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ORIGINAL ARTICLE

The Effect of *P. Amarus* Leaf Extract on The Electrolyte Profile Levels of Alloxan- Induced Diabetic Wistar Rat

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ABSTRACT

This study was designed to investigate the effect of Phyllantus amarus(PA) leaf extract on plasma electrolyte profile levels in alloxan induced diabetic albino wistar rats. A total of 30 albino wistar rats each weighing 100g were assembled and divided into 3 groups (A-C) consisting of 10 rats. Group A received PA treatment, B received ethanol treatment while group C served as the control group. 400mg/kg of aqueous extract of PA leaf was administered orally to the rats in group A but not in group B while group C received only water for 7 days. Blood samples were collected into lithium heparin containers for estimation of biochemical parameters (Na^+,K^+, Cl^-, $HCO3^-$) respectively. Plasma Na^+,K^+, Cl^- and $HCO3^-$ were analyzed using Ion selective electrode (ISE). There was a significant increase in the mean plasma Na^+ and K^+ levels (p=0.002; and 0.000 respectively) whereas, there were no significant differences seen in the mean plasma levels of Cl^- and$HCO3^-$ of the rats with Phyllantus amarus treatment than in those with ethanol treatment (p=0.239; 0.952 respectively). However, there was a significant decrease in the mean weight of the rats treated with PA (p=0.000) compared to those rats with ethanol treatment and control group respectively. Therefore, further studies may be required to better understand and elucidate the mechanisms behind these findings.

KEY WORDS: *Phyllantus amarus, Sodium, Potassium, Chloride, Bicarbonate, Weight.*

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INTRODUCTION

Plants that have medicinal properties with an optimum active ingredient in some form or another are regarded as medicinal plants. These are invaluable natural resources; they are exhaustible if overused and sustainable if the juxtaposition of present and future needs takes place within the behavioural pattern of various kinds of users [17]. The world primary means of treating diseases and fighting infections has been the use of medicinal plant species. From ancient times, plants have been rich sources of effective and safe medicines [16]. Traditional herbal medicine has been a constant source of substances for the treatment of a variety of diseases [7]. According to Manadhar, [9] traditional herbal medicine has been used since ancient times in many parts of the world. About 85% of the traditional herbal medicines used for primary health care are derived from plants [6]. In Africa, traditional herbal medicine derived from plants forms an integral part of life in many indigenous communities as a readily available

alternative to allopathic medicines [20]. Plants have been an indispensable source of both preventive and curative traditional herbal medicinal preparations for many people in Africa. Traditional herbal medicine is of great value, and more than 70% of the people in Africa refer to traditional herbal healers concerning health issues [19]. Traditional herbal medicine has flourished in Africa and has continued to be the main source of health in the rural communities and is heavily relied on by the majority of the sub-Sahara African population.

Phyllanthus amarus is a broad spectrum medicinal plant that has received world- wide recognition [3]. In Nigeria, it is called "Oyomokeisoamankedem" in Efik, "Iyin Olobe" in Yoruba and "Ebebenizo" in Bini [5, 11]. *P. amarus* is generally employed to reduce pain, expel intestinal gas, to stimulate and promote digestion, as anti-helminthes to expel intestinal worms and act as a mild laxative. *P. amarus* also has antiseptic, diuretic, antiviral, anti-diabetic, hypotensive and antipyretic properties, and is also used in the treatment of jaundice, diarrhoea, dysentery, wound, ulcers and urogenital diseases [18, 14, 11].

The plants of the genus *Phyllanthus* are widely distributed in most tropical and subtropical countries and have long been used in traditional medicine to treat chronic liver disease [8]. *P. amarus* has been reported to contain, alkanoids, flavonoids, germanuin, tannin, phyllantine, phypohyllanthine, quercetin, isoquercitrin, astraglin, geranin, corilagin, rutin and 3-0-gluscopyyranoside [2]. *P. amarus* is useful in ophthalmia, gonorrhea sores, swelling and itchiness and hypoglycaemic in nature. It promotes liver functions [12, 1]. Therefore, this study seeks to investigate the effect of *P. amarus o*n the electrolyte profile levels of alloxan- induced diabetic wistar rat.

MATERIAL AND METHODS

Study Location

The study was carried out at The Human Biochemistry Laboratory, Nnamdi Azikiwe University. It is located in the suburb of Nnewi - a popular town in Anambra State Nigeria.

Collection and identification of plant

The *Phyllantus amarus* plant was collected from Okofia College of Health Sciences and Technology, Nnamdi Azikiwe University Nnewi campus, Anambra state Nigeria in the month of January, 2016 and identified by Mrs. Aziagba B.O., Department of Botany, Nnamdi Azikiwe University, Akwa.

Animals

Wistar albino rats (100g) of both male and female were obtained from the Institute Animal House and maintained at 25±2 ºC temperature and relative humidity 45-55% under 12:12 h light:dark cycle. Rats were fed with standard rat chow and water *ad-libitum*.

Preparation of the plant extract

The method used is based on the method described by Kalita *et al; (*2013), although with some modification. About 150 g of dried leaves of *Phyllantus amarus* were taken in a 1000 mL of the round bottom flask and extracted for 72 h by a continuous hot percolation process using the solvent ethanol as solvent. The extracts were filtered through the Whatmann filter paper to remove impurities. The extracts were then concentrated by vacuum distillation, cooled and placed in desiccators to remove the excessive moisture.

Alloxan *induced hyperglycemia*

Animals were divided into three groups, each consisting of ten rats. Rats in the first group (A) received 400mg/kg *Phyllantus amarus* dissolved in ethanol while the second group of rats (B) received ethanol. Rats in groups 3 were normal rats and served as the control groups (C).

All the animals received their respective assigned treatment daily for a period of seven days. Rats were daily fasted over night before *Phyllantus amarus* treatment. On day 8, the animals were anesthetized with ether, and blood was collected using cardiac puncture into lithium heparin containers for estimation of biochemical parameters respectively. Plasma electrolytes (sodium, potassium, chloride and bicarbonate) were analyzed using Ion Selective Electrode (ISE).

Ethical Consideration

The protocol was approved by the Faculty of Health Sciences and Technology ethical committee, Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria.

Inclusion and Exclusion criteria

Apparently healthy Wistar rats weighing 100g were included for the study while Unhealthy Wistar rats with weight less or above 100g were excluded from the study in order to ensure accuracy and uniformity in result interpretation.

Statistical Analysis

Statistical package for social science (SPSS) version 20 was employed in the analysis of the result. The results for the parameters studied were expressed as Mean± SD and the data were analyzed for general

group differences using one way ANOVA while post-HOC comparison was used to determine the inter-group differences. Correlation was done using Pearson correlation r and Level of significance was set at $p<0.05$.

RESULTS

The mean plasma levels of Na^+ andK^+ were significantly different, where as, the mean plasma levels of Cl^- and$HCO3^-$ did not differ significantly when compared among the groups using ANOVA (see table 1).

There was a significant increase in the mean plasma Na^+level when compared between rats with *Phyllantus amarus* treatment (A) and those with ethanol treatment (B) (140.30±2.87 Vs 140.20±1.23; p=0.002). Again, the mean plasma Na^+ level was significantly increased in the rats treated with *Phyllantus amarus* compared with the control group (140.30±2.87 Vs 135.30±4.50; p=0.002), (see table 1).

More so, there was a significant increase in the mean plasma K^+ level of the rats with *Phyllantus amarus* treatment than in those with ethanol treatment (8.08±1.85 Vs 7.40±1.98; p=0.000). Again, the mean plasma K^+ level was significantly increased in the rats after *Phyllantus amarus* treatment than in the control group (8.08±1.85 Vs 3.94±0.33; p=0.000), (see table 1).

However, there were no significant differences seen in the mean plasma levels of Cl^- and$HCO3^-$ when the rats with *Phyllantus amarus* treatment were compared between those with ethanol treatment as well as the control group respectively (p>0.05), (see table 1).

Again, following administration of *Phyllantus amarus*, there was significant decrease in the mean weight of the rats (p=0.000) compared to those rats with ethanol treatment and control group respectively (see table 1).

Table 1: Plasma electrolyte profile levels in alloxan induced diabetic rat with *Phyllantus amarus* treatment (A), with ethanol treatment (B) and in control group(C) (Mean ± SD, n = 10).

Group	Na^+(mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	$HCO3^-$ (mmol/l)	Weight (g)
A (n =10)	140.30±2.87	8.08±1.85	100.40±4.09	16.30±1.77	98.80±1.03
B (n= 10)	140.20±1.23	7.40±1.98	103.40±1.07	16.20±0.92	119.40±1.17
C (n=10)	135.30±4.50	3.94±0.33	99.70±7.67	16.50±3.21	100.60±0.84
F (P) –valve	8.176 (0.002)	20.139 (0.000)	1.510 (0.239)	0.049 (0.952)	150.000 (0.000)
A VB	<0.05	<0.05	>0.05	>0.05	<0.05
A VC	<0.05	<0.05	>0.05	>0.05	<0.05
B V C	>0.05	>0.05	<0.05	>0.05	<0.05

All values are expressed as Mean ± Standard deviation (SD) with P<0.05 considered as significant.

Keys: F (P) – Value = Mean ±SD of parameter compared among group A, B and C using (ANOVA); AvB P-Value =Mean ±SD of parameter compared between group A and B using (t-test); BvC P- Value = Mean ±SD of parameter compared between group B and C using (t-test); AvC P- Value = Mean ±SD of parameter compared between group A and C using (t-test).

There was a significant positive correlation between the mean chloride concentration and the mean weight of the rats studied in control group (r=0.649; p=0.042), see table 2.

Table 2: Level of association between plasma levels of sodium, potassium, chloride and bicarbonate studied in control group(C) (Mean ± SD, n = 10).

Parameter	Pearson correlation r	P-value
Cl^- Vs Weight	0.649	0.042

***Statistically significant at p<0.05.**

The mean plasma concentration of potassium and bicarbonate had a significant strong positive correlation (r=0.846; p=0.002), in the rats treated with ethanol, see table 3.

Table 3: Level of association between plasma levels of sodium, potassium, chloride and bicarbonate studied in alloxan-induced diabetic rats with ethanol treatment (B) (Mean ± SD, n = 10).

Parameter	Pearson correlation r	P-value
K Vs $HCO3^-$	0.846	0.002

***Statistically significant at p<0.05.**

Also, the mean plasma concentration of potassium and chloride had a very significant strong positive correlation (r=0.952; p=0.000) in the rats treated with *P. amarus* extract, see table 4.

Table 4: Level of association between plasma levels of sodium, potassium, chloride and bicarbonate studied in alloxan-induced diabetic rats with *P. amarus* treatment (A) (Mean ± SD, n = 10).

Parameter	Pearson correlation r	P-value
K Vs Cl^-	0.952	0.000

***Statistically significant at p<0.05.**

DISCUSSION

Traditional herbal medicine has been a constant source of substances for the treatment of a variety of diseases [7].*P. amarus* has antiseptic, diuretic, antiviral, anti-diabetic, hypotensive and antipyretic properties, and is also used in the treatment of jaundice, diarrhea, dysentery, wound, ulcers and urogenital diseases [18, 14, 11].

In this study,there was a significant increase in the mean plasma Na^+level when compared between rats with *Phyllantus amarus* treatment (A) and those with ethanol treatment (B) (140.30±2.87 Vs 140.20±1.23; p=0.002). Again, the mean plasma Na^+ level was significantly increased in the rats treated with *Phyllantus amarus* compared with the control group (140.30±2.87 Vs 135.30±4.50; p=0.002). This is in consonance with the report of Peters *et al*. who studied the effect of ethanolic leaves extract of *Phyllantus amarus* on cisplatin induced nephrotoxicity in albino rats and reported a significant increase in plasma Na^+ concentration following the treatment of the rats with *Phyllantus amarus* [15]. Also, Onyesom *et al*. [13] Who investigated the antiplasmodial activity of *phyllanthus amarus in* preserving renal function in *plasmodium berghei* infected mice reported a significant increase in the mean serum Na^+ concentration after the *plasmodium berghei* infected mice were treated with *Phyllantus amarus leaf extract* [13]. The mechanism behind this increase is yet unknown.

The present study showed a significant increase in the mean plasma K^+ level of the rats with *Phyllantus amarus* treatment than in those with ethanol treatment (8.08±1.85 Vs 7.40±1.98; p=0.000). This is in contrast with the report of Peters *et al*. who studied the effect of ethanolic leaves extract of *Phyllantus amarus* on cisplatin induced nephrotoxicity in albino rats and reported a significant decrease in plasma K^+ concentration following the treatment of the rats with *Phyllantus amarus* [15]. However, Onyesom *et al*. [13] did report a similar result to our present findings. This may be due to a compensatory response of the renal system in moderating the concentration of Na^+ in order to maintain a balance in the body fluid compartments. Potassium helps regulate the amount of sodium in the blood, thus helping to keep the sodium level in check and thereby lowering the risk of developing high blood pressure (hypertension) and other cardiovascular complications [4].

Furthermore, the present study revealed no significant differences in the mean plasma levels of Cl^- and$HCO3^-$ when the rats with *Phyllantus amarus* treatment were compared between those with ethanol treatment as well as the control group respectively (p>0.05).

However, following administration of *Phyllantus amarus*, there was significant decrease in the mean weight of the rats (p=0.000) compared to those rats with ethanol treatment and control group respectively.

Finally, there was a significant positive correlation between the mean chloride concentration and the mean weight of the rats studied in control group (r=0.649; p=0.042), while the mean plasma concentration of potassium and bicarbonate had a significant strong positive correlation (r=0.846; p=0.002), in the rats treated with ethanol. Also, the mean plasma concentration of potassium and chloride had a very significant strong positive correlation (r=0.952; p=0.000) in the rats treated with *P. amarus extract.*

CONCLUSION

In conclusion, the present study revealed a significant increase in the mean plasma Na^+ andK^+ levels, whereas, no significant differences in the mean plasma levels of Cl^- and$HCO3^-$ were observedwhen the rats with *Phyllantus amarus* treatment were compared between those with ethanol treatment. Also, the mean plasma concentration of potassium and chloride had a very significant strong positive correlation in the rats treated with *P. amarus extract.* Therefore, further studies may be required to better understand and elucidate the mechanisms behind these findings.

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