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The Effect of Active Fractions of the Roots of *Nauclea Latifolia* Smith (Rubiaceae) on Blood Pressure of Normotensive Rabbits

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Abstract: *Nauclea latifolia* has been found to lower blood pressure of both normotensive and hypertensive rats. The chloroform fractrion of this plant was subjected to column chromatography and five fractions were obtained, CF1- CF5. All the fractions were active in lowering blood pressure of normotensive rabbits. Fraction CF1, CF3 ,CF4 and CF5 were active at a dose range of 2.5 -10 mg/kg. At 10 mg/kg, CF1 reduced the Mean Arterial Pressure (MAP) from 97.6 ± 5.6 to 61.6 ± 8.6 mmHg , CF3 reduced the MAP from 95.3 ±5.1 to 68.3 ±3. mmHg, CF4 reduced the MAP from 84.8 ± 4.7 to 58.6 ± 5.0 mmHg while CF5 reduced the MAP from 78.6 ± 7.5 to 53.0 ± 4.6 mmHg. CF2 was effective at the dose range of 0.2 – 0.6 mg/kg. At 0.6 mg/kg CF2 reduced the MAP from 79.0 ± 3.1 to 48.4 ± 3.7. It was thus concluded that *Nauclea latifolia* can be partitioned into 5 major fractions all of which are active in lowering blood pressure, but CF2 was most potent

Key words: Hypertension, active column fractions, *Nauclear latifolia*, rabbits

INTRODUCTION

Nauclea latifolia is from the family Rubiaceae, the plant is commonly known as Pincushion tree. It is a strangling shrub or small tree native to Tropical Africa and Asia. It has sweet scented flower heads. It produces red fruits that resemble a large, rather hard strawberry, with many seeds which have a pleasant taste but act as emetic if eaten in excess[1]. *Nauclea latifolia* is used profusely by traditional medicine practitioners. The bark and root of *Nauclea latifolia* are used in the treatment of malaria in Ghana[2]. The leaves are also used for the treatment of malaria in East Africa[3] and in Nigeria[4]. The roots are also used to induce abortion and as a purgative[5]. The bark is used in the treatment of wounds, coughs and gonorrhoea in Nigeria[6]. The crude extract of the roots have been shown to have antihypertensive effect[7]. The aim of this work is to fractionate the crude extract and find which of the fraction is active.

MATERIAL AND METHODS

Collection of Plant Material: Roots of *Nauclea latifolia* wERE 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 ºC for 48 hours before the sample was reduced to coarse powder and then stored in airtight containers.

Extraction and Isolation: The powdered plant material (500 g) was extracted with 2 litres of 70 % ethanol for 48 hours using Soxhlet extractor.

The ethanolic extract of *Nauclea latifolia* was evaporated to ¼ of the original volume and was then exhaustively partitioned with aliquots of 250 ml chloroform. Both the ethanol and chloroform soluble fractions were concentrated to dryness using a rotary evaporator. A column was packed with 40 g of silica gel G 60 F_{254} made into slurry with n-hexane. After equilibration with 100 ml of n-hexane, 10 g of chloroform fraction earlier adsorbed on silica gel G 60 was loaded on to the column. This was eluted with hexane with increasing concentrations of chloroform until 100% chloroform was reached. Thereafter increasing concentrations of methanol were introduced until chloroform – methanol 65: 35 was reached to complete elution. A total of 70 fractions were obtained. Each fraction was examined using analytical thin-layer chromatography (TLC) and those fractions with similar spots were pooled together resulting in 5 fractions coded CF1, CF2, CF3, CF4 and CF5.

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Laboratory Animals: Matured male rabbits were 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 rabbits were housed, two in a cage. The animals were exposed to 12 hour light-dark cycle and were handled according to standard protocol.

Blood Pressure Measurements: Five male rabbits (1.5 – 2.2 Kg) were anaesthetised with pentobarbitone sodium (40 mg/kg) intraperitoneally. The trachea was exposed and cannulated to facilitate spontaneous respiration. The ear 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 minutes 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 chloroform fractions were dissolved in 50 % dimethyl sulfoxide (DMSO) before administering to the rats. The effects of 50 % DMSO) followed by graded doses (2.5 – 10 mg/kg) of CF1, CF3, CF4 and CF5 on the basal blood pressures were investigate. Doses of CF2 used were 0.2 – 0.6 mg/kg.

Statistical Analysis: All data were expressed as mean ± SEM (standard error of the mean) and n represents the number of animals used. Data were compared using one way analysis of variance (ANOVA), Graph Pad InstantR version 2.05 software (UK) was used for all data analysis. $P < 0.05$ was regarded as indicating significant difference in all cases.

RESULTS AND DISCUSSION

All the chloroform fractions reduced the blood pressure of normotensive rabbits in a dose dependant manner. Chloroform fraction 1 (CF1) at 10 mg/kg reduced the systolic blood pressure from 113.1 ± 7.1 to 71.0 ± 6.8 mm Hg. It also reduced the diastolic pressure from 91.2 ± 4.9 to 57.0 ± 10 and MAP from 97.6 ± 5.6 to 61.6 ± 8.6 mm Hg. These changes were significant at $p< 0.01$ compared to the basal values (Fig 1). The actual decrease in systolic pressure was of 42.1 mm Hg (37.2 %), was greater than the decrease in diastolic pressure (34.2 mm Hg, 37.5 %) and mean arterial pressure (35.6 mm Hg, 36.4 %).

Chloroform fraction 2 (CF2) was most potent. At 0.6 mg/kg reduced the systolic pressure from 101.8 ± 4.9 to 64.8 ± 6.2 mm Hg. It also reduced the diastolic pressure from 67.8 ± 3.4 to 40.0 ± 3.0 mm Hg and MAP from 79.0 ± 3.1 to 48.4 ± 3.7 mm Hg. These changes were significant at $p< 0.01$ compared to the basal values (Fig. 2). This fraction like fraction 1 caused a greater decrease in actual systolic pressures (37 mm Hg, 36.3 %), compared to diastolic (27.8 mm Hg, 41.0 %) and mean arterial pressures (30.6, 38.7 %)

The effect of chloroform fraction 3 (CF3) at 10 mg/Kg was reduction of the systolic pressure from 107.0 ± 4.6 to 77.6 ± 3.9 mm Hg. Similar effect occurred on the diastolic and MAP pressures, where it reduced them from 91.0 ± 6.5 to 63.4 ± 3.5 and from 95.3 ± 5.1 to 68.3 ± 3.6 mm Hg respectively (Fig. 3). These changes were significant at $p < 0.01$. The difference in the decrease in systolic pressure caused by this fraction (33.8 mm Hg, 31.5 %) is slightly more compared to that caused in diastolic (27.6 mm Hg, 30.0 %) and mean arterial pressures (27.0 mm Hg, 28.3 %).

Chloroform fraction 4 (CF4) at 10 mg/Kg reduced the systolic pressure from 100.0 ± 5.2 to 69.4 ± 5.5 mm Hg. It also reduced the diastolic pressure from 77.6 ± 4.9 to 53.2 ± 5.2 and MAP from 84.8 ± 4.7 to 58.6 ± 5.0 mm Hg (Fig. 4). These changes were significant at $p< 0.01$ compared to the basal values. There is just a slight difference in the actual decrease in systolic (30.6 mm Hg, 30.6 %), diastolic (24.4 mm Hg (31.8 %) and mean arterial pressure (25.5, 30.0 %) caused by this fraction.

Chloroform fraction 5 (CF5) at 10 mg/kg reduced the systolic pressure from 104.5 ±2.6 to 77.2 ± 6.0 mm Hg ($p < 0.01$). It also reduced the diastolic pressure from 64.5 ± 6.6 to 45.5 ± 4.9 mm Hg ($p < 0.05$)and MAP from 78.0 ± 7.5 to 53.0 ± 4.6 mm Hg ($p < 0.05$, Fig. 5). This fraction is the least potent in lowering blood pressures, but it caused a greater decrease in systolic pressure (27.3 mm Hg, 26.1 %), compared to diastolic pressure (19 mm Hg, 29.4 %). There was no difference between the actual decrease in systolic and mean arterial pressures (25.0 mm Hg 32.0 %).

Discussion: Plant extracts are partitioned into organic solvents with the sole aim of narrowing down the active constituents into a particular fraction. This ensures separation of the inactive from active constituents. Chromatographic separation of chloroform fraction of *Nauclea latifolia* gave five fractions CF1 to CF5 all of which were effective in lowering blood pressure. It is unique that all fractions from this plant are active. These fractions from *Nauclea latifolia* do not contain the same constituents according to their R_f values.

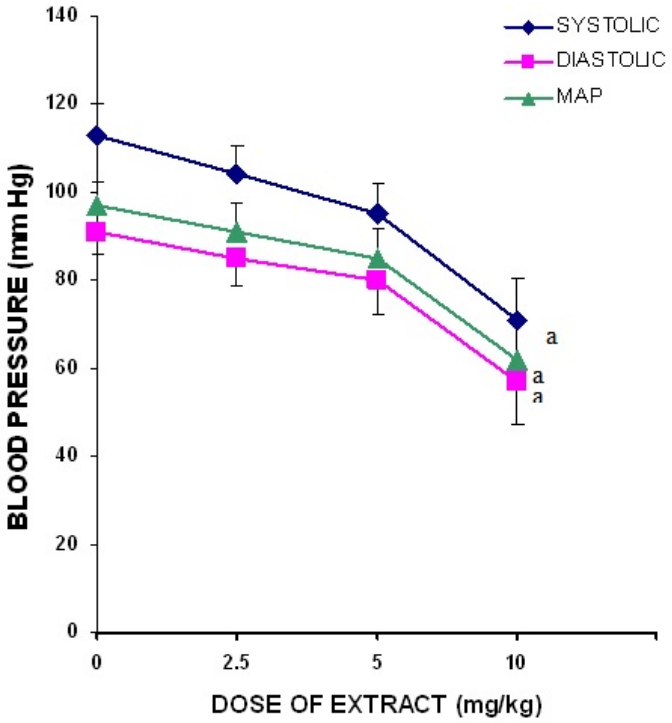

Fig. 1: Effects of chloroform fraction 1 (CF1) of Nauclea latifolia on blood pressure (mean ± SEM mm Hg) of normotensive rabbits. n = 5, ap < 0.01, compared to the respective basal values for the three curves

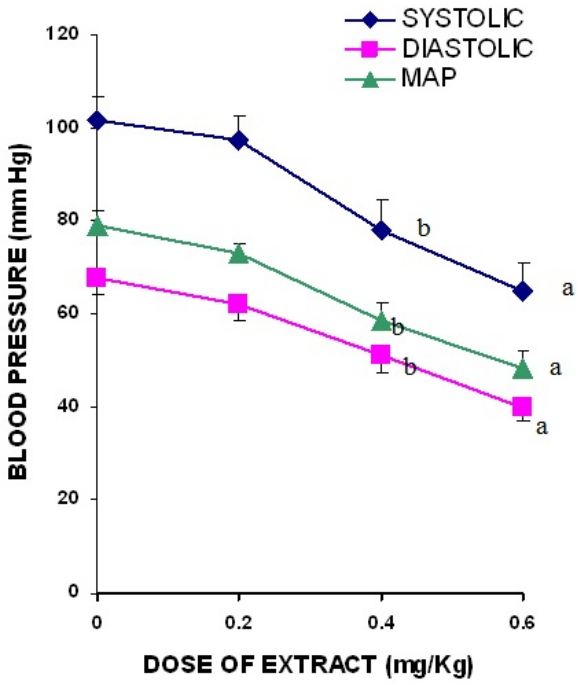

Fig. 2: Effects of chloroform fraction 2 (CF2) of Nauclea latifolia on blood pressure (mean ± SEM mm Hg) of normotensive rabbits. n = 5, ap < 0.01, bp < 0.05 compared to the respective basal values for the three curves

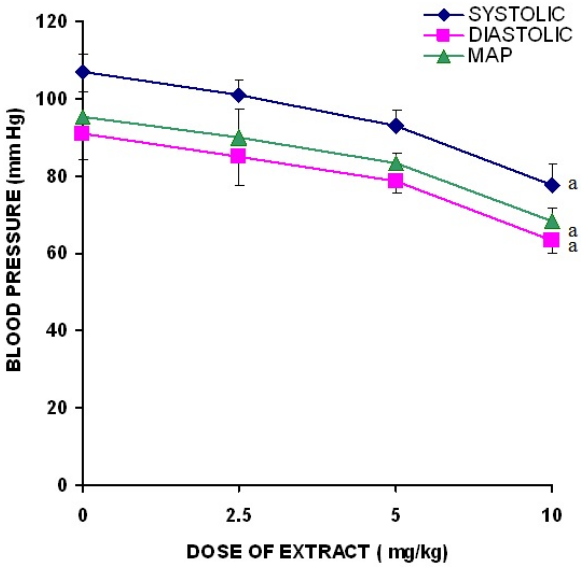

Fig. 3: Effects of chloroform fraction 3 (CF3) of Nauclea latifolia on blood pressure (mean ± SEM mm Hg) of normotensive rabbits. n = 5, ap < 0.01, compared to the respective basal pressure for the three curves

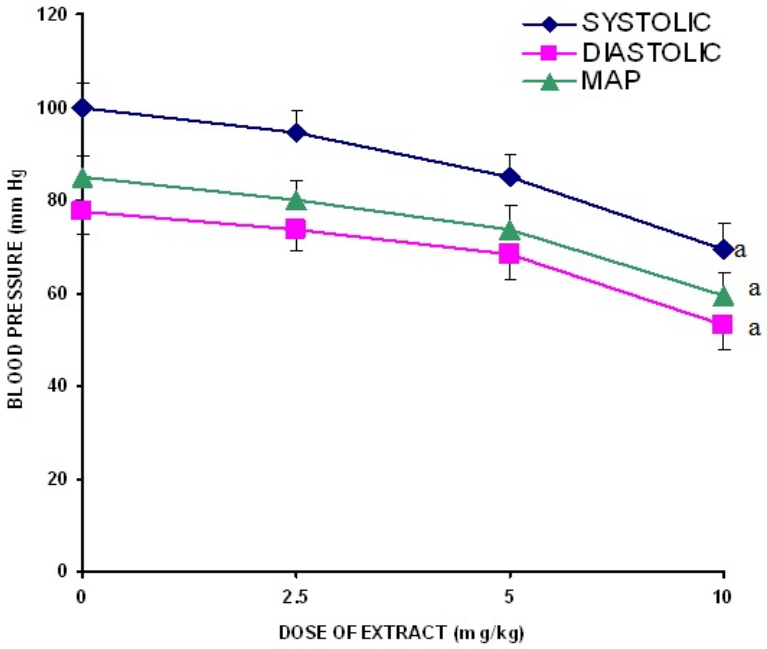

Fig. 4: Effects of chloroform fraction 4 (CF4) of Nauclea latifolia on blood pressure (mean ± SEM mm Hg) of normotensive rabbits. n = 5, ap < 0.01, compared to the respective basal pressure for the three curves

CF2 which was most potent produced its effect at a dose range of 0.2 - 0.6 mg/kg it also produces a much greater decrease in systolic pressure than diastolic pressure. CFI, CF3, CF4 and CF5 produced their effect at a dose range of 2.5 -10mg/kg. Even though these four fractions had the same dose range the extent of blood pressure lowering effect differed. For example at 10 mg/kg CF1 caused a reduction in systolic blood pressure of 42 mmHg compared to 33.8, 30.6 and 27.3 mmHg caused by CF3. CF4 and CF5 respectively. The changes in diastolic pressures followed a similar pattern. CF1 caused a reduction of 34.2 mmHg compared to 27.6, 24.4, and 19.2 caused by CF3 CF4 and CF5 respectively. This shows that in terms of potency CF2 is most potent followed by CFI, CF3, CF4.and CF5.

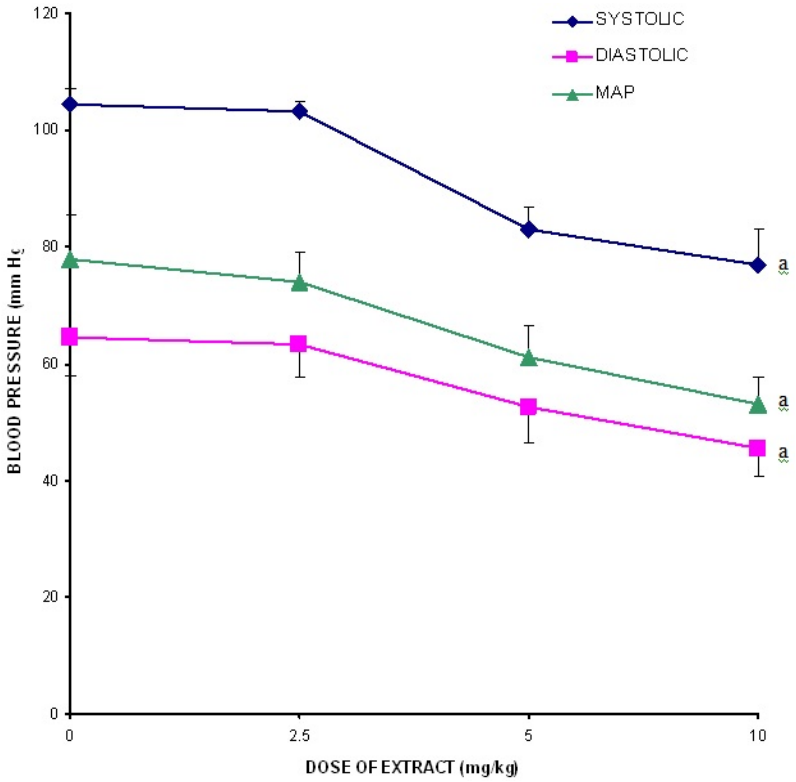

Fig. 5: Effects of chloroform fraction 5 (CF5) of Nauclea latifolia on blood pressure (mean ± SEM mm Hg) of normotensive rabbits. n = 5, ap < 0.01, bp < 0.05 compared to the respective basal pressure for the three curves

All the fractions reduced the systolic blood pressure more than they did the diastolic pressure. Epidemiological evidence has shown that the cardiovascular risk of hypertensive patients relates, probably to a greater extent, to systolic blood pressure values than diastolic blood pressure values (Grassi, 2003). A reduction in systolic blood pressure is accompanied by a clear-cut decrease in cardiovascular morbidity and mortality. This indicates that these fractions will be usefull in reducing cardiovascular risk associated with hypertension as they decrease the systolic pressure.

Conclusion: This study has shown that *Nauclea latifolia* has 5 fractions all of which are active in lowering blood pressure.and that chloroform fractrion 2 was most potent

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