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Kidney Function Evaluation in Wistar rats after *Phyllanthus amarus* Aqueous Leaf Extract Oral AdministrationOduola, T.^{1*}, Oyeniyi, Y.J.², Okanlawon, S.O.¹, Tajudeen, M.¹, Aiyelabegan, F.¹*Department of Chemical Pathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto¹, Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto²**Author for Correspondence*: oduola.taofeeq@udusok.edu.ng/ +234- 803-396-8785***Abstract**

Aqueous leaf extract of *Phyllanthus amarus* is being employed in the treatment of different diseases in North Western part of Nigeria, and because plants phytochemistry differs from one region to another as a result of variation in soil constituents. The aim of this study was to assess the effect of ingestion of aqueous leaf extract of *Phyllanthus amarus* obtained from this region on kidney functions in Wistar rats. The oral acute toxicity of the extract (LD₅₀) was determined in 9 Wistar rats divided into 3, 3 rats per group. Group 1 was the control and received distilled water. Doses of 2000mg/kg and 5000mg/kg were administered in a single oral dose to the study groups (2 and 3) respectively, one animal per day. A sub-chronic toxicity study was carried out in 25 Wistar rats, divided into 5 groups of 5 rats each. Group 1 served as the control and received distilled water and standard rat pellets. The experimental groups (2, 3, 4 and 5) were administered with different doses of 250mg/kg, 500mg/kg, 750mg/kg, 1000mg/kg of *Phyllanthus amarus* aqueous leaf extract daily respectively for 28 days. At the end of the experiment, the rats were sacrificed and blood samples taken for kidney function assessment and kidney for histological examination using standard techniques. No death was recorded in the rats after 24hours and up to 14 days post oral treatment in acute toxicity study, in sub-chronic toxicity study, the result did not reveal dose-response relationship in all the parameters, as the values obtained for the control and experimental animals were fluctuating within the reference range. Histology of the kidney reveals normocytic normochromic cells. The findings

have shown that aqueous leaf extract of *Phyllanthus amarus* is relatively safe and may not be nephrotoxic in Wistar rats, and thus may not produce kidney damage in human.

Key words: *Phyllanthus amarus*, kidney, toxicity, wistar rats, ingestion

Introduction

The *Phyllanthus amarus* belongs to the family *Euphorbiaceae*, it is an erect annual herb of not more than one and half feet tall and has small leaves and yellow flowers. *Phyllanthus amarus* has been reportedly used in traditional medicine for its hepatoprotective, anti-diabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties (Oluwafemi and Debiri, 2008; Adeneye *et al.*, 2006a, b). The plant is also used in the treatment of stomach disorders, skin diseases and cold (Iwu, 1993; Kokwaro, 1976). It has anti-diarrhea effect (Odetola and Akojenu, 2000). Its anti-viral activity against hepatitis B virus has been established (Wang *et al.*, 1995; Thyagarajan *et al.*, 1988) anti-carcinogenic (Joy and Kuttan, 1998) and antimutagenic activities (Joy and Kuttan, 1998). It also has anti-nociceptive, anti-inflammatory activities and antimycotic properties (Ahmed and Sani, 2013; Kassuya *et al.*, 2003) antidiabetic and antilipidaemic potentials (Adeneye *et al.*, 2006a, b). It has been documented that leaf extracts of *P. amarus* may possess protective effect against oxidative stress in human (Oduola *et al.*, 2018). Ingestion of *P. amarus* leaf extract has also been reported to have no toxic effect on liver function and structure in Wistar rats (Oduola *et al.*, 2018).

The kidneys are involved in vital functions associated with homeostatic processes of the human body, such as filtration of blood, regulation of acid, water and electrolyte balance, regulation of hormone levels in the blood, and removal of waste products and unwanted substances from plasma (Henry, 2007).

Plants phytochemistry differ from one region to another due to variation in soil contents; though adverse effect on kidney function among the users of this plant in Sokoto and its environs have not been observed, no scientific proof have been documented to support this, hence this present study was designed to assess effect of *P. amarus* aqueous leaf extract ingestion on kidney function and structure in Wistar rats.

Materials and Methods

Plant Collection and Identification

Fresh leaves of *Phyllanthus amarus* were collected from Fadama dundaye area of Tambuwal LGA of Sokoto, Sokoto State, Nigeria. The plant was identified and authenticated at the herbarium unit of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The plant was given herbarium voucher number PCG/ UDUS/ PHYL/001 and was deposited in the herbarium.

Preparation and Extraction

Fresh leaves of *Phyllanthus amarus* were collected and air dried at room temperature for a period of six weeks. It was then crushed manually using mortar and pestle. 1kg of the grinded leaf was soaked in 5 litres of 80% methanol for 24 hours on a mixer to ensure maximum extraction by percolation method using maceration technique under room temperature. This was then followed by periodic stirring. The resulting crude extract was filtered using Whatman no 1 filter paper and the filtrate was concentrated to dryness in an oven at 40°C to obtain crude extract. The dried crude extract was stored at 4°C in sterile beakers at the Chemical Pathology Laboratory of the School of Medical Laboratory Science, UDUS, until required for use (Ahmed and Sani, 2013).

Experimental Animal

Male and Female albino rats of Wistar strains weighing between 150g – 170g were purchased from the animal house of the Faculty of Pharmaceutical Sciences, ABU, Zaria. They were left to acclimatize for a period of two weeks. They were fed and maintained in clean metabolic cage sand, placed in a well-ventilated room, conditioned with a temperature of 26°C - 28°C, photo periods of 12 hours light and 12 hours darkness; humidity of 40 to 60%. The rats were maintained on rat pellet feeds, which were obtained from the animal house of the Faculty of Pharmaceutical Sciences, UDU, Sokoto and had access to drinking water regularly. Cleaning of the animal cages was carried out daily. Experimental guidelines were followed as stipulated by our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (NIH,1992) and also in accordance with the recommendations of the International Association for the Study of Pain (Zimmerman, 1983).

Experimental Designs

Acute Oral Toxicity Studies

Acute oral toxicity study, Limit Test, was performed in accordance with the procedures outlined by the Organization for Economic Co-operation and Development Guidelines, OECD 423 (OECD, 2008a). Eighteen Wistar rats of both genders were used for this study. The rats were randomly divided into three groups of six rats per group with the first group serving as control. The extract was administered to the rats by intra gastric gavage using oral cannula (a feeding needle), one animal per day starting with group 1 (control) which was given distilled water only, and groups 2 and 3 were administered 2000mg/kg and 5000mg/kg of the extract respectively. The animals were observed within the first 4 hours and subsequently 24 hours for toxic symptoms. Also, behavioural parameters and mortality were closely monitored for 14 days.

Sub-chronic Toxicity Study

Sub-chronic toxicity study was carried out following OECD 407 guidelines. Forty Wistar

rats of both sexes were divided into five groups of eight rats per group. Group 1 served as the control and received distilled water as vehicle. Graded doses of crude extract were administered orally to the rats daily for 30 days as follows: group 1 (control): distilled water, group 2: 250mg/kg, group 3: 500mg/kg, group 4: 750mg/kg and group 5: 1000mg/kg. All the rats had free access to feed and water throughout the period of the experiment and they were observed daily for general symptoms of toxicity and mortality (OECD, 2008b).

Blood sample collection

Blood was collected by cardiac puncture from chloroform anaesthetized rats into heparinized bottles. The rats were later sacrificed using lumbar dislocation and their kidneys were removed. The blood samples collected were centrifuged at 4000 revolutions per minute (4000 rpm) for 10 minutes. The plasma of each sample was separated and transferred into cryovial and stored (frozen) at -20°C until required for analysis (Aniagu *et al.*, 2005).

Histological examination

The kidneys of the animals were fixed in 10% formalin labeled bottles after which the tissues were processed routinely using automatic tissue processor. This was immediately followed by embedding in paraffin wax. Sections of 5-6 μm thick were cut, stained with haematoxylin and eosin and examined under the light microscope (Orchard and Nation, 2012).

Determination of biochemical parameters

The biochemical analyses were performed at the Chemical Pathology Laboratory, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto. The separated plasma were used for the assays of electrolytes Na^+ , K^+ , HCO_3^- , Cl^- , (Henry *et al.*, 1974), urea and creatinine (Delaney *et al.*, 2013).

Data Analysis

The data obtained in this study were analysed using the statistical package for social sciences (SPSS) for windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The data were represented as the mean \pm standard deviation (S.D). Student t-test at 95% confidence interval was used to evaluate the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant when $p < 0.05$.

Result

Acute Oral Toxicity Study (Median Lethal Dose, LD_{50})

Table 1. Shows the result of median Lethal Dose determination of *phyllanthus amarus* leaf extract after a single oral dose administration of 2000mg/kg and 5000mg/kg of the leaf extract to groups 2 and 3 respectively. No death or any sign of toxicity were documented in the two groups after 24 hours and up to 14 days post oral administration. This reveals that LD_{50} is greater than 5000mg/kg.

Figures 1 and 2 shows the results of the effects of intake of *Phyllanthus amarus* leaf extract on kidney functions in Wistar rats in acute oral toxicity study.

Sub-Chronic Toxicity Study.

Figures 3 and 4 show the results of the effects of oral administration of *Phyllanthus amarus* leaf extract on kidney functions in Wistar rats.

Histopathological Results.

Figures 5, 6 and 7 shows kidney tissues (H & E x 100) in acute oral toxicity studies.

Figures 8, 9, 10, 11 and 12 shows kidney tissues (H & E x 100) in sub-chronic toxicity studies.

Table 1: Acute oral toxicity study of *Gliricidia sepium* leaf extract in Wistar rats

Groups	No. of death or abnormal behaviour (24 hours)	No. of death or abnormal behaviour (72 hours)	No. of death or abnormal behaviour (14 Days)
Group 1(control)			
Day 1:1 st rat	0	0	0
Day 2:2 nd rat	0	0	0
Day 3:3 rd rat	0	0	0
Group 2(2000mg/kg)			
Day 1:1 st rat	0	0	0
Day 2:2 nd rat	0	0	0
Day 3:3 rd rat	0	0	0
Group 3(5000mg/kg)			
Day 1:1 st rat	0	0	0
Day 2:2 nd rat	0	0	0
Day 3:3 rd rat	0	0	0

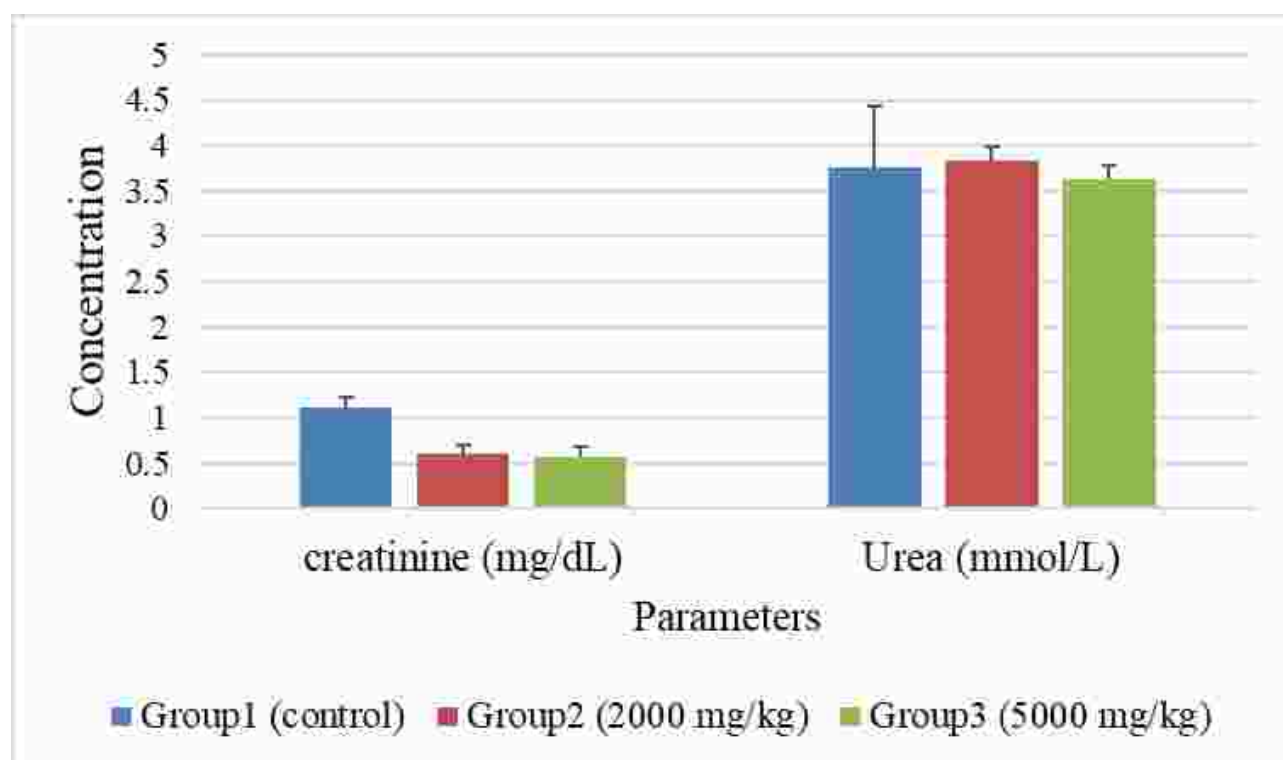


Figure 1. Effect of *phyllanthus amarus* aqueous leaf extract on kidney functions (Creatinine and Urea) in Wistar rats in acute toxicity study.

Values are in Mean ± S.D.

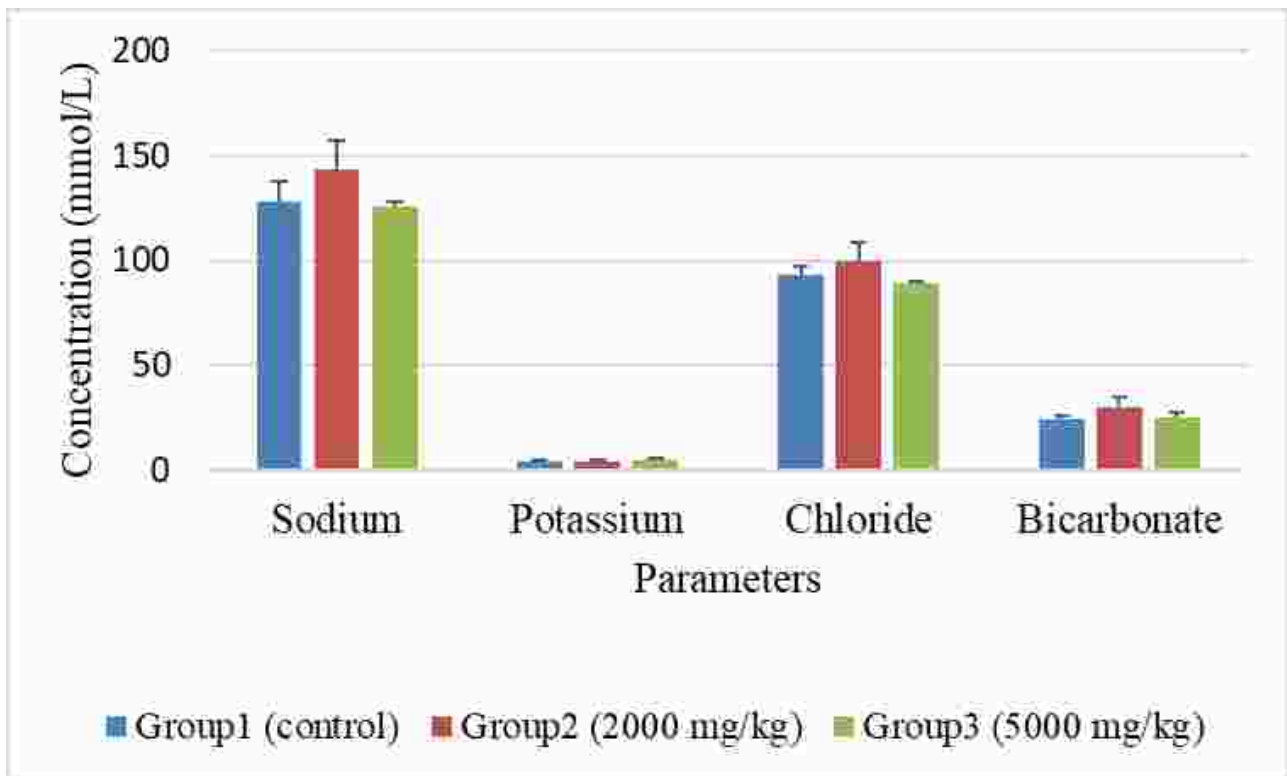


Figure 2. Effect of *phyllanthus amarus* aqueous leaf extract on kidney functions (Electrolytes) in Wistar rats in acute toxicity study.
 Values are in Mean \pm S.D.

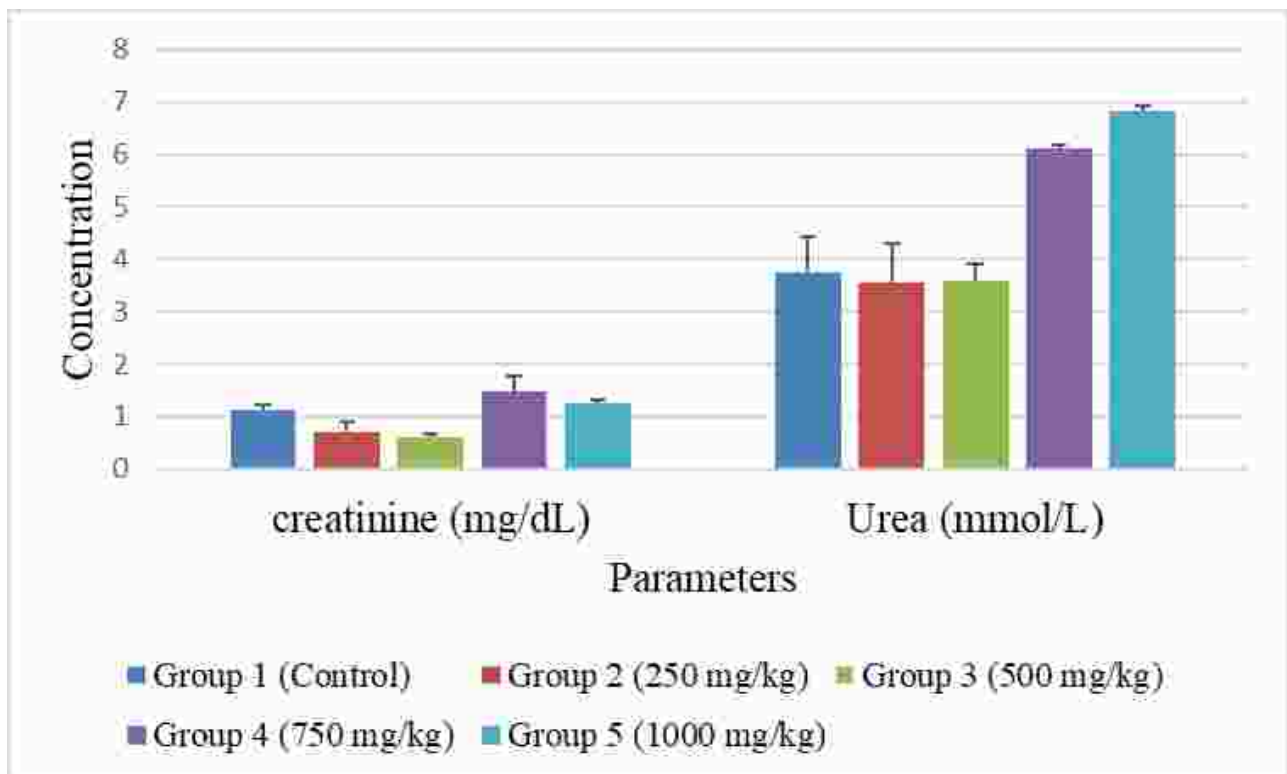


Figure 3. Effect of *phyllanthus amarus* aqueous leaf extract on kidney functions (Creatinine and Urea) in Wistar rats in sub-chronic toxicity study.
 Values are in Mean \pm S.D.

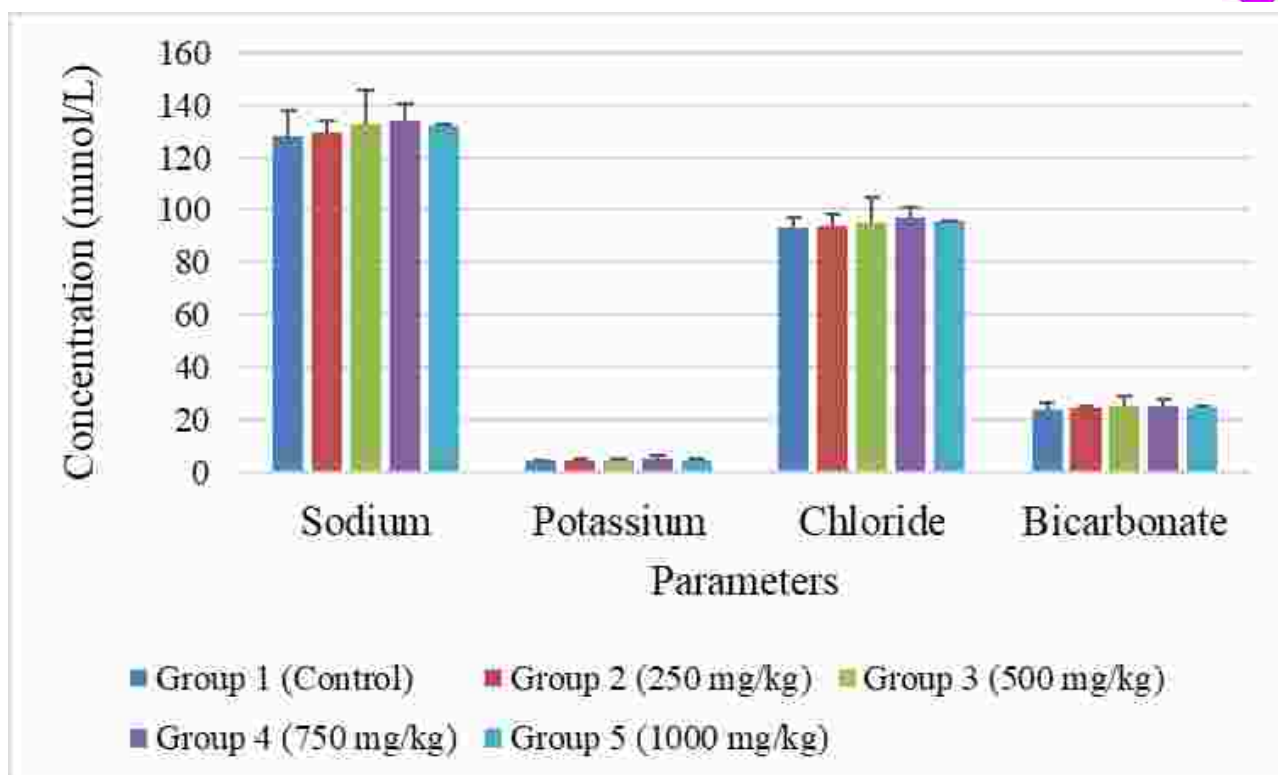


Figure 4. Effect of *phyllanthus amarus* aqueous leaf extract on kidney functions (Electrolytes) in Wistar rats in sub-chronic toxicity study.

Values are in Mean \pm S.D.

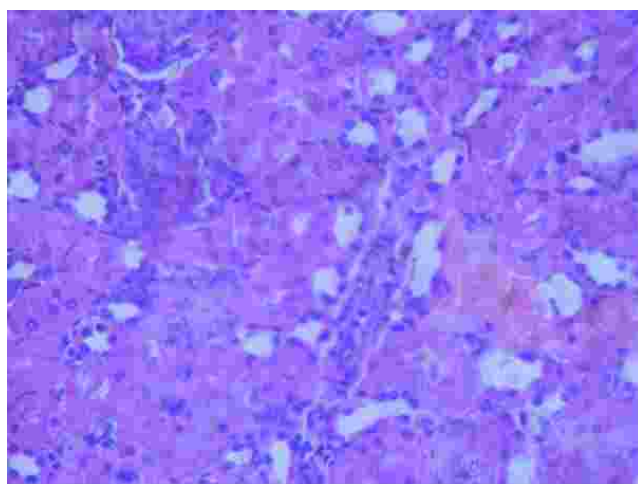


Fig. 5. Photomicrograph of kidney cells (H & E X100) of group 1 (control) in acute oral toxicity study

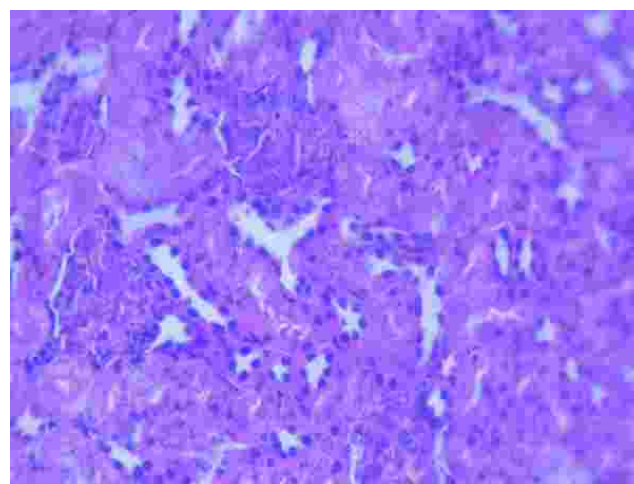


Fig. 6. Photomicrograph of kidney cells (H & E X100) of group 2 (2000 mg/k) in acute oral toxicity study

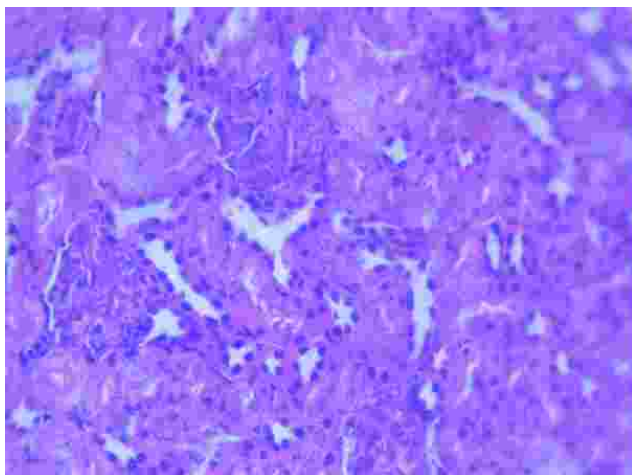


Fig. 7. Photomicrograph of kidney cells (H & E X100) of group 3 (5000 mg/k) in acute oral toxicity study

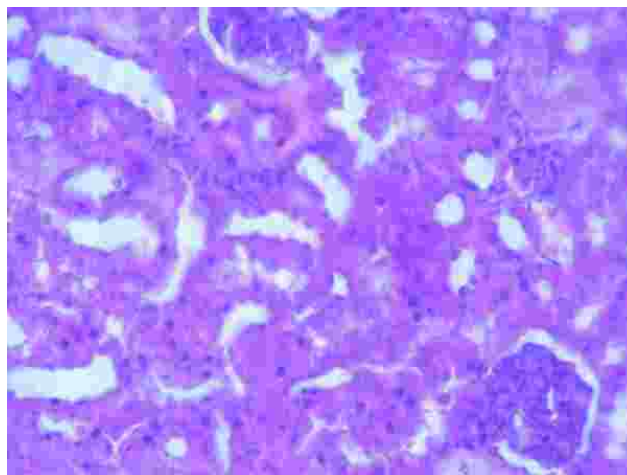


Fig. 8. Photomicrograph of kidney cells (H & E X100) of group 1 (control) in sub-chronic toxicity study

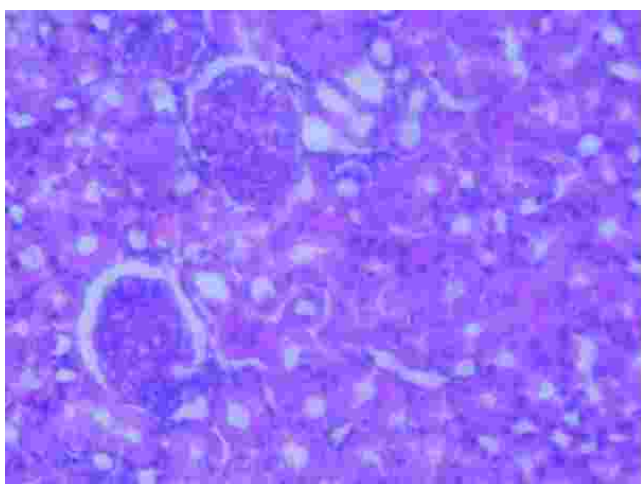


Fig. 9. Photomicrograph of kidney cells (H & E X100) of group 2 (250 mg/kg) in sub-chronic toxicity study

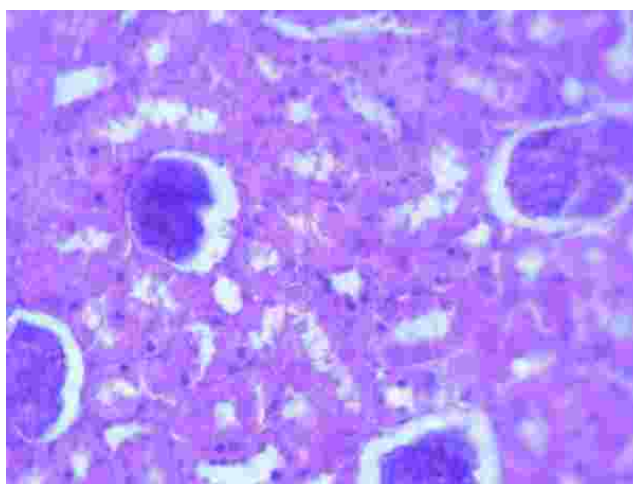


Fig. 10. Photomicrograph of kidney cells (H & E X100) of group 3 (500 mg/kg) in sub-chronic toxicity study

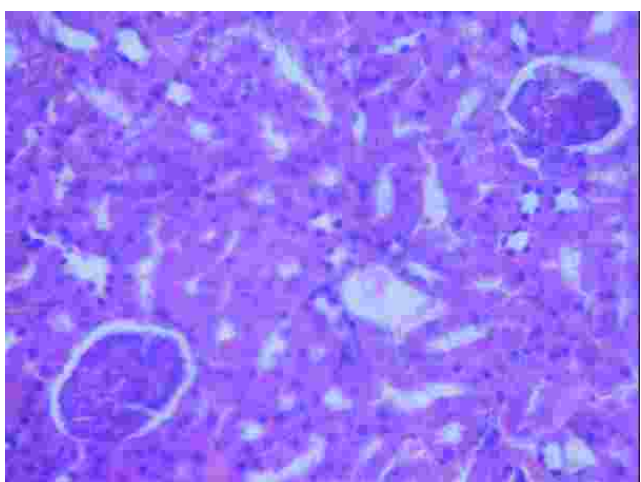


Fig 11. Photomicrograph of kidney cells (H & E X100) of group 4 (750 mg/kg) in sub-chronic toxicity study

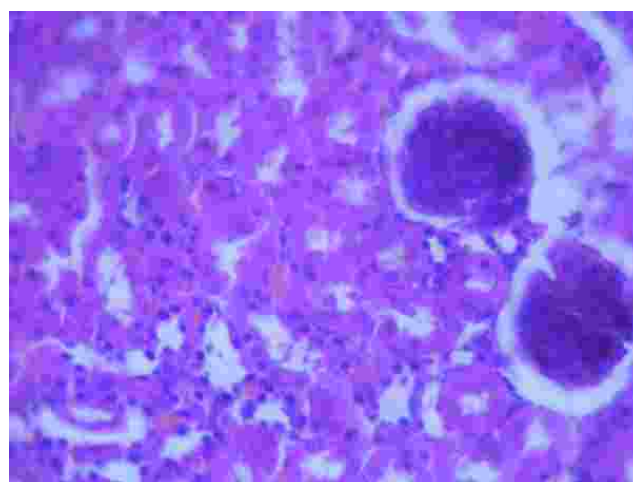


Fig 12. Photomicrograph of kidney cells (H & E X100) of group 5 (1000 mg/kg) in sub-chronic toxicity study

Discussion

In our present study, during acute toxicity experiment, the animals did not show any symptoms of toxicity. Fur, skin, eyes and nose appeared normal and there were no tumors, convulsion, salivation and diarrhea. There was also no mortality or morbidity observed in the rats after 24 hours and throughout the fourteen days period following single oral administration of selected doses (2000mg/kg and 5000mg/kg body weight) of *Phyllanthus amarus* aqueous leaf extracts (Table 1). This thus indicates that the median acute lethal dose (LD₅₀) of the plant is greater than 5000mg/kg body weight. Therefore, this is in accordance with the recommendations of the Globally Harmonized system of classification and labeling of chemicals that a chemical is not classified as toxic, fatal or harmful if the LD₅₀ is greater than 5000mg/kg. It should also be noted that Zbinden and Roversi (1981) suggested that conditions such as animal species, strain, gender, diet, bedding, ambient temperature, caging condition and time of the day, can affect all the LD₅₀ values gotten and are thus considered as uncertainties in extrapolating the LD₅₀ obtained from one species to other species. The electrolytes (sodium, potassium, chloride and bicarbonate), creatinine and urea are markers of kidney function. Maintenance of water homeostasis is essential to life for all organisms. In mammals, the maintenance of osmotic pressure and water distribution in the body fluid compartments is basically a function of the four major electrolytes (sodium, potassium, chloride and bicarbonate) (Tietz, 2012). The kidneys are thus responsible for the regulation of these electrolytes in the body (Satyanarayana and Chakrapani, 2008). Sodium (Na⁺) is the major cation of the extracellular fluid and thus is responsible for about half of the osmotic strength of plasma thereby is central in the maintenance of normal distribution of water and osmotic pressure in the extracellular fluid (Tietz, 2012; and Ochei and Kolhatkar, 2007). Potassium (K⁺), on the other hand, is the major intracellular cation and is useful for heart and other muscle activity (Ochei and Kolhatkar, 2007).

Sodium and potassium are freely filtered by the glomeruli while the tubules (proximal, distal and

loop of henle) regulate their reabsorption, secretion, and excretion. Any damage to the kidney either acute or chronic can affect their regulations in the body. Chloride (Cl⁻) is the major extracellular anion, chloride function alongside sodium and is also regulated by the kidney alongside sodium. Bicarbonate (HCO₃⁻) is the major intracellular anion and also serves as a buffering system in the body (Satyanarayana and Chakrapani, 2008). Creatinine (Cr) is a nitrogenous product which is produced from the metabolism of creatine and creatine phosphate in the skeletal muscles. It is produced endogenously and released into body fluids at a constant rate and its plasma concentration is maintained within narrow limits predominantly by glomerular filtration. Plasma creatinine concentration and its renal clearance is the most widely used markers of the glomerular filtration rate (Tietz, 2012; and Ochei and Kolhatkar, 2007). Urea constitute about half of the non-protein nitrogen (NPN) of the blood. It is formed as the end product of protein and amino acid catabolism and has been measured for years as a marker of kidney function, though creatinine provides better information in this respect (Tietz, 2012).

In this study, there was no statistically significant difference ($p > 0.05$) in creatinine levels of rats that ingested aqueous leaf extract of *P. amarus* in oral acute toxicity study when compared with their corresponding control (Figure 1). However, the urea value of group 2 (2000mg/kg body weight) was significantly higher than the control group but the value was within the reference range. Furthermore, the urea value of group 2 is also higher than that of group 3 which received 5000mg/kg body weight of the extract. This observation implied that the increase may not be due to ingestion of *P. amarus* aqueous leaf extract in the rat, because if the increase was due to the extract, value for group 3 (500mg/kg body weight) ought to be higher than that of group 2 (2000mg/kg body weight). Therefore, the increase did not follow a definite pattern as the values fluctuated within the reference range, hence there was no dose-response relationship (Figure 1).

There was also no statistically significant difference ($p > 0.05$) in the sodium, potassium,

bicarbonate, and chloride levels of rats that ingested aqueous leaf extract of *P. amarus* in oral acute toxicity study when compared with their corresponding control (Figure 2).

In sub-chronic toxicity study, effects of repeated exposure to *P. amarus* aqueous leaf extract were assessed in the experimental rats. In this research work, there was no statistically significant difference ($p > 0.05$) in creatinine levels, of rats that ingested aqueous leaf extract of *P. amarus* in oral sub-chronic toxicity study when compared with their corresponding control (Figure 3). However, the urea values of groups 2 and 3 (250mg/kg and 500mg/kg body weight) were significantly lower than control group, whereas the urea values of groups 4 and 5 (750mg/kg and 1000mg/kg body weight) were significantly higher than the control group but the values were within reference range (Figure 3).

In this research work, there was no statistically significant difference ($p > 0.05$) in the sodium, potassium, bicarbonate, and chloride levels, of rats that ingested methanolic leaf extract of *P. amarus* in oral sub-chronic toxicity study when compared with their corresponding control (Figure 4).

Normal plasma levels of the electrolytes (sodium, potassium, chloride and bicarbonate), creatinine and urea in both acute (Figure 1 and 2) and sub-chronic (Figure 3 and 4) toxicity studies are an indicator of normal kidney function and thus imply that the aqueous leaf extract of *Phyllanthus amarus* is not nephrotoxic. The kidney histology also reveals normocytic normochromic cells in all the experimental rats, no lesion or tumor was observed in any group.

Conclusion

It may thus be concluded from this study that *Phyllanthus amarus* aqueous leaf extract is relatively safe, and did produce adverse effect on kidney function and structure in wistar rats. In all the doses administered, *P. amarus* aqueous leaf extract did not produce any toxic signs or evident symptoms at both acute and sub-chronic toxicity studies in wistar rats. *P. amarus* aqueous leaf extract did not cause any lethality or produce any lesion in the kidney tissues.

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Conflict of Interest

The authors declared no conflict of interest.

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References

- Adeneye, A.A., Benebo, A.S. and Agbaje, E.O. (2006). Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on alcohol-induced hepatotoxicity in rats. *West African Journal of Pharmacology and Drug Research*; 22(23): 42-50.
- Adeneye, A.A., Amole, O.O. and Adeneye, A.K. (2006). Hypoglycemic and hypocholesterolemic activities of the aqueous leaf and seed extract of *Phyllanthus amarus* in mice. *Fitoterapia*; 77: 511-514.
- Ahmed, R. N. and Sani, A. (2013). Antimycotic activity and toxicological effects of stem bark extract of *Vitellaria paradoxa* in Wistar Rats. *Science International (Lahore)*; 25(1): 91102.
- Aniagu, S.O., Nwinyi, F.C., Akumka, D.D., Ajoku, G.A., and Dzarma, S. (2005). Toxicity Studies in Rats fed nature cure bitters. *African Journal of Biotechnology*; 4: 72-78
- Delaney, M.P., Price, C.P., and Lamb, E.J. Kidney Function and Disease. (2013) In: Burtis CA, Ashwood ER, Bruns DE. Tietz fundamentals of clinical chemistry, 6th Edition. Saunder Elsevier, Texas.
- Henry, F.R.J., Canon, D.C., and Winkelman, J.W. (1974). Clinical Chemistry, Principles and Techniques, 2nd edition: 28.
- Henry, O. O. (2007). Renal Function Tests: A Clinical Laboratory Perspective. *Labmedicine*; 38(5): 295 – 304.
- Iwu, M.M., (1993). Modalities of Drug Administration: Hand Book of African Medicinal Plants. CRC Press Inc., Florida, 309-330.

- Joy, K.L. and Kuttan, R. (1998). Inhibition by *Phyllanthus amarus* of hepatocarcinogenesis induced by N-nitrosodiethylamine. *Journal of Clinical Biochemistry and Nutrition*; 24: 133-139.
- Kassuya, C.A., Silerstre, A.A., Rehder, V, and Calixto, J.B. (2003). Anti allodynic and antioedematogenic properties of the lignan from *Phyllanthus amarus* in models of persistent inflammatory and neuropathic pain. *European Journal of Pharmacology*; 478: 145-153.
- Kokwaro, J.O., (1976). Medicinal Plants of East Africa. 1st Edn., East Africa Literature Bureau, Nairobi: 58-59.
- National Institute of Health. Institutional Animal Care and Use Committee Guidebook, NIH Publication (1992). No. 92 – 3415, Washington, D. C. U.S. Government Printing Office
- Ochei, J., Kolhatkar, A. (2007). Medical Laboratory Science: Theory and Practice, 6th edition: 112-118.
- Odetola, A.A. and Akojenu, S.M. (2000). Anti-diarrhoeal and gastro-intestinal potentials of the aqueous extracts of *Phyllanthus amarus* (Euphorbiaceae). *African Journal of Medical Science*; 29: 119-122.
- Oduola, T., Abubakar, A.A., Ayelabegan, F., Tajudeen, M., Okanlawon, S.O. (2018a). Hepatotoxic Assessment of *Phyllanthus amarus* leaf extract in Wistar rats. *European Journal of Medicinal Plant*; 23(4):1-11.
- Oduola, T., Kakako, S.L., Tajudeen, M., Ayelabegan, F., Okanlawon, S.O., Isa, L.O. (2018b). Effect of intake of *Phyllanthus amarus* aqueous leaf extract on lipid peroxidation and some antioxidants factors in Wistar rats. *Journal of Pharmacognosy and Phytochemistry*; 7(4): 2660-2666
- Oluwafemi, F. and Debiri, F. (2008). Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. *African Journal of Biomedical Research*; 11: 215-219.
- Orchard, G. and Nation, B. (2012). Oxford Histopathology Fundamentals of Biomedical Science; New York, 55: 73-74.
- Organization for Economic Cooperation and Development (OECD) (2008a). Toxicity test. In: Organization for Economic Cooperation and Development guidelines for testing chemicals, No 423, Paris, France.
- Organization for Economic Cooperation and Development (OECD)(2008b). Toxicity test. In: Organization for Economic Cooperation and Development guidelines for testing chemicals, No 407, Paris, France.
- Thyagarajan, S.P., Subramanian, S., Thirunalasundari, T., Venkateswaran, P.S. and Blumberg, B.S. (1988). Effects of *Phyllanthus amarus* on the chronic carriers of hepatitis B virus. *Lancet*; 2: 764-766.
- Tietz. (2012). Textbook of Chemical Pathology and Immunology. 5th edition: 650-652.
- Satyanarayana, U., and Chakrapani. (2008). Biochemistry Third Edition, Arunabhasen Books and Allied (P) LTD, Kolkata.
- Wang, M., Cheng, H, Li, Y., Meng, L., Zhao, G. and Mai, K. (1995). Herbs of the genus *Phyllanthus amarus* in the treatment of Chronic Hepatitis B: Observations with three preparations from different geographic Sites. *Journal of Laboratory and Clinical Medicine*; 126: 350-352.
- Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*; 16: 109-110.
- Zbinden, G., Roversi, F. (1981). Significance of the LD₅₀ test for the toxicological evaluation of chemical substances. *Archives of Toxicology*; 47(2): 77-99.

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