

Full Length Research Paper

## Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats

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Roots of *Nauclea latifolia* are used in Nigeria for the treatment of hypertension. Ethanolic extract of the root of *N. latifolia* was administered to anaesthetised rats through the jugular vein while the blood pressure was measured through the carotid artery. The extract reduced systolic, diastolic, and mean arterial pressure in normotensive and in one kidney one clip hypertensive rats in a dose dependant manner. 10 mg/kg of the extract reduced the mean arterial pressure from  $115.7 \pm 0.4$  to  $99.6 \pm 3.8$  mm Hg, while it reduced the systolic and diastolic from  $125.6 \pm 0.5$  to  $102.4 \pm 4.0$  and from  $110.5 \pm 0.3$  to  $95.0 \pm 3.2$  mm Hg respectively. The changes caused in hypertensive rats were much greater. The same dose of the extract reduced the mean arterial pressure from  $157.4 \pm 4.7$  to  $72.0 \pm 3.6$  mm Hg, while it reduced the systolic and diastolic blood pressures from  $180.0 \pm 5.7$  to  $90.1 \pm 2.6$  and from  $146.2 \pm 3.3$  to  $63.0 \pm 4.9$  mm Hg respectively. The extract (10 mg/kg) also reduced the heart rate of normotensive rats from  $365 \pm 12.8$  to  $280 \pm 6.0$  beats/min, while it reduced the heart rate of hypertensive rats from  $550 \pm 22.5$  to  $120 \pm 8.0$  beats/min. The reduction in blood pressure and heart rate was not affected by prior treatment with atropine or promethazine.

**Keywords:** Blood pressure, *Nauclea latifolia* root, phytochemical.

### INTRODUCTION

Hypertension is one of the leading causes of disability or death. It affects 50 million adults in U.S of America but only 68% are aware of their condition and only 27% have it under control (Black, 1999). Each new year, 2 million cases of hypertension are diagnosed (Mayo Foundation, 1999). Prevalence of hypertension in Benin City, Nigeria, is 14% in males and 10% in females (Oviasu and Okupa, 1980), while in Ibadan it is 10.4% in males and 7.1% in females (Kadiri et al., 1999). Hypertension is a major risk factor for coronary heart disease and cerebrovascular disease (WHO, 1974; Stamler et al., 1993). The risk of hypertension increases with age in both sexes (Whelton and Klag, 1989).

In Benin City, Nigeria, roots of *Nauclea latifolia* Sm. (Rubiaceae) are claimed by some traditional medicine practitioners to have antihypertensive effect. *N. latifolia* is

used profusely by traditional medicine practitioners. The bark and root of *N. latifolia* are used in the treatment of malaria in Ghana (Boye, 1990). The leaves are also used for the treatment of malaria in East Africa (Kokwaro, 1976) and in Nigeria (Akubue and Mittal, 1982). The roots are also used to induce abortion and as a purgative (Vasileva, 1969). The bark is used in the treatment of wounds, coughs and gonorrhoea in Nigeria (Madibunyi, 1995). The roots are used in the treatment of hypertension (Akubue and Mittal, 1982).

*N. latifolia* is active against *Plasmodium falciparum* (Traore-Keita et al., 2000), *Bacillus subtilis*, *Escherichia coli*, (Omer et al., 1998), *Salmonella enteritidis*, *Pseudomonas aeruginosa*, (Hussain and Deen, 1991) and *Klebsiella pneumoniae* (Tona et al., 1999). The fruit extract was shown to be active against Human Immune deficiency Virus (Hussein et al., 1999).

The aim of this work is to assess the phytochemical constituents and pharmacologically validate the antihypertensive claim of the roots of this plant in normotensive and hypertensive rats.

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## METHODS

### Collection of plant material

*N. latifolia* was collected around Ugbowo campus of University of Benin, in March. The plant was initially identified by the Chief technologist of Department of Pharmacognosy, Faculty of Pharmacy, University of Benin and later authenticated at Forest Research Institute of Nigeria, Ibadan where a herbarium specimen No FHI16938 was deposited. The plant material was washed and dried at room temperature first, then put in an oven at 50°C for 48 h before the sample was reduced to coarse powder and then stored in airtight containers.

### Phytochemical tests

The powdered plant material was used in portions for the tests for reducing sugars, glycosides, saponins, alkaloids and flavonoids using standard phytochemical screening methods (Sofowora, 1982; Harborne, 1983; Evans, 1989)

### Extraction

The powdered plant material (500 g) was extracted with 2 l of 70% ethanol for 48 h using Soxhlet extractor. The crude extract was evaporated to dryness using rotary evaporator. The percentage yield was calculated, with reference to the dried powder used.

### Laboratory animals

All the rats used were matured males obtained from the Animal House of the Department of Pharmacology and Toxicology of University of Benin. They were maintained with rat chow (Bendel Feeds and Flour mills Plc) and water *ad libitum*. The rats were housed, two in a cage. The animals were exposed to 12 h light-dark cycle and were handled according to standard protocol.

### Blood pressure measurements

Five male rats (180 - 200 g) were anaesthetised with pentobarbitone sodium (30 mg/kg) intraperitoneally. The trachea was exposed and cannulated to facilitate spontaneous respiration. The jugular vein was cannulated for administration of drugs, while the carotid artery was cannulated and connected via a Bentley Physiological Pressure Transducer to a twin channel Ugo Basil (Gemini 7070) recorder for recording of blood pressure and heart rate. Both cannulas were filled with normal saline but the one connected to the pressure transducer was heparinised (20 U/ml). Calibration was done using mercury sphygmomanometer. The animal's body temperatures were kept constant by a 100 watt bulb from an overhead lamp and were allowed 20 min to stabilize before starting the experiments. The initial systolic (SBP) and diastolic (DBP) blood pressures before addition of any drugs (basal blood pressures) were noted. The crude ethanolic extract was dissolved in 50% dimethyl sulfoxide (DMSO) before administering to the rats. The effects of 50% DMSO alone followed by graded doses (2.5 – 20 mg/kg) of crude ethanolic extract on the basal blood pressures were investigated. The effects of a specific dose of the extract before and after administration of 1 mg/kg each of atropine and promethazine were also investigated. The mean arterial pressure was calculated mathematically from SBP and DBP according to the following formula  
$$\text{MAP} = \text{DBP} + 1/3 (\text{SBP} - \text{DBP}).$$

### Induction of renal hypertension

Rats weighing 200 - 250 g were anaesthetized with 35 mg/kg pentobarbitone sodium intraperitoneally. Nephrectomy was carried out on the right kidney while the left kidney was compressed with a figure-of-eight by a ligature to induce renal hypertension (Grollman, 1944). The rats were left to recover from anaesthesia. Thereafter, the rats were given 0.9% sodium chloride solution instead of ordinary drinking water for 6 weeks. Only those rats that had a systolic blood pressure above 140 mm Hg (under pentobarbital anaesthesia) were considered hypertensive. The effect of the extract on the blood pressure of these rats was studied as described under blood pressure measurement.

### Statistical analysis

All data were expressed as mean  $\pm$  SEM and one way analysis of variance Anova statistical test using Graph pad instant<sup>R</sup> version 2.05 was used to test for significance.  $P < 0.05$  was considered significant

## RESULTS

### Phytochemical screening

The roots of *N. latifolia* showed the presence of sugars, saponins and flavonoids. The phytochemical screening also showed that there were no alkaloids, tannins, or cardiac glycoside. (Table 1)

The yield (w/w) of ethanolic crude extract of *N. latifolia* was 15.8%. This extract of *N. latifolia* reduced the systolic, diastolic and mean arterial pressures in both normotensive and hypertensive rats (Table 2 and 3). At a dose of 2.5 mg/kg this extract reduced the basal mean arterial pressure of normotensive rats from  $115.7 \pm 0.4$  to  $110.0 \pm 2.6$  mm Hg, while in hypertensive rats the change was from  $160 \pm 4.7$  to  $152.6 \pm 3.2$  mm Hg. At 10 mg/kg the mean arterial pressure was further reduced to  $99.6 \pm 3.8$  mm Hg in normotensive rats. While in hypertensive rats this dose reduced the blood pressure to  $72.0 \pm 3.6$  mm Hg. These last changes were both significant at  $p < 0.01$ . The actual reduction in the systolic blood pressure at the dose of 10 mg/kg in normotensive rats was 23.2 mm Hg (18.47%), while those of diastolic and mean arterial pressures were 15.5 mm Hg (14.0%) and 16.1 mm Hg (13.9%) respectively. These values are much lower compared to what was obtained in hypertensive rats. The actual reduction in systolic blood pressure caused by 10 mg/kg of the extract in hypertensive rats was 89.9 mm Hg (49.9%), while those of diastolic and mean arterial pressures were 83.2 mm Hg (56.9%) and 85.4 mm Hg (54.8%) respectively.

The extract (10 mg/kg) also reduced the heart rate of normotensive rats from  $365 \pm 12.8$  to  $280 \pm 6.0$  beats/min (Table 2). The same dose reduced the heart rate of hypertensive rats from  $550 \pm 22.5$  to  $120 \pm 8.0$  beats/min (Table 3). The blood pressure lowering effect of the crude extract was not affected by 1 mg/kg of atropine or promethazine (Figure 1).

**Table 1.** Results of phytochemical tests.

Test	Observation	Inference
<b>Fehling test for reducing Sugars</b>	<b>Brick – Red precipitate</b>	<b>Presence of reducing sugars</b>
FeCl <sub>3</sub> test for tannins	No blue, green/black ppt	Tannins absent
Keller Kilian test for deoxy sugars in Cardiac glycoside	No brown ring at interphase	Cardiac glycosides absent
Frothing test for saponins	Thick persistent froth	Saponins present
Dragendoff's reagent for alkaloids	No orange yellow ppt	Alkaloids absent
Hager's reagent for alkaloid	No yellow ppt	Alkaloids absent
Mayer's reagent for alkaloids	No cream ppt	Alkaloids absent
Wager's reagent for alkaloid	No reddish brown ppt	Alkaloids absent
Lead acetate test for flavonoid	Yellow solution	Flavonoids present

**Table 2.** Effects of crude extract of *Nauclea latifolia* roots on basal pressure (mean ± SEM, mm Hg) of normotensive rats

Dose of extract (mg/kg)	Systolic	Diastolic	Mean arterial	Heart rate Beats/min
Basal pressure	125.6 ± 0.5	110.5 ± 0.3	115.7 ± 0.4	365 ± 12.8
50 % DMSO	125.6 ± 0.5	110.5 ± 0.3	115.7 ± 0.4	365 ± 12.8
2.5 extract	120.3 ± 2.2	105.2 ± 2.8	110.0 ± 2.6	345 ± 10.0
5.0 "	115.6 ± 3.8	100.5 ± 5.1	105.3 ± 4.7	320 ± 8.5
10 "	102.4 ± 4.0	95.0 ± 3.2	99.6 ± 3.8	280 ± 6.0
20 "	95.2 ± 5.2	80.4 ± 5.1	85.0 ± 5.1	200 ± 4.6

n = 5, \* p < 0.01 compared to control.

**Table 3.** Effects of crude extract of *Nauclea latifolia* roots on basal pressure (mean ± SEM, mm Hg) of hypertensive rats

Dose of extract (mg/kg)	Systolic	Diastolic	Mean arterial	Heart rate Beats/min
Basal pressure	180.0 ± 5.7	146.2 ± 3.3	157.4 ± 4.7	550 ± 22.5
50 % DMSO	180.0 ± 5.7	146.2 ± 3.3	157.4 ± 4.7	550 ± 22.5
2.5 extract	174.6 ± 3.3	142.3 ± 2.5	153.0 ± 3.2	450 ± 16.0
5.0 "	160.2 ± 2.8	140.5 ± 1.8	146.5 ± 2.3	300 ± 13.5
10 "	90.1 ± 2.6	63.0 ± 4.9	72.0 ± 3.6	120 ± 8.0

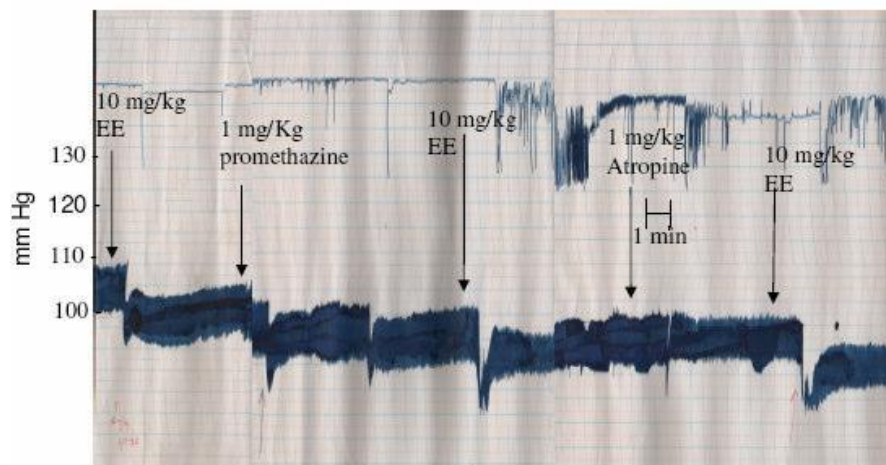
n = 5, \* p < 0.01 compared to control

## DISCUSSION

Extracts obtained from organic solvents extraction of plant materials are a function of the secondary metabolites found in various tissues of the plants. The extent to which they are extracted depends largely on the method of extraction employed, their concentration in the plant tissues and the extraction period. Using continuous hot extraction method in Soxhlet apparatus, *N. latifolia* yielded 15.8%. This was much lower than the 26% that was obtained by Udoh and Lot (1998). This could be due to the fact that in that study, methanol was used while ethanol was used in this study. The main reason for this is to mimic the way the plant was extracted by the traditional medicine practitioners.

Phytochemical analysis of *N. latifolia* showed the pre-

sence of sugars, saponins and flavonoids. This is in agreement with the work of Udoh and Lot, (1998). Saponins and flavonoids have been reported to have hypotensive activities in laboratory animals. For example tea leaf saponins (Peng, 1999) and tenurifolic saponins (Segesaka-Mitaane et al., 1996) have been found to decrease blood pressure. Flavonoids from Hippophae rhamnoides (Zhu et al., 2005) and Astragalus complantus (Xue et al., 2002) also reduce blood pressure. Furthermore, flavonoids have been widely described in the literature as vasodilator compounds (Duarte et al., 1993; Fitzpatrick et al., 1993; Herrera et al., 1996) It is possible that the saponins and flavonoids in *N. latifolia* contributed to its blood pressure lowering effect. Hideyuki et al. (2003) reported the presence of indole alkaloids in the roots of *N. latifolia*, but no alkaloids were found in this



**Figure 1.** Effects of promethazine and atropine on blood pressure lowering action of ethanolic extract (EE) of *N. latifolia*. Neither drugs blocked the effect of the extract.

study. This may probably be due to environmental variation in the areas where the plants were obtained (since the methods used for testing for alkaloids were the same).

*N. latifolia* was effective at lowering blood pressure at a dose of 2.5 – 20 mg/kg in normotensive rats and 2.5 – 10 mg/kg in hypertensive rats. The fact that *N. latifolia* had blood pressure lowering effect seems to justify its use as antihypertensive agent by the traditional medicine practitioner. The extract also decreased the heart rate dose dependently.

Magnitude of response produced by the extract of *N. latifolia* was higher in hypertensive rats than in normotensive rats. This is in support of the general finding that hypertensive animals have exaggerated depressor responses to depressor stimuli (Bunang et al., 1975). The logical reason that was given was that this difference is caused by sympathetic over activity.

Blood pressure can be lowered by stimulation of muscarinic receptors in the heart and the vessels. This would cause decrease in heart rate and also cause vasodilation. The fact that blood pressure lowering effect of *N. latifolia* was not affected by atropine a standard muscarinic antagonist indicates that this effect is probably not mediated through muscarinic stimulation. Alternatively blood pressure can also be lowered as a result of action of histamine released by some drugs. The fact that promethazine which is a histamine antagonist did not affect the extract's blood pressure lowering effect shows that the extract does not produce its effect through histamine. Further work is needed to ascertain the blood pressure lowering mechanism of this plant.

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