OPEN ACCESS

Haya: The Saudi Journal of Life Sciences

Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Biochemical and Histomorphological Assessment of Lead-Induced Renal Damage in a Male Wistar Rat Model

Opeyemi A. Adetunji[1], Oluwapelumi M. Ajiboye[2], Bayo O. Adeoye[3*], Oyedayo P. Akano[4], Kehinde A. Ademoye[5], Halliyah C. Adeshina[3], Ayodeji D. Adeoye[4], Nnenna L. Nwobi[6], Sanmi T. Ogunsanya[1], Chigbogu J. Nwobi[3], Iyanuoluwa O. Adebayo[4], Adetomiwa E. Adeogun[4], Samson O. Oyewumi[4], Omowumi O. Akinnawo[3], Cornilluis B. Achor[3], Sandra O. Ajaere[3], Akin-Akanbi Funmilayo Bimbola[7], Osundina Oluwaseun Babatunde[8]

[1]Department of Anatomy, School of Basic Medical Sciences, Babcock University, Ilisan-Remo, Ogun State, Nigeria
[2]Department of Basic Sciences, School of Science and Technology, Babcock University, Ilisan-Remo, Ogun State, Nigeria
[3]Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ogun State, Nigeria
[4]Department of Physiology, School of Basic Medical Sciences, Babcock University, Ogun State, Nigeria
[5]Department of Physiological Sciences, Obafemi Awolowo University, Ile-Ife, Osun-State, Nigeria
[6]Department of Chemical Pathology, School of Basic Clinical Sciences, Benjamin S. Carson (Snr.) College of Health and Medical Sciences, Babcock University, Ogun State, Nigeria
[7]Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria
[8]Department of Chemical Pathology, Faculty of Basic Clinical Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria

DOI: https://doi.org/10.36348/sjls.2025.v10i02.003 | **Received:** 01.08.2024 | **Accepted:** 06.09.2024 | **Published:** 19.02.2025

***Corresponding author:** Adeoye Bayo Olufunso
Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ogun State, Nigeria

Abstract

Introduction: Lead (Pb) is a hazardous metal that presents substantial health hazards to both humans and animals. Extended exposure to lead can result from various causes, such as contaminated water, soil, and industrial emissions, causing nephrotoxicity and kidney damage. ***Methods***: This study investigated the long-lasting impact of lead acetate on the kidneys of Wistar rats. The rats were categorized into four distinct experimental groups, consisting of one control group and three treatment groups. The treatment groups were exposed to varying amounts of lead acetate (2.5%, 3.0%, and 3.5%). The rats had a 35-day treatment period during which their weights was closely monitored, and after sacrificing, kidney function, and histological alterations were assayed. ***Findings***: The data revealed a significant increase in weight among the groups receiving therapy, which can be attributed to the accumulation of water and edema. The 2.5% lead acetate treatment group exhibited higher creatinine levels, suggesting kidney damage. The histological examination showed evidence of kidney injury, including glomerular lesions, fibrotic lesions, and the buildup of leukocytes. ***Conclusion***: Lead acetate induces nephrotoxicity and weight gain in Wistar rats, resulting in a substantial effect on both kidney function and structure. The study emphasizes the significance of taking into account several biomarkers and histological observations in order to comprehend the effects of toxic chemicals such as lead acetate. The results indicate that exposure to lead acetate can result in the development of chronic kidney disease, highlighting the importance of appropriately managing and disposing of items that contain lead.

Keywords: Lead-Acetate, Nephrotoxicity, Heavy Metals, Glomerular Lesions.

INTRODUCTION

Lead (Pb), a toxic metal, is present in the air, water, and soil, and poses substantial health hazards to both humans and animals (Jomova *et al*., 2023; DalCorso *et al*., 2019). It is a dense and heavy metal particle `that may get into the body through inhalation, ingestion, or absorption into the skin (Rahman *et al*., 2019). Lead exposure is a worldwide health concern since it is found in agricultural areas, contaminated food and soil from industrial waste, and it disrupts metabolic activity in organ systems (Alengebawy *et al*., 2021). Prolonged exposure to lead can occur through multiple sources, including as polluted water, soil, and industrial discharges (Akano *et al*., 2024).

Lead toxicity is defined by the presence of oxidative stress, the disruption of enzyme functioning,

and the interference with physiological functions (Jomova *et al*., 2023). Lead toxicity is characterized by nephrotoxicity, which arises from an imbalance between the generation of reactive oxygen species (ROS) and the body's ability to eliminate them (Collin *et al*., 2022; Patra *et al*., 2011; Bennett, 1985). As a result, the kidneys can be harmed, causing conditions such as hyperuricemia and gout. These conditions occur because the body's ability to eliminate uric acid is reduced and there are alterations in the rate at which blood is filtered by the kidneys (glomerular filtration rate or GFR) (Lee *et al*., 2021). Lead can trigger lipid peroxidation, deplete DNA, and disrupt cellular integrity, leading to cell death and tissue damage (Jomova *et al*., 2023; Renu *et al*., 2021).

Prolonged exposure to lead can result in many types of kidney disease, such as tubular dysfunction, glomerular damage, and interstitial fibrosis (Balali-Mood *et al*., 2021; Derouiche *et al*., 2020; Afsar *et al*., 2018). Chronic kidney disease (CKD) is a condition marked by a progressive decline in kidney function (Adeoye *et al*., 2022; Kalantar-Zadeh *et al*., 2021). Previous studies on lead-induced nephrotoxicity in animal models have confirmed that exposure to lead can result in the deterioration of renal tubules, the development of interstitial fibrosis, and the production of scars in glomeruli (Balali-Mood *et al*., 2021; Boskabady *et al*., 2018; Bennett, 1985). The duration and intensity of lead exposure are crucial factors in determining the extent of kidney dysfunction. The objective of this study is to explore the long-lasting impact of lead acetate on the kidneys of Wistar rats, with a particular focus on alterations in tissue structure, markers for kidney function, and evidence of oxidative stress.

MATERIALS AND METHODS

Analytical grades of lead acetate (BDH chemical limited), Aluminium potassium sulphate, Haematoxylin, Eosin, Citric Acid, Acetic Acid, Chloral hydrate, Ferric Chloride, Formaldehyde, EDTA bottles, microscope, absolute alcohol, Plastic cages. All the reagents and chemicals used are of standard analytical grade.

Animal Housing and Experimental Design

A total of twenty eight (28) adult male Wistar rats weighing between 130-180g were obtained from Babcock University Ilishan-Remo, Ogun state. The animals were housed and allowed to acclimatize for 7 days, in a well-aerated plastic cage under a controlled environmental condition (12 hrs light/dark cycle) with free access to standard feed and water. The animals were cared for according to the rules and regulations in animal research and the teaching approved by the institute of Laboratory Animal Resources, National Research Council DHHS (pub no. NH86-23, 1885).

The rats were divided into four experimental groups (n = 7) A, B, C, and D; with Group A as the control. Group B, C, and D were administered a daily dose of Lead Acetate dissolved in distilled water (150 mg/kg body weight) at 2.5%, 3.0% and 3.5% concentration, respectively. The treatment was administered orally using gastric gavage for 35 days (5 weeks). The weight of the rats were monitored twice per week throughout the duration of the treatment administration, to determine the percentage weight gain or loss in each group.

Collection and Analyses of Blood and Kidney

The animals were fasted overnight (12 hrs) on the last day of treatment and euthanized by cervical dislocation. The blood samples were collected from the rats through capillary tube using retro orbital sinus method. The blood samples were stored in EDTA bottles and centrifuged for 15 mins. The kidneys were excised and rinsed in normal saline, weighed and kept in sample bottles filled with 10% formalin saline solution for histological and histochemical analysis. The animal carcasses were collected and disposed in accordance to the European regulation (EC No. 1069/2009) for treatment and disposal of animal by-product. The serum creatinine and urea assays were carried using standard kits according to manufacturer described procedures (Randox Laboratories Ltd, United Kingdom) (Tijani *et al*., 2022). The kidney tissue were subjected to histological analysis using Haematoxylin and Eosin stains to access the histopathological and histoarchitecture changes (Lana, 1992). The kidney tissues were prepared by fixing them and then cutting them into sections that were 5 microns thick. These sections were then embedded in paraffin and stained with Hematoxylin and Eosin (H&E) (Adeoye *et al*., 2023; Daniyan *et al*., 2023). Finally, the sections were examined under a light microscope for any morphological changes. Each kidney specimen was also stained using Masson Trichrome (Li *et al*. 2017; Adetunji *et al*., 2024). To identify collagen deposition or alteration in the kidney, and Periodic Acid Schiff (PAS) (Chen *et al*. 2016; Adebola *et al*., 2022). To detect glomerular protein deposit or glomerular damage caused by lead acetate administration.

Statistical Analysis

The data was expressed as mean ± Standard Error Mean (S.E.M). One-way Analysis of Variance (ANOVA) was used for the statistical analysis using Graph Pad Prism (Version 6.0) statistical package tool.

RESULTS

The result presented in figure 1 shows the effect of lead acetate on the percentage weight gain or loss in the experimental rats. The result shows a slight weight increase between the initial and final weight of the control (Initial = 117±16.74; Final = 136.9±12.68) and 2.5% lead acetate treatment group (Initial = 134.2±10.84; Final = 153.6±3.512), indicating a 17.1% and 14.4% weight increase, respectively. However, a significant percentage weight increase (34.5% and 26.7%) was observed in the 3.0% and 3.5% lead acetate treatment

group, respectively. This indicate a significant difference in the percentage weight gain between the 3.0% and 3.5% lead acetate treatment group when compared to the control group.

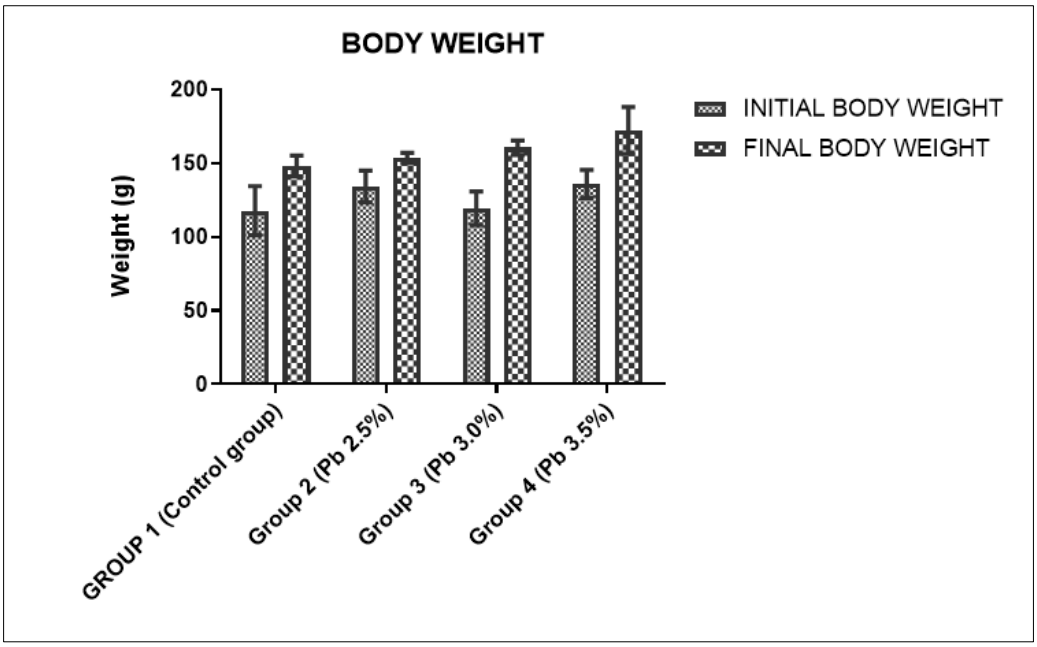

Figure 1: Bar graph showing the initial and final body weight of animals across the experimental groups. Values are mean ± SEM of data p≤0.05.

As shown below in figure 2 representing the chronic effect of lead acetate on urea, there was a slight elevation in the urea level across the entire group when compared with the control group. The creatinine level was also assayed in this study and the results (figure 3) indicate a significant ($p<0.05$) elevation level of creatinine in the 2.5% lead acetate treatment group when compared with the control. Additionally, there was no significant difference observed in the 3.0% and 3.5% lead acetate treatment group when compared with the control group. This implies that the exposure to lead acetate may indicate renal impairment.

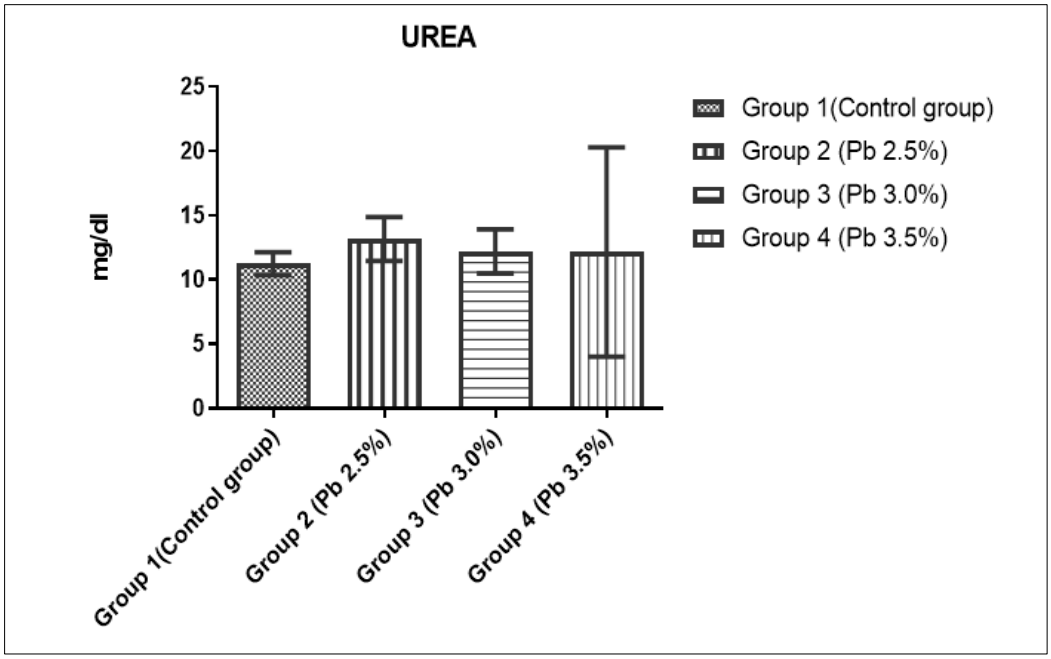

Figure 2: Bar chart showing the urea test across the experimental groups. Values are mean ± SEM of data p≤0.05.

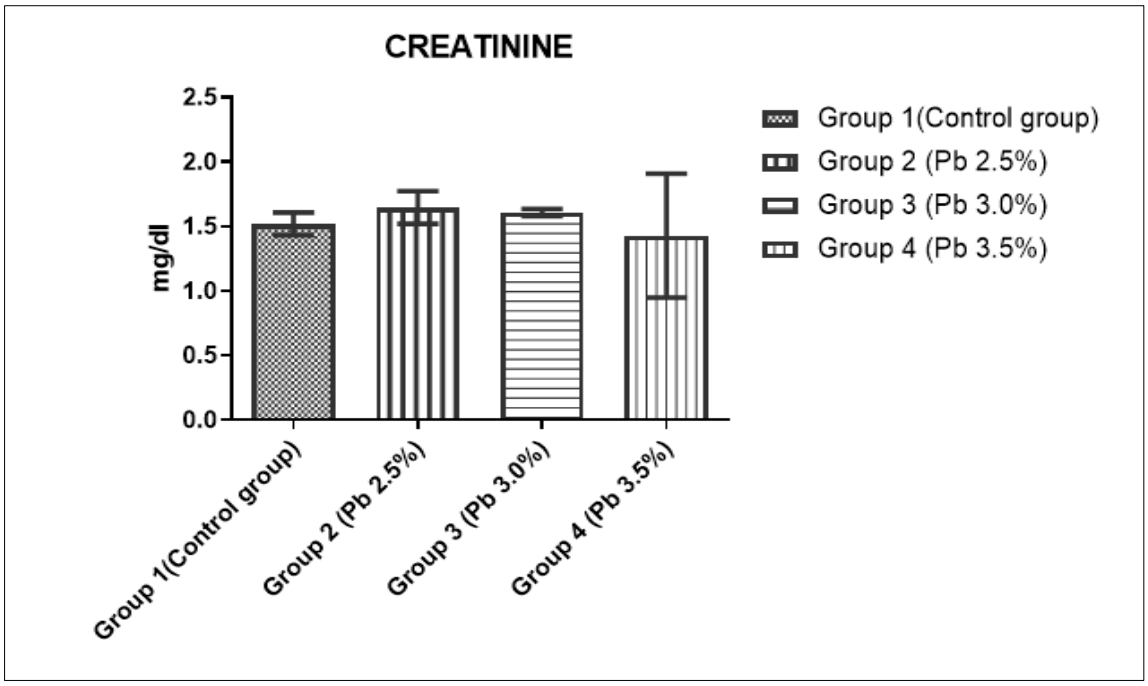

Figure 3: Bar chart showing the creatinine test across the experimental groups. Values are mean ± SEM of data p≤0.05.

PHOTOMICROGRAPHS

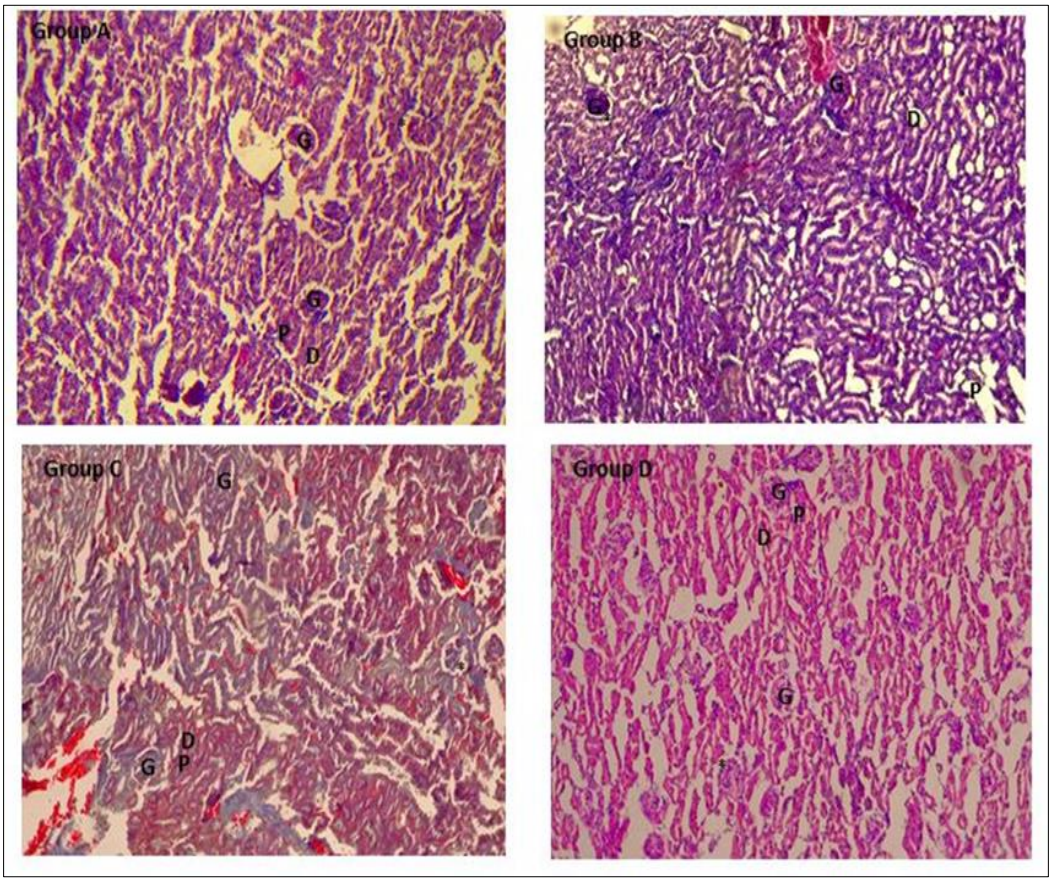

Plate 1: Photomicrograph of the general structure of the kidney of rat exposed to graded dose of Lead Acetate at lower magnification. The plates reveal the general overview across all groups identifying the G= glomerular; P= Proximal convoluted tubule; D=Distal convoluted tubule H&E X100

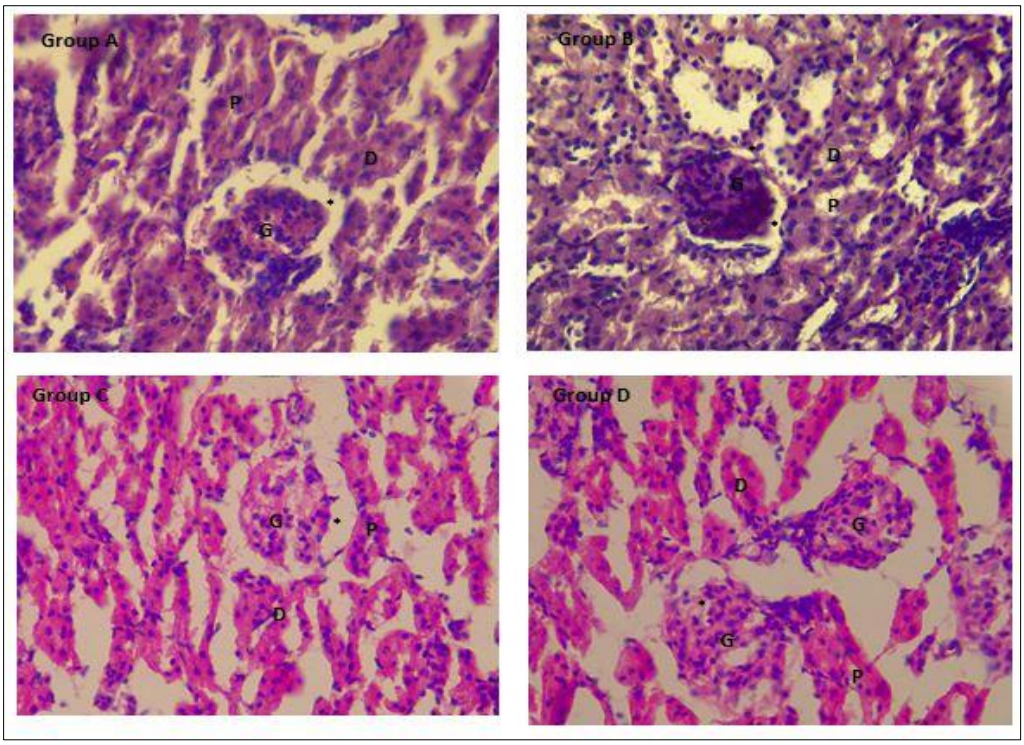

Plate 2: H&E Photomicrographs of the kidney of wistar rat exposed to lead acetate. The control group showed normal histoarchitecture of the normal G= glomeruli, P=proximal convoluted tubule, D= distal convoluted tubule. H&E X400

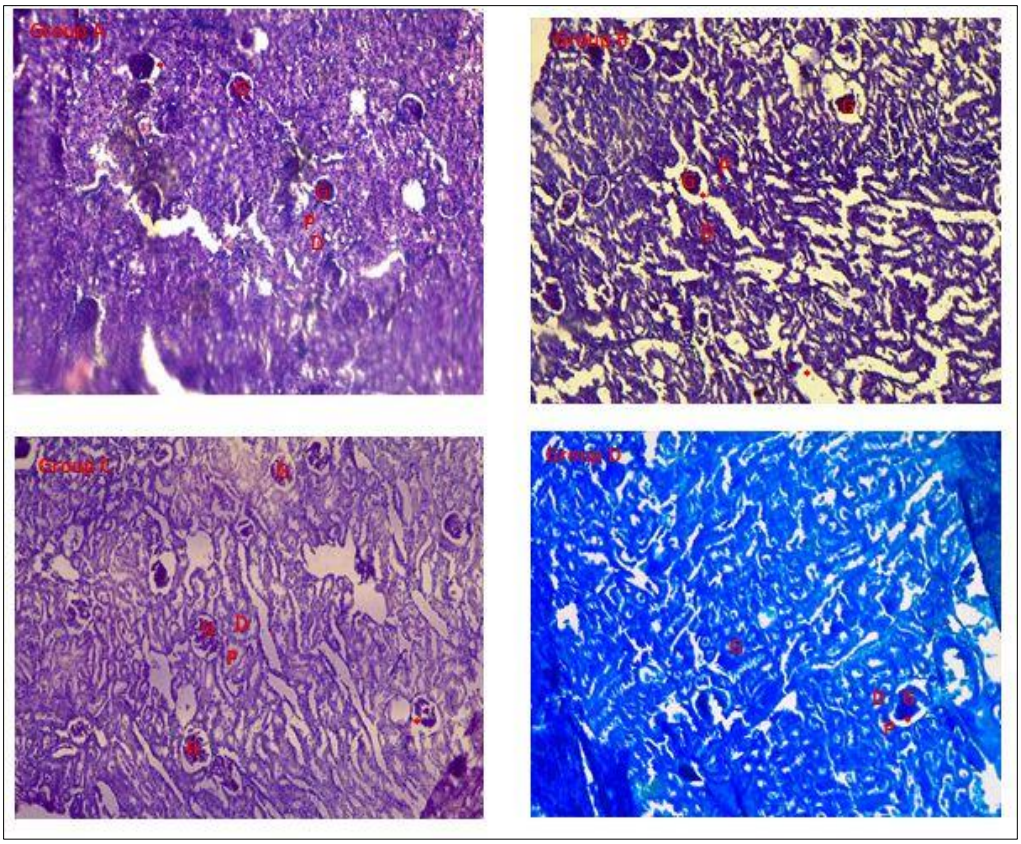

Plate 3: Photomicrograph of the general structure of the kidney of rat exposed to graded dose of Lead Acetate, stained with periodic acid Schiff. G= glomerulus; P= proximal convoluted tubules; D= Distal convoluted tubule PAS X100.

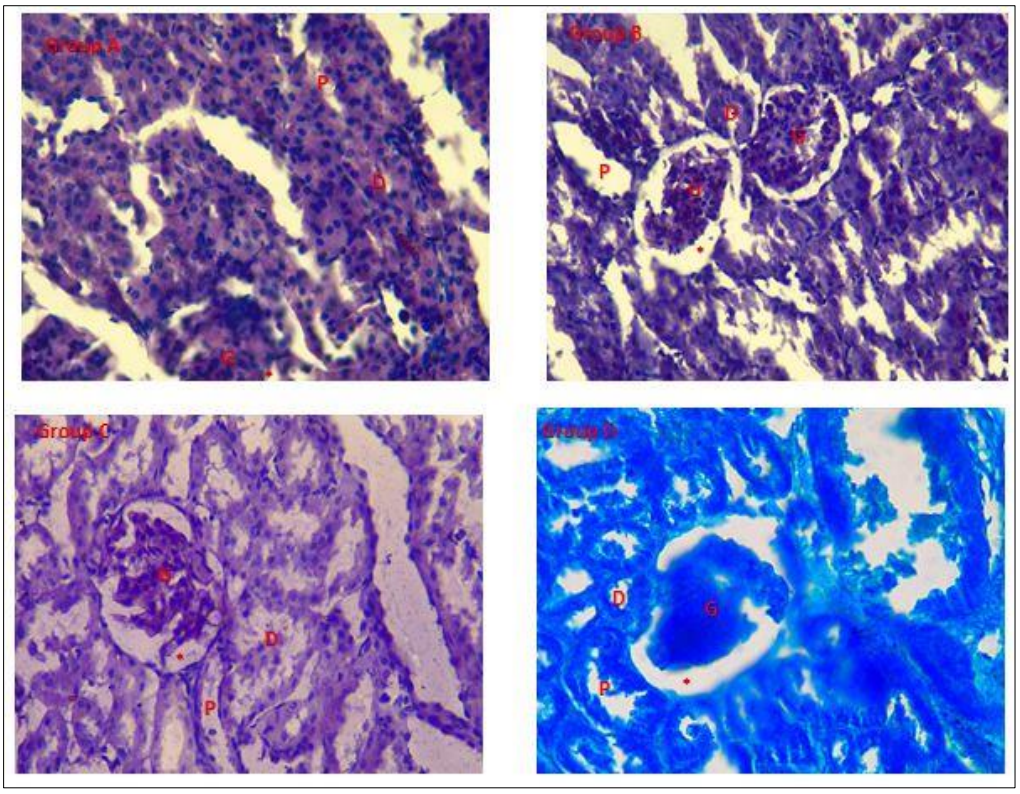

Plate 4: Photomicrographs of the kidney of wistar rats exposed to Lead acetate. The control group showed normal histoarchitecture of; G= glomerulus; P= proximal convoluted tubules; D= Distal convoluted tubule Periodic Acid Schiff (PAS) staining in renal tissues of Group B, C & D developed more severe glomerular lesion such as glomerular hypertrophy, global mesangial expansion and basement membrane thickening. The absence of glomerular and mesangial alterations in carbohydrate restricted Group A. PAS X400

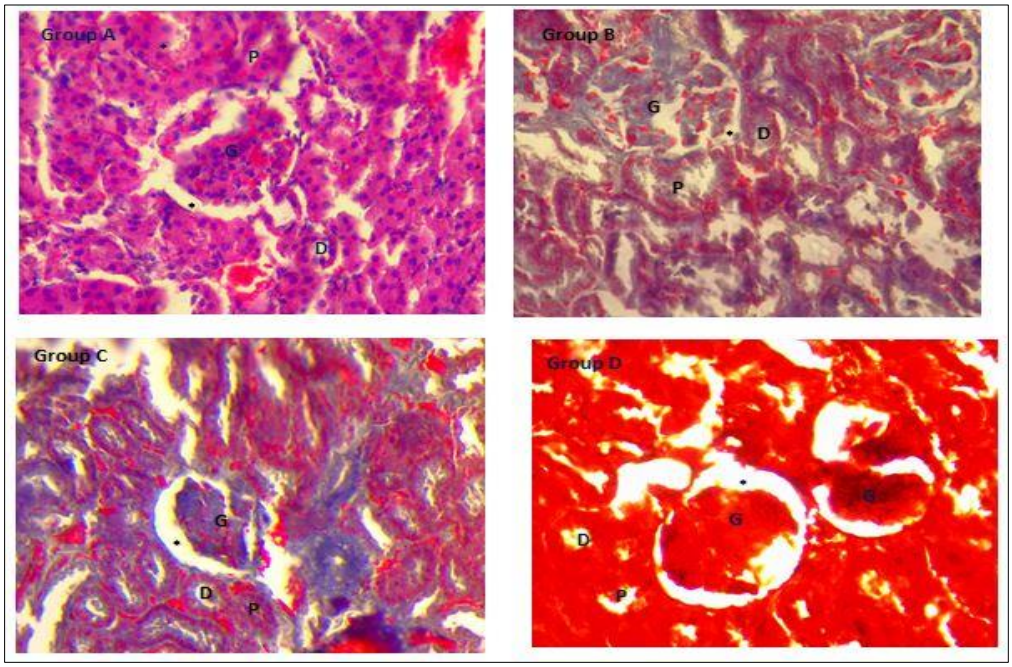

Plate 5: Masson's Trichrome Photomicrographs of kidney sections of the kidney of wistar rats exposed to lead acetate. The blue-appearing collagen. Group a showed normal glomeruli, tubules, interstitium, and vasculature. Group B, C & D Shows that there were large areas of local fibrotic lesion and accumulation of leukocytes. The epithelial lining of cortical collecting duct segments was variable in morphology, ranging from areas where the cells are greatly thinned to regions occupied by large, hypertrophic cells with enlarged nuclei.

DISCUSSION

The morphological study showed a significant increase in weight throughout the entire treatment group, suggesting a remarkable beneficial effect of lead acetate on the physical well-being of Wistar rats. The present finding opposes previous work that has reported considerable weight reduction in rats subjected to lead acetate (Manojlović *et al*., 2019). The weight gain observed in this study can be attributed to the increased water retention and edema caused by lead acetate-induced nephrotoxicity, as reported by Busari *et al*., (2021) and Naimi *et al*., (2011). Nevertheless, it is necessary to understand that this increase in weight does not necessarily indicate good health, as exposure to lead acetate has also been proven to result in substantial kidney damage and impairment. Moreover, the significant increase in weight implies that the medication caused a compensating reaction in the rats, which could potentially conceal the hidden harmful effects. The observed variation in body weight in these studies may be linked to the presence of harmful ions and other related variables that cause metabolic disruption (Alissa & Ferns, 2011). Therefore, it is necessary to take into account the morphological analysis along with the biochemical and histological findings in order to gain a comprehensive understanding of the impacts of chronic lead acetate nephrotoxicity.

The biochemical and histological findings of this investigation indicate that the weight gain observed in the treatment group may be attributed to the nephrotoxic effects of lead acetate. The significant elevation in serum creatinine levels, along with the histological confirmation of kidney damage, suggests that lead acetate treatment resulted in nephrotoxicity.

Urea is the ultimate by product of protein metabolism. Its measurement enables the assessment of the overall metabolism of amino acids derived from proteins, often occurring in the liver through the urea cycle. The kidneys are the primary organs responsible for excreting urea once it is present in the bloodstream (Adeyomoye *et al*., 2022). Following glomerular filtration, approximately 40% to 60% of the filtered substance is reabsorbed at the tube level, serving as an indicator of renal function (Zhang & Mahler, 2023). The significant elevation in urea levels among all treatment groups in comparison to the control group (Figure 2) implies that lead acetate did not have significant impacts on the production or elimination of urea in the kidneys. The 2.5% lead acetate treatment group exhibited a significant increase in creatinine levels compared to the control group (Figure 3), suggesting that lead acetate might have induced kidney injury or dysfunction, resulting in elevated creatinine levels. This finding coincides with previous study that has shown elevated urea and creatinine levels as a result of lead exposure (Andjelkovic *et al*. 2019). Changes in urea and creatinine levels indicate that after exposure to toxic metals, the excretory function of the kidney might be impaired. Similar results of changes in the levels of urea and creatinine were observed in other studies (Yildirim *et al*., 2017; El-Boshy *et al*., 2017).

The slight disparity between the urea and creatinine results suggests that lead acetate has varying effects on different aspects of renal function, with creatinine levels being more susceptible to damage caused by lead exposure. This assertion is supported by previous work that has proven creatinine as a more responsive indicator of renal injury compared to urea (Akpotaire & Seriki, 2023). The absence of significant differences between the treatment groups receiving 3.0% and 3.5% lead acetate, in comparison to the control group, implies that the impact of lead acetate on creatinine levels might be contingent on the dosage. This finding aligns with other research that has reported the impact of lead on kidney function in a manner that is directly proportional to the dosage administered (Andjelkovic *et al*., 2019).

The study's biochemical analysis indicates that continuous exposure to lead acetate may have a mild effect on kidney function, with creatinine levels being more susceptible to impairment caused by lead. These findings correlate with previous investigations and emphasize the significance of evaluating various biomarkers of renal function when examining the impacts of toxic substances such as lead acetate.

The administration of lead acetate orally in rats resulted in significant changes in the glomerular, proximal convoluted tubule, and distal convoluted tubule of the kidneys. The structural changes found in this study are consistent with the known effects of lead acetate, as demonstrated in several previous research. Inflammation and renal fibrosis are two histological characteristics of progressive kidney disease, and a specific antibody can be used to quantify the amount of damage done to the kidneys. The hematoxylin and eosin staining technique revealed the histoarchitecture of the kidney, including the glomeruli, as well as the proximal and distal convoluted tubules in all experimental groups.

The periodic acid Schiff stain (PAS) is utilized for the detection of glomerular injury (Su *et al*., 2020). Group B, C, and D exhibited more severe glomerular lesions, including glomerular hypertrophy, global mesangial expansion, and basement membrane thickening, in the renal tissues stained with PAS. Carbohydrate restriction in Group A did not result in any changes or abnormalities in the glomerular and mesangial structures. A study conducted by Kong *et al*., (2019) demonstrated that exposure to lead acetate resulted in glomerular damage, as evidenced by staining with masson trichrome.

The Masson's trichrome stain is employed to distinguish collagen from other types of fibers by highlighting the blue-colored collagen (Moneim *et al*.,

2023). Group A exhibited histologically normal glomeruli, tubules, interstitium, and vasculature. Groups B, C, and D Indicates the presence of extensive localized fibrotic lesions and the buildup of leukocytes. The morphology of the epithelial lining of cortical collecting duct segments showed variation, with some parts showing significant thinning of cells and other sections characterized by the presence of massive, hypertrophic cells with expanded nuclei. Several studies have demonstrated the buildup of leukocytes when exposed to lead acetate (Harshitha *et al*., 2024; Lee *et al*., 2019).

CONCLUSION

Lead acetate causes nephrotoxicity and weight increase in Wistar rats due to water retention and edema, which have a major negative impact on their physical health. Serum creatinine levels elevated after treatment, suggesting kidney dysfunction or malfunction. Kidney damage was confirmed by histological results, which included leukocyte accumulation, fibrotic lesions, and glomerular lesions. According to the study, lead acetate affects renal function in diverse ways, with creatinine levels being particularly susceptible to damage. Understanding the detrimental impacts of toxic substances requires taking into consideration several biomarkers in addition to histology findings, as highlighted in the study.

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