THE STUDY OF HYPOTENSIVE EFFECTS OF AQUEOUS LEAVE EXTRACT OF VERNONIA AMYGDALINA ON NORMOTENSIVE CATS

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Abstract:
The hypotensive effects of aqueous leave extract of Vernonia amygdalina were evaluated on normotensive cats. The cats used for this experiment were injected with the aqueous leave extract of Vernonia amygdalina at doses of 1mg/ml, 10mg/ml and 100mg/ml through a canula inserted in the femoral artery. Acetylcholine has a relaxing effect on the blood pressure. Atropine, a receptor blocker was then administered along with the standard drug and the leave extract in order to determine the potency of its blocking effect on the leave extract and standard drug. The leave extract of Vernonia amygdalina produced a significant decrease in blood pressure. The 1mg/ml of the leave extract exhibited more effective response; however the standard drug Acetylcholine showed a greater potency than the extract. The blood pressure reducing effects of both drug and extract was inhibited by Atropine. Based on our observations, we therefore conclude that the aqueous leave extract of Vernonia amygdalina produced significant reduction in cat blood pressure and could be use in the management of hypertension.

Keywords: Hypotensive, Blood pressure, Vernonia amygdalina, Normotensive, Hypertension, Effects, Aqueous extract, Cats.

Introduction
Hypertension or high blood pressure, generally referred to as blood vessel high blood pressure, could be a chronic medical condition within which the pressure within the arteries is elevated [1]. This requires the heart to work harder than normal to circulate blood through the blood vessels. Blood pressure is summarized by two measurements; beat and pulsation, that depend upon whether or not the heart muscle is acquiring (systole) or relaxed between beats (diastole) and equate to a most and minimum pressure respectively. Normal blood pressure at rest is at intervals; varying of 100-140mmHg systolic (top reading) and 60-90mmHg diastolic (bottom reading). High blood pressure is said to be present if it is persistently at or above 140/90 mmHg.

It is also a chronic medical condition in which the blood pressure (BP) in the arteries is elevated. It is classified as either primary (essential) or secondary. About 90 to 95% of cases are termed primary HTN,
which refers to high BP for which no medical cause can be found[2] The remaining 5 to 10% of cases, called secondary HTN, are caused by other conditions that affect the kidneys, arteries, heart, or endocrine system [3]. Hypertension is one of the growing important threats of the health of adults in Sub Saharan Africa. Various data that have been published from Nigeria and other countries in Sub Saharan Africa have shown a high (and rising) prevalence of hypertension generally, and a consistently higher prevalence in urban than in rural areas. They have also shown low rates of treatment and control; and when not properly controlled, it could lead to life wasting conditions like stroke, and other cardiovascular complications [4].

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, they have served humans well as valuable components of medicines, seasonings, cosmetics and dyes [5].

Approximately, 100 herbs are reported in the literature to have an effect on blood pressure [6, 7, 8]. Although, few of these herbs were described as having potential to increase blood pressure, the majority of the agents show an ability to decrease blood pressure. Ginseng (Panax Ginseng) is the only commonly used herbs that may produce either a hypotensive or hypertensive effect which are dose-dependent.

Vernonia amygdalina, a member of the Asteraceae family, is a small shrub that grows in the tropical Africa. V. amygdalina typically grows to a height of 2–5 m. The leaves are elliptical and up to 20 cm long. Its bark is rough. Vernonia amygdalina is commonly called bitter leaf in English because of its bitter taste. African common names include grawa (Amharic), ewuro (Yoruba), etidot (Ibibio), onugbu (Igbo), ityuna (Tiv), oriwo (Edo), chusar-doki (Hausa), muluza (Luganda), labwori (Acholi), and olusia (Luo) Ndolé (cameroon) [9,10].

Vernonia amygdalina is well known as a medicinal plant with several uses attributed to it, including for diabetes, fever reduction and recently a non-pharmaceutical solution to persistent fever, headache and joint pain associated with AIDS (an infusion of the plant is taken as needed) [11]. The purpose of this experiment was to evaluate the hypotensive effects of aqueous leaf extract of Vernonia amygdalina on normotensive cats.

**MATERIALS AND METHODS:**

Chemicals and Drugs: The chemical and drugs used in this experiment were of analytical grade Heparin, sodium Thiopentane, Atropine, Acetylcholine, syringes were obtained from Zaria.

**Plant materials:** The leaves of Vernonia amygdalina were collected within Ahmadu Bello University, Samaru, Zaria and were identified by Taxonomist in the Department of Biological Sciences and authenticated voucher samples Voucher no 9176; and later deposited in the research laboratory of National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria.

**Extract preparation:** The Vernonia amygdalina leaves were washed thoroughly, shade dried and grounded into powder. The aqueous extraction was done with the aid of Soxhlet extractor in the research laboratory of National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria.

**Experiment Design:** The cats used were anaesthetised by injecting thiopentane sodium through intraperitoneal route and soon lost consciousness within 10 to 25 minutes after administration. They were properly brought to the dissection table and their limbs were tied. Intravenous injection was made into the left femoral vein and blood pressure was recorded from the left femoral artery. The left femoral vein was exposed and tied with peripheral ligature; and also the right carotid artery was exposed and cannulated for blood pressure measurement. Heparin was injected through before cannulating the right carotid artery in order to act as anti-coagulant, thereby preventing blood clot. The basal blood pressure was recorded on a filter paper of the micro-dynamometer after the administration of the heparinized normal saline to prevent blood clotting. The right Carotid artery was also exposed and cannulated for the blood pressure measurement. Normal saline, Atropine, Acetylcholine (Ach) and the extract was administered through the canula inserted through the left femoral vein. The speed and sensitivity of the machine were 95 mm/minute and 1 respectively. The dosages of Vernonia amygdalina extract administered for each cat was at 1 ml/mg, 10 ml/mg and 100 ml/mg. Flushing was properly done after every administration of extract and drugs (Ach and Atropine) until it was brought back to normal. Mean Arterial Blood pressure (MAP) was calculated as = [(2 x diastolic) + systolic]/3.

**Statistical Analysis:** All data were expressed as Mean ±S.E.M. The data obtained were analyzed using one way analysis of variance (ANOVA) and Turkey-Kramer posthoc test for multiple comparisons. The P-Value less than 0.05 (P<0.05) was to be accepted as statistically significant [12].
RESULTS AND DISCUSSION:

Table 1: Comparison between Normal Basal Rhythm, Acetylcholine and Aqueous leave extract of *Vernonia amygdalina*.

<table>
<thead>
<tr>
<th></th>
<th>Normal Basal rhythm (Mean±SEM)</th>
<th>Acetyl choline (Mean±SEM)</th>
<th>Aqueous extract of <em>Vernonia amygdalina</em> (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1mg/ml</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>64.5 ± 3.5</td>
<td>40.0±0.88*</td>
<td>32.6±0.45*</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>38.0±3.5</td>
<td>22.5±0.35*</td>
<td>20.0±1.85*</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>26.5±3.5</td>
<td>17.5 ±0.35*</td>
<td>12.6±0.63*</td>
</tr>
<tr>
<td>Mean Arterial Blood pressure (MAP)</td>
<td>46.8±2.3</td>
<td>28.3±0.35*</td>
<td>24.2±1.33*</td>
</tr>
</tbody>
</table>

* means Significant at $P \leq 0.05$; **NS** means Not Significant at $P \leq 0.05$ ;

Mean Arterial Blood Pressure (MAP) = $[(2 \times \text{diastolic}) + \text{systolic}] / 3$.

OR MAP = diastolic pressure + $1/3$ pulse pressure

Table 2: Drug Antagonistic Studies

<table>
<thead>
<tr>
<th>Drug + aqueous extract (0.1ml Atropine + 0.05 ml Ach (After 3 min interval) Mean±SEM)</th>
<th>0.1 ml Atropine + 0.8 ml extract [1mg/ml] (After 3 min interval)(Mean±SEM)</th>
<th>0.1 ml Atropine + 0.8 ml extract [1mg/ml] (No time interval) Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>34.50±0.35</td>
<td>32.5±0.35</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>23.0±0.71</td>
<td>28.5±3.19</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>11.5±0.35</td>
<td>4.0±0.35</td>
</tr>
<tr>
<td>Mean Arterial Blood(MAP) pressure</td>
<td>26.83±0</td>
<td>29.83±0.25</td>
</tr>
</tbody>
</table>

Mean Arterial Blood Pressure (MAP) = $[(2 \times \text{diastolic}) + \text{systolic}] / 3$ ; OR MAP = diastolic pressure + $1/3$ pulse pressure
NB = Normal basal rhythm;

Fig. 1: Tracing of Normal Saline

P = Point of administration
NB = Normal basal rhythm

Fig. 2: Tracing of 1mg/ml of aqueous leave extract of vernonia amygdalina with volumes of 0.8mls

P=Point of administration; NB=Normal basal rhythm;

Fig. 3: Tracing of 10mg/ml of aqueous leave extract of vernonia amygdalina with volumes of 0.8mls.

P = Point of administration; NB= Normal basal rhythm

Fig. 4: Tracing of 100mg/ml of aqueous extract of Vernonia amygdalina with volume of 0.8mls

Blood pressure values: The blood pressure values of the cat for normal saline, the injected standard drugs and aqueous leave extract of Vernonia amygdalina as obtained from the micro-dynamometer are shown in the Tables 1-2 and Figures. 1-6. One centimeter (1cm) of the standard ruler used in measuring the systolic and diastolic blood pressure change corresponds to 10mmHg pressure change in glass sphygmomanometer. On the tracing the values from the baseline to the lowest border of the tracing represent the diastolic pressure while from the baseline to upper border represent the systolic pressure. The results of blood pressure values were analyzed by comparing the mean ± SEM of normal basal rhythm, acetylcholine and aqueous leave extract of Vernonia amygdalina using one way analysis of variance as shown in the tables 1&2.
The blood pressure systolic for normal basal rhythm was 64.5 ± 3.5, for Acetylcholine was 40.0±0.88; and for aqueous leaf extract of *Vernonia amygdalina* at doses of 1mg/kg, 10mg/kg and 100mg/kg was 32.4±0.45, 43.1±0.45 and 48.4±3.11 respectively. There were statistically significant differences in the observed decrease in systolic when compared with the control except for the extract at the dose of 10mg/kg that had a decrease value that was not statistically significant (Table1).

The diastolic blood pressure for the normal basal rhythm was 38.0±3.5, that of Acetylcholine was 22.5±0.35; and for aqueous leaf extract of *Vernonia amygdalina* at doses of 1mg/kg, 10mg/kg and 100mg/kg were 20.2±1.85, 30.9±1.1 and 24.25±3.41 respectively. There were statistically significant differences in the decrease observed in the diastolic blood pressure when compared with normal basal rhythm at P ≤ 0.05 except for the extract at the dose of 10mg/kg that had a decrease value that was not statistically significant at P≤ 0.05 (table1).

The pulse pressure for normal basal rhythm was 26.5±3.5, that of Acetylcholine was 17.5±0.35; and for aqueous leaf extract of *Vernonia amygdalina* at doses of 1mg/kg, 10mg/kg and 100mg/kg were 12.6±0.63, 12.2±1.76 and 24.2±5.13 respectively. The decrease observed in the pulse pressure was statistically significant at P≤ 0.05 except for the extract at the dose of 100mg/kg that was not statistically significant at P≤ 0.05(Table 1).

The mean arterial blood pressure for normal basal rhythm was 46.8±2.3, that of Acetylcholine was 28.3±0.35; and for aqueous leaf extract of *Vernonia amygdalina* at doses of 1mg/kg, 10mg/kg and 100mg/kg were 24.2±1.33, 35.0±2.28 and 32.3±3.0 respectively. The decrease in the mean arterial blood pressure observed for acetylcholine and the extract at doses 1mg/kg, 10mg/kg and 100mg/kg were statistically significant at P≤ 0.05 when compared with the normal basal rhythm (Table 1).

In the drug antagonist studies in Table 2, Atropine showed the blood pressure lowering effects (hypotensive) of *Vernonia amygdalina*.

Furthermore, the majority of the populations in most of these countries especially those in the rural areas do not have access to conventional medical facilities, so they rely mainly on traditional medicine for their health care needs. The most widely used antihypertensive drugs have been criticized, particularly in diabetics, because of their various adverse effects [14]. Reports showed that *Vernonia amygdalina* had hypoglycaemic effects in human and animal experimental subjects [15,16,17].

Blood pressure depends on several factors which include heart rate, stroke volume, and peripheral resistance. Agents that lower blood pressure do so via one or a combination of several blood pressure regulatory mechanisms. The results of this study revealed that aqueous leaf extracts of *Vernonia amygdalina* produced a dose-dependent decrease in arterial blood pressure (had significant blood pressure lowering effects) of normotensive cats (Tables 1, Figures 2-4).

The blood pressure (systolic and diastolic), pulse pressure and mean arterial blood pressure in all three doses (1, 10 and 100mg/ml) of the aqueous leaves extract of *Vernonia amygdalina* decreased significantly at P< 0.05 when compared with the normal basal rhythm with the exceptions of the diastolic and systolic pressures of 10mg/ml of the extract and the pulse pressure of 100mg/ml of the leave extract that was not statistically significant at P > 0.05 when compared with the normal basal rhythm (Table 1). The tracings in Figures 2-4 showed the blood pressure lowering effects (hypotensive) of *Vernonia amygdalina*.

The standard drug (Acetylcholine) also compared with the normal basal rhythm decreased significantly in the blood pressure (systolic and diastolic), pulse pressure and mean arterial blood pressure as shown in Table 1 and Figures 1&5 above.

In the drug antagonistic studies in Table 2 above, Atropine blocked the effects of the leaf extract, as well as, that of acetylcholine but with very little difference. This indicates that atropine has blocking effects on both the drug and the leaf extract as seen in Figures 6&7.

The standard drug, Acetylcholine, when compared with the normal basal rhythm, the blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure decreased statistically significant at P<0.05(see Table 1, Figures 1&5). The lowering blood pressure effects observed via the extract was completely blocked by Atropine (Fig. 6). Therefore, the hypotensive action of *Vernonia amygdalina* may be mediated through cholinooceptors as cholinergic antagonist, because Atropine blocked the effects of leave extract (Fig.6).

From this study, it can be inferred that, *Vernonia*
Amygdalina leave extract has an appreciable blood pressure lowering effects (hypotensive) on normotensive cats.

CONCLUSION:

Based on our observations, we therefore conclude that the aqueous leave extract of Vernonia amygdalina produced significant reduction in cat blood pressure (hypotensive) and could be a useful tool in the management of hypertension.

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REFERENCES: