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# ANTIHYPERTENSIVE AND ELECTROLYTE MODULATORY POTENTIAL OF VERNONIOSIDE E (STEROIDAL SAPONIN) FROM *VERNONIA AMYGDALINA* (*ASTERECEA*) IN ADULT ALBINO WISTAR RATS

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## ABSTRACT

Western lifestyle, urban diets and physical inactivity among urban dwellers in Nigeria have caused increase in incidences of hypertension among people of 40 years and above. Drugs for its treatment are expensive, while ethnopharmacology seem to provide alternative and cheaper options. Aqueous leaf extracts of *Vernonia amygdalina* (Bitter leaf) has been successfully used for centuries in Nigeria to treat hypertension, other cardiovascular diseases, stomach pains, fever and malaria. The present study evaluates the antihypertensive potential of Vernonioside E (VE) and crude extract (CE) of *Vernonia amygdalina* leaf, *and the efficacy* of VE and CE in reversing serum electrolyte concentrations, lipid/cholesterol, and enzyme activities implicated in the aetiology and manifestation of hypertension using Albino Wistar rats previously induced with 40mg/Kg bw hypertensive agent (*N*ω-Nitro-L-arginine methyl ester hydrochloride, L-NAME) as study model. The $LD_{50}$ of VE was also determined, while $LD_{50}$ of CE has been previously determined and known. The $LD_{50}$ of VE was determined to be 0.70 mg/kg. Vernonioside E (105.25± 2.29 mEq/L), the crude extract (108.55± 2.41 mEq/L) and Captopril (112.99± 2.30 mEq/L) produced significant ($p<0.05$) decreases in serum sodium concentration, when compared to the hypertensive control (165.72±15.05 mEq/L). The chloride levels in treated groups were slightly decreased while the potassium level was slightly increased compared to control groups. There was significant ($p<0.05$) decrease in total cholesterol levels in treatment groups, VE (56.14±2.15mg/kg), CE (57.40±2.20mg/kg) and Captopril (61.44±0.25mg/kg) compared to hypertensive control (77.63±2.97mg/kg). There were significant ($P<0.05$) decreases in triacylglycerol, low density lipoprotein (LDL), and very low density lipoprotein (VLDL) concentrations of treated groups when compared to hypertensive control. Also, there was significant ($p<0.05$) elevation of high density lipoprotein (HDL) concentration in treated and normal control groups when compared to hypertensive control. There was a significant ($p<0.05$) elevation of two key serum enzymes (AST and ALT) concentraions. It was concluded that, the biochemical changes observed suggests that Vernonioside E (VE) and crude extract (CE) of *Vernonia amygdalina* leaf are potential antihypertensive agents, and this confirms the ethnopharmacological claims of the use of aqueous extracts of the leaves of this plant in the treatment of high blood pressure and associated cardiovascular diseases.

**KEYWORDS:** Vernonioside E, Lethal dose ($LD_{50}$), *Vernonia amygdalina*, antihypertensive, Serum electrolytes, Serum enzymes, total cholesterol, serum lipids, Captopril.

## 1. INTRODUCTION

Hypertension can be defined as sustained systolic blood pressure of more than 140mmHg and/or diastolic blood pressure more than 90mmHg. It is a major risk factor for coronary heart disease and stroke. In most cases the cause is unknown and it is called essential hypertension. There are many causes of hypertension, including obesity, use of steroid drugs, insulin resistance and high alcohol intake. Rarer secondary causes include renal disease such as polycystic disease, scleroderma, pyelonephritis and renal artery stenosis. Endocrine causes include phaeochromocytoma, Cushing's

syndrome, acromegaly and hyperparathyroidism, aortic coarctation and, pre-eclampsia in pregnancy cases.

Hypertension is one of the most dangerous health problems facing mankind today. Key clinical indicators for its diagnosis include elevated arterial blood pressure and some biochemical parameters such as electrolyte sodium, potassium and chloride concentrations. Hypertension is a complex condition whose etiology is yet to be fully understood. It attacks its patients without any warning signs or symptoms, thus making it difficult to predict and manage at early onset. It has been reported that nearly one billion people or approximately 26% of the adult population of the world had hypertension as at the year 2000 and the figures have been increasing.[1,2] The world Health Organization, reported that 7.1 million deaths per year may be attributable to hypertension.[3] The disease is common to both developed and developing countries, and afflicts the affluent and people with less active lifestyles.[1] Its affliction vary widely in different regions of the world, with rates as low as 3.4% (men) and 6.8% (women) in rural India, and as high as 68.9% (men) and 72.5% (women) in Poland.[2] This variation has been attributed to differences in diet, lifestyle and genetic composition. Currently, hypertension is said to be an intermittent or sustained elevation in systolic blood pressure (> 140 mmHg) or diastolic blood pressure (> 80 mmHg) or a systolic and diastolic pressure 20 mm Hg above the individual's baseline pressure.[4,5,6] High blood pressure is also said to be present in a subject, if it is persistently at or above 140/90 mmHg. This requires the heart to work harder than normal to pump and circulate blood through the blood vessels.[4]

Hypertension can be described into two types; Primary and secondary. Primary hypertension is a type of high blood pressure with no obvious underlying medical cause while secondary hypertension is caused by other conditions affecting the kidneys, arteries, heart or endocrine system.[7] Although mild to moderate hypertension is usually asymptomatic, accelerated hypertension is associated with headache, somnolence, confusion, visual disturbances, nausea and vomiting.[7] Although there is a large information and understanding of the patho-physiology of high blood pressure in 90% to 95% of cases, the etiology and thus its prevention or cure is still largely unknown. Consequently, in most cases, the hypertension is treated non-specifically resulting in a large number of minor side effects and a relatively high non-compliance rate.[8]

The etiology of hypertension has been directly linked to those with a body mass index greater than 25, and to those with salt (sodium) sensitivity, alcohol intake, and vitamin D deficiency. It is also related to aging and some inherited genetic disorders.[9,10] It has been suggested that Renin elevation and sympathetic over-activity are other risk factors implicated in hypertension.[11] Renin is an enzyme secreted by the juxtaglomerular apparatus of the kidney and linked with aldosterone secretion in a negative feedback loop.[12] Insulin resistance which is a component of the metabolic syndrome is also thought to cause hypertension. According to,[13] low birth weight has recently been questioned as a risk factor for adult essential hypertension.

Hypertension is a major risk factor for stroke, myocardial infarction (heart attacks), heart failure, aneurysms of the arteries (e.g. aortic aneurysm) and peripheral arterial disease. These conditions are major clinical presentations in hypertension and predispose chronic kidney diseases,[11] which in turn predispose male erectile dysfunction and perturbations in both male and female hormonal balance,[4,14] and had reported that moderate elevation of arterial blood pressure has been associated with a shortened life expectancy in both male and female subjects.

Several classes of medications, collectively referred to as antihypertensive drugs, are currently available for treating hypertension. Majority of patients require more than one drug to control their hypertension condition, while others may require multiple prescriptions. Joint National Committee on High Blood Pressure, advocates starting treatment with two drugs when blood pressure is >20 mmHg above systolic or >10 mmHg above diastolic targets. Preferred combinations are renin–angiotensin system inhibitors and calcium channel blockers, or renin–angiotensin system inhibitors and diuretics.[15,16] Acceptable combinations include calcium channel blockers and diuretics, beta-blockers and diuretics, dihydropyridine calcium channel blockers and beta-blockers, or dihydropyridine calcium channel blockers with either verapamil or diltiazem.

The use of orthodox drugs in the treatment of high blood pressure and hypertension is expensive and virtually unaffordable to the poor and peasant populations in many countries of sub-sahara Africa. Many plants including *Vernonia amygdalina*, *Vernonia calvaona*, *Vernonia teniana* and *Gongronema latifolia* used as vegetables or medicinal herbs in Nigeria and elsewhere provide options for the treatment of a myriad of diseases (communicable and non-communicable).

Traditional medicine practitioners use *Vernonia amygdalina* as an anti-helminth and anti-malarial, antioxidative properties, laxative, digestive tonic, appetizer, febrifuge, and for the topical treatment of wounds.

The plant extract has severally been reported to elicit hypocholesterolemia,[17,18] hypoglycaemia,[19.20,21] and anti-malarial.[22,23]

It was reported that sick Chimpanzees ate and swallowed the bitter juice from the tissues of the plant to get well. Our observation on disturbed Chimpanzees living around limestone quarry sites at Akamkpa near Calabar (Cross

River State, Nigeria), suggests that *V.amygdalina* may as well have been used by these primates to calm nerves, and as sedatives and anti-hypertensives. It appeared that the primates depended totally on *V.amygdalina* for the treatment of all diseases afflicting their kind.[22,23]

In their review article,[24] summarised some of the scientific research in the last few decades and they scrutinized several claims on *Vernonia amygdalina*, and found that extracts from the plant have numerous phytotherapeutic properties including, antimicrobial (antibacterial, antifungal, antiplasmodial), anti-cancer/tumor, antioxidant,[25] hypoglycemic/anti-diabetic, oxytocic, hepato-protective and nephro-protective effects, serum lipid modulation, and other properties.[19] These properties are believed to be mediated by different phytochemicals found in the plant, acting singly or in concert.[24]

Several compounds from this plant have been isolated and characterized, including luteolin flavonoids and its glycosides,[25] Vernoniosides D and E steroidal saponins,[26] Vernonioside C steroidal saponin,[27] Vernonioside A1, A2, A3 steroidal saponins,[29] Vernolepin and sesquiterpene Lactones.[29,30]

Aqueous leaf extracts of *Vernonia amygdalina* (Bitter leaf) has been successfully used for centuries in Nigeria to treat hypertension, other cardiovascular diseases, stomach pains, fever and malaria. Studies carried out in the past centered on the biochemical activities of the leaf extract, but none on the anti-hypertensive activities of its crude extract or pure compounds.

The present study was aimed at evaluating the antihypertensive activities of Vernoniosides E, including its role in the modulation of some endocrine functions.

## MATERIALS AND METHODS

### Chemicals and Drugs

Biochemical assay kits were purchased from DIALAB Production and Vertrieb Von Chemischtechnischen Produkten und Laborinstumenten Gesellschaff M. B. H, A-1160 Wien-Panikengasse. *N*ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), was purchased from Cayman Chemical Company Inc, 1180 East Ellsworth Road, Ann Arbor, ML 48108, USA. Captopril, was purchased from a Pharmacy shop in Pulawy, Poland. Chloroform, Dimethyl sulphoxide (DMSO), Normal saline and distilled water were obtained from the Chemical store of the Departmental of Biochemistry, Pulawy, Poland. Rat chow was obtained from the Agronomy Department of the Institute.

### Collection and Preparation of Plant Material

*Vernonia amygdalina* leaves were harvested from a garden in Assiga, Yakurr LGA, Cross River State, Nigeria. The plant was authenticated by a taxonomist in the Department of Botany, University of Ibadan and a voucher specimen (No. BCH 10013), was deposited in the Herbarium of the Department of Biochemistry, University of Ibadan, Nigeria.

1000g of the leaves was washed thoroughly with tap water and rinsed with distilled water, and then dried at room temperature. It was blended into fine powder using a Q-link electric blender (Model QBL-18L40). Five hundred grams (500g) of the blended dry leaves was soaked in 2000ml of ethyl alcohol (80% BDH) and agitated on an electro-thermal heater at 50$^{o}$C, and allowed to stand at room temperature for 48 hours. The mixture was first filtered with cheese cloth, then with Whatman No 4 filter paper. The filtrate was concentrated *in vacuo* using a Rotary Evaporator (Model RE52A, China) to 10% of its original volume at 33$^{o}$C. This was concentrated to complete dryness in a thermostatic water bath. The extract obtained was stored under refrigeration until required.

### Extraction, purification and identification of Vernonioside E

Vernonioside E was isolated from 100g of the crude extract using the method earlier described by.[26] 100g of the dried extract was reconstituted in 30% MeOH and subjected to fractionation using column chromatography equipped with a Zalimp peristaltic pump (Poland). The fraction later identified and named as Vernonioside E was purified using a combination of repeated normal and reverse phase HPLC, and monitored by thin layer chromatography. Solvents were supplied isocratically using a linear gradient former. The pure fraction of Vernonioside E was dried to amorphous powder using a freeze dryer to afford 2.4g of Vernonioside E, which was identified by a combination of $R_f$ values from thin layer chromatography, UV/VIS spectrophotometric data, FAB-MS (Figure 1), EI-MS (Figure 2), and data from $^{1}$H-NMR and $^{13}$C-NMR (Figure 3 and table 1).

### Identification of Vernoniside E

Vernonioside E structure was confirmed using a combination of $R_f$ values obtained from Tlc against a standard sample;[26] UV/VIS spectrophotometric data, FABMS, EIMS and $^{1}$H-NMR and $^{13}$C-NMR.[26] The only hexose sugar (glucose) attached to the C-3 position was identified by Tlc and FABMS as the only sugar attached to the aglycone which was identified as β-D-glucopyranoside by $^{1}$H-NMR and $^{13}$C-NMR.[26] NMR data led to the identification of the aglycone moiety as a C29 stigmastane-type sterol, and its glycoside was named Vernonioside E.[26] The negative LSIMS spectrum revealed a molecular ion at m/z 677, together with ions at m/z 633 and 515 corresponding to the loss of an acetyl function and a glucose molecule from the parent ion, respectively to confirm the stigmastane-type aglycone.[26] Vernonioside E gave characteristic UV/VIS spectra absorption maxima peaks at 234, 241 and 251nm, indicating the presence of a chromophoric compound $\Delta^{7,9(11)}$ diene function in the aglycone (7, 12). This chromophore has been shown to be a characteristic

structural feature of stigmastane-type steroidal saponins in *Vernonia amygdalina*; and together with their peculiar side-chain, may confer some biogenetic, chemotaxonomic and pharmacological significance on these compounds.[26]

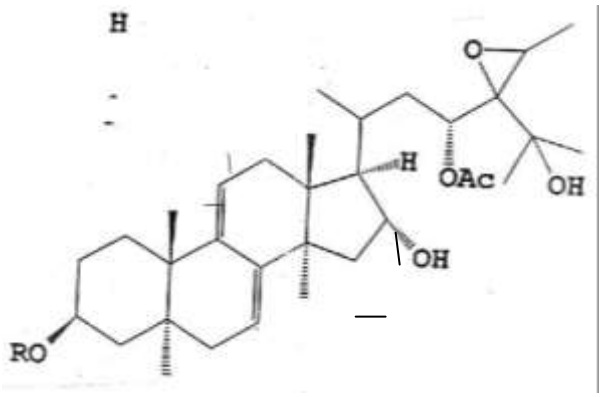


**Figure 1: Chemical Structure of Vernonioside E (also designated Vernonioside E1).**

Thus the molecular formula deduced also by $^{13}C$- and DEPT $^{13}C$-NMR data was $C_{37}H_{58}O_{11}$. The EIMS spectrum of the aglycone showed a molecular ion at m/z 472 and some significant additional peaks corresponding to ions (m/z 268 and 311), indicating a steroid system consistent with a stigmastane sterol. The ion at m/z 472 was interpreted as being a quasi-molecular peak resulting from the loss of an acetyl function from the aglycone molecule. The presence of an acetyl group was confirmed by signals at 21.51 and 172.83 ppm in the $^{13}C$-NMR spectrum (Figure 3). The NMR spectrum also demonstrated (Table 1) that the compound possesses the same carbon skeletal structure of the sterol framework from C-1 to C-19 characteristic of stigmastane aglycone.

**Determination of $LD_{50}$ and acute toxicity test**

The $LD_{50}$ of Vernonioside E (VE) was determined using albino mice, according to the method previously described by.[31] Twenty four (25) albino mice of both sexes, weighing 22-25g,were randomly selected and assigned to 5 metabolic cages of 5 animals per cage (n=4). They were allowed to acclimatize under 12h dark/12h light cycle for one week, while food and water was given *ad lib*. Groups 1, 2, 3 and 4 animals were given 0.20, 0.35, 0.50 and 0.70 mg/kg b.w doses of VE respectively, via intraperitoneal (IP) route of administration from a stock solution of 50mg/ml. The last group (5) which served as the control was given 0.014ml of normal saline IP.

After treatments, the animals were allowed free access to food and drinking water in their respective cages. The animals were observed for any signs of toxicity and mortality for the first 4hrs and till the end of 24hrs and finally for 3 consecutive days adding up to 72hrs after administration of VE. Signs of toxicity to be observed include respiratory distress, diarrhea, paw-licking, body stretching and death. The $LD_{50}$ for VE was determined to be 0.7mg/kg.

**Laboratory Animals**

Albino Wistar rats of both sexes, weighing 180-200g were purchased from the animal house of the Department of Biochemistry, University of Ibadan, and allowed to acclimatize for 1 week. The animals were housed in metabolic cages at 12 hour light/dark cycle and maintained with rat chow (Vital Feeds Ltd, Ibadan) and water was given *ad libitum*.

**Experimental Design and treatment**

At the end of 1 week, the animals were shared into 5 groups of 6 animals each. Group 1 received 50% DMSO and served as normal control (NC) and was not made hypertensive. Groups 2, 3, 4 and 5 were made hypertensive by giving each animal a dose of 40 mg/kg bw *N*ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) for 14 days. Groups 1 and 2 were not treated with anti-hypertensive agents. Group 3 was treated with 20mg/kg bw Vernonioside E, group 4 was treated with 300 mg/kg b.w crude extract, while group 5 was treated with 20mg/kg b.w Captopril (Standard drug). Groups 2, 3, 4 and 5, simultaneously received another 40mg/Kg b.w of the hypertensive agent (*N*ω-Nitro-L-arginine methyl ester hydrochloride, L-NAME) for another two weeks, while the normal control group 1, received 50% Dimethylsulphoxide (DMSO), for the same period (14 days).

**Table 1: Experimental animal groupings and treatments.**

| Group | Treatment | No of animals | Administration of drug per day |
|---|---|---|---|
| 1 | NC | 6 | 50% DMSO |
| 2 | DC | 6 | 50% DMSO |
| 3 | VE | 6 | 20mg/kg bw |
| 4 | CE | 6 | 300mg/kg bw |
| 5 | Captopril | 6 | 20mg/kg bw |

NC = normal control, DC = hypertensive control, VE = Vernonioside E, CE = Crude Extract of *Vernonia amygdalina* leaf, Captopril = Standard drug

**Determination of biochemical parameters**

At the end of the treatment period of 28 days, the animals were anaesthetized using chloroform vapour, sacrificed by cervical dislocation and blood samples collected via cardiac puncture into a plane tubes. The blood was allowed a clotting period of 2h and then centrifuged at 3000rpm for 10 min, using a Model 0412-1 centrifuge (Cole Medical Instrument Co.Ltd, England). The serum of the centrifuged blood was collected into clean plane tubes using hypodermic syringes, and used for the determination of serum lipids, serum enzymes and electrolyte profile using standard analytical kits and an AJ-1222 semi-auto Biochemistry Analyzer (Easy way medical equipments LTD, England).

**Statistical Analysis**

Values were presented as Mean ± SEM, and n=3. The data were statistically analyzed using ANOVA with multiple comparisons versus control groups by Dunnett's method. The values of $p<0.05$ were taken as significant.

**RESULTS**

The results of serum electrolyte concentrations and serum lipid and cholesterol profile of treatment groups are presented in tables 1 and 2. Table 3 shows the result of serum enzyme activities, while table 4 shows the body weight profile of animals during the 14 days of treatment. The $LD_{50}$ of Vernonioside E was deteremined to be 0.70 mg/kg b.w.

**Table 2: Serum Electrolyte concentration of animals treated with Vernonioside E and Crude extract of *Vernonia amygdalina* leaves.**

| Treatment | $Na^+$(mEq/L) | $K^+$(mEq/L) | $Cl^-$(mEq/l) |
|---|---|---|---|
| **Normal control (NC)** | 111.12±1.02 | 7.88±0.25 | 80.15±1.95 |
| Hypertensive control (HC) | 165.72± 15.05 | 6.55±0.30* | 76.27±2.10 |
| VE (20mg/kg) | 105.25± 2.29 | 6.72±0.22*, d | 60.22±7.25*, a, d |
| CE (300mg/kg) | 108.55± 2.41 | 7.25±0.25[a] | 67.75±4.15 |
| Captopril (20mg/kg) | 112.99± 2.30 | 6.87±0.40 | 70.98±2.55 |

Values expressed as mean ± SEM, n = 3
* =P<0.05 compared to the normal control.
a=P<0.05 compared to the hypertensive control.
d=P<0.05 GP4 treated with 300mg/Kg of root extract.

Figure 1 shows the variation of sodium ion concentration between treatment groups, while Figure 2 shows the HDL-C, LDL-C and VLDL-C lipid profile of animals treated with Vernonioside E and Crude extract (CE) of *Vernonia amygdalina* leaves, compared with the group treated with the standard drug (Captopril).

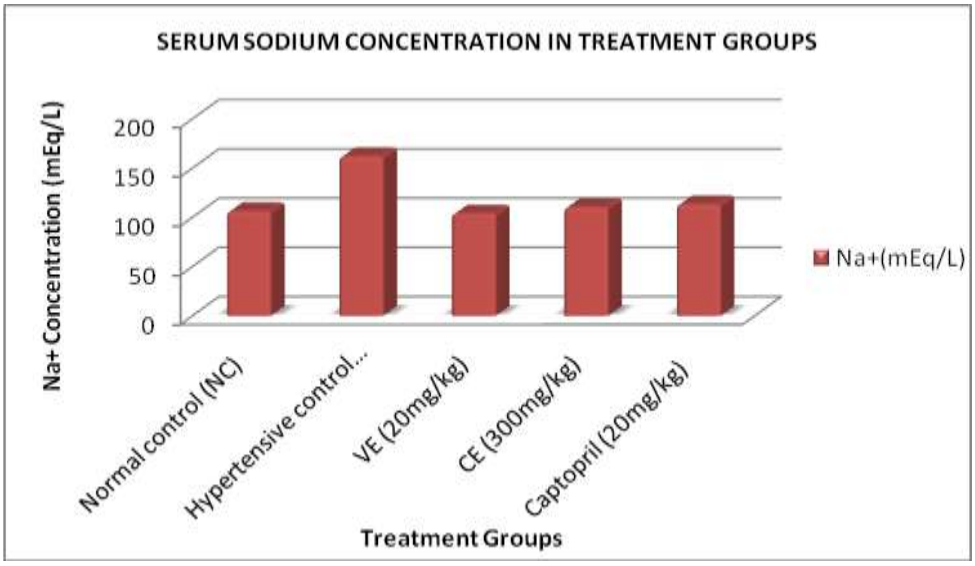


**Fig. 1: Sodium ion concentration of animals treated with Vernonioside E and Crude extract of *Vernonia amygdalina* leaves.**

**Table 3: Serum Lipids concentration of animals treated with Vernonioside E and Crude extract of *Vernonia amygdalina* leaves.**

| Treatment | T-Cholesterol (mg/dL) | Triacylglycerol (mg/dL) | HDL-C (mg/dL) | LDL-C (mg/dL) | VLDL-C (mg/dL) |
|---|---|---|---|---|---|
| Normal control (NC) | 62.09± 2.45 | 89.67±7.55 | 21.52±3.32 | 19.63±6.40 | 3.93±0.66 |
| Hypertensive control (HC) | 77.63±2.97* | 106.24±11.98 | 14.27±0.82 | 30.22±19.80 | 5.84±0.59* |
| VE (20mg/kg) | 56.14±2.15* | 78.39±15.47* | 20.55±0.55[b] | 19.40±14.86 | 6.32±0.22* |
| CE (300mg/kg) | 57.40±2.20* | 15.08±2.57[b] | 20.79±1.20[b] | 24.05±9.23 | 5.45±0.58* |
| Captopril (20mg/kg) | 61.44±0.25* | 95.03±2.67 | 22.21±0.85[a] | 25.98±1.84 | 6.39±1.84* |

Values expressed as mean ± SEM, n = 3
* =P<0.05 compared to the normal control.
a=P<0.05 compared to the hypertensive control

b=P<0.05 compared to the standard treated

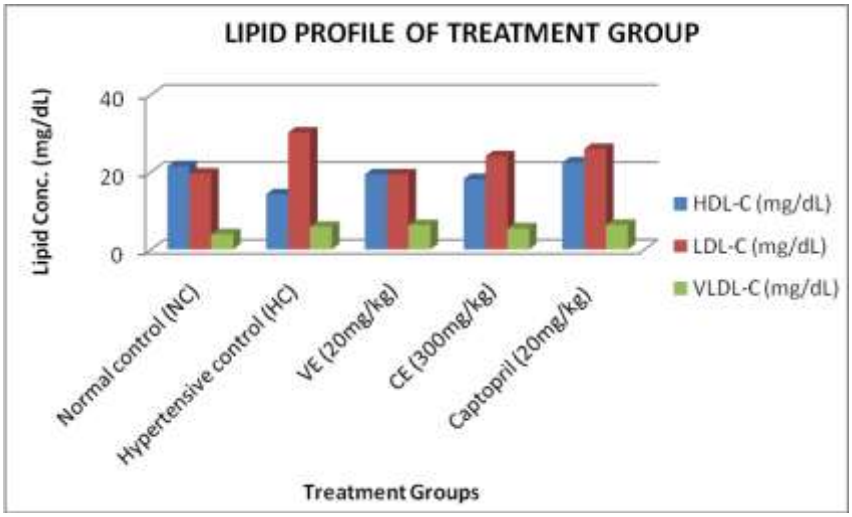


**Fig. 2: Lipid profile of animals treated with Vernonioside E and Crude extract of *Vernonia amygdalina* leaves.**

**Table 4: Serum Plasma Total Cholesterol to HDL Cholesterol Ratio in animals treated with Vernonioside E and Crude extract of *Vernonia amygdalina* leaves to predict Hepertension.**

| Treatment | T-Cholesterol (mg/dL) | HDL-C (mg/dL) | LDL-C (mg/dL) |
|---|---|---|---|
| Normal control (NC) | 62.09± 2.45 | 21.52±3.32 | 19.63±6.40 |
| Hypertensive control (HC) | 77.63±2.97* | 14.27±0.82 | 30.22±19.80 |
| VE (20mg/kg) | 56.14±2.15* | 20.55±0.55[b] | 19.40±14.86[a] |
| CE (300mg/kg) | 57.40±2.20* | 20.79±1.20[b] | 24.05±9.23[b] |
| Captopril (20mg/kg) | 61.44±0.25* | 22.21±0.85[a] | 25.98±1.84 |

Values expressed as mean ± SEM, n = 3
* =P<0.05 compared to the normal control.
a=P<0.05 compared to the normal control
b=P<0.05 compared to the standard treated

**Table 5: Serum Enzyme concentration of animals treated with Vernonioside E and Crude extract of *Vernonia amygdalina* leaves.**

| Parameter | Normal Control (NC) | Hypertensive Control (HC) | VE (20mg/kg) | CE (300mg/kg) | Captopril (20mg/kg) |
|---|---|---|---|---|---|
| AST(mg/dL) | 18.62±7.26 | 20.42±7.47 | 18.82±5.42[a] | 18.22±6.25[a] | 18.60±7.22[a] |
| ALT(mg/dL) | 16.74±8.33 | 17.48±7.73 | 16.85±5.44[b] | 17.52±5.20* | 16.95±6.21 |

Values expressed as mean ± SEM, n = 3
* =P<0.05 compared to the normal control.
a=P<0.05 compared to the normal control
b=P<0.05 compared to the standard treated

The animals treated with Vernonioside E (VE), and crude extract (CE) of *Vernonia amygdalina* gave significantly (p<0.05) lower sodium electrolyte concentration (105.25± 2.29 mEq/L and 108.55± 2.41 mEq/L), when compared to normal control (111.12±1.02 mEq/L), and standard drug (Captopril) treated group (112.99±2.30 mEq/L). Sodium electrolyte concentration for hypertensive control (165.72±15.05 mEq/L) was significantly high when compared to the treated groups, This showed that VE and CE had a sodium lowering effect on the animals. The Chloride electrolyte concentrations showed that the normal control (81.28±3.11mEq/L) was insignificantly higher (P>0.05) than those of hypertensive control (76.31±3.36 mEq/L) and Captopril treated group (71.72±3.79 mEq/L), but significantly (p<0.05) higher than those of treatment 1 (58.36±14.83 mEq/L) and treatment 2 (68.17±3.38 mEq/L). These results may not be of any serious consequence since there was a lowering effect on the Sodium electrolyte concentrations. On the other hand, the Potassium electrolyte concentration for the hypertensive control (6.17±0.39 mEq/L) was slightly lower than those of normal control, treatment 1, treatment 2 and Captopril treated groups (6.29±0.56, 7.80±0.33, 7.51±0.20 and 6.95±0.10 mEq/L) respectively. These trends suggest that sodium electrolyte, which is considered the main extra cellular electrolyte may be most implicated in hypertensive

condition, and may probably implicate and complicate renal failure and nephro-toxicity in hypertensive condition. The antihypertensive results obtained with Vernonia amygdalina extract and compound, were consistent with results obtained with Nuclea latifolia in an earlier study.[5,6]

The Total Cholesterol levels (mg/dl) of treatment 1 (62.08±1.09) and treatment 2 (58.85±2.90) were significantly lower ($p < 0.05$) than the hypertensive control (77.69±19.80). Also the changes in normal control (65.13±6.40) and standard treatment (70.17±1.84) were significant ($p > 0.05$). The Triacylglycerol level (mg/dl) for treatment 2 (15.08±2.57) was significantly lower ($p < 0.05$) than the hypertensive control (106.24±11.98). Also the decrease in treatment 1 (78.39±15.47) was significantly lower than the hypertensive control (106.24±11.98). The high density lipoprotein levels (mg/dl) for normal control, treatment 1 and standard treatment (19.41±1.22, 18.21±2.57 and 22.47±3.97 respective) were significantly higher ($p < 0.05$) than that of the hypertensive control (14.27±0.82), but the changes in the treated groups were insignificant compared to the normal control. This trend of changes in the lipid profile, especially total cholesterol and high density lipoprotein showed that these lipids are most implicated in hypertensive management, and the extracts have antihypertensive potentials.

The Aspartate aminotransferase levels in the treatment groups (18.82±25.42) treatment 1, (18.22±12.25) treatment 2 and (18.60±12.10) standardard/Captopril treatment were slightly higher than the normal control (18.55±7.18) but lower than the hypertensive control (20.40±17.45). However, these changes were not significant at $p<0.05$. Similarly the Alanine aminotransferase levels for treatments 1, 2 and standard (Captopril) treated (21.41±5.80, 18.21±5.20 and 16.95±6.15 respectively) were insignificantly higher ($p<0.05$) than the normal control (16.75±9.17). However, the treatment 1 was higher than the hypertensive control (17.40±7.71), while treatment 2 and standard treatment were lower. These changes are equally not significant ($p<0.05$). This is a possible indication that these enzymes activities are probably not directly associated with hypertensive pathology.

## DISCUSSION

### Effect on Serum Electrolyte Concentration

Sodium, potassium and chloride ions are some of the electrolyte concentrations commonly used in clinical diagnosis as biochemical indicators for the assessment of hypertension.[32,33] Sodium concentration is the major extracellular electrolyte implicated in hypertension, while Potassium functions in collaboration with other electrolytes such as Calcium and Magnesium for the maintenance of body homeostasis.[33] Extra cellular Sodium electrolyte concentration is implicated in vessel walls contraction.[34] Contraction of blood vessels is said to be a function of sodium electrolyte concentration in plasma. When sodium electrolyte concentration is high, there will be a corresponding increase in contraction of the blood vessels, especially the kidney blood vessels. When this happens, the heart may require a greater force to pump blood, thus presenting hypertensive conditions.[33]

It was shown in this study that there was a significant ($p<0.05$) elevation in sodium level in the hypertensive control group, but a decrease in Chloride level. However, there was no alteration in the Potassium levels. This is in agreement with,[5,34] who reported that the electrolyte alteration in hypertension is not directly linked to Potassium. This elevation was shown to be ameliorated and reversed in anti-hypertensive treatment groups by the administration of VE, CE, and the standard drug (Captopril). This further confirms the work by,[5,35] who reported the anti-hypertensive activities of *Nauclea latifolia*, another vegetable and herb widely used in Nigeria. The Potassium and Chloride levels for the controls and treated groups were within normal range. This may be due to the fact that the VE and CE may possess cellular protection properties, with a normal extracellular potassium level, or it may be due to the fact that these electrolytes are not directly associated with the development of hypertension as earlier reported by.[33] The decrease in Chloride levels caused by the extracts appears not to be dose-dependent.

### Effect on Lipid Cholesterol Concentration

Also, the extracts showed antihypertensive properties as they were able to considerably lower the sodium electrolyte levels in hypertensive animals, compared to the controls. High levels of Cholesterol and Low Density Lipoproteins have been implicated as causative agents of hypertension, as they are directly involved in artherogenicity, while High Density Lipoprotein is noted for its ability to prevent hypertension as it functions as anti-atherogenic lipid.[5,33,36] In this study, there was an increase in all the parameters under study, except HDL which suffered a decrease. The increase produced by the extract (treatments 1 and 2) were non-significant. Also the increase produced by the extract probably had no effect on the TG, LDL and VLDL levels, while there was a significant increase in the HDL levels. These shows that the phytochemical constituents of the extract, including saponins,[17,23] flavonoids,[25] and sesquiterpene lactones[30] may indeed ameliorate hypertensive and hyperlipidemic conditions. Hyperlipidemia is directly related to high cholesterol levels in the blood.[33,36,37] This has a consequent occlusion of the vessel wall, with a resultant increase in the force required to pump and circulate blood through and round the body thus resulting in hypertensive condition. For the extracts to decrease the total cholesterol levels of the blood, it shows that it has anti-hyperlipidemic properties. A similar finding was previously reported by.[38] Also, the low levels of triacylglycerol and low density lipoprotein showed that

VE and CE, and the standard drug (Captopril), have the tendency to ameliorate hypertension. This confirms earlier reports that saponins lower plasma cholesterol and possess anti-hypertensive properties.

**Effect on Enzymes Activities**

This study showed that enzymes activities were not significantly altered in hypertension. This might be due to the fact that the pathological conditions in hypertension is not directly associated with tissue destruction of liver cells at sub-chronic stage. It further shows that a low enzymes concentration in hypertension indicates that the condition is not complicated by diabetes or hepatotoxicity. Enzymes are intracellular proteins, which can only be found in appreciably high levels in plasma in cases of mild to severe tissue destruction,[5,33,36] as has been observed . Thus, the induction of hypertensive condition, and subsequent treatment of this condition with VE and CE in experimental rat models did not cause any significant ($p<0.05$) change in the Aspartate aminotransferase and Alanine aminotransferase activities.

## CONCLUSION

This study confirms earlier reports that saponins lower plasma cholesterol and possess anti-hypertensive properties. The study also showed that enzymes activities were not significantly altered in hypertension and this might be attributable to the fact that the pathological condition in hypertension is not directly associated with tissue destruction of liver cells at sub-chronic stage. It further showed that a low enzymes concentration in hypertension suggests that the condition is not complicated by diabetes and hepatotoxicity. We conclude that VE and CE are not only anti-hypertensive but are also hepatoprotective and nephroprotective.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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