IJPSR (2020), Volume 11, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 June 2019; received in revised form, 28 September 2019; accepted, 25 March 2020; published 01 April 2020

AQUEOUS EXTRACTS OF *VERNONIA AMYGDALINA* AND *OCIMUM GRATISSIMUM* PROTECT AGAINST ELECTROLYTE DERANGEMENT IN SALT-LOADED RATS

Ugochukwu Vincent Igbokwe [1], Ejike Daniel Eze [*2], Moses Dele Adams [3] and Chidimma Felicia Chukwuegbo [1]

Department of Physiology [1], Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
Department of Physiology [2], Faculty of Biomedical Sciences, Kampala International University, Western Campus, P. O. Box 71, Ishaka, Bushenyi, Uganda.
Department of Biochemistry [3], Faculty of Computing and Applied Sciences, Baze University, Abuja, Nigeria.

Keywords:

Vernonia amygdalina, *Ocimum gratissimum*, Asteraceae, Labiatae, Electrolyte, Salt-induced hypertension

Correspondence to Author:
Ejike Daniel Eze

Ph.D, Senior Lecturer,
Department of Physiology,
Faculty of Biomedical Sciences,
Kampala International University,
Western Campus, P. O. Box 71,
Ishaka, Bushenyi, Uganda.

E-mail: daneze4@gmail.com

ABSTRACT: The study aimed at checking the effects of aqueous extracts of *Vernonia amygdalina* and *Ocimum gratissimum* on the electrolyte level of salt-loaded rats. 25 male rats weighing 160-220g were shared into 5 groups of 5 rats. Group 1 animals that were fed standard feed and water served as the control. Animals in group 2-5 which were salt-loaded orally with 2 ml of 4% sodium chloride solution for 2 weeks, were left untreated, treated with 1 ml of 300 mg/kg body weight of aqueous bitter leaf extract, 300 mg/kg body weight of aqueous extract of scent leaf and 300 mg/kg body weight of both extracts in ratio 1:1 respectively. Treatment of salt-loaded animals with the extract was done orally once daily for two weeks after which plasma electrolyte levels were determined. There was significant ($P<0.05$) increase in Na^+ and Cl^- levels in all the salt-loaded groups when compared with the control group. Bitter leaf extracts significantly ($P<0.05$) decreased the sodium level only when compared to the group with no treatment while scent leaf extract did not affect ($P>0.05$) on any of the electrolytes. Treatment with both extracts reduced Na^+ and Cl^- levels significantly ($P<0.05$). Individual and co-treatment with bitter leaf and scent had no change ($P>0.05$) in the levels of K^+ and HCO_3^-. The co-administration of aqueous extract of *Vernonia amygdalina* and *Ocimum gratissimum* has synergistic effect that might be of importance in reduction of blood electrolytes of sodium and chloride in salt-induced derangements. This might be useful in managing salt-induced hypertension.

INTRODUCTION: Globally, according to the World Health Report, hypertension is one of the heart killer illnesses, and this is so especially in rural and developing communities where there are gradual changes in lifestyles to those of more developed and urban societies. Diet poses as the major cause of hypertension in the present-day population, ranging from high fatty foods to high salt intake. Throughout evolution, the human race only consumed sodium and chloride naturally present in food and the daily intake was about 10 mmol/day [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(4).1889-97

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(4).1889-97

Not less than 5000 years ago, salt began to be added to food, and the present intake has increased between 100-400 mmol/day [2]. Dietary salt appears to be an important single factor in raising the blood pressure [3]. Excess dietary salt has harmful effects, which include increasing the mass of the left ventricle, thickening and stiffening conduit arteries

and resistance arteries, including the coronary and renal arteries. It also increases the number of stroke incidence, the severity of cardiac failure and the tendency of platelet aggregation. Salt-induced increase in blood pressure has often been observed to occur within a period of several days to weeks, a time course approximately paralleling the re-establishment of electrolyte balance.

Electrolytes are naturally occurring elements and compounds in the body. They are any substance that produces an electrically conducting solution when dissolved in water. Electrolytes include sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), bicarbonate (HCO^{3-}), magnesium (Mg^{2+}), chloride ($C1^-$), and hydrogen phosphate (HPO_4^{2-}). They control important physiologic functions [4]. These substances are present in the blood, body fluids, and urine and are gotten from food, drinks, and supplements. Excess salt-intake raises the level of electrolytes in the bloodstream and wrecks the delicate fluid balance between the extracellular and intracellular fluids. Electrolytes have been demonstrated by some studies to have an inverse relationship with renal function and cardiovascular events [5]. Electrolyte imbalance due to kidney failure alters the normal physiological functions by altering the level of body fluids and blood thereby increasing the blood pressure; this is directly connected to the kidneys which have a big role to play in the regulation and maintenance of the body electrolytes. The kidneys remove wastes, control the body's fluid balance, and keep the right levels of electrolytes thereby maintaining a constant acid-base balance. Long-time electrolyte imbalance thickens and narrow walls of arteries, damage the kidneys giving rise to kidney diseases, cause stroke, etc which are precursors in the development of hypertension.

Hypertension is defined by a systolic blood pressure that is ≥ 140mmHg and a diastolic blood pressure that is ≥ 90mmHg [6]. High blood pressure is the greatest cause of strokes and heart failure and also a major contributor to coronary heart disease. Essential hypertension *e.g.* salt-induced hypertension also results from a complex interaction of genes and environmental factors such as diet, (high fatty foods intake), age, physical changes, *etc*. Another type of hypertension known as secondary hypertension results from identifiable causes like kidney diseases, endocrine abnormalities, obesity, apnoea, pregnancy, excessive alcohol intake, *etc*. A dispute which lasted about 100 years over the evidence that suggested that hypertension is in part due to the present high intake of salt is now resolved [7]. Hypertension is diagnosed using a device called sphygmomanometer whose cuff is wound around the upper part of the arm above the elbow.

In view of the importance of a healthy heart to the body and the essence of the body homeostatic mechanisms, there is a need to explore available natural products [8]. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drug and the main therapeutic activity depends upon the plant or fungal metabolites which it contains [9].

Vernonia amygdalina, a member of the Asteraceae family, is a shrub or small tree of 2–5m with a petiolate leaf of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odor and a bitter taste. No seeds are produced and the tree is propagated through cutting. It is known in Nigerian local languages as *etidot* (Efik), uzi (Ebira), *onugbu* (Igbo), *ewuro* (Yoruba) and *chusar duki* (Hausa). Elsewhere in Africa, it is called *muop* or *ndole* (Cameroon), *tuntwano* (Tanzania) and *mululuza* (Uganda) [10]. The bitter taste is due to antinutritional factors such as alkaloids, saponins, tannins, and glycosides. The leaf of *Vernonia amygdalina* extract is reported to possess a number of chemotherapeutic potentials [11, 12]. Other activities include antidiabetic [13-16], antioxidant [17- 19], anticancer [20], hepatoprotective [21], cardioprotective [8], antimalarial [22], antihyperglycaemic [23], anti-hypercholesterolemia and antialbuminemia [24], antimicrobial [25], toxicity [26], hepatoprotective [27], antihyperlipidaemic [15], hematological effect [28] as well as amelioration of electrolyte and renal disorders [29].

Ocimum gratissimum is an herbaceous shrub that belongs to the Labiatae family. In Nigeria, the Nupe tribe calls it Tan-motsungi-wawagi, Ebira: Ireru; in the southern part of Nigeria, the plant is called “effinrin-nla” by the Yoruba speaking tribe. It is called “Ahuji or Nchanwu” by the Igbos, while

in the Northern part of Nigeria, the Hausas call it "Daidoya" [30]. The plant contains alkaloids, tannins, phytates, flavonoids, and oligosaccharides [31]. In Nigeria, it is used in treatment of epilepsy, diarrhea, mental illness and fever [32]. Other reported effects are antihyperglycemic [33, 34, 35, 36], anti-hyperlipidemic [37, 38], renal function [39], anti-microbial [40], antidiabetic [41, 42], antioxidant [43, 44], toxicity [30], anti-diarrheal [45] and ameliorate diabetic disorders [46].

The combination of *Vernonia amygdalina and Ocimum gratissimum* has been reported to possess antidiabetic [47], cardioprotective [48], kidney restorative [49, 50] as well as angiotensin-converting enzyme inhibitory, hypolipidemic and antioxidant properties [51] without possible effect of same combination on electrolyte level in hypertensive rats. Therefore, this work seeks to study the effect of aqueous extracts of both plants on electrolyte level of salt-loaded male Wistar rats.

MATERIALS AND METHODS:

Materials:

Plant materials and Authentication: The fresh species of *Vernonia amygdalina* and *Ocimum gratissimum* which were obtained from the Ose Market, Onitsha, Anambra State, Nigeria, were identified and authenticated at the Forestry Research Institute of Nigeria, Ibadan. Voucher Specimen Number of F101863 for *V. amygdalina* and 110026 for *O. gratissimum* was assigned.

Experimental Animals: Male rats weighing (160-220g) were purchased from a commercial supplier. The rats were housed in well-ventilated cages in the animal house of the College. The animals were allowed free access to feed (growers fighter feed) and clean water according to the Barthold's guidelines of the National Research Council (US) Committee for the Update on the Guide for the Care and Use of Laboratory Animals [52]. The animals were acclimatized to the housing and feeding conditions for fourteen days.

Methods:

Ethical Approval: Ethical approval number was allocated under the Animal Research Ethics Review Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria (NAUTH/CS/66/Vol. 2/149) in a letter dated September 15, 2017. This is in conformity with guidelines that are in compliance with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research.

Preparation and Administration of Sodium Chloride Solution: 4 g of sodium chloride was dissolved in 100 ml of distilled water to obtain a concentration of 4% sodium chloride. 2 ml of the 4% sodium chloride solution was orally administered by means of calibrated syringe with attached cannula to the male Wistar rats once a day for two weeks to cause elevated electrolyte levels.

Preparation of Aqueous Extracts of *Veronia amygdalina* and *Ocimum gratissimum*: Large quantities of the fresh specimens of *Veronia amygdalina* and *Ocimum gratissimum* were washed free of soil and debris. The leaves were plucked from the stems and air-dried for three (3) weeks. The dried leaves were milled to powder using a mechanical grinder and kept in airtight containers. *Vernonia amygdalina* and Ocimum *gratissimum* leaf powders (100 g) each was soaked in 1 liter of distilled water. The mixture was allowed to stand for 48 hours with intermittent stirring. Following filtration, the filtrate was stored in a refrigerator after which it is subjected to a lyophilizer yielding the extract which was reconstituted to give 300 mg/kg body weight. The same procedure was adopted to obtain extract individual extract of *Veronia amygdalina* and *Ocimum gratissimum*.

Experimental Design: Twenty-five male Wistar rats were divided into five (5) groups of five rats.

Group A (control) was fed with only feed and water throughout the duration of the experiment.

Group B (negative control) induced with 4% sodium chloride solution for two weeks and was left untreated until the end of the experiment.

Group C received 4% sodium chloride solution for two weeks. Then after, they were treated with 300 mg/kg/day of aqueous extract of *Occimum gratissimum* for two weeks.

Group D received 4% sodium chloride solution for two weeks and then treated with 300 mg/kg/day of aqueous extract of *V. amygdalina* for two weeks.

Group E received 4% sodium chloride solution for two weeks and treated with 300mg/kg/day in ratio 1:1 of combined aqueous extract of *O. gratissimum* and *Veronia amygdalina* for two weeks.

Blood Collection: The rats were placed in a closed cylinder containing a large ball of cotton soaked with chloroform and allowed to stand for a minute for them to lose consciousness. Syringes were then used to collect 2 ml of blood via cardiac puncture into plain bottles and centrifuged at 300 rpm for 10 minutes. The plasma was then collected and kept at 20 ºC until analysis.

Measurement of Electrolyte Level:

Sodium and Potassium Analysis using Flame Emission Spectrometry:

Requirement: Serum sodium and serum potassium were measured against a standard with 140 mmol/L sodium and potassium 5 mmol/L. Stock potassium standard (1.OM) 58.45% NaCl was dissolved in distilled water and made up to 1 liter. Stock potassium standard (1.OM) 74.55% KCl was dissolved in distilled water and made up to 1litre.

Method: The solution was mixed well, and the air compressor was switched on, the air pressure was adjusted, then deionized water was introduced through the auto-miser and the gas turned on, the flame was adjusted to give fine sharp cones as described by [53]. Then appropriate filters were placed for simultaneous sodium and potassium estimation, after which the machine was set at zero with deionized water, then the standard (120/2) adjusted 120.0 for sodium, and 2.0 for potassium were introduced. Then the display of the standard was checked, which showed the exact concentration for sodium and potassium, after which dilute test serum was introduced the readings for sodium and potassium were noted.

Plasma Chloride using Coulometry Method:

Reagents Used: diluting fluid, glacial acetic acid (100ml), concentrated nitric acid (6.4ml), deionized water (1 litre).

Solution: Unflavoured gelatin (6.0g), Thymol blue (0.1g), Thymol (0.1g).

Method: 0.1ml of plasma with 10ml of diluting fluid was put in a beaker, and 2-3 drops of the indicator solution were added as described by [54]. Then the electrolyte was immersed. The machine was switched on, and the digital display started. The concentration of chloride in mmol/L was indicated when the digital display stopped finally, the procedure was repeated with 0.1ml of standard to confirm the accuracy of the result.

Plasma Bicarbonate Test: Reagents used: Saline (1%), dissolved 1g of NaCl in 100mls of distilled water, HCl, 0.01N:

Method: The method of [55] was adopted. Briefly, 0.1ml of plasma bicarbonate was added to 1.5 ml of 1% saline and 2 drops of phenol red indicator and they were mixed well.4.0 ml of 1% saline was put in another tube and then 1.0 ml of 0.1N HCl, was added.0.1ml of plasma bicarbonate was added to 2 drops of phenol red indicator and titrated against 0.01N NaOH till the color changed from yellow to red.

Statistical Analysis: Data analysis was done using the SPSS (Version 20.0) software. The results were expressed as mean value ± SEM of five determinations. Differences between mean and the main effects of treatment group were determined by the one-way analysis of variance (ANOVA) with Tukey's posthoc test. The values of $P<0.05$ was considered statistically significant.

RESULTS:

Acute Toxicity for both Extract: The aqueous leaf extract of *Veronia amygdalina* and *Ocimum gratissium* have median lethal dose of 288.5mg/kg body weight in rats when administered orally.

TABLE 1: ELECTROLYTE LEVELS OF THE DIFFERENT TEST GROUPS (GROUP 2-5) AND THE CONTROL GROUP (GROUP 1) AFTER SALT LOADING

Groups	Na^+ Mean ± SEM	P-Value	Cl^- Mean ± SEM	P-Value	HCo_3^- Mean ± SEM	P-Value	K^+ Mean ± SEM	P-Value
1	141.20±1.07		101.8±1.28		21.4±1.33		5.761±0.16	
2	146.40±0.93	0.000*	107.2±0.71	0.001*	25.4±0.75	0.01*	5.84±0.15	0.707
3	142.60±0.87	0.334	105.4±0.81	0.014*	24.0±1.30	0.079	5.50±0.15	0.230
4	146.60±0.51	0.000*	106.0±0.95	0.005*	23.8±0.80	0.103	5.42±0.13	0.121
5	146.40±0.81	0.000*	106.4±0.87	0.003*	23.6±0.51	0.133	5.74±0.15	0.925

Result is significant at p=<0.05

From **Table 1**, there was a significant (P<0.05) increase in sodium ion level in groups 2, 4, and 5 when compared with group 1 after salt-loading except in group 3. Significant (P<0.05) increase was noticed in chloride ion level in all the salt-loaded groups (2, 3, 4, and 5) and increase in bicarbonate ion level in group 2 only and none for the other groups. There were no statistically significant (P>0.05) changes in potassium ion levels in all the salt-loaded groups.

TABLE 2: ELECTROLYTE LEVELS OF THE DIFFERENT TEST GROUPS (GROUP 2-5) AND CONTROL GROUP (GROUP 1) AFTER ADMINISTRATION OF EXTRACTS

Groups	Na^+ Mean ± SEM n=5	P-Value	Cl^- Mean ± SEM n=5	P-Value	HCo_3^- Mean ± SEM n=5	P-Value	K^+ Mean ± SEM n=5	P-Value
Control	141.2±0.58		101.8±0.92		21.4±1.33		5.76±14	
No extract	146.4±0.87	0.000*	106.20±0.58	0.001*	23.80±1.02	0.010*	5.74±16	0.921
Bitter leaf	142.6±0.81	0.205	105.6±1.03	0.014*	24.0±1.74	0.079	5.56±0.12	0.325
Scent leaf	146.6±0.87	0.000*	106.20±0.86	0.005*	23.80±1.02	0.103	5.42±0.12	0.102
Both	141.8±0.58	0.581	101.20±0.58	0.003*	23.80±1.02	0.133	5.40±0.15	0.084

Result is significant at p=<0.05

From this table, it is shown that there was a significant (P<0.05) decrease in sodium ion, chloride ion, and bicarbonate ion levels and none for potassium in the untreated group when compared with the positive control. Significant (P<0.05) decrease occurred only in chloride ion level in group administered with bitter leaf and no change (P>0.05) in sodium ion, bicarbonate ion, and potassium ion. Also there was a significant (P<0.05) decrease in sodium ion and chloride ion and none for potassium ion and bicarbonate ion in groups treated with scent leaf. The group treated with both extracts showed a decrease (P<0.05) in chloride ion level only.

TABLE 3: ELECTROLYTE LEVELS OF THE TREATED GROUPS (GROUP 3-5) AND UNTREATED GROUP (GROUP 2) AFTER ADMINISTRATION OF EXTRACTS

Groups	Na^+ Mean ± SEM	P-Value	Cl^- Mean ± SEM	P-Value	HCo_3^- Mean ± SEM	P-Value	K^+ Mean ± SEM	P-Value
No extract	146.6±0.93		107.0±0.7		25.4±0.75		5.84±0.15	
Bitter leaf	142.4±0.87	0.001*	105.4±0.81	0.20	24.0±1.3	0.33	5.5±0.15	0.11
Scent leaf	146.6±0.51	1.000	106.0±0.95	0.42	23.8±0.8	0.27	5.42±0.13	0.05*
Both	146.4±0.81	0.854	106.4±0.87	0.63	23.6±0.51	0.21	5.74±0.15	0.63

Result is significant at p=<0.05

From the table above, the result showed a significant (P<0.05) decrease in sodium ion level in group treated with bitter leaf extract. No Significant (P>0.05) changes occurred for the other electrolyte levels of the other groups.

TABLE 4: ELECTROLYTE LEVELS OF TEST GROUPS (GROUP 2-5) BEFORE AND AFTER ADMINISTRATION OF EXTRACT

Group	Stage	Na^+ Mean ± SEM	P-Value	Cl^- Mean ± SEM	P-Value	HCo_3^- Mean ± SEM	P-Value	K^+ Mean ± SEM	P-Value
No extract	Pre	146.6±0.93	0.9	107±0.9	0.46	25.4±0.7	0.28	5.8±0.15	0.03*
	Post	146.4±0.9		106.2±0.9		23.8±1.02		5.7±0.16	
Bitter leaf	Pre	142.4±0.5	0.9	105.4±0.8	0.89	24±1.3	0.94	5.5±0.15	0.21
	Post	142.6±0.8		105.6±1.03		23.8±1.7		5.6±0.12	
Scent leaf	Pre	146.6±0.5	1.00	106±0.51	0.90	23.80±1.02	1.00	5.4±0.5	1.00
	Post	146.6±0.9		106.2±0.9		23.80±0.8		5.4±0.9	
Both	Pre	146.4±0.8	0.026*	5.74±0.8	0.01*	23.60±0.51	0.87	5.74±0.15	0.06
	Post	141.8±0.6		5.4±0.6		23.8±1.02		5.4±0.15	

Result is significant at p=<0.05

From the table, the result shows that a significant (P<0.05) decrease occurred in sodium ion and chloride ion levels in the group treated with both extracts only. Results also show no significant (P>0.05) change for bicarbonate ion level in all the test groups, but there was a significant (P<0.05) decrease in potassium ion in the untreated group.

DISCUSSION: Studies have indicated a positive association between dietary salt intake, level of BP and prevalence of hypertension in humans and animals [7]. High salt intake raises the blood pressure by causing the kidney to retain sodium and water thereby increasing the blood volume. This study investigated the effect of aqueous extracts of *Ocimum gratissium* and *Vernonia amygdalina* on electrolyte level of salt-loaded male Wistar rats.

Electrolytes regulate the nerve, muscle function, body hydration, blood pressure and rebuilding of damaged tissues [56]. Nerves, heart, muscles all use electrolytes to maintain voltages across their cell membranes in order to carry electrical impulses to other cells. Electrolyte imbalance can, therefore, result in blood pressure alterations [56]. Sodium-ion is the major cation of extracellular fluids [57]. The rate of Na^+ excretion by the kidney is related to the glomerular filtration rate (GFR). When the GFR falls, less Na^+ is excreted and vice versa [58]. Potassium ions play an important role in the way in which nerve impulses are propagated along the nerve cells and transmitted to receptor cells [59]. The significant increase in sodium and chloride ion levels in group 2, 4 and 5 when compared to group 1 after salt-loading except in group 3 which showed no increase following administration of 2 ml of 4% of sodium chloride solution once per day for two weeks comes as no surprise and could be as a result of retention and sodium overload culminating in elevated sodium levels, as variable increase in salt-intake from 1-8% for 1-16 weeks increases the blood volume [60]. The observed significant increase in sodium and chloride ion level after salt-loading with NaCl may also imply that it is possible 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 [61].

The absence of increase in the sodium level in group 3 is suggested to be a result of the degree of salt-sensitivity which varies widely among individuals [62]. According to [63], high salt-intake increases the ECF volume which in turn causes an increase in blood volume. This increases the mean circulatory filling pressure and venous return of blood to the heart resulting in an increase in cardiac output and arterial blood pressure.

Chloride ion is essential for water balance, regulation of osmotic pressure and acid-base balance. Since Na^+ and Cl^- act almost parallel, abnormalities of sodium metabolism are generally accompanied by abnormalities in chloride metabolism [58]. Serum bicarbonate is a measure of the base that remains after all acids, stronger than carbonic acid, have been neutralized. It represents the reserve of alkali available for the neutralization of such strong acids, and it has been termed the alkali reserve. The rise in blood HCO_3^- is compensated by increased renal excretion of HCO_3^- [64]. Chloride, potassium, and bicarbonate showed no significant changes after salt-loading as a way of compensating for the increase in sodium and chloride levels (salt retention by the kidneys). Observational studies that demonstrated there is an inverse relationship between potassium intake and BP [65] were not observed in this study. Recent meta-analyses indicated that adequate intake of minerals, *e.g.* potassium and probably calcium, rather than restriction of sodium, should be the focus of dietary recommendations in salt-induced hypertension.

Limitation of sodium chloride in food has historically been considered the critical change for reducing blood volume that might lead to hypertension. The reduction in the serum levels of sodium and chloride ions after the two weeks of salt withdrawal correlated with the study of the three meta-analyses by [60, 66, 67] which revealed that a reduction in sodium intake over periods ranging from few days to years lowers blood pressure.

Treatment with *Vernonia amygdalina* showed a significant decrease in serum level of sodium ion in group 3 without a resultant change in chloride, potassium or bicarbonate levels. Group 4 treated with *Ocimum gratissimum* had no significant decrease in all the electrolyte levels. There was a significant decrease in the sodium and chloride levels in the group treated with both extracts. This might be a result of their phytochemical components which chemically triggered the reduction in the serum levels of sodium and chloride ion, thus are regarded as been synergistic.

In summary, the result of this study showed that taking *Vernonia* amygdalina alone has a positive role to play in reduction of sodium ion levels in any case of electrolyte imbalances [68, 69]. Also that

combining both leaf extracts of *Vernonia amygdalina* and *Ocimum gratissimum* can be beneficial in management and treatment of salt-induced hypertension because of their impact in reducing the plasma levels of sodium and chloride ions which are the major contributor to salt-induced elevation of blood pressure [4, 70].

CONCLUSION: This study has thus demonstrated that the combination of the aqueous leaf extracts of *Vernonia amygdalina* and *Ocimum gratissimum* has synergistic effect that might be of importance in reduction of blood electrolytes of sodium and chloride in salt-induced derangements. This might be of use in the management of salt-Induced hypertension. However, further studies are required to be carried out to explain the mechanism of action of the leaf extracts.

ACKNOWLEDGEMENT: The authors of this work wish to acknowledge the technical assistance of the chief technologist and other laboratory assistants of the Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria, towards ensuring that this research study was completed in good record time. Indeed, we are sincerely grateful.

CONFLICTS OF INTEREST: The authors have not declared any conflict of interest.

REFERENCES:

1. Easton SB and Konner M: Paleolithic nutrition. N Eng J Med 2005; 312: 283-89.
2. Adshead SAM: Macmillan Academic and Professional Ltd: London, Salt and Civilization 1992; 31(5): 569-71.
3. Tuomilehto J, Jousilahti P, Rastenyte D, Moltchanov V, Tanskanen A, Pietinen P and Nissinen A: Urinary sodium excretion and cardiovascular mortality in Finland: A prospective study. Lancet 2001; 357: 848-51.
4. Zilva JF, Panmall PR and Mayne PD: Clinical Chemistry in diagnosis and treatment. Arnold Medical Publishers Inc, Fifth edition, 1991.
5. Haddy FJ and Pamnani MB: Role of dietary salt in hypertension. J Am Coll Nutr 1995; 14: 428-38.
6. Mancia G, de-Backer G, Dominiczak A, Cifkova R and Fagard EA: Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2007; 25: 1105-87.
7. Law MR: Epidemiologic evidence on salt and blood pressure. American Journal of Hypertension 2007; 10, 42S-45S.
8. Olaiya CO, Choudhary MI, Ogunyemi OM and Nwauzoma AB: Nutraceuticals from Bitter Leaf (*Vernonia amygdalina* Del.) protects against cadmium chloride induced hypertension in albino rats. Nature and Science 2013; 11(6): 136-45.
9. Elujoba AA, Odeleye OM and Ogunyemi CM: Traditional medicine development for medical and dental primary health care delivery system in Africa. Afr J Trad CAM 2005; 2(1): 46- 61.
10. Mbang A, Owolabi I, Jaja SI, Oyekanmi OO and Opeyemi J: Evaluation of the antioxidant activity and lipid peroxidation of the leaves of *V. amygdalina*. Journal of Complementary and Integrative Medicine 2008; 5(1): 21.
11. Akah PA, Okoli CO and Nwafor SV: Phytotherapy in the management of diabetes mellitus. Journal of Natural Remedies 2002; 2: 59-65.
12. Farombi EO: African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African Journal of Biotechnology 2003; 2: 662-71.
13. Akah PA and Okafor CL: Blood sugar lowering effect of *Vernonia amygdalina* Del in an experimental rabbit model. Phytotherapy Research 1992; 6: 171-73.
14. Ekpo A, Eseyin OA, Ikpeme AO and Edoho EJ: Studies on some biochemical effects of *Vernonia amygdalina* in rats. Asian Journal of Biochemistry 2007; 2(3): 193-97.
15. Oboh FOJ and Enobhayisobo EI: Effect of aqueous extract of *Vernonia amygdalina* leaves on plasma lipids of hyperlipidemic adult male albino New Zealand rabbits. Afr Sci Res 2009; 10(4): 11-19.
16. Asante DB, Effah-Yeboah E, Barnes P, Abban HA, Ameyaw EO, Boampong JN, Ofori EG and Dadzie JB: Antidiabetic effect of young and old ethanolic leaf extracts of *Vernonia amygdalina*: a comparative study. J. Diabetes Res 2016; 8252741.
17. Nwanjo HU: Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. Nig J Physiol Sci 2005; 20: 39-42.
18. Owolabi MA, Jaja SI, Oyekanmi OO and Olatunji J: Evaluation of the antioxidant activity and lipid peroxidation of the leaves of *Vernonia amygdalina*. J Compl Integr Med 2008; 5: 1.
19. Farombi EO and Owoeye O: Antioxidant and Chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. Int J Environ Res Public Health 2011; 8(6): 2533-55.
20. Lifiani R, Harahap U, Hasibuan PAZ and Satria D: Anticancer effect of African leaves (*Vernonia amygdalina* Del.) to T47D cell resistant. Asian Journal of Pharmaceutical and Clinical Research 2018; 11(1): 4-7.
21. Sitorus P and Nerdy N: Hepatoprotective activity of *Vernonia amygdalina* leaf ethanolic extract in white rats induced by Paracetamol. Asian Journal of Pharmaceutical and Clinical Research 2018; 11(10): 562.
22. Masaba SC: The antimalarial activity of *Vernonia amygdalina* Del (Compositae). Trans R Soc Trop Med Hyg 2000; 94: 694-95.
23. Modu S, Adeboye AE, Maisaratu A and Mubi BM: Studies on the Administration of *Vernonia amygdalina* Del (bitter leaf) and Glucophage on Blood Glucose level of alloxan-induced diabetic rats. Medicinal Plants and Administrative Medicine 2013; 1(1): 13-19.
24. Uhegbu FO and Ogbuehi KJ: Effect of the aqueous extract (crude) of leaves of *Vernonia amygdalina* on blood glucose, serum cholesterol and serum albumin levels in alloxan induced diabetic albino rats. Global J. Pure Applied Sci 2004; 10: 189-94.
25. Akinpelu DA: Antimicrobial activity of *Vernonia amygdalina* leaves. J Study Med Plants 1991; 70: 432-35.

26. Zakaria Y, Azlan NZ, Fakhuruddin N, Hassan N and Muhammad H: Phytochemicals and acute oral toxicity studies of the aqueous extract of *Vernonia amygdalina* from State of Malaysia. J Med Plants Stud 2016; 4: 1-5.
27. Iwalokun BI, Alibi-Sofunde JA, Odunala T, Magbagbeola OA and Akinwande AI: Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. J Med Food 2006: 9(4): 526-30.
28. Akah PA, Alemji JA, Salawu OA, Okoye TC and Offiah NV: Effects of *Vernonia amygdalina* on biochemical and hematological paramaters in diabetic rats. Asian J Med Sci 2009; 1: 108-13.
29. Onyema-iloh OB, Meludu SC, Iloh EO, Dioka CE and Obi-Ezeani CN: Effects of methanolic extract of *Vernonia amygdalina* on electrolytes and renal biomarkers in NaCl–induced hypertensive male wistar rats. Journal of Pharmaceutical Research International 2018; 23(1): 1-7.
30. Effraim KD, Jacks TW and Sodipo OA: Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of Rabbits. Afr J Biomed Res 2003; 6: 21-25.
31. Ijeh II and Ekpe CECC: Current Perspective on the Medicinal Potential of *Vernonia amygdalina* Del. J Med Plant Res 2011; 5(7): 1051-61.
32. El-said F, Sofowora EA, Malcolm SA and Hofer A: An investigation into the efficacy of *Ocimum gratissimum* as used in Nigerian native medicine. Planta Medica 1969; 17: 195-99.
33. Aguiyi JC, Obi CI, Gang SS and Igweh AC: Hypoglycaemic activity of *Ocimum gratissimum* in rats. Fitoterapia 2000; 71(4): 444-6.
34. Egesie UG, Adelaiye AB, Ibu JO and Egesie OJ: Safety and hypoglycaemic properties of aqueous leaf extract of *Ocimum gratissimum* in streptozotocin-induced diabetic rats. Niger J Physiol Sci 2006; 21: 31-35.
35. Mohammed A, Tanko Y, Okasha MA, Magajo RA and Yaro AA: Effects of aqueous leave extract of *Ocimum gratissimum* on blood glucose levels of streptozotocin-induced diabetic Wistar rats. African J Biotech 2007; 6(18): 208-09.
36. Onaolapo AY, Onaolapo OJ and Adewole OS: Ethanolic extract of *Ocimum gratissimum* leaves rapidly lowers blood glucose levels in diabetic wistar rats. Macedonian Journal of Medical Science 2011; 4(4): 351-57.
37. Nwanjo HU and Oze GO: Hypolipidaemic and antioxidant properties of *Ocimum gratissimum* on diabetic rats. Plant Prod Res J 2007; 11: 1-4.
38. Ayinla MT, Dada SO, Shittu ST, Olayaki LA, Akiode AO and Ojulari SL: Anti-hyperlipidemic effect of aqueous leaf extract of *Ocimum gratissimum* in alloxan induced diabetic rats. Int J Med Med Sci 2011; 3: 360-63.
39. Ogundipe DJ, Akomolafe RO and Sanusi AA: Effects of two weeks administration of *Ocimum gratissimum* leaf on feeding pattern and markers of renal function in rats treated with gentamicin. Egypt J Basic Appl Sci 2016; 3: 219-31.
40. Orafidiya LO, Oyedele AO, Shittu AO and Elujoba AA: The formulation of an effective topical antibacterial product containing *Ocimum gratissimum* leaf essential oil. Int J Pharmacol 2001; 244(2): 177-84.
41. Oguanobi NI, Chijioke CP and Ghasi S: Anti-diabetic effect of crude leaf extracts of *Ocimum gratissimum* in neonatal streptozotocin-induced type-2 model diabetic rats. Int. J. Pharm. Pharm. Sci 2012; 4: 77-83.
42. Okoduwa SIR, Umar IA, James BD and Inuwa MH: Anti-diabetic potential of *Ocimum gratissimum* leaf fractions in fortified diet-fed streptozotocin treated rat model of type-2 diabetes. Medicines (Basel) 2017; 4(4): 73.
43. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM and Farombi EO: Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Sci. Res. Essay 2007; 2(5): 163-66.
44. Arfa MM and Rashed AM: The modulative biochemical effect of extract of *Ocimum gratissimum* as anti-oxidant on diabetic albino rats. Egypt. J Comp Path & Clinic Path 2008; 21(3): 69-87.
45. Ezekwesili CN, Obiorah KA and Ugwu OP: Evaluation of anti-diarrheal effect of *Ocimum gratissimum* crude extract on albino rats". Biokemistri 2004; 16(2): 122-31.
46. Okon UA, Owo DU, Udkang NE, Udokang JA and Ekpenyong CE: Oral administration of aqueous leaf extract of *Ocimum gratissimum* ameliorates polyphaghia, polydipsia and weight loss in streptozotocin-induced diabetic rats. Am J Med Med Sci 2012; 2: 45-49.
47. Adewoga TOS, Sebiomo A and Fagbemi FT: The effect of Vernonia amygdalina and *Ocimum gratissimum* on alloxan-induced diabetic rats. African Journal of Cellular Pathology 2014; 2: 75-82.
48. Asuquo O, Igiri A, Akpan J and Akpaso M: Cardioprotective potential of *Vernonia amygdalina* and *Ocimum gratissimum* against streptozotocin (Stz)–induced diabetes in wistar rats. The Internet Journal of Tropical Medicine 2009; 7(1): 1-7.
49. Agbai EO, Ofoego UC, Nwodo FN and Nwanegwo CO: Synergistic effect of methanolic extract of *Vernonia amygdalina* Del and *Ocimum gratissimum* on kidney functions in streptozotocin-induced diabetic wistar rats in comparison with insulin. Journal of Medical and Applied Biosciences 2013; 5(1): 116-31.
50. Izunwanne DI, Aduema W and Okonkwo OC: Effect of crude extracts of *Ocimum gratissimum* and *Vernonia amygdalina* on urea and creatinine in non diabetic and diabetic rats. MAYFEB Journal of Biology and Medicine 2017; 1: 43-52.
51. Abdulazeez MA, Ibrahim K, Bulus K, Babvoshia HB and Abdullahi Y: Effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* extract on the activity of angiotensin-converting enzyme, hypolipidemic and antioxidant parameters in streptozotocin-induced diabetic rats. African Journal of Biochemistry Research 2013; 7(9): 165-73.
52. Barthold SW, Bayne KA, Davis MA, Everitt JI, Fox JG and Garnett NL: National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. The National Academies Press Publishers, Eighth Edition 2011.
53. Vogel AI: A Textbook of Quantitative Inorganic Analysis. Longman Group Ltd. London. 3rd Edition, 1960; 882-85.
54. Schales O and Schales SA: Simple and accurate method for the determination of chloride in biological fluids. Journal of Biochemistry 1941; 140: 879-84.
55. Segal MA: A rapid electrotitrimetric method for determining CO_2 combining power in plasma or serum. American J of Clinical Pathology 1955; 25(10): 1212-16.
56. Nordquist C: What are electrolytes? What causes electrolyte imbalance? Medical News Today. Allen Publishers Ltd, Seventh Edition 2016.
57. Smyth A, Dunkler D and Gao P: The relationship between estimated sodium and potassium excretion and subsequent renal outcomes. Kidney Int 2014; 86: 1205-12.
58. Naik P: Biochemistry. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India Second Edition, 2007; 379-82, 502-08.

59. Vasudevan DM, Sreekumari S and Vaidyanathan K: Textbook of biochemistry for medical students. Sixth Edition. Jaypee Brothers Medical Publishers (P) Ltd New Delhi, India 2011; 342-43, 361-63.
60. Storm BL, Yakline AL and Oria M: Sodium intake in populations. Washington (DC): National Academies Press (US); 2013.
61. Haddy FJ: Role of dietary salt in hypertension. Life Science 2006; 5(3): 244-49.
62. Fujita M, Ando K, Kawarazaki H, Kawarasaki C, Muraoka K, Ohtsu H, Shimizu H and Fujita T: Sympatho-excitation by brain oxidative stress mediates arterial pressure elevation in salt-induced chronic kidney disease. Hypertension 2012; 59: 105-12.
63. Guyton AC and Hall JE: Blood pressure control-special role of the kidneys and body fluids. Science 1991; 252: 1813-16.
64. Widmaier E, Raff H and Strang K: The kidneys and regulation of water and inorganic ions. Vander's Human Physiology (13th ed.). New York, NY: McGraw-Hill 2014; 446-89.
65. Intersalt Cooperative Research Group: Intersalt: an International Study of electrolyte excretion and blood pressure. Results for 24 h urinary sodium and potassium excretion. British Medical Journal 2012; 297, 319-28.
66. Midgley PJ, Matthew AG, Greenwood CMT and Logan AG: Effects of reduced dietary sodium on blood pressure. A meta-analysis of randomized controlled trials. Journal of the American Medical Association 2006; 275: 1590-97.
67. Graudal NA, Galloe AM and Garred P: Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterols, and triglyceride, a meta-analysis. J of the Amer Med Assoc 2008; 279: 1383-91.
68. Kadiri O and Olawayo B: *Vernonia amygdalina*: An underutilized vegetable with Nutraceutical potential-A Review. Turkish Journal of Agriculture, Food Science and Technology 2016; 4(9): 763-68.
69. Ogah OS, Okpechi I and Chukwuonye II: Blood pressure, prevalence of hypertension and hypertension-related complications in Nigeria Africans: A review. World Journal of Cardiology 2012; 4(12): 327-40.
70. Adediran OS, Okpara IC and Adeniyi OS: Hypertension prevalence in an urban and rural area of Nigeria. Journal of Medicine and Medical Sciences 2013; 4(4): 149-54.

How to cite this article:
Igbokwe UV, Eze ED, Adams MD and Chukwuegbo CF: Aqueous extracts of *Vernonia amygdalina* and *Ocimum gratissimum* protect against electrolyte derangement in salt-loaded rats. Int J Pharm Sci & Res 2020; 11(4): 1889-97. doi: 10.13040/IJPSR.0975-8232.11(4).1889-97.