Antihypertensive Effect of Methanol Leaf Extract of Andrographis paniculata in Experimental Cats

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ABSTRACT

The leaves of Andrographis paniculata (Family Acanthaceae) are used locally in Northern Nigerian for the treatment of hypertension. This work was primarily set out to find justification for the local medicinal use of this plant and to scientifically study the pharmacological activity of the plant. Phytochemical analyses of methanolic extract of the plant leaves were carried out using standard methods. The extract was tested for antihypertensive activity in cats by monitoring changes in blood pressure following administration of the extract. The antihypertensive activity was compared with Propranolol (control). Toxicity and histopathological studies were also carried out on the extract. Results of the Phytochemical test showed that the plant extract contained alkaloids, tannins, cardiac glycosides, flavonoids and terpenes. The plant is relatively safe, with an LD₅₀ of 1.90 x 10²⁴ mg/kg body weight which is much higher than those of 0.81mg/kg body weight taken by the local people. Pharmacological studies showed that the methanol extract exhibited greater antihypertensive activity than Propranolol, a standard anti-hypertensive drug. This study suggests that the methanolic leaf extract of Andrographis paniculata contains bioactive constituents that may be beneficial in hypertension and lend pharmacological credence to the ethnomedical claim for the use of the plant in the management of hypertension.

KEYWORDS: Andrographis paniculata; Anti-hypertensive effect; Propranolol; Anaesthezied cats.

INTRODUCTION

Hypertension, defined as a disturbance in hemodynamic function in which there is persistent abnormal elevation of systemic blood pressure, whether it is systolic or diastolic, above the arbitrary level of normal pressure of 140/90 mmHg, is a common disease that cut across all races and the prevalence rate increases with age. [1, 2]. A silent killer among the most vibrant and productive class of the society hypertension has no cure and when discovered the treatment is life-long [3]. Many drugs are available for the control of hypertension but some are not affordable for majority of the people in Africa [4, 5]. Orthodox drugs used in the treatment of hypertension are associated with side effects and drug interactions [6]. Herbal medicines are widely used due to their therapeutic efficacy coupled with least side effects, which initiate the scientific research regarding herbs with the antihypertensive activity. In addition, herbal preparations are most assessable to the majority of the population who cannot afford orthodox drugs [7]. Andrographis paniculata (Fig. 1) (family Acanthaceae), commonly known as “King of Bitters”, is an annual branched erect plant up to 1 m tall that grows abundantly in South Eastern Asia, India and Sri Lanka, Pakistan and Indonesia. It is cultivated extensively in China and Thailand [8], East and West Indies and Mauritius [9]. Due to its ubiquitous nature, AP grows in pine, evergreen and deciduous forest areas and along the roads in villages; it grows in all type of soil, even where almost no other plant can be cultivated. This ruggedness of the plant accounts for its wide distribution. It is therefore not surprising to find it growing in some parts of Northern Nigeria and West Africa. Specifically in Nigeria, it is found in Adamawa and Kaduna states, and the Federal Capital Territory (FCT), Abuja. The plant is known to have proven biological activities and that may be

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the basis of its wide use for various ailments in different parts of the world. *Andrographis paniculata* is a plant with a wide application in traditional medicine especially in Asia and Indonesia. [10]. The plant has been shown to possess anti-clotting, antimicrobial, anti-malarial, anti-filarial, anti-tubercular, anti-inflammatory and immune-stimulant activities as well as Protection on the Liver and Gall Bladder [11-20]. It has been used for centuries in Asia to treat gastrointestinal tract and upper respiratory tract infections, fever, herpes, sore throat and a variety of chronic and infectious diseases [21]. In India it is called ‘Kalmeh’ It is found in the Indian Pharmacopoeia and is prominent in at least 26 Ayurvedic formula. In traditional Chinese medicine (TCM), it is an important “cold remedy” herb [10]. It is used to rid the body of heat as in fevers and also dispel toxins from the body. In the Scandinavian countries, it is commonly used to treat and prevent common colds. It has been found to be an immune-stimulant [22] and used in treatment of cold and colic pains in children [23]. In folkloric medicine, it has been proved to possess antihypertensive properties. This study was undertaken to substantiate its traditional use by the natives in Garkida, Adamawa state of Nigeria. The natives usually take ten to fifteen of the fresh leaves of *Andrographis paniculata* to chew once or twice a day with a glass of cold water and this dose is enough to lower blood pressure continuously. Orthodox drugs used for the treatment of hypertension for example propranolol at various dosage forms e.g. 40mg and 80 mg. The use of traditional medicine as substitute or complementary to modern medicine has been documented by several authors [24]. Herbs such as *Andrographis paniculata* are also employed to treat mild hypertension at dose of 500mg of the crude leaf which is equivalent to 60 - 70 mg per daily dose. Medicinal plants used for the therapy of hypertension, in traditional medicine have been shown to possess promising antihypertensive activities in animal models of antihypertensive screening [10,25-30]. The objective of this study was to evaluate the antihypertensive effect of methanol leaf extract of *Andrographis paniculata* in experimental cats.

**EXPERIMENTAL**

**Materials**

The following reagents were used as procured without further purification: Methanol (BDH Chemicals Ltd., Poole, England), Ferric chloride solution (BDH Chemicals Ltd., Poole, England), Mayers reagent (BDH Chemicals Ltd, Poole, England), Vanillin Sulphuric Acid spray reagent BDH Chemicals Ltd England, Dragendorff’s reagent (BDH Chemicals Ltd, Poole, England), Fehling’s solution A and B (BDH Chemicals Ltd, Poole, England), Potassium carbonate, Sodium Chloride (Dangote Nigeria), Chloroform (Prolab. 12 Rue Pelle, Paris,France), Petroleum ether (Hopkins and Williams, Chadwell Health, Sussex, U.K), Ethanol (BDH Chemicals Ltd., Poole, U.K.), Tween 80 (BDH Chemicals Ltd., Poole, U.K), Lead Acetate (BDH Chemicals Ltd., Poole, U.K) and Distilled water (Sterile laboratory, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria).

**Experimental animals**

The animals were procured from the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria, and were allowed to acclimatize to the new environment for a period of two weeks prior to the study. The mice, maintained on standard rodent feed and water ad libitum, were housed in metallic cages at room temperature throughout the study and were maintained under standard conditions of humidity, room temperature and 12 h light/12h darkness cycle. The animal experiments were conducted in accordance with Ethical Guidelines of Animal Care and Use Committee (Research Ethics Committee) of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

**Preparation of plant material and extracts**

The leaves of *Andrographis paniculata* were collected from Zaria, Kaduna State of Nigeria and properly identified. The voucher specimens were deposited in the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD). The herbarium number is NIPRD 5558. The leaves of *Andrographis paniculata* were shade-dried and pulverized using an electrically operated mill. A 0.5 kg of shade-dried pulverized leaves was extracted with 2 L of methanol using a Soxhlet apparatus, concentrated under vacuum and kept in an airtight container until further use.

**Phytochemical tests of the plant extracts**

Various phytochemical tests were carried out on the extract using standard procedures to determine the presence of saponins, flavonoids, glycosides, steroids, triterpenes, alkaloids, cardenolide aglycone and tannins following standard procedures [31-33]. Each test was qualitatively expressed as
negative (-) or positive (+); the intensity of the characteristic colour was expressed as (++) or (+++).

**Anti-hypertensive effects of the extract on anaesthetized cats**

The anti-hypertensive effect of the extract was carried out on anaesthetized cats. Briefly, a concentrated solution of the extract was prepared by dissolving 100 mg in 10 ml of normal saline to obtain a 10 mg/ml stock concentration. The cats were stabilized in the laboratory for 24 hours before the experiment. In each case, the cat was anaesthetized with pentobarbitone sodium BP (Vet) 60 mg/ml at a dose of 27.5 mg/kg body weight for the general anaesthesia. 1.35 ml of the 60 mg/ml was administered intraperitoneally. Heparin sodium 6.8 mg/ml and 0.2 ml which was equivalent to 1.3 mg/1.8 kg body weight was given to prevent blood clot. The anaesthetized cat was placed on the dissecting board and secured in a supine position to the board using threads. A midline incision was made in the neck and the vagus vein and right carotid artery were separated and short ligatured. An incision was made in the carotid artery large enough to insert a cannula. The cannula was held and secured in the artery by a second ligature. The cannula from the artery was connected to the one end to a microdynamometer for the measurement of the mean arterial blood pressure. Another incision was made in the left femur, the left femoral vein was exposed, cannulated and ligated as was done for the carotid artery. The cannulated femoral vein was the site for the administration of the drug and test samples. Blood clotting was prevented by the injection of heparinised normal saline (N/S) intravenously into the femoral venous cannula. After about 30 min equilibrium, the control blood pressure was recorded after which 2 ml of normal saline was administered and the mean arterial blood pressure recorded.

Thereafter, the following studies were carried out on the anaesthetised cat and the point of administration was the femoral vein.

i. The effect of Acetylcholine (Ach) in the dose of \( 2.0 \times 10^{-4} \) mg/kg and \( 4.0 \times 10^{-4} \) mg/kg were administered respectively and the response on the blood pressure was observed.

ii. Adrenaline of \( 8.0 \times 10^{-3} \) mg/kg was also administered and the response on the cat blood pressure was observed.

iii. The effect of the extract on the cat blood pressure was also observed. Various doses of the extract (0.04, 0.083, 0.330, 0.412, 0.826 and 1.651 mg/kg) were administered and the response on the cat blood pressure recorded.

iv. The antagonistic effect of the extract against Atropine was also observed at dose of \( 4 \times 10^{-3} \) mg/kg, followed immediately with dose of 1.615 mg/kg of the extract and the response on the cat blood pressure observed.

v. The comparism of the extract with standard drug, Propranolol was determined on the cat blood pressure. The same doses of both the extract and Propranolol were administered. The response of the drug on the cat blood pressure was observed.

**Toxicological and histopathological studies**

The safety of the extract to the animals (rats) was evaluated using the probit method [34]. Autopsy was carried out on animal groups where death was recorded. The organs examined were the kidneys, livers and heart. Histopathology was also carried out on these organs and also on the control group. Briefly, small pieces of each tissue in each group were collected in 10 % neutral buffered formalin for proper fixation for 24 h. These tissues were processed and embedded in paraffin wax. Sections of 5-6 μm in thickness were cut and stained with hematoxylin and eosin (H & E). These sections were examined photomicroscopically at a magnification of x400.
Statistical analysis
The experimental results were expressed as the Mean ± SEM for the animals in each group. Difference between means were determined statistically using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (DMCT). P value of < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION
The present study was undertaken to evaluate the anti-hypertensive effect of Andrographis paniculata, a commonly used plant in Nigerian traditional medicine for treatment of hypertension. Ethnopharmacological data has been one of the useful ways for the discovery of biologically active compounds from plants [35]. Ethnopharmacological use of plants could form the basis for phytochemical and phytopharmacological investigation. Preliminary phytochemical tests of the extracts of Andrographis paniculata revealed the presence of phytoconstituents as presented in Table 1. Phytochemistry of the extract showed that it contains high amounts of tannins, flavonoids, alkaloids, saponins, anthraquinones, terpenes and steroids. Phytoconstituents like triterpenoids and steroids are known to possess anti-hypertensive activity [36]. Perhaps the anti-hypertensive effect of the extract could be attributed to the presence of these phytoconstituents present in the plant extract. These are the sources of the most active component of the leaves which contain the andrographolide [37].

![Fig. 2: Effect of the various concentrations of Methanolic extract on cat blood pressure](image1)

![Fig. 3: Effect of the various concentrations of Propranolol on cat blood pressure.](image2)
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Fig. 4: Comparison of the effect of Methanolic extract and Propranolol on cat blood pressure.

Fig. 5: Effect of the various concentrations of Acetylcholine (Ach) on cat blood pressure.

Fig. 6: Effect of the various concentrations of Adrenaline on cat blood pressure.

Fig. 7: Effect of the concentrations Atropine and Methanolic extract of Andrographis paniculata on cat blood pressure.
Toxicological and histopathogical studies

Table 2 and Fig. 8 showed the graph of acute toxicity of the extract given intra-peritoneally from which the LD$_{50}$ (1.9 x 10$^4$ mg/kg body weight) was extrapolated. This implies that doses below this could be safe while doses above it could be detrimental to the human system [38]. The LD$_{50}$ was higher than the dose used by the natives which was approximately 0.8mg/kg body weight of the crude extract. This is in agreement with previous studies [16, 17]. The acute toxicity results indicated that the extract was practically non-toxic acutely [38] and thus provided a guide in the choice of doses for further in vivo studies. This high safety profile might have contributed to the widespread use of Andrographis paniculata in different ethno-therapeutic interventions, particularly as an anti-hypertensive agent. Behavioural signs of toxicity observed in treated rats include increased breathing rate, ataxia and muscular fatigue, reduced activity and paw licking. However, in the negative control group that received normal saline, none of the rats changed their behaviour and none died either.

Histopathological results (Table 3 and Figs. 9-14) indicate that all of extract used were hepatotoxic, nephrotoxic and were also toxic to the heart and lungs only at very high concentration. However these doses were extremely very high as compared with the dose used by the natives to reduce Blood Pressure which was approximately 0.8mg/kg body weight and the LD$_{50}$ was 1.9 x 10$^4$mg/kg body weight. In the control group (Group 9) no significant histological findings were observed in the major organs. These results are consistent with previous reports on Andrographis paniculata [9, 13-15, 17, 18]. Intravenous andrographolide (a constituent of Andrographis paniculata) showed no abnormal cardiovascular response; liver enzyme test and the heart, liver, kidney and spleen were normal [15, 36]. More so, it has a very short half life, 80% of it is removed within eight hours via the kidney and gastro intestinal tract and 90% is eliminated within forty-eight hours [37].

Table 2: Results of determination of the LD$_{50}$ of aqueous extracts of AP in rats using Probit method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Concentration (mg/ml)</th>
<th>Dead</th>
<th>Survival</th>
<th>Dead</th>
<th>Corrected %</th>
<th>Probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.6 x 10$^3$</td>
<td>1.0 x 10$^3$</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>2.08</td>
<td>2.95</td>
</tr>
<tr>
<td>2</td>
<td>1.1 x 10$^4$</td>
<td>2.0 x 10$^3$</td>
<td>2</td>
<td>10</td>
<td>16.6</td>
<td>16.6</td>
<td>4.026</td>
</tr>
<tr>
<td>3</td>
<td>1.4 x 10$^4$</td>
<td>2.5 x 10$^3$</td>
<td>3</td>
<td>9</td>
<td>25</td>
<td>25.0</td>
<td>4.326</td>
</tr>
<tr>
<td>4</td>
<td>1.7 x 10$^4$</td>
<td>3.0 x 10$^3$</td>
<td>5</td>
<td>7</td>
<td>41.6</td>
<td>41.6</td>
<td>4.786</td>
</tr>
<tr>
<td>5</td>
<td>1.9 x 10$^4$</td>
<td>3.5 x 10$^3$</td>
<td>6</td>
<td>6</td>
<td>50.0</td>
<td>50.0</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>2.2 x 10$^4$</td>
<td>4.0 x 10$^3$</td>
<td>6</td>
<td>6</td>
<td>50.0</td>
<td>50.0</td>
<td>5.0</td>
</tr>
<tr>
<td>7</td>
<td>2.5 x 10$^4$</td>
<td>4.5 x 10$^3$</td>
<td>9</td>
<td>3</td>
<td>75.0</td>
<td>75</td>
<td>5.0</td>
</tr>
<tr>
<td>8</td>
<td>2.8 x 10$^4$</td>
<td>5.0 x 10$^3$</td>
<td>12</td>
<td>0</td>
<td>100</td>
<td>97.91</td>
<td>5.674</td>
</tr>
<tr>
<td>9</td>
<td>6.923 x 10$^1$</td>
<td>9.0</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>7.054</td>
</tr>
</tbody>
</table>
**Table 3:** Histopathological studies of the various organs of the rats

<table>
<thead>
<tr>
<th>Group</th>
<th>OBSERVATION ON LIVER</th>
<th>OBSERVATION ON KIDNEY</th>
<th>OBSERVATION ON HEART</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2 &amp; 3</td>
<td>The liver had diffuse areas of necrosis with mononuclear cellular infiltration, haemosiderosis, dilated sinusoids and congested blood vessels.</td>
<td>Necrosis of the glomerulus; proximal and distal convoluted tubules and collecting tubules with mononuclear cellular infiltration; there was purplish pigment in the medulla of the kidney.</td>
<td>Necrosis; fibrosis and mononuclear cellular infiltration; myocarditis.</td>
</tr>
<tr>
<td>4</td>
<td>Areas of necrosis with mononuclear cellular infiltration, dilated sinusoids and congested blood vessels.</td>
<td>Necrosis of glomerulus, proximal and distal convoluted tubules involving the collecting tubules; mononuclear cellular infiltration and proliferation of fibrous connective tissue; fibrosis in medullar and pelvis.</td>
<td>Necrosis in the heart with mononuclear cellular infiltration and myocarditis.</td>
</tr>
<tr>
<td>5 &amp; 6</td>
<td>Had areas of necrosis; mononuclear cellular infiltration, dilated sinusoids and haemosiderosis.</td>
<td>Necrosis of glomerulus, proximal and distal convoluted tubules involving the collecting tubules with mononuclear cellular infiltration.</td>
<td>Had focal areas of necrosis with mononuclear cellular infiltration.</td>
</tr>
<tr>
<td>7 &amp; 8</td>
<td>The liver of the rats had areas of necrosis, dilated sinusoids, mononuclear infiltration and congested blood vessels.</td>
<td>The kidneys had necrosis of the glomerulus and distal convoluted tubules involving the collecting tubules with mononuclear cellular infiltration and congested blood vessels.</td>
<td>The hearts had areas of necrosis and mononuclear cellular infiltration.</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>No significant histopathological findings.</td>
<td>No significant histopathological findings.</td>
</tr>
</tbody>
</table>

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**Fig. 8:** Graph of acute toxicity of methanol extract of *Andrographis paniculata* after intra-peritoneal (IP) administration on rats

- **Probit** vs. **Log Dose**
Fig. 9: Photomicrograph of a section of untreated liver of cat which served as control [Note the normal sinusoids (S) and normal hepatic cords (H). H&E stain x400].

Fig. 10: Photomicrograph of a section of a liver from a cat treated with methanol extract of *Andrographis paniculata* at $2.2 \times 10^4$ mg/kg for two hours. [Note the areas of necrosis of the hepatic cells (arrow heads) the dilated sinusoidal spaces the atrophied (shrunked) hepatic cords (A) and the central vein (CV). H & E stain X 400].
Fig. 11: Photomicrograph of a section of untreated kidney of cat which served as control. [Note: The normal glomerular (G) and normal Renal tubular epithelial cells (RT)].

Fig. 12: Photomicrograph of a section of the kidney of a cat treated with methanol extract of *Andrographis paniculata* at 2.2 x 104 mg/kg from two hours. [Note the glomerular(g) and renal tubular arrow head (necrosis). H & E stain x400].
Fig. 13: Photomicrograph of a section of untreated heart of cat which served as control. [Note: Distinct nuclei of myocardial cells (N). H & E stain x 400].

Fig. 14: Photomicrograph of a section of the heart treated with methanol extract of *Andrographis paniculata* at 2.5 x 10⁴ mg/kg from 10 minutes. [Note the areas of necrosis of the myocardial cells (arrow heads) and the mononuclear cells (M). H & E stain x 400].
CONCLUSIONS
The present investigation has shown that Andrographis paniculata methanolic leaf extract exerted significant dose-dependent reduction of the blood pressure of anaesthetized cats. This justifies the continuous folkloric use of Andrographis paniculata leaf as a remedy for hypertension by the natives in Garkida, Adamawa state of Nigeria. The natives usually take ten to fifteen of the fresh leaves of Andrographis paniculata to chew once or twice a day with a glass of cold water and this dose is enough to lower blood pressure continuously. The antihypertensive effect of the extracts was significantly higher than that of Propranolol, a standard antihypertensive drug. The antihypertensive effect of the plant extract may be attributed to the cardiac glycosides, terpenes and steroids principles present in the plant, possibly andrographolide. However, further identification and elucidation of the structures of the actual constituents responsible for this activity is underway.

REFERENCES


