Supported by tetfund TERTIARY EDUCATION TRUST FUND

In-vitro and *in-vivo* Therapeutic Assessment of Leaf Fraction of *Phyllanthus amarus* on Induced Nephrotoxicity in Wistar Rats

Ujah, O. Frederick[2], [1*], Mohammad, Y. Yusurf[2] and Tyoapine, A. Daniel[1]

[1]Department of Chemical Sciences, College of Science and Education, University of Mkar, Mkar, P.M.B. 017, Benue State, Nigeria.

[2]Department of Biochemistry, Faculty of Basic Medical Sciences Bayero University Kano, Kano State, Nigeria.

Corresponding Author: oyiujah2004@yahoo.com, foujah@umm.edu.ng

Received: March 20, 2022 **Accepted:** June 18, 2022

Abstract: The study was designed to assess the therapeutics of ethyl acetate fraction of *Phyllanthus amarus* leaves on induced nephron toxicity. Arsenic used for *in-vivo* induction, is a toxic heavy metal. It occurs as environmental and food/feed contaminant causing public and animal's health hazards and Doxorubicin was used as *in-vitro* comparative cytotoxic study with *P. amarus* fraction on kidney epithelial and fibroblast cells. Results showed significant elevation of kidney function test (KFT) parameters alongside the antioxidant enzymes which were greatly decreased. The renal tissue histogram showed severe degeneration and necrosis accompanied by severe congestions and multifocal hemorrhages in the intoxicated rats. But from the treatment groups, the post administration of high dose (300mg/kg), provided significant decrease in the kidney function test indexes and increased activity of the enzymes. Furthermore, it was observed that, the cytotoxic effect of the sample fraction on kidney epithelial cells was lesser with IC50 of 47.0±0.02 to that of doxorubicin (1.8±0.04) and no toxic effect on fibroblast as compared with Doxorubicin (5.0±0.01). Histopathological examination showed mild necrosis on the treated kidney architecture. Conclusively, by the *in vivo* evaluation of antioxidant activity, KFT assessment with promising anti-cytotoxic and cell viability demonstrated by *P. amarus* leaves fraction against arsenic and doxorubicin, the plant fraction showed antinephropathic potential.

Keywords: Cytotoxicity, Cell viability, Histopathology, Nephron toxicity, *Phyllanthus amarus*, Therapeutics,

Introduction

Arsenic has found its way into prominence as a toxicant of grave health risk out of the many naturally occurring elements found abundantly distributed in the earth's crust (Tchounwou *et al*., 2012). A heavy metal, specifically classed as a metalloid naturally produced during processes such as volcanic eruptions and biodegradation of other organic minerals and rocks (Oyagbemia *et al*., 2017). Due to the increased dependency on arsenic among other heavy metals for anthropogenic activities, there has been widespread release of arsenic by-products into the environment and human exposure is hence unavoidable, not just from occupational cases, but also from atmospheric pollution, ingestion of contaminated food and water sources and from contact with certain finished industrial products (ATSDR, 2016). Tchounwou *et al*., (2012) reported that each individual has an average arsenate intake of 50µg per day which is critical and can pose a considerable adverse heath effect. This toxicant is found to accumulate more in the liver than other tissues after a little period of exposure, when administered orally and subcutaneously. However, a more prolonged period of exposure is found to accumulate more in the kidney than in the liver or other tissues (Ezedom *et al.*, 2016). Even at low exposure level, arsenate is shown to cause multiple organ damage by generating reactive oxygen species (ROS) and promoting oxidative stress, (Jomova *et al*., 2011). The kidney been a susceptible organ to ROS because of polyunsaturated fatty acids abundance in its cellular compositions, ROS may induce pathological mechanisms which cause glomerulosclerosis, tubulointerstitial fibrosis, tubular cells apoptosis, senescence, and also deactivate cellular regenerative pathways, leading to renal failure (Small *et al*., 2012). Despite the epidemiological and experimental studies on the various carcinogens, the attempt to resolve arsenic renal toxicity through the use of potential natural plant materials like *Phyllanthus amarus* have rarely been considered. *P. amarus* is reported to have a wide number of therapeutic uses; hence assessment of anti-renal toxicity potential of the plant is needful as very large populations are at risk (Shah *et al.*, 2017).

Materials and Methods

Plant Material

Fresh leaves of *Phyllanthus amarus* were collected around Mkar hill behind University of Mkar Benue State, Nigeria and were identified by Mr. Alfred Ozioko, a taxonomist at the Centre for Ethno-Medicine and Drugs Development Nsukka Enugu State.

Preparation of Plant Extract

Fresh plants of *Phyllanthus amarus* were air-dried under room temperature for 21 days until constant weight was obtained. The leaves were separated from the whole plant and sieved to remove other particles (Stem, Roots, and Seeds). 40g of *Phyllanthus amarus* leaves were macerated in 800ml of water, shaken for 10 minutes and allowed to stay for 72 hours at room temperature to achieve maximum extraction (Ujah et al., 2018). The extract was sieved with a filter cloth and the juice was filtered using cotton wool and later dried in oven at 35^{o} C. The dried filtrate was used to run column chromatography using silica gel (MESH200) for which 100% ethyl acetate fraction was obtained (100E). This showed better *in vitro* antioxidant potential (Ujah *et al.*, 2021). The dried fraction was later reconstituted in warm distilled water to the required dosage for administration.

Experimental Animals

Thirty (30) adult Wister rats of both sexes weighing 120-180g were obtained from the Animal housing unit of Chemical Sciences Department of University of Mkar, Benue State, Nigeria and were allowed to acclimatize for one week, housed in standard cages with wire mesh top, exposed to natural light-dark corner and handled according to standard protocol. They were fed with standard laboratory chow (Vital feed) and water *ad libitum*. Approved experimental protocol by the Institutional Animal Ethics Committee (IAEC) was dully followed; animals care was taken in accordance with the guidelines of European convention for the protection of Vertebrate animals used for experimental and other scientific purposes ETS-124. The research was carried out following the experimental design in table 1.

Table1 Experimental Design

Group	No of Animal	Extract Administration(dose)
A. Normal control	5	Vital feed/ distil H_2O
B. Negative control	5	Arsenic administration for 10days
Treatment Groups		
C. Pre-treatment	5	C1. Low dose (100mg/kg) Fraction administered for 10days followed with Arsenic for 10days
	5	C2. High dose (300mg/kg)
D. Post-treatment	5	D1. Low dose (100mg/kg) Arsenic administered for 10days followed with
	5	D2. High dose (300mg/kg) fraction for 10days

Determination of Renal Antioxidant Activity

Nephron anti-oxidant activity of *P. amarus* leaves fraction was evaluated by estimating the levels of renal anti-oxidant markers such as Catalase (CAT), Glutathione (GPH) and Superoxide dismutase (SOD) and Malondialdehyde (MDA). Prior to homogenization, the kidneys were washed with phosphate buffered saline (pH: 7.4) to remove blood. The homogenates were prepared by churning the kidneys in ice cold assay buffer (provided with assay kits) solution separately (10-20% of tissue homogenates were prepared for different assays as per the instructions) using a tissue homogenizer (Glass-Teflon Potter Elvehjem Tissue Homogenizer) and centrifuged at 14000×g (10-30 minutes at 4°C; time of centrifugation varies from one enzyme to another) in a ultra-freeze-centrifuge. The supernatants (containing cytosolic and mitochondrial fractions) were assayed directly for renal anti-oxidant markers according to the procedures indicated in kits using a Micro-plate reader (Sperctra-Max-340).

Determination of Serum Electrolytes

Urea concentration was estimated by Urease-Bertholet method as described by Weatherburn (1967) using Randox diagnostic kit. Na^+ K^+, Cl^-, HCO^-_3 and creatinine were also assessed.

Histological Analysis

Kidneys from each rat were dissected and washed with dexterous saline and stored in sterilized sample bottles containing formalin (10% formaldehyde) for histological studies, following the procedure used by Strate et al., (2005).

Statistical Analysis

Data obtained were analyzed and expressed as Mean ± Standard Deviation (SD) by One-way ANOVA followed by Tukey-Kramer multiple comparison tests, using Statistical Package for Social Sciences (SPSS). The values at P=0.05 were regarded as significant as compared with appropriate control.

Results

Results of amelioration of nephron toxicity in rats with arsenic administration are presented in table 2, 3 and plate1-6 while table 4 shows the cytotoxic effect of ethyl acetate fraction of *P. amarus* leaves on Kidney cell lines

In table 2, there was a significant (P=0.05) rise in the level of Malondialdehyde (MDA) with concomitant decrease in levels of glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) in the arsenic-induced untreated rats compared with normal control group. However, all treatments with *P. amarus* fraction significantly reversed the deplorable status of the antioxidant system.

Table 2. Effect of Ethyl Acetate Leaves Fraction of *P. amarus* on Kidney Antioxidants and other Biomarkers in Rats with arsenic Poison.

EXPERIMENTAL GROUP	SOD (µm/min)	CAT (µm/min/mg)	GSH (µg/mL)	MDA (mm/mg)
A. Positive Control	31.51±2.56^b	42.45±1.49^b	13.25±1.16^b	1.89±0.34^b
B. Negative control	19.73±1.56^h	30.69±2.60^h	8.60±0.14^h	3.50±0.24^h
C_1.Pre-treatment (100mg/kg)	27.79±6.24^a	43.95±10.86^a	10.45±0.44^a	2.25±0.18^a
C_2.Pre-treatment (300mg/kg)	25.73±0.66^a	38.59±6.55^a	10.00±0.25^a	2.89±0.63^b
D_1.Post-treatment(100mg/kg)	28.26±4.05^a	44.83±1.79^a	9.80±0.67^a	2.69±0.15^a
D_2.Post-treatment(300mg/kg)	28.85±3.23^a	43.93±5.90^a	10.75±0.36^a	2.68±0.25^a

Result are expressed as mean ± standard deviation, (n=5), at *P= 0.05.* Values in the same column having different superscript differ significantly at P<0.05

KEY

MDA: Malondialdehyde, SOD: Superoxide Dismutase, CAT: Catalase, GSH: Glutathione.

Table 3 present results on effect of ethyl acetate leaves fraction of *Phyllanthus amarus* on serum electrolytes (Na^+, K^+, Cl^- and HCO_3^-, urea, and creatinine) levels. Administration of arsenate caused significant (P=0.05) elevation of all the measured parameters in the serum compared with group A (normal control) indicating arsenic poisoning. However, both pre and post treatment with *Phyllanthus amarus* leaves fraction significantly (P=0.05) reversed the elevated parameters close to that obtained in the normal control.

Table 3. Effect of Ethyl Acetate Leaves Fraction of *P. amarus* on serum electrolytes levels and other Kidney markers in Rats with Arsenic Poison.

Experimental Group	Urea (mm/L)	Creatinine (Um/L)	Na^+	K^+	Cl^-	HCO_3^-
A. Positive Control	10.50±0.01 [b]	55.83±0.01[b]	122.42±0.19[b]	6.26±0.11[b]	65.82±32.02[b]	22.54±0.01[b]
B. Negative control	16.12±0.05[j]	95.65±0.03[j]	133.34.1.36[j]	9.31±1.33[j]	114.24±3.88 [j]	25.09±0.48
C_1. Pre-treatment (100mg/kg)	7.42±0.49[a]	40.65±1.12[a]	125.66±3.97	6.96±0.28[a]	70.78±3.68[a]	21.19±1.15[a]
C_2. Pre-treatment (300mg/kg)	7.09±0.69[a]	50.52±4.06[a]	121.49±8.00[a]	7.17±0.56[a]	79.00±2.89[a]	21.38±2.80[a]
D_1. Post treatment (100mg/kg)	9.30±0.63[a]	51.69±1.47[a]	126.76±2.68	5.20±1.48[a]	82.70±5.15[a]	21.01±0.44[a]
D_2. Post treatment (300mg/kg)	8.39±0.53[a]	49.08±1.37[a]	122.50±7.85[a]	5.70±0.90[a]	79.33±5.80[a]	22.58±1.20[b]

Result are expressed as mean ± standard deviation, (n=5), at *P= 0.05.* Values in the same column having different superscript differ significantly at P<0.05.

KEY: Sodium ion (Na^+), Potassium ion (K^+), Chlorine ion (Cl^-) and Bicarbonate ion (HCO_3^-)

Table 4 Cytotoxic Effect of Ethyl Acetate Fraction of *P. amarus* Leaves on Kidney Cell Lines

SAMPLE	LLCPK1	VERO
	IC50(µg/mL)	
P. amarus	47.0±0.02	NC
Doxorubicin	1.8±0.04	5.0±0.01

Values are expressed in Mean ± SE (n=3)

Key: LLCPK1-Kidney epithelial cells; VERO- Kidney fibroblast; NC- No cytotoxic effect

Discussion

Plants make up 80 percent of the earth, although human activity over the years had reduced this. This accentuate the importance of plant to life and truly many of these plants have been evaluated for antioxidant property which is essential to limit the progression and risk of certain acute and chronic diseases (Fatma *et al*., 2013) attributed to the richness in secondary metabolites.

According to John *et al*., (2019), arsenic (As) is ranked first in the list of 20 hazardous substances by the Agency for Toxic Substances and Diseases Registry. This epidemiologically important metalloid is currently poisoning tens of millions of people worldwide due to it's assessed in drinking water and food causing liver disease and kidney failure. The study examined the therapeutic and/or ameliorative effect of ethyl acetate fraction of *P. amarus* leaves on arsenic toxicity in kidneys of rats. The rats treated with arsenate showed significant deplorable effects in kidney functions and structure. This was unriddled as indicated by significant elevated urea and creatinine levels as well as electrolytes (Na^+, K^+ and Cl^-) except for bicarbonate ion (HCO_3^-) which increased but insignificant (P=0.05) in the serum of these experimental animals. These findings are in consonance with earlier findings by Yocout *et al*., (2012) and the significant increase in serum urea, creatinine and electrolytes levels can be attributed to damaged nephron structure integrity (Khan and Siddique, 2012). Creatinine for instance, is a waste product produced by the muscles. It is typically removed from the body in urine by the kidneys, a process known as creatinine clearance, thus, high level is an indication of chronic kidney disease (CKD)

The study also accessed the level of 2, 2-diphenyl-1-picrylhydrazyl reactive substance such as Malondialdehyde (MDA) and the activity of antioxidants like glutathione (GSH), superoxide dismutase (SOD) and Catalase (CAT) which are very sensitive indexes in free radical induced renal toxicity (Mohajeri *et al*., 2011). The significant (P=0.05) elevation in the level of MDA in the kidney tissue of the negative group may be attributed to enhanced membrane lipid per-oxidation by free radicals generated by arsenate and the failure of the body antioxidant defense mechanism to reduce the excessive detrimental effects of As^- radicals is probably because the antioxidants have been overwhelmed (Kim et al., 2010). This was observed by the significant reduction in activity of GSH, SOD and CAT in the kidney tissues. This high concentration of free radicals generated by arsenate (As^-) may have led to inactivation and/or probably inhibition of the synthetic pathways of the antioxidant enzymes, hence the low turnover (Showkat *et al*., 2010). The consequence of this is that, the low levels of catalase and SOD can cause hydrogen peroxide and superoxide radicals to build up, causing damage to cells. Nevertheless, both pre and post treatments with ethyl acetate fraction of *P. amarus* significantly restored the levels of GSH, SOD, and CAT activities near to normal as well as decreases MDA level confirming the therapeutic property of the ethyl acetate fraction of *P. amarus* leaves against arsenic poison. Furthermore, the significant reduction in urea, creatinine and electrolytes levels suggests amelioration of the glomerular and tubular cells thus improving the renal functions of the damaged kidney. Finally, arsenic as low as 10mg/kg exhibited

severe alterations in histology of the renal tissues as showed in plate 2. Moreover, the post treatment of high dose of the fraction ameliorated the histopathology of the tissues through facilitating recovery of the hemorrhagic and inflammatory conditions. This protective effect of *P. amarus* may be due to its reported bioactive constituents like polyphenols, (Umbare *et al.*, 2009, Ujah *et al.*, 2021). Polyphenols have been reported to possess membrane stabilizing activity by inhibiting the generation of ROS induced by toxicants and maintain cell membrane structural integrity.

The cytotoxic effect of the sample fraction on kidney epithelial and fibroblast cells were compared with Doxorubicin and the IC50 calculated as shown in table 4.25. IC50 provide quantitative information regarding specific dosage of drugs and their effects on cell lines. The IC50 of the *P. amarus* was higher to that of Doxorubicin (drug use as combined therapy for various cancer treatments) and according to studies, the higher the IC50, the lower the potency to cause toxicity, thus *P. amarus* is less toxic on kidney epithelial cells and no cytotoxic effect on the kidney fibroblast compared to the standard drug.

Conclusion

In conclusion, administration of arsenic (10mg/kg bw) for 10 days caused significant (p = 0.05) elevation in kidney function test indices. Whereas the administration of ethyl acetate fraction (100E) of *P. amarus* leaves for 10days restored these (urea, creatinine and electrolytes) to normal. As regard to antioxidant system, the significant decline due to arsenic radicals' visa vice elevated MDA level in serum was also restored. This was further elucidated by the amelioration of severe alterations in histology of the renal tissues, especially in the post treatment group with 300mg/kg bw of *P. amarus* leaves fraction. The plant leaves also showed less cytotoxic effects compared to anticancer drug (doxorubicin), thus it is a promising source for antinephropathic drug.

Recommendation

This result showed promising therapeutic activity of ethyl acetate fraction of the plant leaves. Hence, it is becomes imperative to give further preclinical trial on higher animals and to take close look at the mechanism it's antinephropathic property.

Acknowledgement

We wish to acknowledge the staff of Hematology Department, Federal College of Veterinary and Medical Laboratory Technology, Vom for their assistance during the bench work.

Competing Interests

Authors have declared that no competing interests exist.

Monogram on kidney showing Ameliorative Effect of Ethyl Acetate Fraction of *P. amarus* Leaves in Arsenic Administered Rats:

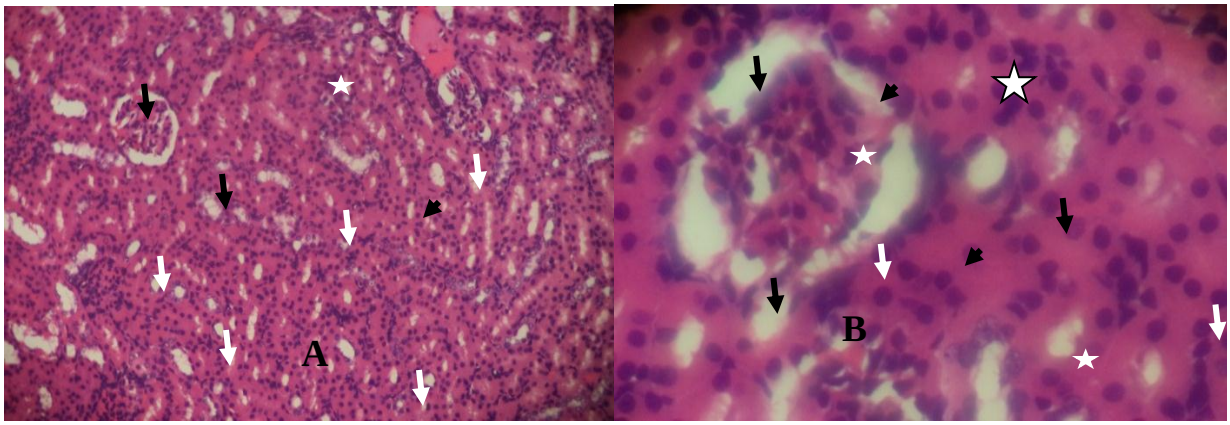

Plate 1. (NORMAL CONTROL): Kidney of Wistar rat given vital feed and water for 10 days, presenting with normal tissue morphology as demonstrated by cells maintaining their architectural integrity. White arrows= nuclei of cells, white stars= glomeruli, black arrows= capsular space, black arrowheads= glomerular capsule, H&E A: X100 B: X400.

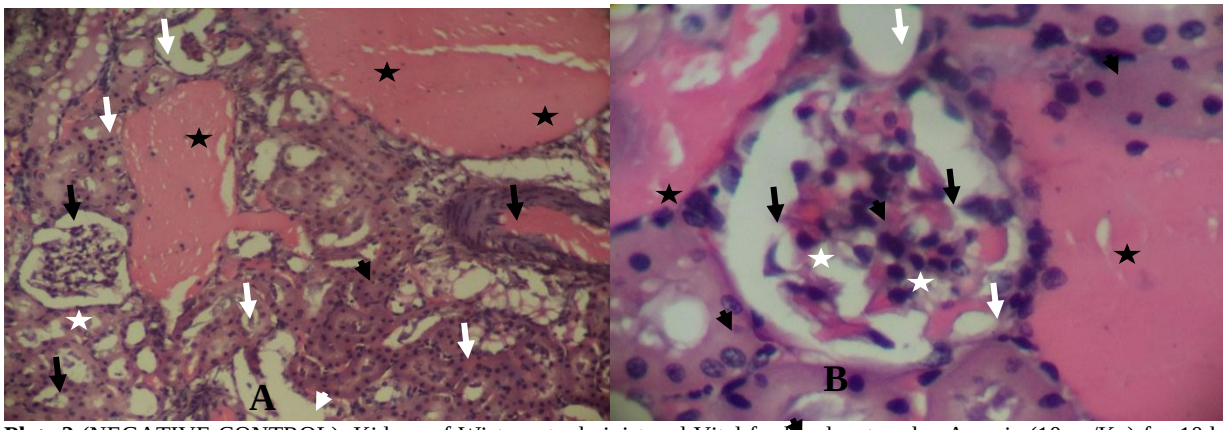

Plate.2 (NEGATIVE CONTROL): Kidney of Wistar rat administered Vital feed and water plus Arsenic (10mg/Kg) for 10days showing massive tissue congestion as demonstrated by the massive presence of red blood cells (black stars) within the tissue. The capsular space (black arrows) is clear and the glomerular capsules (black arrowheads) remain intact. White arrowheads= nuclei of cells, white arrows= kidney tubules. H&E A: X100 B: X400.

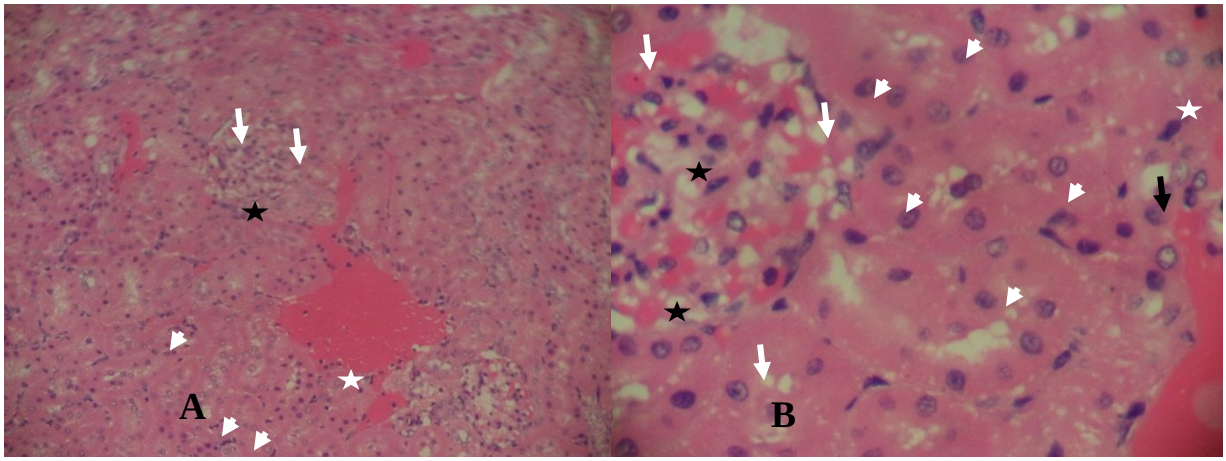

Plate 3 (PRE-TREATMENT 100mg): Kidney of an Wistar rat administered Vital feed and water plus *Phyllanthus amarus* fraction (100mg/Kg) for 10 days. Arsenate (10mgh/kg) for 10days. Show severe congestion as demonstrated by the massive presence of red blood cells (white stars) and loss of capsular space thereby presenting glomeruli (black stars) that have direct contact with the capsular space (white arrows). White arrowheads= cell nuclei H&E A: X100 B: X400

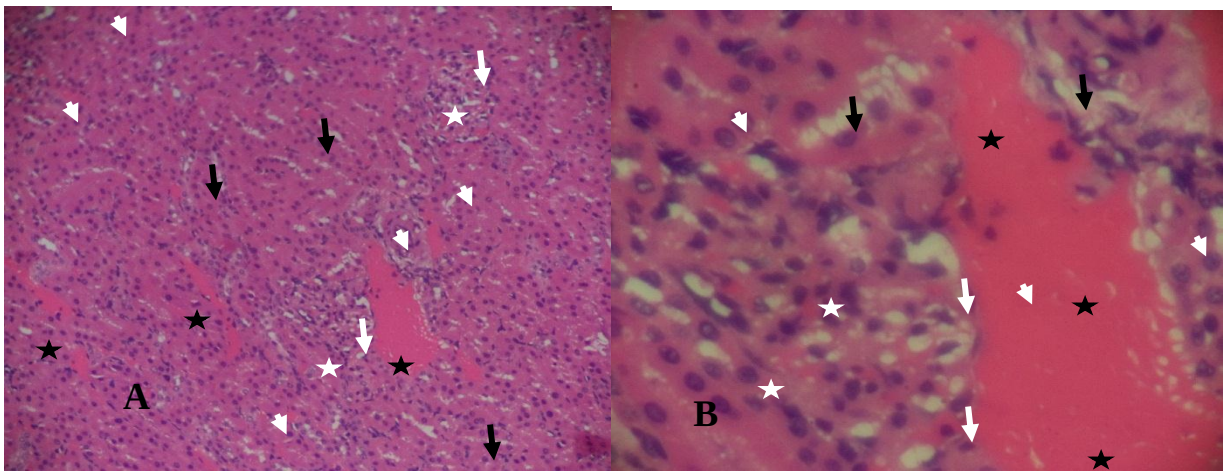

Plate 4 (PRE-TREATMENT 300mg): Kidney of Wistar rat administered Vital feed and water plus *Phyllanthus amarus*fraction (300mg/Kg) and arsenate (10mgh/kg) for 10days, showing congestion as seen by the massive presence of red blood cells (black stars) within the tissue and loss of capsular space, making the glomeruli (white stars) to have direct contact with the glomerular capsule (white arrows). There is a great reduction of interstitial spaces (black arrows). The nuclei (white arrowheads) remain normal. H&E A: X100 B: X400

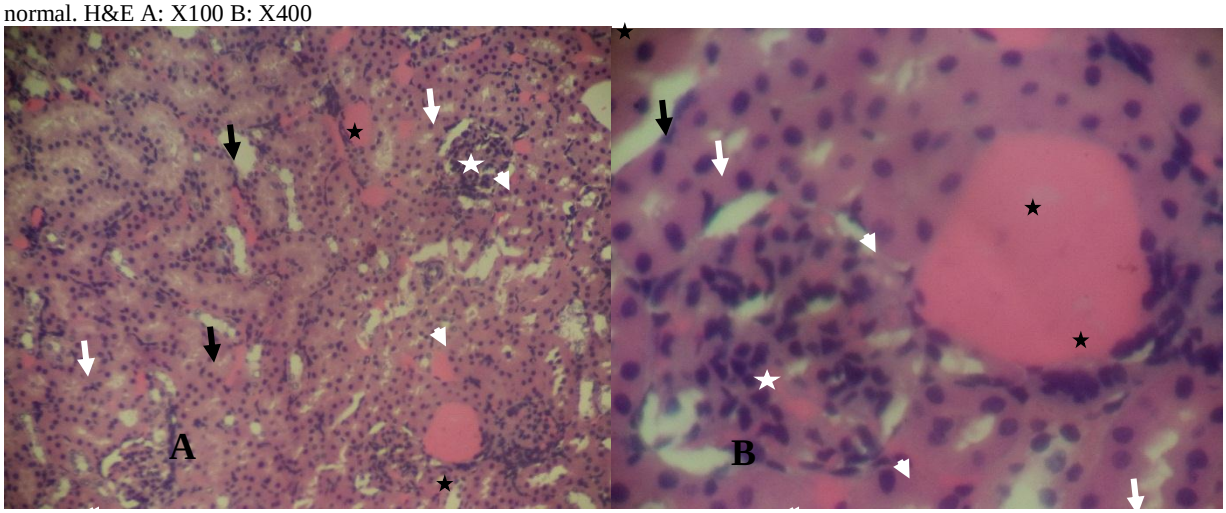

Plate 5 (POST-TREATMENT 100mg): Kidney of Wistar rat administered Vital feed and water and Arsenate (10mgh/kg) for 10days.*Phyllanthus amarus*fraction (100mg/Kg) for 10days, showing congestion as demonstrated by the massive presence of red blood cells (black stars) within the tissue and massive reduction in capsular space, thereby making the glomeruli (white stars) to have a direct contact with the glomerular capsule (white arrowheads). Black arrows= Kidney tubules, white arrows= nuclei of normal cells. H&E A: X100 B: X400.

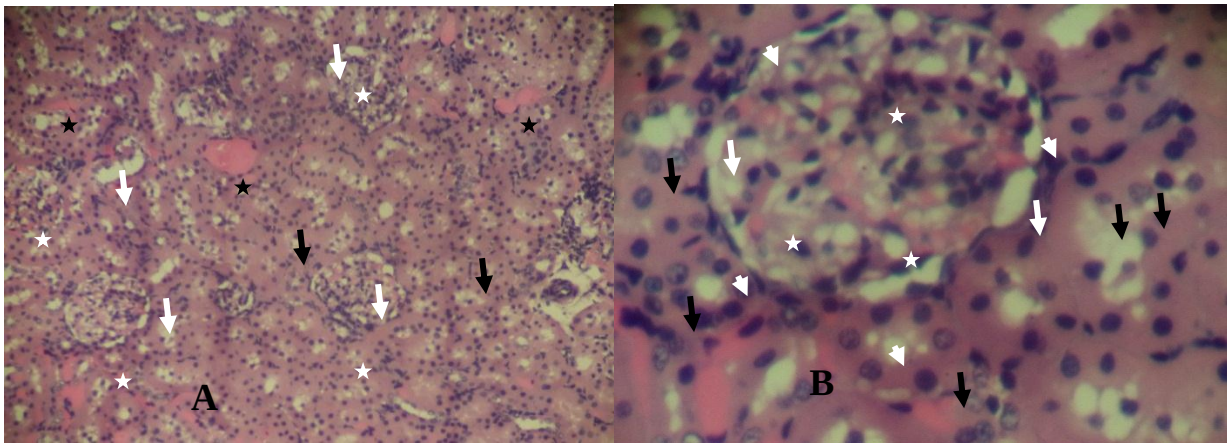

Plate 6 (POST-TREATMENT 300mg): Kidney of Wistar rat administered Vital feed and water plus Arsenate (10mgh/kg) for 10days.*Phyllanthus amarus*fraction (300mg/Kg) for 10days, showing mild congestion as seen by the pres ɔnce of red blood cells (black stars) within the tissue an loss of capsular spaces as seen the glomeruli (white stars) having a direct contact with the glomerular capsules (white arrowheads), black arrows= nuclei, white arrows= tubules. H&E A: X100 B: X400.

References

ATSDR Arsenic Toxicity Case Study: Where Is Arsenic Found? | ATSDR – Environmental medicine and environmental health education CSEM (2000) Available from: http://www.atsdr.cdc.gov/csem/csem.asp?csem=1&po=5 (Accessed 10 March 2016).
environment, Mol. *Clin. Environ. Toxicol*. 3 133–164 In: Luch A, ed.

European treaty series, European Convention for the Protection of Vertebrate Animals and other Scientific Purposes. 2005. ETS-124.

Ezedom T, Asagba SO 2016. Effect of a controlled food-chain mediated exposure to cadmium and arsenic on oxidative enzymes in the tissues of rats. Toxicol. Rep., 3. 708-715.

Fatma A, Sokindra K & Shah AK 2013. Estimation of total phenolic content, *in vitro* antioxidant and anti-inflammatory activity of flowers of *moringa oleifera*. Asian Pacific Journal of Tropical Biomedicine, 3(8): 623-627.

John OF, Adeleke GE, Badmus JA, Afolabi OK, Adedosu OT, Kehinde SA, & Adekunle AS 2019. Time–Course of Sodium Arsenate induced hepatotoxicity and nephrotoxicity in male wistar rats. Microcirculation. 9(4).

Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ & Valko M 2011. Arsenic: toxicity, oxidative stress and human disease, J. Appl. Toxicol., 31(2) 95–107.

Khan MR. & Siddique F 2012. Antioxidant effects of citharexylum spinosum in CCl4 Induced Nephrotoxicity in Rat. Experimental and Toxicological Pathology, 64, 349-355. http://dx.doi.org/10.1016/j.etp.2010.09.009.

Kim HY, Kim JK, Choi JH, Jung JY, Oh WY, Kim DC, Lee HS, Kim YS, Kang SS, Lee SH, & Lee SM 2010. Hepatoprotective effect of pinoresinol on carbon tetrachloride-induced hepatic damaged in mice. Journal of Pharmacological science, 112(1):105-112.

Mohajeri D, Amouoghli TB, Doustar Y & Nazeri M 2011. ProtectiveEffect of Turnip Root (*Brassica Rapa*. L) Ethanolic Extract on Early Hepatic Injury in Alloxanized Diabetic Rats. Australian Journal of Basic and Applied Science. 5(7): 748-756.

Oyagbemia TO, Omobowaleb ER, Asenugac JM, Afolabib OA et al 2017. Effect of arsenic acid withdrawal on hepatotoxicity and disruption of erythrocyte antioxidant defense system. Toxicological Reports 4 521-529.

Shah AA, Funk WC, & Ghalambor CK 2017. Thermal acclimation ability varies in temperate and tropical aquatic insects from different elevations. Integrative and Comparative Biology, *57*(5), 977-987.

Showkat P, Zaidi Y, Asghar S, & Jamaluddin S 2010. In vitro Propagation and Callus Formation of Bacopa monnieri (L.) Penn. Plant tissue culture and biotechnology, 20(2), 119-125.

Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC 2012. Oxidative stress, anti-oxidant therapies and chronic kidney disease. Nephrology. 17:311-21.

Strate T, Mann O, Kleighans H, Rusani S, Schneider C, Yekebas & Izbicki JR 2005. Micro Circulatory Function and Tissue Damage Improved After The Therapeutic Injection of Hemoglobin in Server Acute Rodent Pancreatitis. Pancreas Apr. 30(3):254-259.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ 2012. Heavy metal toxicity and the
environment, Mol. Clin. Environ. Toxicol. 3 133–164 In: Luch A, ed.

Ujah OF, Nenge HP, Berinyuy EB, Ujah IR, & Ankala DE 2018. Effect of interaction of anti-retroviral drug (TDF) and m*oringa oleifera* leaf extract on hepato-renal status of wistar rats. IJHPR 7(2):22-30.

Ujah OF, Mohammad YY & Dewua CM June, 2021. Quantification, antioxidant and free radical scavenging potentials of polyphenols from crude extracts of phyllanthus amarus leaves. ChemSearch Journal, Publication of Chemical Society of Nigeria, Kano Chapter 12(1): 97 – 107.

Umbare RP, Mate GS, Jawalkar DV, Patil SM & Dongare SS 2009. Quality evaluation of phyllanthus amarus (Schumach) leaves extract for its hypolipidemic activity. Biology and Medicine, 1(4): 28-33.

Weatherburn MW 1967. Urease-berthelot colorimetric method for in vitro determination of urea. Analytical Chemistry. 39: 971-974.

Yacout GA, Elguindy NM & El-Azab EF 2012. Hepatoprotective effect of basil (*OcimumBasilicum*L.) on CCl_4-induced liver fibrosis in rats. African Journal of Biotechnology, 11 (90): 15702-15711.