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Effects of Methanolic Extract of *Vernonia amygdalina* on Electrolytes and Renal Biomarkers in NaCl – Induced Hypertensive Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OBO designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors SCM and CED managed the analyses of the study. Author EOI performed the phytochemical and biochemical analysis. Author CNOE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Vernonia amygdalina popularly called bitter leaf is used in traditional medicine to treat fever, malaria, diarrhoea, dysentery and diabetes. They serve as laxatives and fertility inducer. They are commonly used as vegetable. The effects of methanolic extract of *Vernonia amygdalina* on electrolytes and renal biomarkers in NaCl- induced hypertensive male wistar rats were assessed. Forty wistar rats (120-160) g were assigned to 5 groups of eight rats each. Group 1,2,3,4 and 5

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constitute the normal, hypertensive group, VA (200 mg/kg bwt) group, VA (400 mg/kg bwt) group and reference drug (lisinopril, 30 mg/kg) group respectively. Group 3 and 4 were given the extract through oral gavage for 4 weeks while group 5 was given the reference drug. All groups except group 1 were induced with 8% NaCl for 0- 4weeks before treatment with VA and reference drug for 4-8weeks. Phytochemical analysis of *Vernonia amygdalina*showed the presence of phenol, alkaloids, flavonoids, saponins and steroids. The biochemical results showed significant (p=0.05) decrease in serum sodium and chloride after treatment with VA (200 mg/kg bwt), VA (400 mg/kg bwt) and lisinopril. Serum urea and creatinine showed significant (p=0.05) increase after induction of hypertension when group 1 (at 4 weeks) was compared with the treatment groups: VA (200 mg/kgbwt), VA (400 mg/kg bwt) and lisinopril (30 mg/kg). The increase in urea and creatinine decreased after treatment with the VA extract but not in reference group. In conclusion, VA extract contains useful phytochemicals that may enhance the proper functioning of the kidney when supplemented in hypertensives.

Keywords: Vernonia amygdalina(VA); phytochemical analysis; electrolytes; urea; creatinine; NaCl hypertension.

1. INTRODUCTION

According to world health organization [1], one in three adults worldwide has raised blood pressure known as hypertension. Hypertension is a chronic medical condition with a repeatedly elevated systolic blood pressure reading of 140 mm/Hg and diastolic blood pressure reading of 90 mm/Hg using a sphygmomanometer [2]. Hypertension is divided into two main groups: primary and secondary hypertension. Primary (essential) hypertension does not have a specific cause and make up to 90–95% of hypertensive patients. Secondary hypertension has a known cause and make up to 5–10% of hypertensive patients. Hypertension is a serious public health problem which has been identified as a leading cause of cardiovascular mortality [3]. Hypertension and other non-communicable diseases are currently responsible for at least 20% to 60% of the patients admitted into the wards of most tertiary hospitals in Nigeria [4]. Hypertension is more prevalent in the urban area than in the rural area [5,6].

The overall prevalence of hypertension in Nigeria ranges from 8% to 46% depending on the study target population, type of measurement and cut off value for defining hypertension [7]. The increasing prevalence and mortality associated with hypertension emphasizes the necessity of approaches to reduce its life–threatening complications such as hypertensive encephalopathy, cerebrovascular accidents (stroke), hypertensive cardiomyopathy (heart failure), retinopathy and nephropathy (kidney failure).

Presently, increasing application of natural plant products are being investigated for their chemotherapeutic potentials. Hence, the need to look at our indigenous plant *Vernonia amygdalina,* used as vegetables for their possible chemotherapeutic properties.

Vernonia amygdalina is a bitter plant that is widely distributed in West African and Central African countries. It belongs to family of Asteraceae. It is called onugbu, shikawa and ewuro by Igbos, Hausa and Yoruba respectively. The parts most commonly used are the leaves, roots and stems [8]. *Vernonia amygdalina*is popularly used in the preparation of popular 'Onugbu' soup. *Vernonia amygdalina*has been shown to exhibit anti-diabetic effect [9], anti-bacterial effect [10], hepatoprotective, antioxidant activities [11] and anti-cancer activities [12]. In this study, the possible changes in electrolyte and renal biomarkers associated with the use of *Vernonia amygdalina* in the management of hypertension were investigated.

1.1 Aim

To investigate the renal effect of *Vernonia amygdalina* in NaCl-induced hypertensive male wistar rats for possible therapeutic potentials through the assessment of serum electrolytes and renal biomarkers as indices of hypertension.

2. MATERIALS AND METHODS

2.1 Methanolic Extraction of *Vernonia* Leaves

The *Vernonia amygdalina* leaves were collected and authenticated. Fresh leaves were air-dried at room temperature. Air-dried leaves of the plant were milled into powder, the powdered leaves were weighed and macerated into methanol (100

g) of the plant material to 300 ml of methanol) for 5 days with occasional shaking to ensure sufficient extraction of the active component. The methanolic extract was filtered using No. 1 filter paper, and the filtrate (solvent) concentrated to dryness under reduced pressure at 60 ± 10°C in a rotary evaporator. The resultant methanolic extract was weighed and used appropriately.

2.2 Animal Procurement

Forty male wistar rats (120–160 g) obtained from University of Nigeria Nsukka were used for the research. The rats were allowed to acclimatize for 2 weeks during which they were fed ad-libitum with rat chow and clean water. The rat local restrainers were included in their different cages to prepare the rats for blood pressure measurement. The rats were maintained under good laboratory conditions at a temperature of 22±2°C, relative humidity of 50±5% and photoperiod of 12 hours.

2.3 Animal Groupings

The animals were allocated into five different groups (1, 2, 3, 4, 5) of eight rats each after their body weight measurement. Group 1 was the normal group, Group 2 was the hypertensive group , Group 3,4 and 5 were the groups given 200 mg/kg bwt (VA), 400 mg/kg bwt (VA) and the reference group (lisinopril=30 mg/kg bwt) respectively. The extracts were given through oral gavage daily for 4 weeks, following 4 weeks of hypertension-induction period.

Phytochemical analysis was determined by the method described by Sofowara [13]

2.4 Biochemical Assay

A) Electrolyte Determination
Electrolyte was determined using electrolyte analyser [14].

Principle: At equilibrium, the membrane potential is mainly dependent on the concentration of the target ion outside the membrane and is described by the Nernst equation. The measured voltage is proportional to the logarithm of the activity (effective concentration) of the ions in solution.

B) Determination of Urea Level
Blood urea was determined by the method described by Kassirer [15].

Principle: Urea is decomposed by urease to form ammonia and carbon dioxide. Ammonia combines with 2-oxo-glutarate in the presence of glutamate dehydrogenase and NADH to form L-Glutamate and NAD. The rate of NAD formation measured at 340 nm is directly proportional to the amount of blood urea.

C) Determination of Creatinine Level
Serum creatinine was estimated by Jaffe's method as described by Laron, 1972 [16].

Principle: Serum creatinine in alkaline medium reacts with Picric acid to produce orange coloured complex that absorbs light at 492 nm. The rate of increasing absorption is directly proportional to the amount of creatinine in the sample.

creatinine + Sodium Picrate $\xrightarrow{\text{Alkaline pH}}$ Creatinine-Picrate complex (yellow-orange)

2.5 Statistical Analysis

All data were expressed as mean± standard deviation using ANOVA and difference between the groups considered using Post-Hoc test and the level of significance at P= 0.05.

2.6 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.

LD 50: Acute toxicity studies of methanolic extract of *Vernonia amygdalina* (MEVA).

The acute toxicity of MEVA was determined. Rats were divided into two phases. In the first phase of the study, 9 rats were divided into 3 groups of 3 rats each and they were treated with MEVA by oral gavage at the doses of 10, 100 and 1000 mg/kg respectively. The rats were observed for behavioural, neurological and lethality in the first 24 hours.

In the second stage, 3 rats were divided into 3 groups of 1 rats each and they were treated with MEVA by oral gavage at the doses of 1600, 2900 and 5000 mg/kg bwt. The general behavior of the animals was observed continuously for 1 hour after treatment and then intermittently for 4 hrs, then hourly for the next 24 hrs. The LD_{50} was determined using the formula

$$LD_{50} = \sqrt{a \times b}$$

Where

a = least dose that killed a rat or minimal lethal dose
b =highest dose that did not kill any rat or maximal survival dose

$MEVALD_{50} = \sqrt{a \times b} = \sqrt{2900 \times 5000} => \sqrt{14500000}$ =3807 mg/kg

3. RESULTS

The result of the study showed that sodium, chloride, urea and creatinine significantly increased while potassium decreased.VA treatment reversed the changes after 8 weeks.

4. DISCUSSION

Phytochemicals are natural occurring bioactive compounds known for their health benefits. The presence of phytochemicals such as saponin, flavonoids, phenol, glycosides in the leave extract of *Vernonia amygdalina* was in agreement with work done by other researchers [17,18]. Electrolytes regulate our nerve and muscle function, our body's hydration, blood pressure and rebuilding of damaged tissues [19]. Our heart, muscles and nerves all use electrolytes to maintain voltages across their cell membrane and to carry electrical impulses to other cells. Hence, electrolyte imbalance can lead to blood pressure changes [19]. Fruits and vegetables are good sources of electrolyte; therefore, they can be used to balance the differences in the electrolyte.

The observed significant increase (p=0.05) in sodium level after hypertension-induction with NaCl is expected. It is possible that NaCl causes water retention which releases a digitalis-like substance that increases the contractile activity of the heart and blood vessels. It may also be that sodium itself penetrates the vascular smooth muscle cells causing it to contract [20]. After treatment with 200 mg/kg bwt and 400 mg/kg bwt of *Vernonia amygdalina*, there was an observed decrease in sodium level (p=0.05) in a dose dependent manner as well as reduction in those treated with the reference drug, lisinopril. This was in agreement with the work done by Olayia et al.*[*21] who showed a significant reduction in bioactive (BSS, BSSG, BSS:BSSG) extract of *amygdalina*-treated group.

Table 1. Phytochemical component of *Vernonia amygdalina*

Phenol	Present (+)
Steroid	Present (+)
Alkaloids	Present (+)
Phlobatain	Absent (-)
Flavonoids	Present (+)
Tannins	Present (+)
Saponins	Present (+)

Table 2. Serum urea before (baseline) and after (at 4 weeks) NaCl-induced hypertension and following treatment (at 8 weeks) with VA and lisinopril in male wistar rats (n =8 in each group)

Urea(mmol/l)	0 week	4 weeks	8 weeks
Group 1 (normal)	5.63± 0.03	5.61± 0.01	5.62± 0.02
Group 2 (hypertensives)	5.62± 0.02	7.33± 0.67*	7.82± 0.46*
Group 3 (200 mg/kg bwt)	5.63± 0.02	7.20 ± 0.23*	5.71± 0.38
Group 4 (400 mg /kg bwt)	5.63± 0.01	7.11± 0.41*	5.68± 0.33
Group 5 (lisinopril=30 mg/kg bwt)	5.63± 0.01	6.85± 0.57*	6.85 ± 0.56*

Table 3. Serum creatinine before (at baseline) and after (at 4 weeks) NaCl-induced hypertension and following treatment (at 8 weeks) with VA and lisinopril in male wistar rats (n =8 in each group)

Creatinine (µmol /l)	0 week	4 weeks	8 weeks
Group 1 (normal)	47.18± 1.49	47.17± 1.47	47.16±1.47
Group 2 (hypertensives)	47.67± 1.21	63.35± 3.77*	61.17± 4.95*
Group 3 (200 mg/kg bwt)	47.00± 0.89	63.33 ± 1.03*	52.17± 4.21
Group 4 (400 mg /kg bwt)	47.33± 1.21	63.33 ± 1.03*	49.50± 3.72
Group 5 (lisinopril=30 mg/kg bwt)	47.00± 0.89	66.00 ± 2.75*	68.50 ± 1.3.40*,

Table 4. Serum sodium before (at baseline) and after (at 4 weeks) NaCl induced hypertension and following treatment (at 8 weeks) with VA and lisinopril in male wistar rats (n =8 in each group)

Na^+(mmol/l)	0 week	4 weeks	8weeks
Group 1 (normal)	136.67± 1.36	136.62± 1.32	136.67± 1.36
Group 2 (hypertensives)	138.16± 1.94	168.50 ± 3.45*	174.83± 6.49*
Group 3 (200 mg/kg bwt)	138.67± 1.50	180.17 ± 9.35*	156.00± 14.08
Group 4 (400 mg /kg bwt)	137.67± 2.31	178.75± 10.23*	144.16± 7.70
Group 5 (lisinopril=30 mg/kg bwt)	138.67± 1.50	184.00 ± 7.51*	149.50± 7.28

Table 5. Serum Chloride before (at baseline) and after (at 4 weeks) NaCl-induced hypertension and following treatment (at 8 weeks) with VA and lisinopril in male wistar rats (n =8 in each group)

Cl^- (mmol/l)	0 week	4 weeks	8 weeks
Group 1 (normal)	105.33± 2.41	105.31± 2.42	105.32± 2.42
Group 2 (hypertensives)	105.83 ± 0.75	125.00 ± 9.65	130.33± 3.50*
Group 3 (200 mg/kg bwt)	104.83± 1.16	122.00 ± 4.980	103.16± 2.48
Group 4 (400 mg /kg bwt)	105.33 ± 2.42	123.00 ± 4.243	105.50± 3.94
Group 5 (lisinopril=30 mg/kg bwt)	103.67± 2.58	128.00 ± 3.633	128.00± 3.63*

Table 6. Serum potassium before (at baseline) and after (at 4 weeks) NaCl-induced hypertension and following treatment (at 8 weeks) with VA and lisinopril in male wistar rats (n =8 in each group)

K^+(mmol/l)	0 week	4 weeks	8 weeks
Group 1 (normal)	5.53±0.16	5.50±0.125.53±0.16	
Group 2 (hypertensives)	5.53± 0.16	3.400 ± 0.36*	3.20±0.32*
Group 3 (200 mg/kg bwt)	5.44± 0.10	4.88 ± 0.48*	5.61±0.26
Group 4 (400 mg/kg bwt)	5.47± 0.08	4.83± 1.32*	5.65±0.30
Group 5 (lisinopril=30 mg/kg bwt)	5.47± 0.08	4.83± 0.84*	4.82±0.84*

Table 7. Serum bicarbonate before (at baseline) and after (at 4 weeks) NaCl-induced hypertension and following treatment (at 8 weeks) with VA and lisinopril in male wistar rats (n =8 in each group)

HCO_3 (mmol/l)	0 week	4weeks	8weeks
Group 1 (normal)	25.16 ± 1.72	25.13 ± 1.82	25.17±1.78
Group 2 (hypertensives)	25.50 ± 1.64	24.50 ± 0.54	23.17±1.69
Group 3 (200 mg/kg bwt)	26.00 ± 0.89	24.33 ± 3.14	23.78±2.81
Group 4 (400 mg /kg bwt)	25.16 ±1.72	17.16 ± 3.31*	16.63±2.73*
Group 5 (lisinopril=30 mg/kg bwt)	26.00 ± 1.41	20.33 ± 1.86*	20.33±1.86*

Key= asterisk (*) shows significant difference at p=0.05 when compared between group 1 and other groups in the different tables represented

There was significant reduction p=0.05 in potassium after induction which was reversed after treatment with methanol extract of *Vernonia amygdalina* in a dose dependent manner. This effect may have resulted from inorganic constituents of the leaf such as potassium, zinc, calcium, manganese which are believed to possess beneficial effect [14]. The result showed some therapeutic similarity in the group treated with extract and that treated with the reference drug (lisinopril) on electrolyte studies. Results from renal biomarker showed that urea was significantly increased (p=0.05) and creatinine levels were significantly elevated in hypertensive rats. After treatment with the *amygdalina* extract, there was reversal of the increment suggesting that VA has potential nephron-protective activity. The reference drug-treated group showed a slight increase in creatinine which is in agreement with the work done by Olayia et al., [21]. There were no changes in urea levels between 4 weeks and 8 weeks in the group

treated with reference drug. The VA may be used as supplement since it decreases urea and creatinine levels in the groups treated with the extract. The hypertensive rats showed a significant increase (p=0.05) in renal biomarkers which could be a sign of kidney impairment that was restored through treatment with VA extract. These results suggest a therapeutic potential of the plant in the management of kidney malfunction as one of the complications of hypertension. The therapeutic role of *Vernonia amygdalina* may be explained by the presence of phytochemicals, inorganic constituents and biochemicals present in the plant that may work synergistically at the molecular and cellular level.

5. CONCLUSION

The result of the study confirmed that hypertension altered some biochemical parameters in wistar rats. Methanolic extract of *Vernonia amygdalina* significantly attenuated the altered biochemical parameters when the electrolytes and renal biomarkers were assessed as one of the indices of hypertension management. It is suggested, that VA contains phytochemicals and inorganic components that account for the demonstrated amelioration of deleterious changes in hypertensive rats. Therefore, *Vernonia amygdalina* may be used as a supplement in hypertensive conditions associated with altered renal parameters and electrolyte imbalances.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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