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The Effect of Methanolic Extract of *Vernonia Amygdalina (MEVA)* and *Ocimum Gratissimum (MEOG)*on Blood Pressure, Blood Volume, and Angiotensin Converting Enzyme Activities in Hypertensive Male Wistar Rats

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ABSTRACT

Background*:* High blood pressure is a significant risk factor for cardiovascular diseases and can lead to life-threatening complications when not managed properly. Antihypertensive drugs are used in the management of hypertension but with some side effects.

Aim: To determine the effect of methanolic extract of *Vernonia amygdalina (MEVA)* and *Ocimumgratissimum (MEOG)*on blood pressure, blood volume, and angiotensin converting enzyme activities in hypertensive male wistar rats.

Methodology: Fifty-six wistar rats (100-110)g were assigned to seven groups of eight rats each. Group 1-7 constitutes the normal, hypertensive group, MEVA (200 mg/kg bwt) group, MEVA (400 mg/kg bwt), MEOG (200 mg/kg bwt) group, MEOG (400 mg/kg bwt) group and reference drug (lisinopril, 30 mg/kg) group respectively. The extract and reference drug were given through oral gavage. All groups except group 1 were induced with 8% NaCl from 0-4weeks before treatment with extract and reference drug from 5-8 weeks.Angiotensin converting enzyme (ACE) activities was assayed using spectrophotometric method.

Results: At 0 week (before induction), there were no significant differences ($p<0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups. At 4 weeks (after induction), there were significant increase ($p<0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups except group 1. At 8 weeks (after treatment), there were significant decrease ($p<0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups except group 1.

Conclusion: The decrease in elevated blood pressure, blood volume and serum angiotensin converting enzyme activities showed that MEVA and MEOG may possess angiotensin converting enzyme inhibitory effect and diuretic effect. Hence may be useful in hypertensive conditions.

Key words: Methanolic extract of *Vernonia amygdalina* (MEVA), methanolic extract of *Ocimumgratissimum* (MEOG), blood pressure, blood volume, angiotensin converting enzyme (ACE) activities.

INTRODUCTION

Hypertension is the most common risk factor for cardiovascular disease and affects nearly two-thirds of adults aged 60 years or older (1). It is estimated that uncontrolled hypertension is responsible for 7.5 million deaths per year worldwide (2) and in USA, hypertension accounts for over 47 billion dollars spent in health care services, medications and absent

workforce (3). Despite various advances in the field, it was projected that 1.56 billion people may have hypertension by 2025 (2). Hypertension can lead to life-threatening complications when not managed properly. Although antihypertensive drugs are used in the treatment of hypertension, some of these drugs are faced with the problem of being fake and adulterated, drug abuse, high cost of drugs and drug side effects such as erectile dysfunction, extra urination, weakness and asthma symptoms. Presently, increasing application of natural plant products are being investigated for their therapeutic potentials. Traditionally, there are some plants (ginger, garlic, avocado leaves, *Hibiscus sabdariffa*) that are used as antihypertensives without much scientific evidence. In Nigeria, there is high prevalence of hypertension (28.0%) which has been projected to increase to 30.8% by 2030 (4). In other to reduce the increasing prevalence, there is need to look at our indigenous plants used as vegetables like bitterleaf, *Vernonia amygdalina (MEVA)* and Scent leaf, *Ocimumgratissimum (MEOG)*because they are natural, readily available at almost no cost when compared with conventional drugs for their possible therapeutic properties that may aid in the management and control of hypertension.

Aim and objective of the study: To determine effect of methanolic extract of *Vernonia amygdalina (MEVA)* and *Ocimumgratissimum (MEOG)*on blood pressure, blood volume, and angiotensin converting enzyme activities in hypertensive male wistar rats.

METHODOLOGY

Methanolic extraction of the experimental leaves- Maceration technique.
Fresh leaves of bitter leaf and scent leaf were air-dried at room temperature. Air-dried leaves of the plant were milled into powder using a blender- pyramid brand with model number PM-Y44B3.

The powdered leaves were weighed and macerated (soaked) in methanol (500g of the plant material to 2.5liters of methanol in a stopper) for 3 days with frequent agitation to soften and break the plant cell wall to ensure sufficient extraction of the active phytochemicals **(**5). At the end of three days, the methanolic extracts were filtered using whatman No.1 filter paper and the filtrate concentrated to dryness under reduced pressure at 60^0 C in a rotary evaporator at 45^o C, weighed and stored frozen until use. The exact weight of dried extract from 500g powder was 88.4g which gave a percentage yield of 17.68% for *Vernonia amygdalina* while 87.8g gave a percentage yield of 17.56% for *Ocimumgratissimum*. The extracts were dissolved and were given to the animals through oral gavage at graded doses of 200mg/kg body weight and 400mg/kg body weight for *Vernonia amygdalina and Ocimumgratissimum.*

Procurement and Care of the Animals:
Fifty-six (56) male wistar rats weighing 100-110g, that are up to 3months old were obtained and housed in seven cages of eight rats each. They were fed with clean water and rat chow *ad libitum*. The rat chow, pelletized feed was produced by vital feeds, Nigeria. The rats were allowed to acclimatize for 2weeks during which the rat local restrainer that served as a cone was included in their cages to prepare the rat for blood pressure measurement. The cone was used to restrain the movement of the rat (immobilize) before blood pressure measurement. The rats were maintained according to the national institutes of health (NIH) guidelines for care and use of laboratory animals.

Animal grouping:
Fifty-six wistar rats (100-110)g were assigned to seven groups of eight rats each. Group 1-7 constitutes the normal, hypertensive group, MEVA (200 mg/kg bwt) group, MEVA (400 mg/kg bwt), MEOG (200 mg/kg bwt) group, MEOG (400 mg/kg bwt) group and reference drug (lisinopril, 30 mg/kg) group respectively. The extract and reference drug were given through oral gavage. All groups except group 1 were induced with 8% NaCl from 0-4weeks before treatment with extract and reference drug from 5-8 weeks

Induction of Hypertension and Blood Pressure Measurement in Rats
Induction of hypertension was achieved in the rats through oral administration of 8% sodium chloride in water for a period of 4 weeks. The induction process was according to the modified protocol of Rini*et al.,* (6), who reported an increase in blood pressure of rat using NaCl solution for 14 days and they were treated with the test material without stopping the induction for another 14 days. In this study, hypertension was induced with 8% NaCl for 28 days (4weeks) before treatment with the extract. A rat weighing 120g was orally given 0.12mls of 8% NaCl (freshly prepared) once daily between 8-10a.m in the morning. The oral dosing of 8% NaCl was performed without anesthesia and the blood pressure reading was monitored and recorded weekly. Noninvasive method of measurement of blood pressure through the tail was used in the study.Blood pressure was measured in rat using Volume pressure recording (VPR) non-invasive blood pressure monitoring system (CODA-6 Kent Scientific, Torrington, CT, USA).

Procedure for the blood pressure measurement: At the start of the measurement cycle, blood was pushed from the tail by the Volume pressure recording (VPR) cuff and then the occlusion cuff inflates to prevent blood flow back into the tail. When the occlusion cuff deflates, blood begins to flow back into the tail, increasing the tail volume. The occlusion cuff pressure at which the tail volume increases is the systolic blood pressure. The tail volume will continue to increase as the occlusion cuff deflates until blood flow into and out of the tail equalizes; the occlusion cuff pressure at this point is the diastolic blood pressure.

Precautions: The following factors were considered to avoid experimental variability in measurement of blood pressure:

1) Time of the day of measurement of the blood pressure: the measurement of blood pressure was done in the morning between 8am to 10am.
2) Operator handling of each animal: A Veterinary Doctor assisted in handling and measurement of the animals.
3) Differences between strains: the same strain of rat bred specially for hypertension studies were used in the study.

DETERMINATION OF ACE ACTIVITY (ELABSCIENCE, USA)

Principle:
The colorimeteric method is based on the use of angiotensin converting enzyme to enzymolyze N – (3[2 –furyl] Acryloy) – Phe – gly – gly into FAP and GG which leads to the declining change in absorbency. The activity of angiotensin converting enzyme in the sample is calculated by measuring the change rate of absorbency at 340nm.

$$FAPGG \xrightarrow{ACE} FAP + GG$$

Procedures:
After adding 1000µL of working reagent (FAPGG) in the blank tube and sample tube, 125 µL of distilled water was added to blank tube and 125 µL of sample (serum) was added to sample tube. The mixture was allowed to incubate at 37^0C for 180 seconds. The spectrophotometer was zeroed with blank tube at 340nm. The absorbance variation was monitored continuously for 300 seconds.

Calculation:

$$A\ C\ E \text{ activity (U/L)} = (\triangle A/\min_{sample} - \triangle A/\min_{blank}) \times F\ (9100)$$

$$F = \frac{\text{Total volume of reaction liquid (mL)} \times 1000}{\text{Sample volume (mL)} \times \text{millimolar extinction coefficient} \times 1.0}$$

Where $\triangle A/\min$ = average absorbance change per minute

1000 = conversion coefficient of U/mL to U/L

0.989 = millimolar extinction coefficient of FAPGG at 340nm.

Ethical Approval
The ethical approval for this study was obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi with approval number: NAUTH/CS/66/VOL.9/145/2016/119.

Statistical Analysis
The version 23 of Statistical Package for Social Sciences (SPSS) was used in statistical analysis. The variables were expressed as Mean±SD. The independent student t-test was used to assess significant mean difference between two independent groups while paired t-test was used to assess the mean difference between two related groups. Analysis of Varience (ANOVA) was also used and POST HOC was used to determine the significant difference within the groups in animal study. Sigma plot statistical package was used for graphical representation of the data. The level of significance was considered at $P<0.05$. Graphpad prism version 7.05 was used for scientific graphing of some of the data obtained from the animal study after SPSS statistical analysis.

Results: At 0 week (before induction), there were no significant differences ($p<0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups. At 4 weeks (after induction), there were significant increase ($p<0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups except group 1.

At 8 weeks (after treatment), there were significant decrease ($p<0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups except group 1.

Systolic blood pressure of NaCl induced hypertensive wistar rats from 0week to 8weeks The mean levels of systolic blood pressure at baseline (week) in Group 1 to 7 (81.00 ± 11.23, 83.50 ± 10.32, 82.66 ± 11.20, 84.83 ± 11.35, 82.16 ± 11.40, 85.32 ± 10.98, 82.23 ± 19.66) did not differ significantly when compared within groups.

At 4 weeks, after induction of hypertension, the mean levels of systolic blood pressure Group 2 to 7 (162.33± 6.12, 174.17 ± 13.39, 178.16 ± 13.15, 180.50 ± 9.03, 171.00 ± 9.01, 168.66 ± 6.88) respectively showed significant increase (P =0.001) when compared with 0 week.

At 8 weeks, after treatment with methanolic extract of *Vernonia amygdalina*, methanolic extract of *Ocimumgratissimum* and lisinopril, the mean levels of systolic blood pressure significantly decreased (P =0.001) in Group 3 – 7 (123.83± 3.48, 92.66 ± 6.94, 97.50 ± 4.27, 87.17 ± 9.60, 104.50 ± 7.76) when compared with 4 weeks. fig 4.1

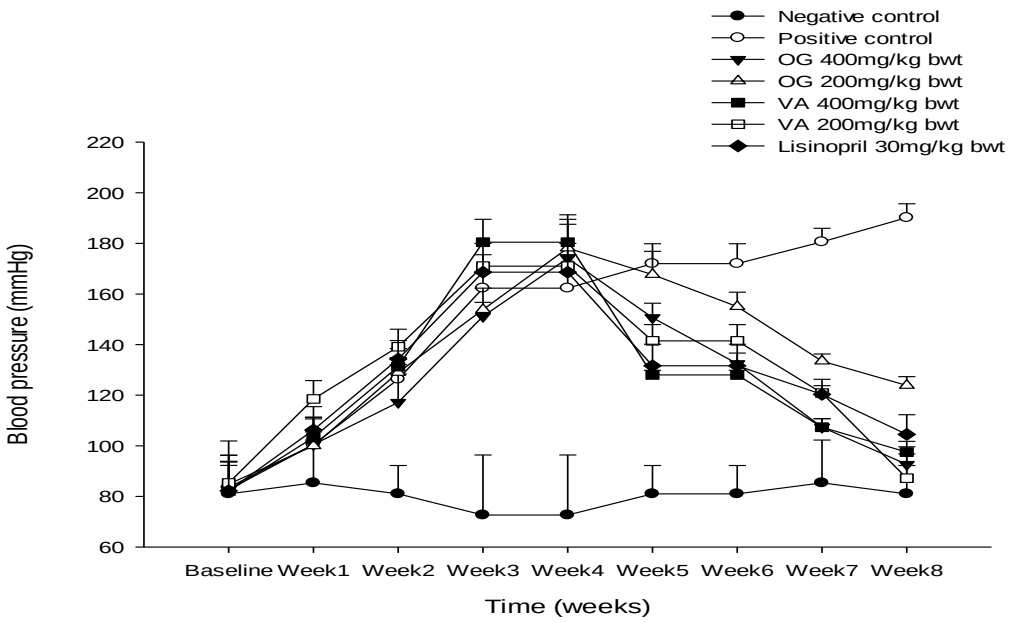

Fig 4.1: Systolic blood pressure of NaCl induced hypertensive wistar rats.

Diastolic blood pressure of NaCl induced hypertensive wistar rats from 0week to 8weeks
The mean levels of diastolic blood pressure at baseline (0 week) in Group 1 to 7 (60.66 ± 11.91, 61.50 ± 8.57, 61.16 ± 8.70, 63.83 ± 5.60, 52.33 ± 11.70, 63.80 ± 9.57, 52.67 ± 19.35) did not differ significantly when compared within groups.

At 4 weeks, after induction of hypertension, the mean levels of diastolic blood pressure Group 2 to 7 (124.66± 16.35, 127.66 ± 13.4, 126.67 ± 13.78, 127.66 ± 13.41, 124.67 ± 16.35, 119.67 ± 15.87) respectively showed significant increase (P =0.001) when compared with 0 week.

At 8 weeks, after treatment with methanolic extract of *Vernonia amygdalina*, methanolic extract of *Ocimumgratissimum* and lisinopril, the mean levels of diastolic blood pressure significantly decreased (P =0.001) in Group 3 – 7 (63.00 ± 8.62, 74.00 ± 8.83, 69.67 ± 5.82, 63.50 ± 11.13, 74.66 ± 4.63) when compared with 4 weeks. fig 4.2.

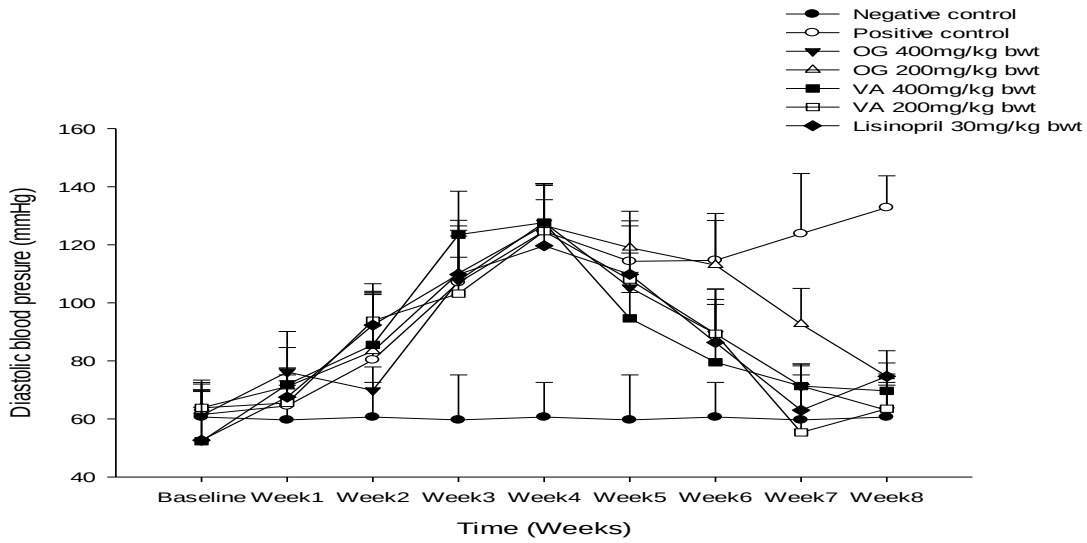

Fig 4.2: Diastolic blood pressure of NaCl induced hypertensive wistar rats

Mean blood pressure of NaCl induced hypertensive wistar rats from 0week to 8weeks

The mean levels of mean blood pressure at baseline (0 week) in Group 1 to 7 (67.33 ± 11.07, 66.67 ± 10.07, 66.83 ± 9.94, 67.16 ± 11.30, 68.83 ± 11.17, 68.67 ± 10.07, 66.33 ± 19.07)did not differ significantly when compared within groups.

At 4 weeks, after induction of hypertension, the mean levels of mean blood pressure of Group 2 to 7 (137.33± 10.36, 143.00 ± 10.09, 142.83 ± 12.54, 144.50 ± 10.85, 139.68 ± 11.74, 135.00 ± 11.97) respectively showed significant increase (P =0.001) when compared with 0 week.

At 8 weeks, after treatment with methanolic extract of vernoniaamygdalina, methanolic extract of ocimumgratissimum and lisinopril, the mean levels of mean blood pressure significantly decreased (P =0.001) in Group 3 – 7 (72.00 ± 8.53, 91.33 ± 6.62, 70.50 ± 10.93, 78.66 ± 2.73, 91.33 ± 6.62,) when compared with 4 weeks. fig 4.3

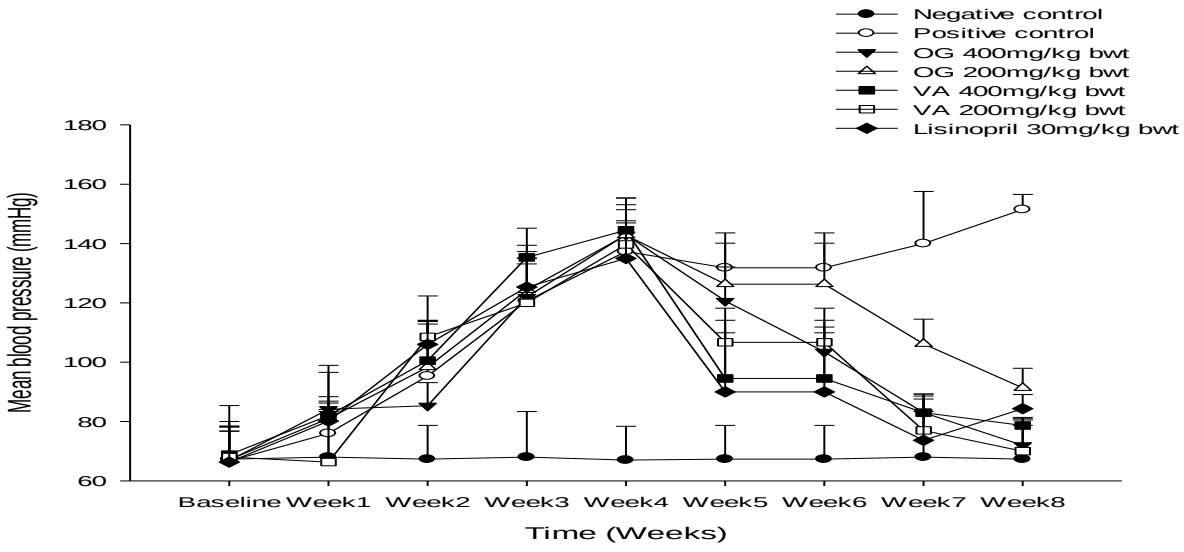

Fig 4.3: Mean blood pressure of NaCl induced hypertensive wistar rats

Blood volume of NaCl induced hypertensive wistar rats from 0week to 8weeks

The mean levels of blood volume at baseline (0 week) in Group 1 to 7 (20.17 ± 3.66, 23.83 ± 5.98, 22.00 ± 5.44, 21.66 ± 5.75, 23.84 ± 4.75, 22.66 ± 5.05, 20.50 ± 2.74)did not differ significantly when compared within groups.

At 4 weeks, after induction of hypertension, the mean levels of volume Group 2 to 7 (76.50± 6.63, 72.83 ± 8.77, 61.67 ± 18.61, 70.00 ± 9.18, 73.33± 12.37, 70.50± 5.92) respectively showed significant increase (P =0.001) when compared with 0 week.

At 8 weeks, after treatment with methanolic extract of *Vernonia amygdalina*, methanolic extract of *Ocimumgratissimum* and lisinopril, the mean levels of blood volume significantly decreased (P =0.001) in Group 3 – 7 (38.83 ± 5.23,21.17 ± 8.28, 35.33 ± 5.27, 41.33 ± 4.88, 25.50 ± 7.05) when compared with 4 weeks. fig 4.4

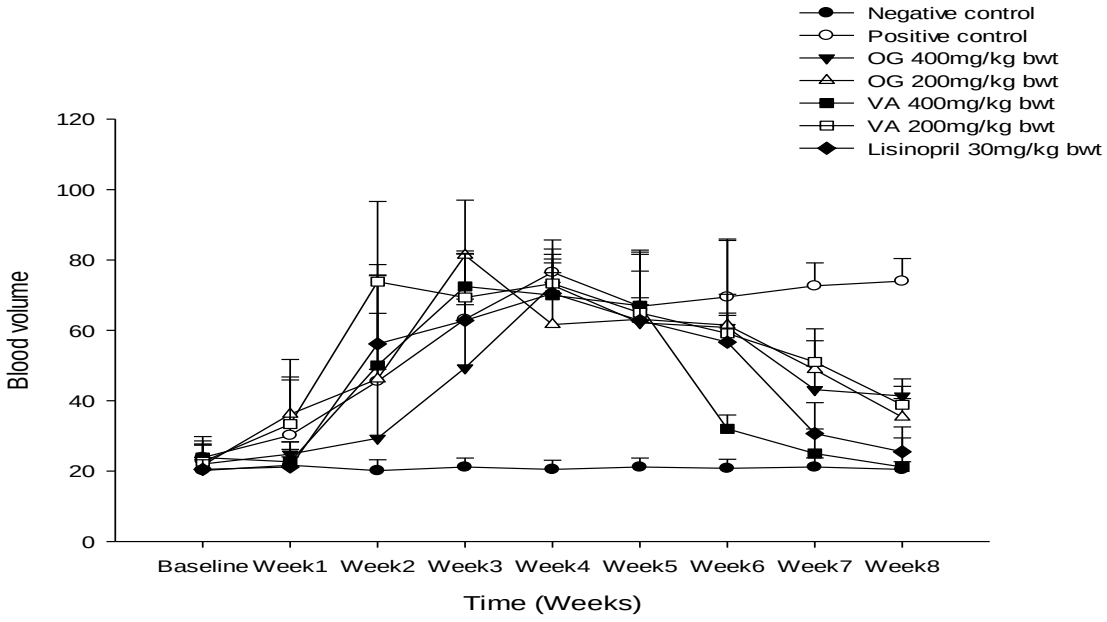

Fig 4.4: Blood volume of NaCl induced hypertensive wistar rats.

Serum levels of angiotensin converting enzyme (ACE) before induction (0 week), after NaCl induction of hypertension (4 weeks) and after treatment with methanolic extract of *Vernonia amygdalina* (MEVA), methanolic extract of *Ocimumgratissimum* (MEOG) and lisinopril in wistar rats expressed as mean ± SD

The mean serum levels of angiotensin converting enzyme did not differ significantly (P=0.986) at 0 week from group 1 to 7 (12.36 ± 0.40, 12.38 ± 0.05, 12.35 ± 0.04, 12.36 ± 0.05, 12.38 ± 0.03, 12.37 ± 0.02 and 12.38 ± 0.05) respectively. After induction of hypertension at 4 weeks, the mean serum levels of angiotensin converting enzyme significantly increased (P =0.001) when compared with 0 week in group 2 -7 (14.03 ± 0.05, 14.01 ± 0.06, 14.03 ± 0.07, 13.89 ± 0.04, 13.92 ± 0.05, 13.88 ± 0.05) respectively. Group 1 (control group) did not differ from 0 week.

At 8 weeks, after oral administration of methanolic extract of *Vernonia amygdalina* (MEVA), methanolic extract of *Ocimumgratissimum* (MEOG) and lisinopril, the mean serum levels of angiotensin converting enzyme significantly decreased (P =0.001) in group 3 – 7 (11.95 ± 0.05, 11.54 ± 0.04 , 11.83 ± 0.06 , 11.52 ± 0.05 , 10.42 ± 0.04) respectively when compared with 4weeks. There was no significant change in group 1 (control group) and group 2 (untreated hypertensive group). Fig 4.5

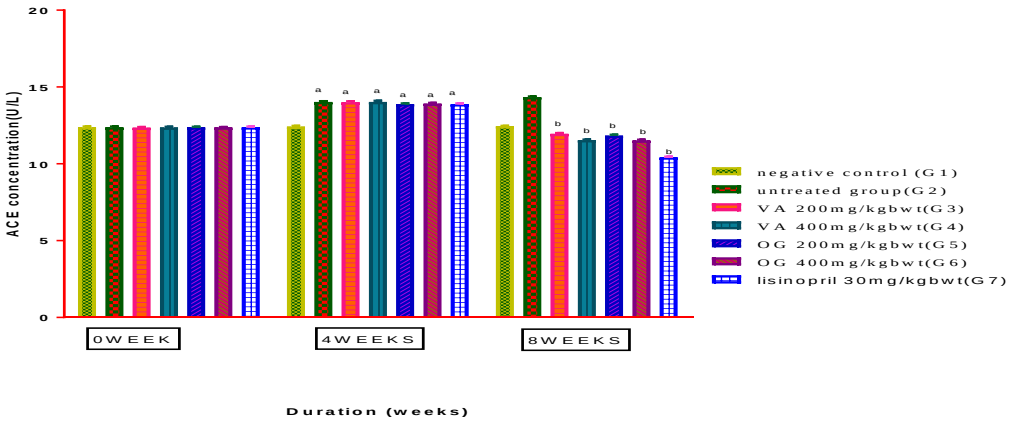

Fig 4.5 Serum levels of Angiotensin converting enzyme(ACE) before induction (0 week), after NaCl induction of hypertension (4weeks) and after treatment with MEVA, MEOG and lisinopril in wistar rats expressed as mean ± SD.

Key: a=significant when compared between 0week and 4 weeks $p<0.05$

b=significant when compared between 4week and 8weeks $p<0.05$

DISCUSSION

Hypertension is as a result of increased salt intake (7,8), physical inactivity (9,10), weight gain (11), high fat diet (12), alcohol intake (13), decreased potassium intake (14), and overall healthy dietary pattern (15). In this study, hypertension was induced in male wistar rat to look at the potential effect of methanolic extract of *Vernonia amygdalina*(VA) and *Ocimumgratissimum*(OG) on blood pressure and biochemical indices of hypertension in comparison with a reference drug, lisinopril (an angiotensin converting enzyme inhibitor) that is used in the management of hypertension.

Induction of hypertension was achieved in the rats through oral administration of 8% sodium chloride in drinking water for a period of 4 weeks and subsequent treatment of the hypertension for another period of 4 weeks. The induction process was in agreement with the work done by Rini*et al.,* (6) who reported an increase in blood pressure using NaCl solution for 14 days and the rats were treated with the test material without stopping the induction for another 14 day.

After administration of the graded methanolic leaf extract of *Vernonia amygdalina* and *Ocimumgratissimum,* the systolic blood pressure, diastolic blood pressure, mean blood pressure, and blood volume significantly decreased. These findings suggest the antihypertensive nature of these leaves. The mineral content of these leaves may have worked synergistically to reduce the blood pressure. Angiotension converting enzyme (ACE) is a central component of renin-angiotensin aldosterone system (RAS), which controls blood pressure by regulating the volume of fluids in the body. It converts the hormone angiotensin I to the active vasoconstrictor angiotensin II. Therefore, ACE directly increases blood pressure by causing blood vessels to constrict. The decrease in angiotensin converting enzyme seen in the study suggests that*Vernonia amygdalina* and *Ocimumgratissimum,* may be an angiotensin converting enzyme inhibitor.They may not increase the production of angiotensin II (a vasoconstrictor), thereby decreasing the systolic blood pressure in the test group. ACE inhibitors are widely used as pharmaceutical drugs for treatment of high blood pressure and heart failure. ACE inhibitors inhibit ACE competitively which results in the decrease formation of angiotensin II and decrease metabolism of bradykinin, which leads to systemic dilation of the arteries and veins and a decrease in arterial blood pressure. In addition, inhibiting angiotensin II formation diminishes angiotensin II – mediated aldosterone secretion from the adrenal cortex, leading to a decrease in water and sodium reabsorption and a reduction in extracellular volume seen in the animal study.

CONCLUSION

The decrease in elevated blood pressure, blood volume and serum angiotensin converting enzyme activities showed that MEVA and MEOG may possess angiotensin converting enzyme inhibitory effect and diuretic effect. Hence may be useful in hypertensive conditions.

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