ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Kidney Homeostasis and Histology: The Role of Alcoholic Extract of *Phyllantus amarus*.

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Manuscript Info

Manuscript History:

Received: 14 December 2015
Final Accepted: 19 January 2016
Published Online: February 2016

Key words:
Tubal, dilation, histology, biochemistry, sodium heamatoxylin

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Abstract

Phyllantus amarus is an ethnobotanical plant widely use in Africa for management of various ailments of the kidney. We aimed at evaluating the effect of ethanolic extract of *Phyllantus amarus* on kidney homeostasis and morphology in alloxan induced diabetes in adult Wistar rat. Wistar Rats of both sex (n=20), averaging 150-250g were randomly assigned into four groups: A, B, C and control of n=5 respectively. Group A, B, and C served as experimental groups. The experimental groups received 50mg/kg, 100mg/kg, and 200mg/kg of alcoholic extract of *Phyllantus amarus* orally via orogastric tube for 21days. Sodium, potassium, chloride, bicarbonates, glucose, urea and Creatinine were analyses using ion selective electrode and spectrophometric method. Kidney morphology was evaluated using 10% formal saline fixed, paraffin embedded processed tissues and heamatoxylin and eosin staining techniques. Biochemical assay shows antidiabetic properties, significant increase in chloride and bicarbonates ions in group A, B and C and potassium ion only in group A compared with control. Urea and Creatinine were normal in the groups with corresponding increase in body weight. Histological findings show several portions of mild necrosis and effacement of renal corpuscles compared with control group. The finding indicated that the administration of ethanolic extract of *Phyllantus amarus* has adverse effects on body homeostasis and kidney morphology despite its tradomedicinal importance. The current findings suggest that *Phyllantus amarus* consumption should be control to prevent renal impairment.

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Introduction:-

Phyllantus amarus is an ethnobotanical plant distributed in almost all the tropical regions including America, Indian and Nigeria and its medicinal use cited since the 18th century. It is a common weed that grows well in moist, shady and sunny places (6). It is of the family of euphorbiaceous with about 800 species found in tropical and subtropical countries of the world.

Phyllantus amarus is a branching annual glabrous herb which is 30-60cm high and slender with sub sessile, elliptic oblong, obtuse round base leaves and flowers are yellowish, whitish and greenish flowers (7, 9) *Phyllantus amarus* is generally employed in range of illness including its use in the treatment of jaundice, diarrhea and dysentery, wound healing and ulcers, and other urogenital diseases (3,10). Pytochemical analysis of *Phyllantus amarus* confirmed the presence of tannins, saponin, lignids, polyphenolyic compounds, tetracyclic triterpenoids and steroids (9). It has been classified among plants with low potential for toxicity with an LD50 averaging 2000mg/kg/day.

The kidney is highly vascularised organ that function to maintain the composition of body fluids and homeostasis (15). It also regulates the body fluids and electrolytes and the production or renin and erythropoietin responsible for the regulation of blood pressure and erythrocytes respectively (13). Electrolytes such as potassium, sodium, bicarbonates, and chlorides help to regulate heart and neurological function, fluid balance, oxygen delivery and acid base balance and however electrolytes imbalance can occur due to certain disease condition of the kidney (15). The effect of *Phyllantus amarus* on the kidney has been widely studied and the traditional use of *Phyllantus amarus* in the treatment of kidney and gall bladder stones has been validated by clinical research (3, 5) with little or no emphasis on homeostasis thus the justification for this study on the kidney morphology and homeostasis.

Diabetic mellitus is a group of metabolic disease in which the body experience hyperglycemia either because the body does not produce enough insulin or because cells do not respond to circulating insulin. Classic symptoms are polyuria (frequent urination), polydipsia (increase thirst) and polyphagia (increased hunger) (2). According to world health organization (WHO) there are approximately 160,000 diabetics worldwide and due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes is a major medical concern. The aim of this work is to evaluate the homeostatic, anti-diabetic properties, and histological alteration of *Phyllantus amarus* in the kidney of diabetic and non diabetic adult wistar rats.

Materials and Method

Plant Identification and Extraction

The *Phyllantus amarus* leaves were identified and authenticated in the Department of pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island Bayelsa State. Cold extraction of *Phyllantus amarus* leaves was carried out at room temperature of 18^0C-22^0C (1). 500g of the dried coarse leaves was introduced into a standard volumetric flask and dissolved with equal volume of distilled water and 95% ethanol, stopper with cotton wool and allowed to stand at room temperature for 48hrs for complete extraction. The aqueous extraction was filtered off into pre-weighed evaporating dishes. The filtrates were evaporated into a syrupy residue using a rotary extractor at 40^0C. The extracts were pooled together into an airtight container and stored refrigerated at -4^0 C until required for use.

Extract administration

Fresh preparations were made on each day of the experiment and the resulting solution inserted orally using orogastric tube into adult albino wistar rat.

$$\text{Administered volume} = \frac{\text{Effective Dose (ED)}}{\text{Stock solution}}$$

Effective dose = Dose assigned to each group

Stock solution = mg/ml of distilled water (8).

Experimental Design

Experimental Animals

Albino rats weighing 150 –200g were obtained and kept in the animal house of Medical Laboratory Science Department, Niger Delta University Bayelsa State, Nigeria. The animals were housed under controlled conditions with 12:12 hour light/dark cycle in standared cages with access to standared feed (Vita feed®, Ibadan) and water *ad libitum.* The animals were acclimatise for 2 weeeks in the laboratory and fasted for 24 hour before the assay. The experiment were executed after the approval of the protocol by the Niger Delta University wilberforce Island Animal Ethics Committee and were executed in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigation in conscious animals.Twenty adult albino rats were randomly divided into four groups (A, B, C, and D) and the initial and final weights of all animals were recorded using Saltun EK 5055max weighing balance. Group A, B and C were administered *Phyllantus amarus* leaves extract orally using orogastric tube with 50mg, 100mg, and 200mg per kilogram body weight of the extract for 21 days respectively while the control were on normal feeds.

Induced- diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan monohydrate at the dose of 15mg kg−1 in normal saline to the control group after an overnight fast. Diabetes was confirmed using Accu -check test after 72 hours and blood glucose range of 102 -110mg/dl was ascertained.

Blood samples collection

Blood samples was collected by Cardiac puncture under chloroform anesthesia into fluoride oxalate contains (for blood sugar) and into sterile container. The blood samples in the sterile bottles were allowed to clot and retracted at room temperature. Sera were separated into plastic vials and stored in the freezer (- 20^0C) and allowed thaw at room temperature before biochemical assay. Animal sacrifice was by cervical dislocation and the kidneys were removed at necropsy and fixed in 10% buffered formalin saline.

Instrumentations and equipments

Automatic tissue processor (Leica TP 1020), Embedding panel (Leica EG 1160), Microtome (Leica RM 2125), Spectrophotometer (Kayto RT-920 semi auto chemistry analyzers), Water bath(Leica), Digital weighing balance(Metar MT-301), conical flask, Digital microscope(UNISCO), Dissecting sets, Automatic pipettes, Ion selective electrodes and histochemical stains.

Histological methods

Tissues were sectioned at 4µm on a Rotary microtome (Leica RM 2125) and stained with Erhlich's heamatoxylin and eosin staining technique using the method of Aviwioro, 2002(16). The tissues were processed in Niger Delta University Okolobiri while photomicrographs were taken in the Department of Anatomical Pathology, Niger Delta University, and Wilberforce Island Bayelsa State.

Biochemical assay

Glucose oxidase method using spectrophotometer method was use for glucose estimation while Creatinine and Urea estimation was by kinetic method (di-acetyl- monoxime method). Sodium, potassium, chlorides and bicarbonates electrolytes were estimated using ion selective electrode method.

Statistical analysis:-

One-way analysis of variance and the Tukey's post hoc test were used to assess the significance of differences between groups. Values were expressed as mean ± SD. A *P* value < 0.05 was considered to be significant. Analysis of variance was performed using Graph-Pad Prism 5 (GraphPad Software, San Diego, CA).

Result:-

Table 1 Mean Weight (g) of the Adult Wistar Rats

GROUP	INITIAL WT(G)	FINAL WT(G)	P.VALUE	COMMENT
CONTROL	240.6±27.190	259±33.675	P>0.05	NS
A	208±35.007	222±31.249	P>0.05	NS
B	187.6±24.100	216.8±16.649	P>0.05	NS
C	225.8±21.545	226.6±17.401	P>0.05	NS

Values represented as Mean ± standard deviation. Values are statistically different from the initial weight at P<0.0.5* using one way analysis of variance (ANOVA) + Turkey-Kramer multi comparison test.

Table 2 Fasting Blood Sugar (mmol/l) Among Groups Studied.

	Control	Group A	Group B	Group C
Day 0	93.75±9.179	87.2± 12.696	93.4 ± 10.597	72.8 ±9.338*
Day 3	93.5 ± 8.185	182.2 ± 13.217***	182.8 ± 13.700***	174.8±8.585***
Day 21	94 ± 8.907	63 ± 7.969**	59.4 ± 13.740***	53 ± 7.681***

Values represented as mean ± standard deviation. Values are statistically different from the initial weight at P<0.0.5*, **0.01 and ***0.001 using one way analysis of variance (ANOVA) + Turkey-Kramer multi comparison test.

Table 3 Renal Function Test and Electrolytes of Adult Wistar Rats Studied.

Parameter (Mmol/L)	Control	Group A	Group B	Group C
Potassium	0.8250±0.4193	0.4000 ± 0.07071*	0.4800 ± 0.1483	0.4600 ± 0.05477
Sodium	106.50 ± 5.000	116.20 ± 20.142	98.400 ± 1.817	114.60 ± 4.506
Chloride	90.750 ± 5.252	68.800 ± 2.950***	74.000 ± 4.000***	71.600 ± 3.647***
HCO_3^-	4.000 ± 1.826	19.400 ± 3.050 ***	11.000 ± 2.000**	25.400 ± 2.608 ***
Urea	6.125 ± 0.2217	7.840 ± 1.815	8.700 ± 1.384	8.020 ± 1.443
Creatinine	47.500 ± 3.416	66.400 ± 3.130	54.000 ± 27.083	52.600 ± 26.548

Values represented as mean ± standard deviation. Values are statistically different from the initial weight at P<0.0.5*, **0.01 and ***0.001 using one way analysis of variance (ANOVA) + Turkey-Kramer multi comparison test.

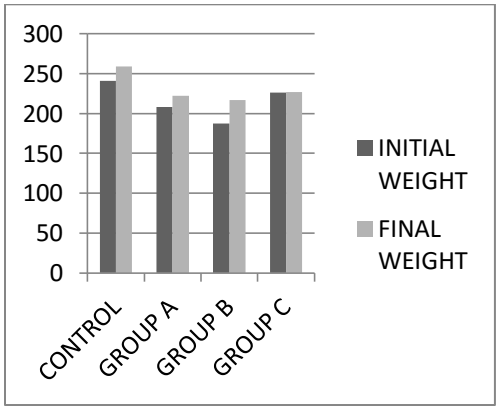


Figure 1 mean initial and final body weight (kg) of the adult Wistar rat before and after administration of *Phyllantus amarus*

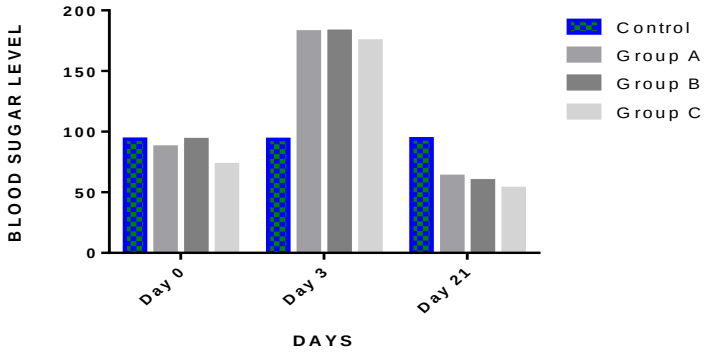


Figure 2: Blood Sugar Per day (mmol/l) after administration of *Phyllantus amarus*

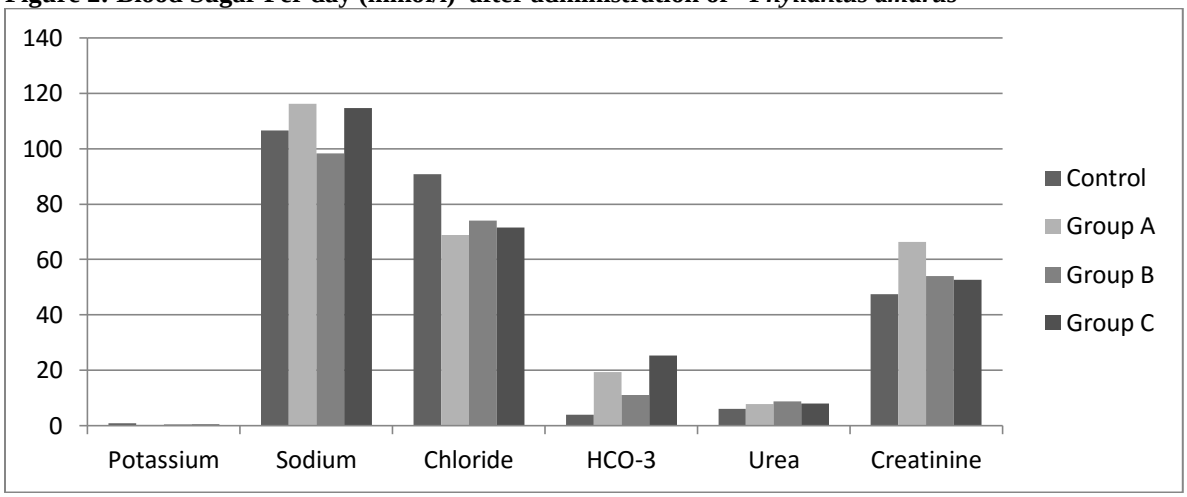
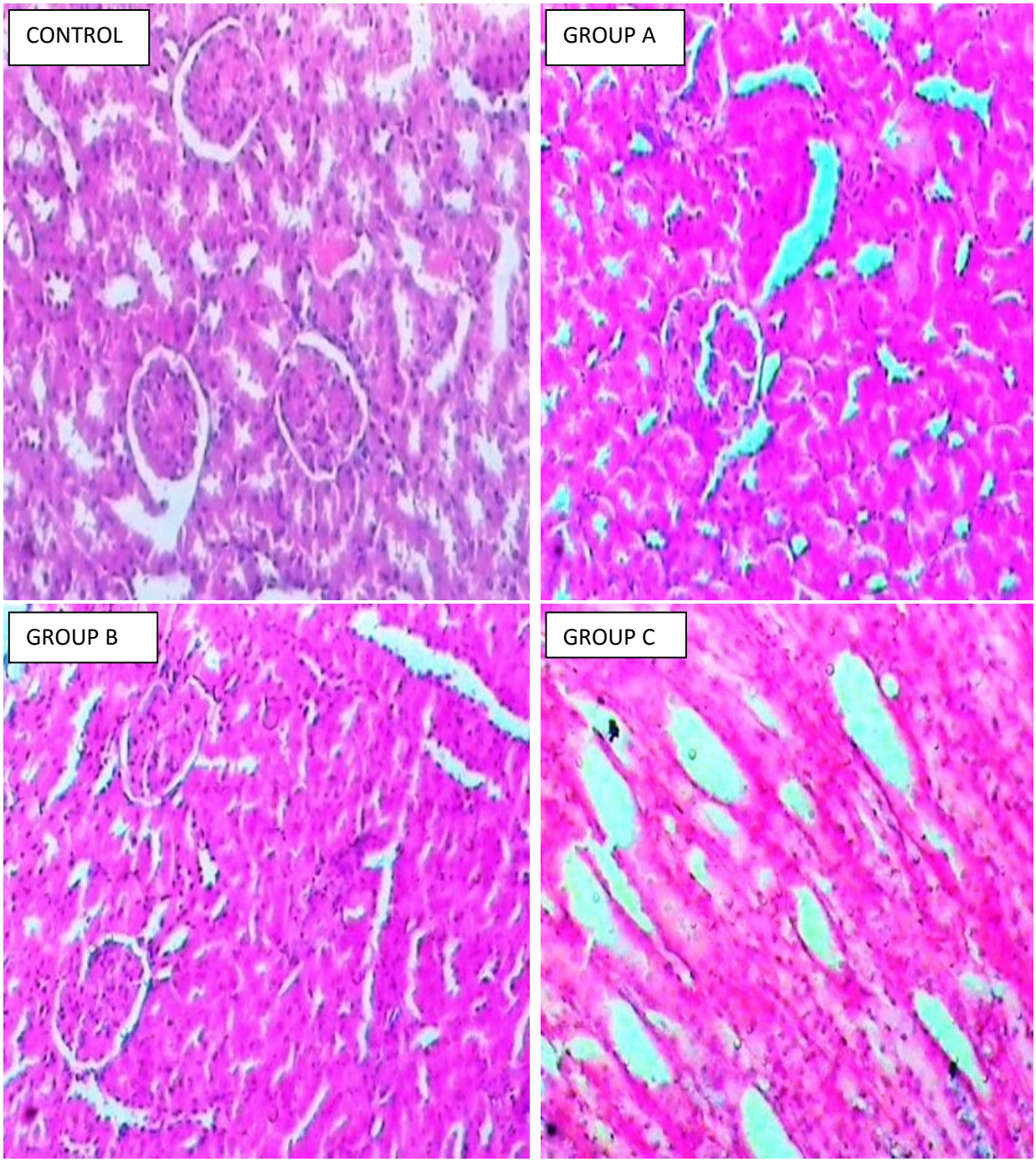


FIGURE 3 serum electrolytes after 21 days administration of *Phyllantus amarus*

Photomicrographs:-

Control show a normal kidney with intact glomeruli. The glomeruli exibit normal bowman capsule and urinary space. The proximal and distal convulated tubules together with the blood vessels are normal. This group was given only distilled water and standard diet.Group A show a section of kidney administred with 50mg/kg of *Phyllantus amarus*, showing normal glomeruli and mild dilation of proximal tubules. Group B show a section of kidney administred with 100mg/kg of *Phyllantus* amarus showing normal glomeruli tubules with increase cellularity (Heamatoxylin and Eosin X400)**.** Group C show a section of atrophic kidney administred with 200mg/kg of *Phyllantus amarus*. Section show prominent loss of glomeruli and dilation of both proximal and distal convulated tubules and increase cellularity. The blood vessels are abnormal. (Heamatoxylin and Eosin X400)


CONTROL
GROUP A
GROUP B
GROUP C

Discussion

Phyllantus amarus is an ethno botanical plant that is distributed in almost all tropical regions including America, Indian and Nigeria and its medical use is cited since the 18th century. Administration of aqueous extract for 21 days causes an increase in body weight and was statistically different which could be attributed to anabolic steroid found in the extract (Table 1). De-piccolo *et al.*, 1991(4) reported that steroid are used to stimulate bone marrow and growth, lean body mass and also play role in the prevention of bone loss even in the elderly and is agreement with the present study.

Oral administration of ethanolic extract of *Phyllantus amarus* lowers blood glucose concentration even at the first three days of administration with a direct proportionality in dosage, the effect was more pronounce on group C(200mg/kg) for 21 days duration(94±8.907 in control and 53±7.681 in Test group C).

Electrolytes play a vital role in maintaining the body homeostasis, regulatation of the heart and neurological function, fluid balance, oxygen delivery to tissues and acid base balance of the body. However electrolytes imbalance can develops due to reduction of electrolytes, excessive ingestion, and the most common had been kidney failure. This study shows electrolytes alterations in both the intracellular fluids and extracellular fluids except for sodium following administration of the extract. The electrolytes imbalance was more pronounced in chloride and bicarbonates levels and minimal in potassium. This bears a direct effect on the effacement of glomeruli as seen in the renal histology. Urea and creatine regarded as parameters for evaluating the kidney integrity were not statistically significant compared with the control thou a substantial difference in their values.

Renal histology result (heamatoxylin and eosin) revealed that administration of *Phyllantus amarus* can caused varying degree of Cyto-architectural distortion and vasculogenic effect on the kidney which affected blood vessel and inflammatory cells seen in the treatment group compared with the normal. Degenerative and mild necrotic changes were observed more in groups that received higher doses (200mg/kg) of *Phyllantus amarus* resulting in areas of mild necrosis with effacement of renal corpuscles in the cortex. The histological effect observed in this experiment is in consonance with the report of Manjreka *et al.*, 2008 who observed that *Phyllantus amarus* induced deleterious changes on the renal tubules and testes of male rats (4, 11). It is noteworthy that *Phyllantus amarus* contains alkaloids and lots of antioxidants (7, 9, 14) and these antioxidant are associated with toxicities especially if taken arbitrarily (12). The important of this report lies on the potential adverse effect of *Phyllantus amarus* on the microanatomy of the kidney despite its medicinal relevance. *Phyllantus amarus* has been indicated to cause necrosis and protein cast in the tubules (11) in addition to tubal dilations and effacement of glomeruli seen in this present work. The necrosis observed in this present study could be attributed to higher doses of *Phyllantus amarus* on the kidney. However the exact mechanism by which *Phyllantus amarus* induced cellular degeneration on the kidney tissue in this experiment requires further studies on immunohistochemistry and other molecular markers studies.

Conclusion

The result obtained in this study revealed that administration of *Phyllantus amarus* can affect the histology of the kidney of adult Wistar rat causing tubal dilation, mild necrosis with effacement of renal corpuscles resulting in distortion of the Cyto –architecture of the kidney despite its tradomedicinal important in the treatment of gall stones, kidney stones and other related kidney related ailment. This result suggests that renal function may be impaired by administration of *Phyllantus amarus* the extract. It is therefore recommended that consumption of *Phyllantus amarus* leave extract should be control especially for individual with renal impairment.

Acknowledgment:-

We wish to acknowledge MLS Charles Agu of the department of histopathology NDUTH Okolobiri bayelsa state.
Research was self sponsor.
Source of funding- None.
Conflict of interest- Nil

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