






Original Research

Comparative Effects of *Phyllanthus Amarus* on the Serum Electrolyte Level of Hypertensive and Normotensive Male Wistar Rats

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ABSTRACT

Background. High blood pressure, also known as hypertension, is one of the most well-known major risk factors for cardiovascular disease (CVD) and stroke. **Aims.** This present study tried to compare the effect of *P.amarus* leaves on normotensive and hypertensive male Wistar rats. **Materials and method.** 15 male Wistar rats were divided into three groups of five rats each (Control group, Control + Extract group, and Hypertensive+ Extract group). One of the groups of rats (group 3) was induced with hypertension using 0.5mg/kg for 5 days of Dexamethasone. Then, the normal/control group and the hypertensive were treated with 200mg/kg of aqueous Extract of *Phyllanthus amarus* (AEPA) for 28 days. Fasting venous blood samples were collected for the measurements of serum electrolytes. Serum potassium, sodium, and chlorine levels were measured by the ion-selective electrode method. **Results.** In this experiment, for the normotensive and hypertensive treatment groups respectively, there was an increase in K⁺ level and decrease in K⁺ level compared to the control rats, decrease in Na⁺ level, and decrease in Na⁺ level. Also, a decrease in Cl⁻ of normotensive rats while there was no change in the Cl⁻ level of hypertensive group when compared to control group. The p values for all results were found to be non-significant. *Phyllanthus amarus* has an effect on serum electrolyte imbalance. Its reducing effect on sodium and chloride levels implies that it can be used to manage hypertension, thereby reducing the risk of developing cardiovascular diseases.

Highlights:

1. It discusses effects of *phyllanthus amarus* on serum electrolyte which results in an imbalance.
2. The reducing effect on sodium and chloride levels implicate a new chance in managing hypertension.

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Introduction

High blood pressure, often known as hypertension, has become a common disease in the modern world. It is a non-communicable disease that is characterized as a long-term state of hypertension and a risk factor for cardiovascular conditions including heart attack, stroke, and the likes. Concern over hypertension has spread across the globe^[1-2]. Hypertension affects almost a billion people worldwide and is known as the "silent killer" since it first exhibits no specific signs or symptoms.^[3]

One of the major factors responsible for the development of hypertension is the diet we consume. Most meals and fluids contain minerals that play essential roles and when these minerals exceed or are below the adequate amount needed, it leads to electrolyte imbalance. Sodium, potassium, and chloride are the significant electrolytes along with magnesium, calcium, phosphate, and bicarbonates. Research done by Mane^[4], revealed that serum sodium levels and serum potassium were significantly associated with the risk of development of hypertension.

Sodium is an important mineral which, besides its functions in fluid balance, action potential generation, digestive secretions, and absorption of many nutrients, also plays an important role in blood pressure regulation. There are several physiological explanations for the association between high sodium intake and blood pressure like enhanced

reabsorption and retention of filtered sodium through the renal tubules^[5] or activation of the brain renin–angiotensin–aldosterone system (RAAS), which is suggested to increase blood pressure through angiotensin II and aldosterone promoting locally oxidative stress and activating the sympathetic nervous system^[6]. Also, based on the “vasodysfunction theory” of salt induced hypertension, salt loading results in subnormal decreases in systemic vascular resistance leading to an increase in blood pressure^[7]. In this regard salt sensitivity, which varies among individuals, is suggested to play an important role^[6,8]. Independent of body weight, sex and age, too much dietary salt (sodium chloride) is regarded as an established risk factor for hypertension.^[9]

Therefore, reduced sodium intake is being associated with a reduction in systolic and diastolic blood pressure^[8]. Like sodium reduction, higher potassium intake or supplementation has also been repeatedly shown to reduce the blood pressure of especially hypertensive persons^[10]. The findings of Shrimanker and Bhattaraj^[11], confirmed that sodium (salt) reduction and a higher intake of potassium have convincing blood pressure lowering effects. Numerous research and meta-analyses revealed that potassium supplements lower blood pressure and the risk of hypertension in addition to modifying sodium intake^[12,13]. Potassium may lower blood pressure by several processes, including improved

endothelial function and NO release, vasodilation caused by a decrease in cytosolic smooth muscle cell calcium, an increase in natriuresis, and a reduction in sympathetic nervous system activity^[10]. Furthermore, sodium alteration in conjunction with potassium has been shown to support blood pressure lowering effects^[13]. Increased reabsorption of water and Cl⁻ in the loop of Henle may contribute to the blunted natriuretic capacity and hence lead to hypertension in the rats.

Dexamethasone was used in this research work to induce hypertension in the experimental rats. This is a synthetic glucocorticoid known for its potency in demonstrating glucocorticoid activity. Despite its efficacy as an anti-inflammatory agent, the use of glucocorticoids for a long period of time has been associated with various side effects including hypertension^[14]. The hypertensive effect of dexamethasone has been demonstrated in many studies^[14]; this is hypothesized to be via depleting the supply of Nitric oxide NO and subsequently causing vasoconstriction through the following mechanisms: up regulation of ROS level, reaction with Nitric oxide (NO), reduction in its bioavailability and inhibition of expression of reaction with NO synthase at the transcription level^[15]. Also, Muhamed *et al.*^[16] recorded that dexamethasone decreases potassium concentration in blood while Kenyon *et al.*^[17] stated that dexamethasone treatment increases total body sodium amount. This can be an

explanation for its effect in increasing blood pressure.

The management of hypertension involves change in lifestyle, exercise, consumption of healthy meals and sometimes, drugs that keep the blood pressure moderate. Several antihypertensive drugs are in existence and most of them are synthetic in nature but the use of herbal products to treat various human diseases has become popular due to their easy access and low cost, compared with advanced Western synthetic medicines^[18-20]. The World Health Organization (WHO) has reported that about 80% of population in the developing countries such as Nigeria, depends on herbal medicines or phytomedicines for the treatment of a number of diseases^[21,22]. One of such herbs is *Phyllanthus amarus*.

Phyllanthus amarus has a long history of use in herbal medicine in every country where it is grown. In traditional medicine, it is used for its hepatoprotective, anti-diabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties^[23]. A study carried out by Eweka and Enogieru^[24] recorded that rats in their treatment groups received 500mg/kg body weight of aqueous extract of *Phyllanthus amarus* orally for twenty-eight days. The kidneys of these animals revealed some level of cyto-architectural distortion of the cortical structures as compared to the control despite the fact that *Phyllanthus amarus* is known to eliminate kidney

stone and other kidney related problems. Hence, this study used 200mg/kg of *Phyllanthus amarus* for 28 days to reduce possible organ damage.

The purpose of this study is to ascertain whether *Phyllanthus amarus* affects serum electrolyte levels in a way that helps maintain normal blood pressure. Additionally, to contrast *Phyllanthus amarus*' impact on normotensive and hypertensive patients.

Materials and Method

Materials

The following materials were used during this study: Lithium heparin bottles, Medical gloves, Capillary tubes, Automated Electrolyte Analyzer (SFRI ISE6000-France).

Drug

Dexamethasone (4mg/ml) was purchased from a pharmaceutical store in Shagamu, Ogun State. The experimental group was given daily injections of water-soluble dexamethasone (0.5 mg/kg of body weight for 5 days. This dose was sufficient to elevate blood pressure in Wistar rats.

Method

In this study, the animals used had a body weight of about 100 to 150 g. They were divided into batches of five (5) rabbits (male or female) per cage, acclimated for two (2) weeks and fed daily with 200g of rat chow used as nutrient maintenance.

Dexamethasone (0.5mg/kg rat per day for 5 days) was administered via subcutaneous injection at 2400

h daily. On the 28th day after an overnight fasting, the blood of the rats was drawn retro-orbitally with the use of capillary tubes.

Collection of Plant Material and Preparation of Extract

Fresh areal parts of *Phyllanthus amarus* were collected from a local farm land in Ikenne local government area of Ogun State and was confirmed and authenticated by Federal Research Institute Ibadan, Nigeria and was allocated with voucher number 112938 and sample was deposited at the Federal Research Institute Herbarium. The plant was thoroughly cleaned with running tap water and air-dried on the laboratory bench under normal atmospheric temperature for some days. The dried leaves were grounded to fine powder using a grinding mill. Aqueous extract was prepared by soaking 100 g dried powder in 1L of water for 72 h and shaking intermittently. This was filtered with a muslin cloth. The filtrate was concentrated and refrigerated till it was needed.

Animals

Fifteen male Wistar rats were purchase from Olabisi Onabanjo University Animal house. Before starting the experiments, rats were isolated and acclimated for 14 days. They were maintained under standard laboratory conditions (25 ± 2 °C) with dark and light cycle (12/12 h) and had free access to a standard dry pellet diet and water *ad libitum*. Experiments were

performed in accordance to the Guidelines for experiments involving animals ²⁵.

Experimental Design

After the acclimatization period, the rats received treatments as shown below.

Group I: Normal control rats, received distilled water alone

Group II: Control rats, received a single dose of 200 mg/kg body wt/day of AEPA

Group III: Hypertensive rats treated with 200mg/kg body wt /day of AEPA

Fifteen male adult Wistar rats weighing 100-150 g were distributed randomly into three groups (I-III) of five rats per group. Group I served as control and received distilled water throughout the duration of the experiment. Group III was administered 0.5mg/kg b. wt of dexamethasone to induce hypertension in the rats.

Thereafter, group II and III were treated with single dose of 200 mg/kg body wt/day of AEPA for a consecutive period of 28 days. All administrations of drugs and extract to the rats were carried out by oral gavage.

Procedures

Blood collected in lithium heparin tubes was then taken to the laboratory for evaluation of serum electrolytes. These blood samples were centrifuged and allowed to settle. Sera obtained after settling were then frozen to -20 °C and used to determine the

values of sodium, potassium and chlorine using an automated electrolyte analyzer (SFRI ISE6000-France).

Statistical analysis

The Mean \pm S.E.M of all values was calculated, and change observed between the treatment groups and control was subjected to analysis of variance (ANOVA). Differences between groups were considered significant at $p < 0.05$.

Result

The effect of the *Phyllanthus amarus* leaves extract on some serum electrolytes levels is depicted in the tables below. There was a non-significant ($p > 0.05$) difference between the rats administered with extract and the control rats.

Table 1. Effects of *Phyllanthus amarus* on potassium level in adult male Wistar rats

Group	Mean \pm S.E. M (mmol/L)	P- value
1 (Control)	6.4 \pm 0.9	
2 (Control + Aepa)	6.5 \pm 0.2	0.76
3 (Hypertensive + Aepa)	5.85 \pm 0.65	

AEPA- Aqueous Extract of *Phyllanthus amarus*, K⁺ = Potassium ($p < 0.05$) is significant

Table 2. Effects of *Phyllanthus amarus* leave extract on sodium level in adult male Wistar rats

Group	Mean \pm S.E. M (mmol/L)	P- value
1 (Control)	149 \pm 9.0	
2 (Control + Aepa)	141.5 \pm 1.5	0.56
3 (Hypertensive + Aepa)	140 \pm 4.0	

AEPA- Aqueous Extract of *Phyllanthus amarus*, Na⁺ = Sodium, (p<0.05) is significant

Table 3. Effects of *Phyllanthus amarus* on chlorine level in adult male Wistar rats

Group	Mean ± S.E. M (mmol/L)	P- value
1 (Control)	95.5 ± 5.5	
2 (Control + Aepa)	97.5 ± 0.5	0.86
3 (Hypertensive + Aepa)	95.0 ± 2.0	

AEPA- Aqueous Extract of *Phyllanthus amarus*, Cl⁻ = Chlorine, (p<0.05) is significant

The table 2 below showed that there was a non-significant decrease in Na⁺ level of Group 2 and Group 3 rats when compared to Control group.

Discussion

Amongst many other causes, abnormal serum electrolyte level is said to be one of the major causes of hypertension^[12]. Serum sodium and serum potassium levels were significantly associated with the risk of development of hypertension.^[4]

This study was carried out to determine whether *Phyllanthus amarus* has an effect on serum electrolytes level which could result in an evident change in blood pressure. Also, to examine if there is any difference between the effects of *Phyllanthus amarus* on a hypertensive subject compared to its effect on the electrolyte level of normotensive subjects.

In this study, there was a non-significant (p>0.05) increase observed in amount of serum K⁺ of

normotensive rats treated with *Phyllanthus amarus* when compared to the control group and a non-significant decrease in K⁺ level of hypertensive rats compared to the normotensive group. This decrease in K⁺ level of hypertensive could be as a result of dexamethasone that was used to induce hypertension. As dexamethasone has being found out to decrease potassium level^[16]. Also, Hu *et al.*^[27] suggested that serum potassium level was lower in the hypertension group compared with the non-hypertension group. Therefore, this result implies that AEPA has no effect on the potassium levels in the blood.

For the sodium, there was a non-significant decrease in Na⁺ level of both treatment groups when compared to control group. This implies that *Phyllanthus amarus* could result in the decrease of sodium levels in the blood. This findings agree with Yakubu *et al.* (2022) whose result recorded a decrease (p>0.05) in sodium ion (Na⁺) concentration between groups administered with *Phyllanthus amarus* extract of 200 mg compared to normal/control and their result was significant The further decrease in Na⁺ level of the hypertensive rats can imply that *Phyllanthus amarus* has more effect on the sodium level of hypertensive subjects than the normotensive ones.

As for chlorine, there was a non-significant increase in Cl⁻ level of normotensive rats compared to control and there was no difference in the chlorine level of

hypertensive rats compared with the control rats. De Bacquer *et al.* [28] stated that serum $\text{Cl}^- < 100$ mEq/L was associated with an increased risk of all forms of diseases. Hence it can be deduced from the result that *Phyllanthus amarus* has an effect on preventing cardiovascular and non-cardiovascular diseases.

When comparing the effect of *Phyllanthus amarus* on each treatment group (normotensive and hypertensive group respectively) with the control group, the findings of this experiment shows that there was about 1.5% increase in K^+ level and 9.4% decrease in K^+ level compared to the control rats, 5.3% decrease in Na^+ level and 6.4% decrease in Na^+ level as well as 11.8% decrease in Cl^- and 2.7% decrease in Cl^- .

Conclusion

The aqueous extract of *Phyllanthus amarus* leaves result in a non-significant difference in serum sodium, potassium and chlorine levels which have been associated with the risk of developing hypertension. Higher sodium level and lower potassium also has been found to be responsible for the development of hypertension. Therefore leaves that have hypotensive effect such as *Phyllanthus amarus*, can be taken to adjust electrolyte imbalance that leads to hypertension. Hence, this may go a long way in preventing the development of high blood pressure and cardiovascular diseases.

It can be concluded that administration of *Phyllanthus amarus* extract up to 200 mg/kg body weight can be useful in managing the serum electrolytes to a moderate amount due to its effect on Na^+ and Cl^- level in the blood. But it is also important to consume this extract in small amounts as it has a K^+ lowering effect in the blood.

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Authors Contribution

Osonuga Ifabunmi Oduyemi and Bello Solape Faderera conceived, designed the study and participated in data collection and analysis, Olukade Baliqis Adejoke and Ogunlade Albert Abiodun conducted literature search, Ezima Esther Nkechi and Adegbesan Bukunola Oluyemisi conducted data analysis, Olalekan Samuel Oluwadare participated in data collection.

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Ethics approval

This study was conducted upon ethical consideration approval that was received from Ethical Committee of Animal Experiment in Olabisi Onabanjo University, OOU, Ago-Iwoye, Nigeria.