Rats kidney morphological particularities and functions post-treatment with *vernonia amygdalina* extract and low-dose lead acetate

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SUMMARY

This study was conducted to evaluate the effect of *Vernonia amygdalina* extract on Pb-induced kidney toxicity in Wistar rats. This investigation was carried out using 25 Wistar rat of both sexes, and the animals were divided into five groups: 5 rats per group. Group A served as the negative control group and was orally gavaged with 5mg/kg body weight of normal saline daily. Group B served as the positive control and was treated with daily intraperitoneal (IP) injection of 8 mg/kg body weight of Pb acetate (Pb). Group C was treated with daily intraperitoneal (IP) injection of 8 mg/kg body weight of Pb along with 20 mg/kg body weight of *Vernonia amygdalina* extract orally. Group D was treated with daily intraperitoneal (IP) injection of 8 mg/kg body weight of Pb along with 40 mg/kg body weight of *Vernonia amygdalina* extract orally. Group 5 was treated with daily intraperitoneal (IP) injection of 8 mg/kg body weight of Pb along with 60 mg/kg body weight of *Vernonia amygdalina* extract orally. All treatments were done for a period of 28 days. The animals were sacrificed on the 29th day by cervical dislocation, then blood was collected by cardiac puncture, and kidneys were collected for histological profile. Lipid peroxidation (MDA), creatinine and urea level were all determined. There was marked elevation in MDA level with a concomitant depletion in urea and creatinine content in the group treated with only Pb when compared with the negative control group. There was a significant increase in proximal tubular area, distal tubular area, glomerular membrane thickness, area, perimeter and feret's diameter and a significant decrease in proximal tubule, distal tubule ratio and cellularity in this group of rats when compared to the negative control. Oxidation and histological changes in the kidneys were successfully prevented by the pre- administration of *Vernonia amygdalina* as evidenced by creatinine and urea and MDA level. These were made evident as the morphological scores across all experimental groups were significantly different from those of the positive control (group 2). Based on the current findings, it can be concluded that *Vernonia amygdalina* successfully minimizes the deleterious effects in kidney function and histological coherence associated with nephrotoxicity by strengthening the antioxidant defense system, suppressing oxidative stress, and mitigating apoptosis.

Key words: *Vernonia amygdalina* – Kidney – Morphology – Toxicity – Oxidative stress

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INTRODUCTION

In the environment, Pb is a common natural element. It is one of the most significant and prevalent environmental contaminants (Joworaski, 1968). Pb can harm humans and other living things by moving up the food chain and harming them. It is one of the environmentally harmful metals and negatively affects the majority of human organs (Duruibe et al., 2007; Alwaleedi, 2016).

Pb poisoning is linked to a variety of physiological, morphological, and biochemical changes, including liver dysfunction (ATSDR, 1993; Elayat and Bakheelf, 2010), hematological diseases, and impairment of renal system functions (Mugahi et al., 2003; Suradkar and Ghodasara, 2009; Alwaleedi, 2016).

Reactive oxygen species (ROS) generation, depletion of intracellular antioxidant reserves and free radical scavengers, and inhibition of antioxidative enzymes are all well-known mechanisms by which heavy metals cause oxidative stress (Shilpi et al., 2014; Jan et al., 2015). Due to ROS damage to lipids, proteins, and DNA, cells under oxidative stress exhibit a variety of dysfunctions (Ercal et al., 2010).

One of the most obvious symptoms of Pb toxicity has been observed to be impaired kidney functions (Chang et al., 1980). Proximal tubular nephropathy, glomerular sclerosis, and interstitial fibrosis are symptoms of Pb-induced nephrotoxicity (Loghman-Adham, 1997; Diamond, 2005).

Enzymuria, low- and high-molecular-weight proteinuria, reduced transfer of organic anions and glucose, and decreased glomerular filtration rate are functional abnormalities in people that have been linked to excessive Pb exposure. A few investigations have revealed the histological signs of renal damage in humans, including interstitial fibrosis, cellular necrosis, and intranuclear inclusion bodies in the proximal tubule (Cramer et al., 1974; Biagini et al., 1977).

Ijeh and Ejike (2011) and Pitot and Dragan (2013) provide extensive documentation of the medicinal benefits of *Vernonia amygdalina* (VA), a shrub growing 2-5 meters tall with petiolate leaves that are around 6 millimeters in diameter and elliptic in form and consumed as a green vegetable in Nigeria.

Due to its bitter flavor, which can be linked to its anti-nutritional components such tannins, alkaloids, glycosides, flavonoids, and saponins, it is commonly referred to as “bitter leaves” (Igile et al., 1994; Johri and Singh, 1997; Ejoh et al., 2007; Ekpo et al., 2007; Eleyinmi et al., 2008).

Nitrogen, phosphorus, calcium, magnesium, sodium, potassium, manganese, copper, and cobalt are the main ions found in VA, along with ascorbic acid and carotenoids (Egedigwe, 2010). (Kupchan et al., 1969; Ayodele, 1999). According to Pitot and Dragan (1996), the plant’s leaves have antibacterial, anti-cancer, antioxidant, antidiabetic, hepatoprotective, hypolipidemic, and anti-fertility characteristics when extracted in water (Innih and Ubhenin, 2021).

Additionally, the abortifacient, antifertility, antimicrobial, antiplatelet and anticoagulant, antimalarial, hepatoprotective, analgesic, anti-inflammatory, anti-pyretic, antimutagenic, and effect on CD4+ cell count (HIV/AIDS) were reported (Pitot and Dragan, 1996). Bitter leaf’s safety had been demonstrated through both solitary administration and administration while in the presence of toxins (Correia et al., 2000; Demirezen and Kadiriye, 2006).

It is known how VA affects kidney toxicity brought on by Pb (Achuba, 2018; Innih and Ubhenin, 2021). On the impact of chronic and sub-Pb poisoning on renal function parameters, there are contrasting studies. According to certain investigations (Afolabi and Oke, 1981; John, 1999; Innih and Ubhenin, 2021), exposure to Pb caused renal hypertrophy and an increase in glomerular filtrate rate (GFR), whilst other studies indicated a decrease in GFR (Khalil-Manesh et al., 1994). According to independent research on low-dose Pb intoxication (Restek-Samarzija and Momcilovic, 1992; Khalil-Manesh et al., 1994), there were no detectable pathological alterations in Pb-intoxicated rats. Given these discrepancies across various research, it is unclear if Pb toxicity can be linked to the length of time and dosage used to induce Pb intoxication.

Additionally, the majority of the research on this area has been mostly on the subjective qualitative approaches of slide interpretation. In cases of toxicity, morphometric and stereological examinations of tissue are extremely effective. There are more techniques for evaluating the total kidney volume and renal function for hypertrophy or shrinking (Almajdub et al., 2008; Abdellatif and Hassan, 2013; Yamashita et al., 2015; Abdurrahman et al., 2018) including stereological technique which involved the exhaustive sectioning of the kidney and then selecting representative sections using systematic uniform random sampling (Nyengaard, 1999).

Relative medullary thickness (RMT) was discovered to have a substantial direct link with the maximum urine concentrating ability of animals. In an investigation on a goat after a nephrectomy, Tejo et al. (2014) reported this link between animal species (Abdellatif and Hassan, 2013). RMT is calculated by comparing the medullary thickness (MT) to the kidney size (KS) of the medulla, measured from the corticomedullary junction to the tip of the papilla (Al-kahtani et al., 2004; Abdurrahman et al., 2018).

This investigation uses morphometry to assess the effects of VA on the kidney of Wistar rats given low doses of Pb in relation to biochemical indicators of kidney function.

MATERIALS AND METHODS

Acquisition of chemicals: Pb

The chemicals used- Lead (Pb) was procured from a laboratory Emole Nigeria limited at high-level makurdi, Benue state.

Acquisition of plant materials

Vernonia amygdalina (Bitter leaf) used in the study was obtained from a farm land close to River Benue at Wurukum, Makurdi-Benue state.

Procurement and maintenance of animals

A total of 25 Wistar rats between 100-150 g of both sexes were obtained from Animal House at College of Health Science, Benue state University, Makurdi. They were fed with normal rat chow for a period of two week for acclimatization.

They were housed in groups of five (5) in plastic constructed cages, in a well-ventilated room at room temperature and fed with normal commercial pellet diet and water. Animal Research Review Panel and Animal Welfare Unit regulations of temperature and lighting systems were maintained with a room temperature of 20-26°C as well as regular light cycles of 12 hours light/dark. All methods and protocols used in the study were observed following established public health guidelines Guide for Care and Use of Laboratory Animals."

Preparation of leaf extracts

The plant samples were washed thoroughly with tap water to remove any form of dirt. The leaves were then air-dried under shade for 14 days at room temperature and pulverized into powder using a blender and stored in airtight plastic containers. One hundred and twenty-five grams (300 g) of the powdered leaves were macerated in separate cold ethanol (Absolute ethanol) and were allowed to stand to at room temperature for 48hour. The mixture was shaken continuously for several times for a uniform mixture and to obtain a maximum extract yield. After the 48 hours, the macerated solution was filtered using a filter paper into a beaker. The filtrate was boiled on a water bath at a temperature of 70°C to separate the ethanol from the crude drug. The crude drug extract was labelled EEVA (ethanolic extract of Vernonia amygdalina) and yield about 61.6g then was transferred into a Petri dish and kept under sun shied to solidify and dry the extract, after which it was kept in a refrigerator at 4°C.

Experimental design

The Wistar rats were separated into 5 different groups containing 5 animals each, labeled A to E. Group A: This group served as the negative control group and was orally gavaged with 5mg/kg body weight of normal saline daily for 5 days.

Group B: This group served as the positive control and was treated with daily intraperitoneal (IP) injection of 8mg/kg body weight of Pb for 28 days (Salem and Salem, 2016).

Group C: This group was treated with daily intraperitoneal (IP) injection of 8mg/kg body weight of Pb along with 20mg/kg body weight of VA extract (Akinyemi, 2016) orally for 28 days.

Group D: This group was treated with daily intraperitoneal (IP) injection of 8mg/kg body weight of Pb along with 40mg/kg body weight of VA extract orally for 28 days.

Group E: This group was treated with daily intraperitoneal (IP) injection of 8mg/kg body weight of Pb along with 60mg/kg body weight of VA extract orally for 28 days.

Animal sacrifice

After 24 hours at the end of the treatment period of 28 days, the animals were sacrificed by cervical decapitation been the 29th day after an overnight fast. Blood samples were collected immediately by cardiac puncture into ETDA tubes and labeled. The blood samples collected were for various biochemical assays. The kidneys also were harvested into labeled containers under cold conditions for histological analysis.

Assessment of biochemical parameters

Experimental Wistar rats were sacrificed, and blood was dispensed into serum separator tubes and then analyzed for biochemical parameters. Kidney urea, creatinine and malondialdehyde (MDA) levels were assayed and analyzed using a standard BS-200E Mindray chemistry Autoanalyzer, PKF Scientific Limited. The urea and creatinine were measured in mg/dl.

Histological and morphometric analysis

Rats were dissected, and the kidneys were taken out and examined for signs of gross pathology. Tissue samples were immediately fixed in 10% formalin, put through an automated tissue processor, and then embedded in paraffin wax for light microscopic inspection. The tissue blocks were then cut into serial sections using the rotary microtome. Following deparaffinization, the sections were stained with hematoxylin and eosin (H&E). Images of each section were captured using a light microscope after the tissue had undergone histological processing. A computerized image analysis system was used for the morphometric study. Hematoxylin and eosin-stained kidney slices were seen in Image J using a digital camera. The morphometric parameters that were measured were as follows: proximal and distal tubule area in square micrometers, nuclear/cytoplasmic ratio of proximal and distal tubule epithelial cells, glomerular membrane thickness (GBM) in micrometers, glomerular area in square micrometers, glomerular cellularity in square micrometers, perimeter in micrometers, Feret's diameter in micrometers, and circularity. At least 50 proximal and distal tubules and glomeruli were counted in every animal. Prior to each study, a spatial calibration using an object micrometer was carried out.

Circularity was determined by the formula: $\frac{(4\pi^{*}\text{area})}{(\text{perimeter}^2)}$

Statistical analysis

Mini tab software (version 17.1.0. cracked) was used for the data analysis. Data were presented as mean ± SEM on bar chart graphs and tables. To compare the biological effects of the treatment, analysis of variance (ANOVA) was used. Values of less than 0.05 were considered statistically significant.

RESULTS

Gross anatomical features

There was no significant change in the body weight, kidney weight and kidney weight and body weight ratio when compared across all group as shown in Table 1.

Biochemical analysis and oxidative makers

Creatinine

The activity level of creatinine in group 2 rats was significantly ($p \leq 0.05$) decreased when compared to Group-1 rats. The rats in Groups 3, 4 and 5 recorded a mean creatinine level when compared to group 2 rats. There were significant ($p \leq 0.05$) increase when compared to group 2 rats. The activity level of Urea in Group

Table 1. Showing the mean ± standard deviation of different gross anatomical features across all group.

	Initial body weight (g)	Final body weight (g)	Body weight difference (%)	Kidney weight (g)	Kidney weight/body weight ratio
Group 1	80.94 ± 6.59	102.56± 9.51	21.62±4.94	1.70 ± 1.30	0.08±0.06
Group 2	106.24± 6.33	128.46± 5.32	22.62±2.02	1.51 ± 0.30	0.08±0.06
Group 3	101.70±13.64	117.60± 14.40	15.9±3.09	1.70 ± 1.01	0.11±0.09
Group 4	101.34±11.15	114.84± 11.76	13.50±4.11	1.82 ± 1.2	0.11±0.06
Group 5	93.62±10.92	107.04± 10.42	13.42±2.09	1.0 ± 0.40	0.13±0.11

Table 2. Showing the mean ± standard deviation of different biochemical analysis and oxidative markers across all groups.

	Creatinine (mg/dl)	Urea (mg/dl)	MDA (nmol/mg pro)
Group 1	0.84 ± 0.04	26.00 ± 1.32	0.64 ± 0.11
Group 2	0.51 ± 0.11*	19.91 ± 1.50*	3.17 ± 0.13*
Group 3	0.85 ± 0.05**	21.88 ± 2.06	1.76 ± 0.50**
Group 4	0.85± 0.09**	22.16 ± 2.37	0.88 ±0.44**
Group 5	0.93 ± 0.12**	21.88 ± 2.06	0.95 ± 0.63**

*and** showed a significance when compared to group 1 and 2 respectively.

2 rats were significantly ($p \leq 0.05$) decreased when compared to group 1 rats. The rats in Groups 3, 4 and 5 recorded a mean urea level that had non-significant ($p > 0.05$) increase when compared to Group-2 rats.

The activity level of lipid peroxidation was significantly ($p \leq 0.05$) increased in Group-2 rats when MDA level was compared to group 1 rats. However, the rats in Groups 3, 4 and 5 recorded a mean MDA level that was significant ($p \leq 0.05$) when compared to Group 2 rats with each.

Histological profile

Macroscopically the kidneys of Group 1 (Fig. 1) appeared to be normal as seen in histological text. The surfaces were granular and there was presence of numerous cortical tissues with corticomedullary closure and devoid of vascular markings. The pyramids were also intact. The kidney surfaces of Group-2 rats (Fig. 2) appear contracted and have a granular surface. The cut surface shows general loss of cortical tissue, corticomedullary demarcation and vascular markings.

The pyramids are small but intact. When Group-2 histomorphology were compared to that of Group 1, there were varying degrees of relatively cellular interstitial nephritis. Areas of dilated tubules alternate with atrophic tubules, rendering a granular appearance to the kidney surface. A large proportion of glomeruli are lost without leaving a trace, which is a characteristic feature.

Level of distortion and disruption of the cytoarchitecture of the renal cortical structure with marked diffuse glomerulonephritis and an enlarged Bowman's space are observed as compared to the control section.

The kidney of Groups 3 to 5 (Figs. 3, 5) also appeared distorted when compared to Group 1. But when you compare them to Group 2 they were better off.

Morphological scores

There was a significant increase in proximal tubular area, distal tubular area, glomerular membrane thickness and a significant ($p \leq 0.05$) decrease in proximal tubule and distal tubule ratio of Group-2 rats when compared to the negative control (Group 1).

The proximal tubular area and distal tubule ratios across all experimental groups were significantly ($p \leq 0.05$) different from those of the positive control (Group 2). For distal tubule area and glomerular membrane thickness,

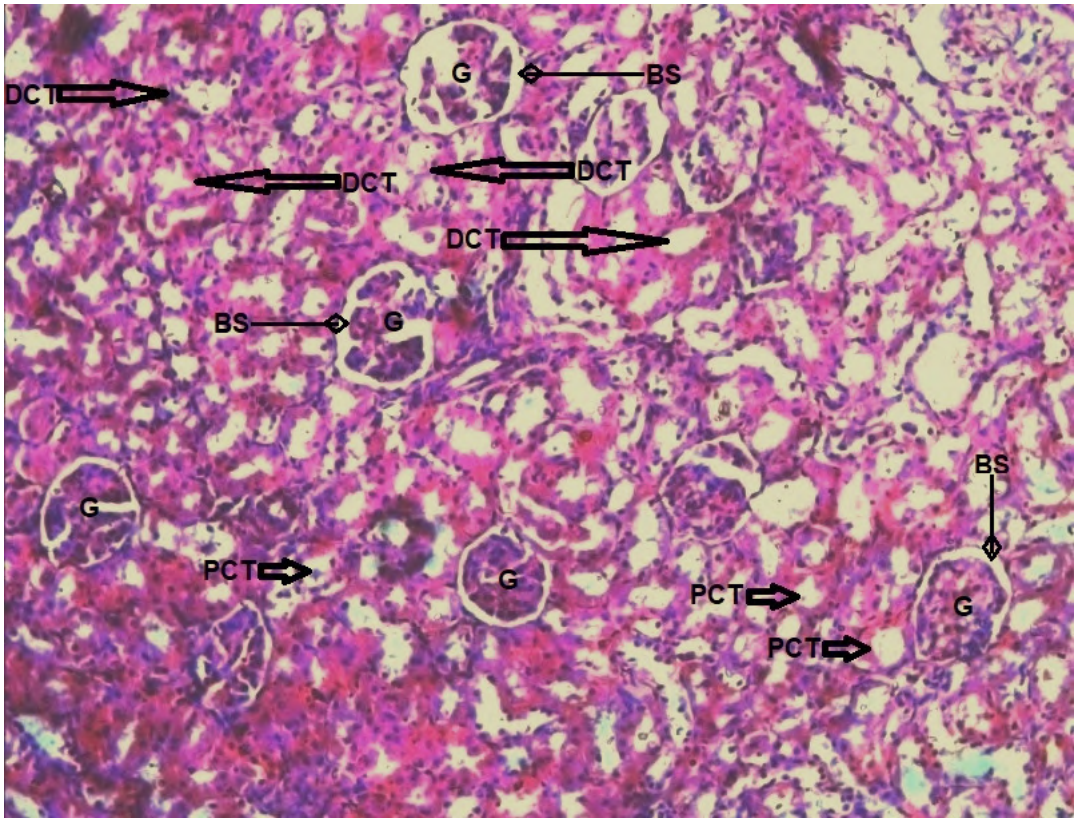

Fig. 1.- Kidney morphology from Group-1 rats (5 ml/kg of normal saline) showing the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), glomerulus (G) and Bowman's space (BS). Magnification: x200. Stain: H&E.

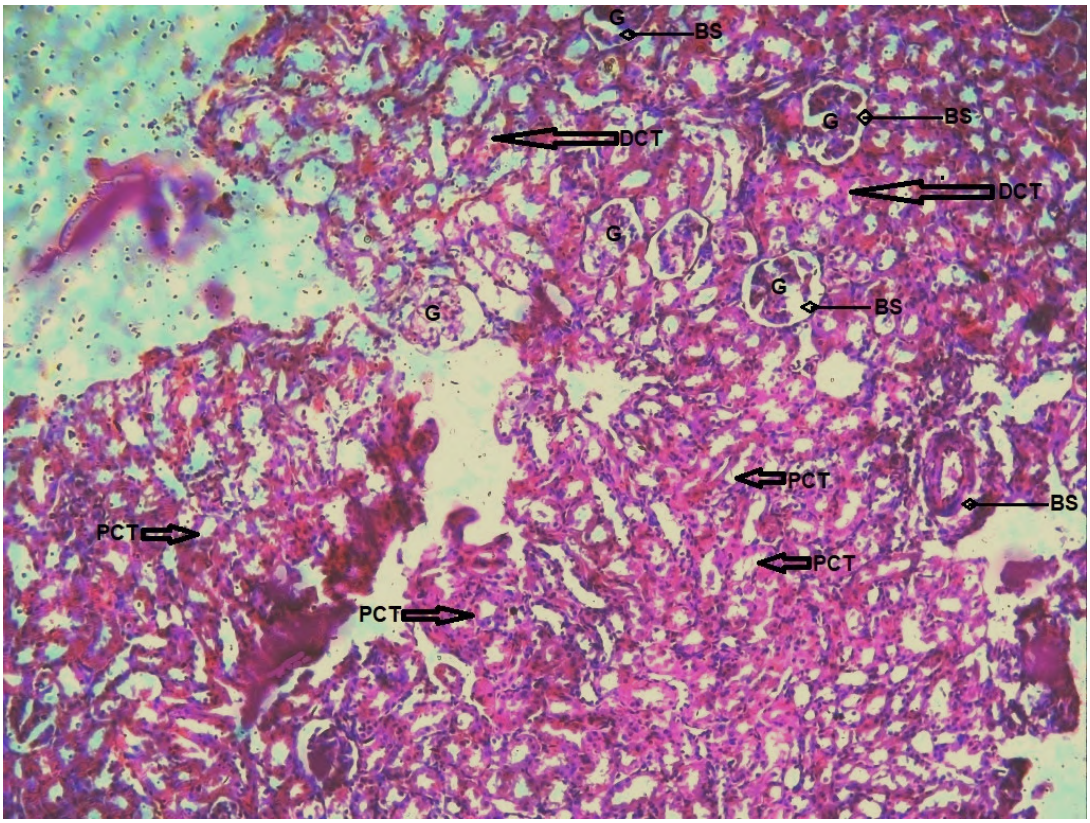

Fig. 2.- Kidney from Group 2 / positive control (20 mg/kg body weight of Pb intraperitoneally) showing the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), glomerulus (G) and Bowman's Space (BS). Magnification: x200. Stain: H&E.

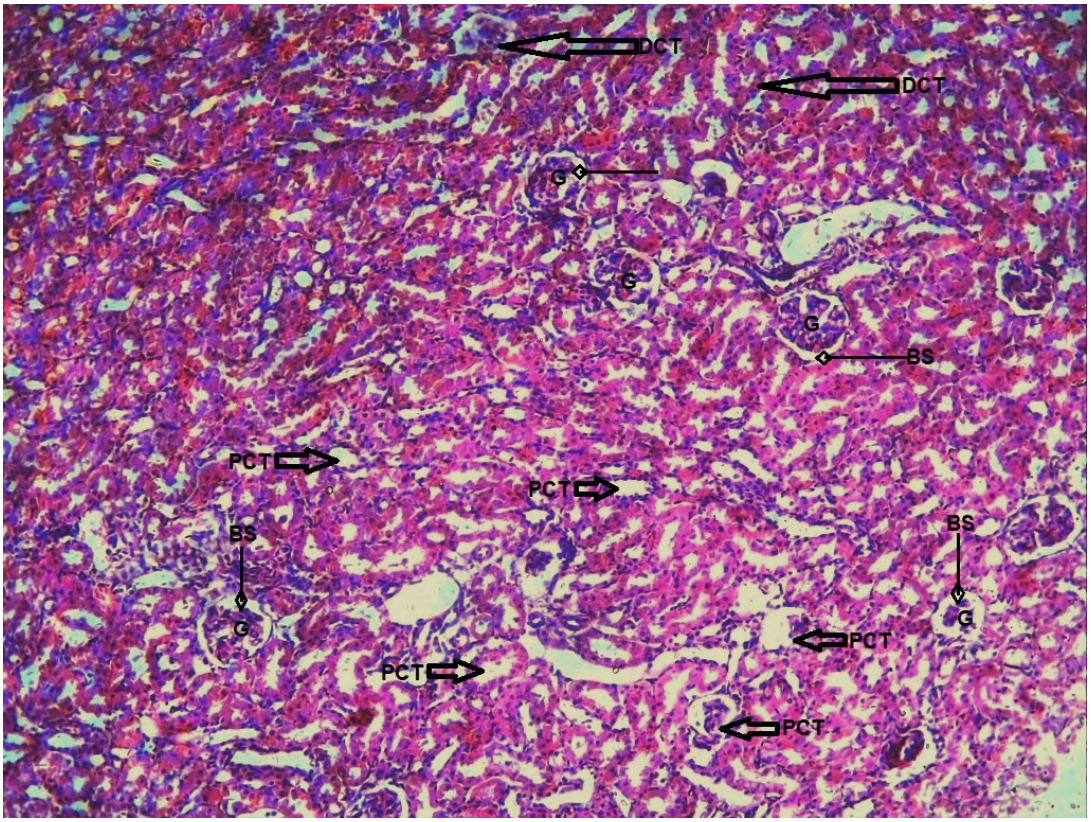

Fig. 3.- Kidney from Group 3 (20 mg/kg body weight of Pb intraperitoneally and 100 mg/kg body weight of *Vernonia amygdalina* extract) showing the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), glomerulus (G) and Bowman's Space (BS). Magnification: x200. Stain: H&E.

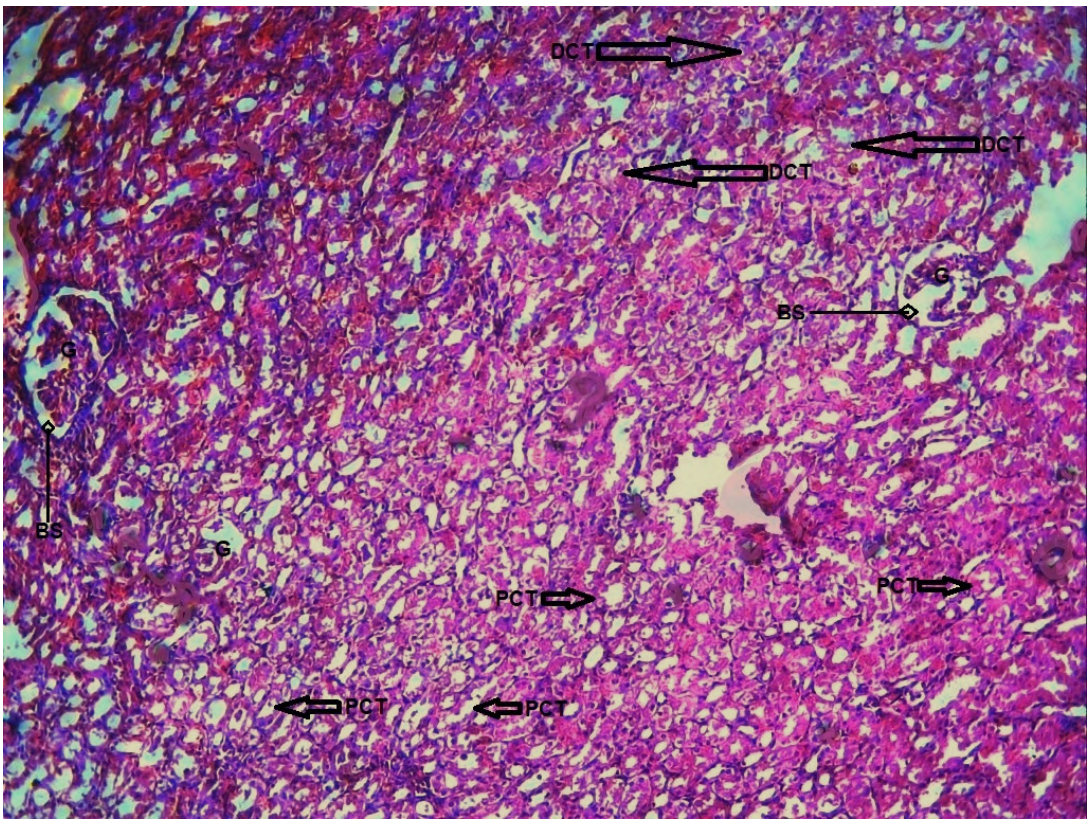

Fig. 4.- Kidney from Group 4 (20 mg/kg body weight of Pb intraperitoneally and 200 mg/kg body weight of *Vernonia amygdalina* extract), showing the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), glomerulus (G) and Bowman's Space (BS). Magnification: x200. Stain: H&E.

only Groups 4 and 5 were significantly ($p \leq 0.05$) different from that of the positive control (Group 2). Also, with proximal tubule ratio only Group 5 was significantly ($p \leq 0.05$) different from that of the positive control (Group 2) (Table 3).

There was a significant ($p \leq 0.05$) increase in area, perimeter and feret's diameter and a significant ($p \leq 0.05$) decrease in cellularity of Group-2 rats when compared to the negative control (group 1).

Although the area, cellularity and feret's diameter in Groups 4 and 5 rats were significantly ($p \leq 0.05$) different from those of the positive control (Group 2), there was no significant ($p \geq 0.05$) difference between the values of area, cellularity and feret's diameter in Group-3 rats when compared to the positive control (Group 1). Group 4 had no significant difference between the perimeter values when compared to the positive control (Group 1) (Table 4).

Table 3. Showing the mean ± standard deviation of some morphological scores across all groups.

Groups	Proximal tubule Area (µm²)	Distal tubule Area (µm²)	Proximal tubule N/C ratio	Distal tubule N/C ratio	Glomerular membrane thickness (µm)
1	1323±103.04	726.40±23.02	0.614±0.11	0.762±0.23	0.72±0.51
2	3634± 132.1**	1214.2±64.1**	0.271±0.13**	0.523±0.16**	0.91±0.71**
3	2131±102.1*	1204.1±89.3	0.292±1.04	0.663±0.31*	0.82±0.21
4	2021±97.1*	946.3±104*	0.262±1.08	0.681±0.41*	0.71±1.04*
5	1783±131.3*	832.6±132.3*	0.562±2.3*	0.625±0.03*	0.63±0.31*

*and** showed a significance when compared to group 1 and 2 respectively.

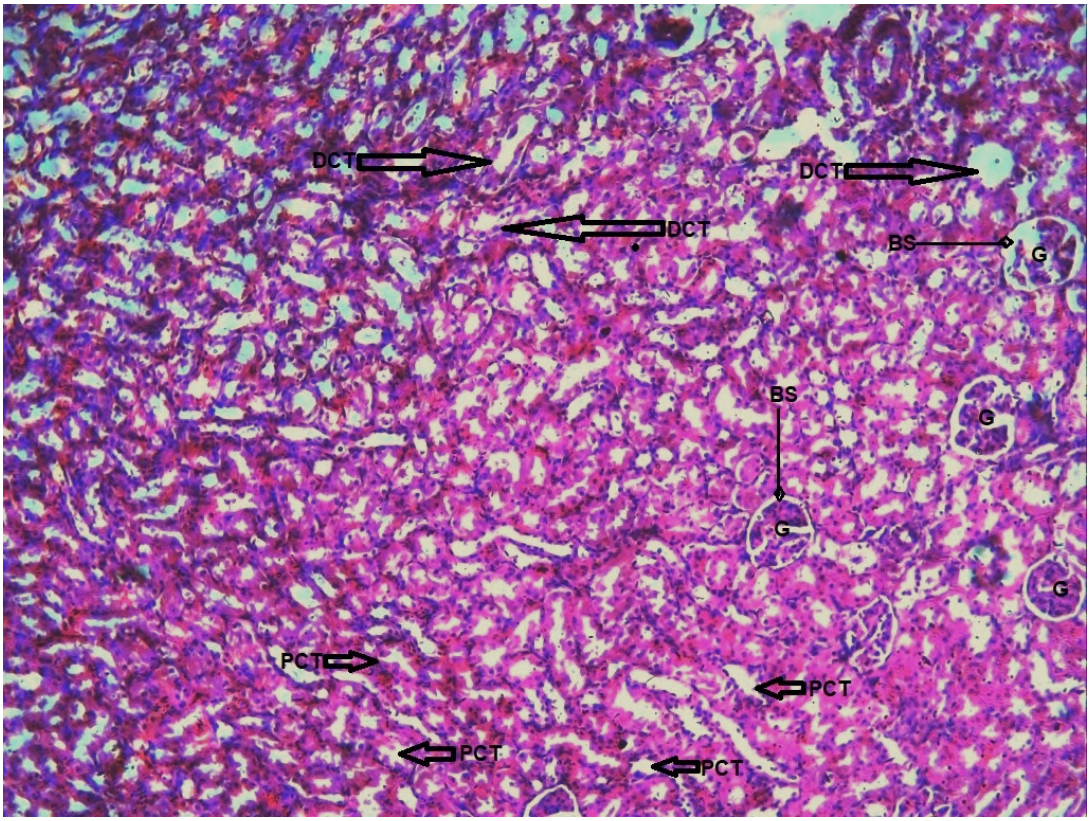

Fig. 5.- Kidney from Group 5 (20 mg/kg body weight of Pb intraperitoneally and 300 mg/kg body weight of *Vernonia amygdalina* extract), showing the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), glomerulus (G) and Bowman's Space (BS). Magnification: x200. Stain: H&E.

Table 4. Showing the mean ± standard deviation of some morphological scores across all groups.

Groups	Area (μm²)	Cellularity (cells/μm²)	Perimeter (μm)	Feret's diameter (μm)	Circularity
1	9321±201.4	0.0092±0.0001	412.3±6.1	130.2±3.1	0.764±0.001
2	12240±140.1**	0.006± 0.0001**	492±21.03**	162.1±1.3**	0723±0.003**
3	11411±110.3	0.007± 0.0002	328±24.3*	151.3±2.8	0721±0.001
4	10121±91.4*	0.008± 0.0001*	472±17.1	142.5±5.7*	0724±0.004
5	91201±124*	0.008± 0.0004*	332±11.5*	129.1±6.2*	0728±0.002

*and** showed a significance when compared to group 1 and 2 respectively.

DISCUSSION

In this study, all groups of rats had a non-significant increase in body weight but not in kidney weight. This finding conflicts with other earlier studies on the impact of Pb on kidney weight (Seddik et al., 2010; Ali et al., 2010). While Flaherty et al. (1986) saw increases in kidney wet weight and dry weight in rats given varied doses of Pb, Goyer (1971) noted an increase in kidney wet weight in rats given a high dose of Pb for prolonged periods of time (Aseth et al., 1995; Teijon et al., 2006). Because of their active growth phase, the rats in this study may have gained weight even in the positive control group (Marija et al., 2004).

In mice, exposure to Pb has been found to increase the generation of reactive oxygen species (ROS), which causes lipid peroxidation and changes to the antioxidant defense mechanisms, leading to oxidative stress (Demirezen and Kadiriye, 2006; Xienia et al., 2000). The byproducts of several degenerative processes in different tissues, known as ROS, damage the cellular components and impair normal metabolism (Foyer and Noctor, 2000). When compared to the positive control, the VA-treated rat groups showed a considerable improvement in their antioxidant system.

The positive impact of antioxidant nutrients through exogenous supplementation of antioxidant molecules may be ascribed to lowering the chance of Pb interacting with important biomolecules and promoting oxidative damage or enhancing the cell's antioxidant defense (Marija et al., 2004). Because flavonoids include numerous hydroxyl groups, which serve as metal chelators by creating a coordination link with Pb, VA may have prevented the accumulation of Pb in the kidney. These biologically active substances may have chelated lead and improved its removal from the body, reducing lead buildup in renal tissue (Sharma et al., 2010; Iwara et al., 2013; Oladele et al., 2021).

Additionally, as compared to the negative control group, animals treated with Pb alone had higher levels of urea and creatine. This is consistent with findings from investigations in rats (Mugahi et al., 2009; Elayat and Bakheelf, 2010), goats (Swarup and Dwivedi, 1992; Haneef et al., 1998), sheep (Mugahi et al., 1998), and goats after oral administration of Pb (Ahmed and Shalaby, 1991).

This could be a symptom of kidney disease and is thought to constitute functional proof of Pb-induced nephrotoxicity (Patterson, 1965; Zook et al., 1972). The increase in serum creatinine level revealed decreased creatinine clearance and a compromise of the glomerular filtration function. Creatinine clearance is a more clinical biomarker of glomerular filtration capacity.

The metabolic byproducts of creatine and amino acids, respectively, are creatinine and urea. They frequently serve as markers of renal function. Due to a decrease in glomerular filtration, damage to functional nephrons can cause a rise in serum creatinine and urea levels (Ellman, 1959; El-Ashmawy et al., 2005). Since serum creatinine and urea cannot be excreted from the blood due to renal failure, their elevated levels indicate glomerular dysfunction (El-Ashmawy et al., 2005). This results in a rise in blood levels and a corresponding decrease in urine. The blood levels of urea and creatinine were raised as a result of this impairment of the renal functions.

In contrast to the negative controls, the rats treated with Pb had lower levels of creatinine and urea in their kidneys, according to the current investigation, whereas Heba et al. (2014) observed

a significant increase in serum creatinine and urea activities. Our findings showed that VA had a nephroprotective effect against Pb-induced nephrotoxicity, as evidenced by improvements in creatinine and urea levels in the pretreated groups.

This might be through its direct action on free radicals of Pb, protecting the kidney from cellular damage by maintaining its membrane integrity. The development of localized tubular necrosis away from the kidney's photomicrograph further supported the pathognomonic symptoms of kidney damage brought on by Pb.

Dystrophic and necrotic processes that manifest as ischemic necrosis of the tubular epithelium and interstitial oedema with connective tissue disorganization complicate pathomorphological processes (Aarabi, 2009; Merets'kyi and Holovata, 2013).

Pb exposure induced increasing glomerular and tubular changes, according to histological studies. These results are consistent with those of earlier studies by Abdel-Moneim et al. (2011), who identified changes in Pb exposure-related kidney histopathology. Heba et al. (2014) previously documented tubular vacuolization, necrosis, and dilation seen in the current research as a result of Pb intoxication. These Pb-induced tubular abnormalities may be the result of hydrolic changes in the renal tissue, which suggests that Pb toxicity leads to a partial breakdown in the transport of ions by tubule cells, resulting in tubular swelling, necrosis, and vacuolization of the tubules.

It should be mentioned that the structure and function of a rat's kidney are comparable to those of a human kidney in the context of the clinical application of the findings of experimental investigations of the effects of Pb on rats. Minor variations are mostly found at the macrostructural level; unlike human kidneys, rats' kidneys only contain one pyramid. The structure of the vascular grid also differs. However, the nephron structure, kidney layer hierarchy, and functioning are comparable to those found in humans.

This closeness has allowed for a number of inferences on the comparability of the pathological changes brought on by Pb in the rat kidney (Prus et al., 2020). When compared to rats in Group 1, rats in Group 2 had larger glomeruli, irregularly thickened glomerular basement membrane, and neutrophil cell infiltration.

There was a considerable Pb-induced apoptotic reaction in the renal glomerular tissue, as evidenced by the reduction in the number of cells in the glomerular area.

According to Kohn et al. (2002), the damage to glomerular cells may be significantly influenced by mitochondrial dysfunction. Barnes et al. (2020) also made this observation. This is the first morphometric investigation of rat kidneys in this experimental setting that we are aware of.

This finding offers proof that the compromised function observed in rats exposed to Pb may be related to renal morphological alterations. We noted a decrease in the content of urea and creatine. It is sufficient to state that this research has provided quantitative microscopic morphometry on the effects of VA on kidney damage caused by Pb in a rat model.

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REFERENCES

AARABI B, SIMARD J (2009) Traumatic brain injury. *Curr Opin Crit Care*, 15(6): 548-553.

ABDELLATIF AM, HASSAN Y (2013) Effects of uninephrectomy on morphological and histological measurements of the remnant kidney in a goat (*Capra hircus*) model. *J Phys Pharm Adv*, 3(3): 94-102.

ABDEL-MONEIM E, DKHIL MA, AL-QURAISHY S (2011) The potential role of flaxseed oil on Pb induced kidney injure in adult male Albino rats. *Afr J Biotechnol*, 10(8): 1436-1451.

ABDULKADIR A, MBAJIORGU EF, NYIRENDA T (2018) Effects of concurrent chloroquine and ethanol administration on the rat kidney morphology. *Pan Afr Med J*, 29: 49.

ACHUBA FI (2018) Role of bitter leaf (*Vernonia amygdalina*) extract in prevention of renal toxicity induced by crude petroleum contaminated diets in rats. *Int J Vet Sci Med*, 6(2): 172-177.

AFOLABI OA, OKE OL (1981) Preliminary studies on the nutritive value of some cereal-like grains. *Nutr Rep Int*, 24: 389-394.

AHMED YF, SHALABY SA (1991) Clinicopathological and histopathological studies on chronic Pb intoxication in male Barki sheep. *Afr J Agric Sci*, 18: 19-37.

ALI MA, AWAL MA, MOSTOFA M, ISLAM MA, GHOSH A (2010) Effects of selenium and vitamin B6 with their combination in Pb induced toxicities in long Evans rats. *Bangl J Vet Med*, 8: 63-73.

AKINYEMI T (2016) Effect of bitter leaf (*Vernonia amygdalina*) saponin-rich extract on streptozotocin-induced diabetes in Wistar rats. Masters Thesis. http://196.220.128.81:8080/xmlui/handle/123456789/3453

AL-KAHTANI MA, ZULETA C, CAVIEDES-VIDAL E, GARLAND JR T (2004) Kidney mass and relative medullary thickness of rodents in relation to habitat, body size and phylogeny. *Physiol Biochem Zool*, 77(3): 346-365.

ALMAJDUB M, MAGNIER L, JUILLARD L, JANIER M (2008) Kidney volume quantification using contrast-enhanced in vivo X-ray micro-CT in mice. *Contrast Media Mol Imaging*, 3(3): 120-126.

ALWALEEDI SA (2016) Hematobiochemical changes induced by Pb intoxication in male and female albino mice. *Natl J Physiol Pharm Pharmacol*, 6: 46-51.

ASETH J, JACOBSEN D, ANDERSEN O, WICKSTROM E (1995) Treatment of mercury and Pb poisoning with dimercaptosuccinic acid (DMSA) and sodium dimercapto-propanesulfonate (DMPS). A review. *Analyst*, 120: 853.

AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY (ATSDR) (1993) Toxicological Profile for Pb (1993) Update. Prepared by Clement International Corporation under contact no. 205-88-060 for ATSDR. Atlanta, GA: US Public Health Services.

AYODELE MS (1999) Karyomorphological studies in some Nigerian species of Vernonia SCHREB. (Asteraceae) with different growth forms. *Feddes Repert*, 110: 541-553.

BARNES P, YEBOAH JK, GBEDEMA W, SAAHENE RO, AMOANI B (2020) Ameliorative effect of *Vernonia amygdalina* plant extract on heavy metal-induced liver and kidney dysfunction in rats. *Adv Pharmacol Pharmac Sci*, 2020: 2976905.

BIAGINI G, CAUDARELIA R, VANGELISTA (1977) Renal morphological and functional modification in chronic Pb poisoning. In: Brown SS (ed). *Clinical chemistry and chemical toxicology of metals*. Elsevier/North-Holland Biomedical Press, pp 123-126.

CHANG LW, WADE PR, OLSON MN (1980) Ultrastructural changes in renal proximal tubules after tetraethyle Pb intoxication. *Environ Res*, 23: 208.

CORREIA PM, OLIVEIRA E, OLIVEIRA PV (2000) Simultaneous determination of Cd and Pb in foodstuffs by electro-thermal atomic absorption spectrometry. *Anal Chim Acta*, 405: 205-211.

CRAMER K, GOYER RA, JAGENBURG R (1974). Renal ultrastructure, renal function and parameters of Pb toxicity in workers with different periods of Pb exposure. *Br J Ind Med*, 31: 113-127.

DEMIREZEN D, KADIRIYE U (2006) Comparative study of trace elements in certain fish, meat and meat production. *Meat Sci*, 74: 255-260.

DIAMOND GL (2005) Risk assessment of nephrotoxic metals. In: Tarloff J, Lash L (eds). *The toxicology of the kidney*. London: CRC Press, pp 1099-1132.

DURUIBE JO, OGWUEGBU MC, EGWURUGWU JN (2007) Heavy metal pollution and human biotoxic effects. *Int J Phys Sci*, 2: 112-118.

EGEDIGWE CA (2010) Effect of dietary incorporation of *Vernonia amygdalina* and *Vernonia colorata* on blood lipid profile and relative organ weights in albino rats. MSc., Dissertation, Dept. Biochem., MOUAU, Nigeria.

EJOH RA, NKONGA DV, INOCENT G, MOSES MC (2007) Nutritional components of some nonconventional leafy vegetables consumed in Cameroon. *Pak J Nutr*, 6: 712-717.

EKPO A, ESEYIN OA, IKPEME AO, EDOHO EJ (2007) Studies on some biochemical effects of Vernonia amygdalina in rats. *Asia J Biochem*, 2: 193-197.

EL-ASHMAWY IM, EL-NAHAS AF, SALAMA OM (2005) Protective effect of volatile oil, alcoholic and aqueous extracts of Origanum majorana on Pb toxicity in mice. *Basic Clin Pharmacol Toxicol*, 97: 238-243.

ELAYAT W, BAKHEELF MS (2010) Effects of chronic Pb toxicity on liver and kidney functions. *J Med Lab Sci*, 1: 29-36.

ELEYINMI AF, SPORNS P, BRESSLER DC (2008) Nutritional composition of Gongrone malatifolium and Vernonia amygdalina. *Nutr Food Sci*, 38: 99-109.

ELLMAN GL (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82: 70-77.

ERCAL N, GURER OH, AKYIN BN (2010) Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr Topics Med Chem*, 1: 529-539.

FOYER CH, NOCTOR G (2000) Oxygen processing in photosynthesis: regulation and signaling. *New Phytol*, 146: 359-388.

HANEEF SS, SWARUP D, DWIVEDI SK, DASH PK (1998) Effects of concurrent exposure to Pb and cadmium on renal function in goats. *Small Rum Res*, 28: 257-261.

HEBA M, ABDOU, MOHAMED A. HASSAN (2014) Protective role of omega-3 polyunsaturated fatty acid against pb-induced toxicity in liver and kidney of female rats. *BioMed Res Int*, 2014: 435857.

IGILE GO, OLESZEK W, JURZYSTA M, BURDA S, FAFUNSO M (1994) Flavonoids from Vernonia amygdalina and their antioxidant activities. *J Agri Food Chem*, 42: 2445-2448.

IJEH II, EJIKE CE (2011) Current perspectives on the medicinal potentials of Vernonia amygdalina. *Del J Med Plants Res*, 5: 1051-1061.

INNIH SO, UBHENIN AE (2021) The protective effect of vernonia amygdalina in Pb-induced nephrotoxicity in Wistar rats. *Niger J Exp Clin Biosci*, 9: 227-233.

IWARA, IA, OUT EA, EFIONG E. IGILE GO, MGBEJE BAI, EBONG PE (2013) Evaluation of the nephroprotective effect of combine extract of vernonia amygdalina and moringa oleifera in diabetic induced kidney injury in albino Wistar rats. *Sch J App Med Sci*, 1(6): 881-886.

JAN A, AZAM M, SIDDIQUI K, ALI A, CHOI I, HAQ Q (2015) Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. *Int J Mol Sci*, 16(12): 29592-29630.

JOHN JB (1999) Estimating plasma in pH. In: Bray John J (ed). *Lecture Notes on Human Physiology*, 4th ed. Lecture Note Series, Blackwell Scientific Publications Lecture Notes, Wiley, New York, United States, p 610.

JOHRI RK, SINGH C (1997) Medicinal uses of Vernonia species. *J Med Aromat Plant Sci*, 19: 744-752.

JOWORASKI Z (1968) Stable and radioactive Pb in environment and human body. Warsaw: Nuclear Energy Information Center, Review Report, 29. pp 17-30.

KHALIL-MANESH F, TARTAGLIA-ERLER J, GONICK HC (1994) Experimental model of Pb nephropathy. IV. Correlation between renal functional changes and hematological indices of Pb toxicity. *J Trace Elem Electrolytes Health Dis*, 8: 13-19.

KOHN S, FRADIS M, BEN-DAVID J, ZIDAN J, ROBINSON E (2002) Nephrotoxicity of combined treatment with cisplatin and gentamicin in the guinea pig: glomerular injury findings. *Ultrastruct Pathol*, 26(6): 371-382.

KUPCHAN SM, HEMINGWAY RJ, KARIM A, WERMER D (1969) Tumour inhibitors XLVII vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from Vernonia amygdalina Del. *J Organic Chem*, 34: 3908-3911.

LOGHMAN-ADHAM M (1997) Renal effects of environmental and occupational Pb exposure. *Environ Health Perspect*, 105: 928-939.

MARIJA V, PIASEK M, BLANUSA M, SARIC M, JURESA D, KOSTIAL K (2004) Succimer treatment and calcium supplementation reduce tissue Pb in suckling rats. *J Appl Toxicol*, 24: 123-128.

MERETS'KYÏ VM, HOLOVATA TK (2013) Morphological changes in internal organs of animals in different periods of craniocerebral trauma on background of diabetes mellitus. *Klin Khir,* 4: 73-75.

MUGAHI MN, HEIDARI Z, SAGHEB HM, BARBARESTANI M (2003) Effects of chronic Pb intoxication on blood indices of male adult rat. *DARU*, 11: 147-151.

NYENGAARD JR (1999) Stereologic methods and their application in kidney research. *J Am Soc Nephrol*, 10(5): 1100-1123.

OLADELE, JO, OYELEKE OM, OLOWOOKERE BD, BABATOPE OD, OLANIYAN MD, AKINDOLIE BO, OLADELE OT (2021) Bitter leaf (Vernonia amygdalina) modulates nitrobenzene-induced renal damage in rats via suppression of oxido-inflammatory activities. *Serbian J Exp Clin Res*, 22(4): 317-324.

PATTERSON C (1965) Contaminated and natural Pb in environments of man. *Arch Environ Health*, 11: 344-360.

PITOT CH, DRAGAN PY (1996) Chemical carcinogenesis. In: Casarett and Doull's (eds). *Toxicology: The Basic Science of Poisons*, 5th edn. McGraw-Hill, New York, pp 201-260.

PRUS R, POKOTYLO P, LOGASH M, ZVIR T (2020) Morphological particularities and morphometry of rats' kidneys under the effect of experimental mild traumatic brain injury. *Folia Morphol*, 80(2): 310-316.

RESTEK-SAMARZIJA N, MOMCILOVIC B (1992) Late changes in renal function after Pb poisoning and chelation therapy-article in Czech. *Arh Hig RadaToksikol*, 43: 321-328.

SALEM NA, SALEM EA (2016) Protective antioxidant efficiency of garlic against lead-induced renal and testicular toxicity in adult male rats. *J Heavy Met Toxicity Dis*, 1: 3.

SEDDIK L, BAH TM, AOUES A, BENDERDOUR M, SLIMANI M (2010) Dried leaf extract protects against Pb-induced neurotoxicity in Wistar rats. *Eur J Sci Res*, 42: 139-151.

SHARMA V, SHARMA A, KANSAL (2010) The effect of oral administration of Allium sativum extracts on Pb nitrate induced toxicity in male mice. *Food Chem Toxicol*, 48(3): 928-936.

SHILPI G, SHILPI S, SHARMA S (2014) Tolerance against heavy metal toxicity in cyanobacteria: role of antioxidant defense system. *Int J Pharm Pharmac Sci*, 7(2): 916.

SURADKAR SG, GHODASARA DJ, VIHOL P, PATEL J, JAISWAL V, PRAJAPATI KS (2009) Haemato-biochemical alterations induced by Pb toxicity in Wistar rats. *Vet World*, 2: 429-431.

SWARUP D, DWIVEDI SK (1992) Changes in blood and cerebrospinal fluid indices in experimental Pb toxicity in goats. *Ind J Animal Sci*, 62: 928-931.

TEIJON C, OLMO R, BLANCO D, ROMERO A, TEIJON JM (2006) Low doses of Pb: effects on reproduction and development in rats. *Biol Trace Elem Res*, 111: 151-165.

TEJO RIQUELME PA, DIAZ ISENRATH GB, ANDINO N, BORGHI CE (2014) Renal intraspecific variation along an aridity gradient detected by new renal indices in a desert herbivorous rodent. *J Exp Zool*, 321(6): 348-356.

XIENIA U, FOOTE GC, VAN S, DEVREOTES PN, ALEXANDER S, ALEXANDER H (2000) Differential developmental expression and cell type specificity of Dictyostelium catalases and their response to oxidative stress and UV light. *Biochim Biophys Acta*, 1492: 295-310.

YAMASHITA SR, ATZINGEN AC, IARED W, BEZERRA ASA, AMMIRATI AL, CANZIANI MEF, D'IPPOLITO G (2015) Value of renal cortical thickness as a predictor of renal function impairment in chronic renal disease patients. *Radiol Bras*, 48(1): 12-16.

ZOOK BC, CARPENTER JL, ROBERTS RM (1972) Pb poisoning in dogs: occurrence, source, clinical pathology, and electroencephalography. *Am J Vet Res*, 33: 891-902.