no significant changes in the lipid profiles and the serum level of sodium (Na^+). There was reduction in the phenylephrine pre-contracted tissue indicating a tendency of these extracts to reduced blood pressure. Also the extract could be mediating its vasodilator effect probably in a cholinceptor pathway. This confirmed earlier report on the cardiovascular activity of this herb. We conclude that the K^+ and Na^+ levels are related to the mechanism of *Nauclea latifolia* in reducing blood pressure. The insignificantly low LDL-cholesterol obtained in this work suggests the possibility of this herb ameliorating cardiovascular conditions. Also Na^+ - K^+ ATPase may be affected since this enzyme is required for the movement of these electrolytes across the membrane. We are investigating further the effect of this herb on the serum apolipoprotein to help us understand better the mechanism of action of this herbal medication.

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