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The effects of sub-acute oral lead administration on the histology of the kidney and some renal parameters in adult Wistar rats

Abdulrashid, Sunusi[1], Oliver Wilson Hamman[2], Sunday Abraham Musa[2], Lawan Hassan Adamu[3]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University, Kano.
[2] Department of Human Anatomy Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria.
[3]Department of Human Anatomy, Faculty of Basic Medical Sciences, Federal University Dutse

Corresponding author: Abdulrashid, Sunusi

E-mail: asunusi.ana@buk.edu.ng; Tel: +2348067667730

ABSTRACT

Newer sources of lead are being discovered on daily bases; likewise, the prevalence of kidney diseases is increasing hence the need to look at the effect of lead as a predisposing factor to kidney diseases. The present study was aimed at determining the effect of lead on the histology of kidney and some renal parameters. A total of 20 adult male Wistar rats were used for the study. The animals were divided into four groups of five animals each. Group I, II, III and IV received 1ml of distilled water, 60mg/kg, 120mg/kg and 180 mg/kg bwt of lead (II) acetate respectively, orally for 28 days. The rats were sacrificed after ketamine injection (50 mg/kg bwt) intraperitonially and blood samples were collected via cardiac puncture for biochemical analysis. Routine histological technique was used for tissue processing and tissue were stain using hematoxylin and eosin stain (H&E) and periodic acid schiff stain (PAS). The histological features showed distortion in normal membrane and glomerulus, fibrosis of mesangial cells and macular denser in a dose-dependent manner. Biochemical analysis showed a significant increase in the concentrations of creatinine, urea, iron and potassium in Groups II, III, and IV. However, a decrease in sodium, calcium, carbonate and total protein was observed. In conclusion, sub-acute oral lead administration caused dose-dependent renal histopathological and biochemical changes in the treated groups in adult male Wistar rats.

Keywords: Histology, Kidney, Lead, Renal parameter and sub-acute

INTRODUCTION

Lead is a naturally occurring element and is a member of Group 14 (IVA) of the periodic table [1]. Lead, which is a soft, grey-blue heavy metal, is a common cause of poisoning in domestic animals throughout the world[2]. Lead is a poisonous metal, which exists in both organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in the environment [3]. It is sometimes found free in nature, but is usually obtained from the ores galena (PbS), anglesite ($PbSO_4$), cerussite ($PbCO_3$) and minim (Pb_3O_4)[4]. Although lead makes up only about 0.0013% of the earth's crust, it is not considered to be a rare element since it is easily mined[5].

Inorganic lead is one of the oldest occupational toxins and evidence of lead poisoning can be traced to Roman times [4]. It has been used in medicines, paintings, pipes, ammunition and as alloys for welding storage materials for chemical reagents [6]. Exposure to lead mainly occurs through the respiratory and gastrointestinal systems [7].

Lead poisoning (also known as plumbism, colics, pictirium, soturnism, devon colic or painter colic) is a type of metal poisoning and a medical condition in humans and other vertebrates caused by increased level of lead in the body [6]. Lead interferes with varieties of body processes and is toxic to many organs and tissues including kidney, heart, bones, intestines, reproductive system and nervous system [8]. It interferes with the development of the nervous system and therefore particularly toxic to children, causing potentially permanent learning and behavioural disorders[7]. Symptoms include abdominal pain, anaemia, headache, irritability, confusion and in severe cases seizures, coma and death [4].

Sources of lead exposure include contaminated air, water, soil, food and consumer goods. Occupational

exposure is a common cause of lead poisoning in adult [4,9]. Absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs [10]. Affected biological activities at the molecular, cellular and intercellular levels as a result of lead toxicity may result in morphological alterations that can remain even after lead level has fallen[11].

MATERIALS AND METHODS

Chemical Material

Analytical grade lead acetate $(CH_3COO)_2Pb.3H_2O$=379.33 was obtained from British Drug Houses (BDH) Chemical Limited, (Poole, England) with the product number 29021. An LD_{50} of 600 mg/kg body weight (bwt) was adopted [12]. The chemical was authenticated at the Department of Chemistry, Ahmadu Bello University (A.B.U). Distilled water was obtained from Department of Chemistry which was used for the dilution of the chemicals. Formal saline used as tissue fixative was obtained from the Department of Human Anatomy A. B. U., Zaria.

Experimental Design

Twenty matured male Wistar rats of average weight of 190g were obtained from the Animal House of Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria. The rats were kept in standard cages and allowed to acclimatize for two weeks in the Animal House of the Department. They were fed on standard animal feed and clean tap water throughout the period of the experiment. All animal handling was in accordance with Ahmadu Bello University, Animal Care Committee guidelines.

Animal Treatment

After two weeks of acclimatization, the rats were weighed using a sensitive balance and randomly divided into four groups of five rats each. Group I, (control), received 2 ml of distilled water, Groups II, III, and IV (treated groups), received 10%, 20% and 30% of the LD_{50} (i.e. 60mg/kg,120mg/kg and 160mg/kg) respectively for four weeks as shown in Table 1. The route of administration was oral through oral intubation.

Table 1: Animal Group, concentration and frequency of administration of Lead Acetate

Group	Dosage	Frequency of administration
I	Normal saline	Once daily
II	60mg/kg	Once daily
III	120mg/ kg	Once daily
IV	180mg/kg	Once daily

Animal Sacrifice

The animals were sacrifice by intraperitoneal injection of ketamine (50mg/kg bwt). Through thoracic incision, the heart was exposed and blood was collected with the aid of 2ml syringe. The blood samples were stored in plain specimen bottles, anticoagulant-free and were allowed to coagulate; the serum was used for biochemical analysis.

Biochemical Analysis

Urea/ Blood Urea Nitrogen

Urease was used to determine blood urea nitrogen (BUN) by breaking down urea into ammonia and carbon dioxide. The ammonia then reacts with hypochlorite and salicylate to form dicarboxyindophenol, a coloured compound in an alkaline medium. The reaction was catalyzed by sodium nitroprusside. The intensity of colour produced was measured photometrically at 578 nm (570-620 nm).

Uric Acid

Uricase was used to react with uric acid, to produce allantoin and hydrogen peroxide. Peroxidase was then used to convert the hydrogen peroxide (proportional to uric acid concentration) to chromogen to coloured complex. The intensity of the colour produced was proportional to uric acid concentration and was measured photometrically at 520 nm (500-550nm or with GREEN filter).

Calcium

The metallochromogen arsenazo III procedure was employed in the analysis of calcium [20]. When calcium ions and arsenazo III interact at pH 6.75, a brightly colored chromophore is formed, the absorbance of which is measured at 630 nm. Arsenazo III exhibits little interference from other cations typically seen in serum or plasma and has a strong affinity for calcium ions.

Sodium ion assessment

Serum sodium was assayed by colorimetric method, in which 1000µl of precipitate reagents (Sodium R1), 10µl of sodium and 10µl of serum were mixed vigorously and allowed to incubate at room temperature for 5 minute and then centrifuged at 3000 RPM to obtain clean supernatant. The supernatant was transferred for standard and test, absorbance was measured at 530nm.

Potassium ion assessment

Serum potassium was estimated by turbidometric method in which 1000 µl of potassium reagent, 25 µl of standard and 25 µl of serum were mixed well and allowed to incubate at room temperature for 5 minutes. Then centrifuged at 3000 RPM to obtain clear supernatant. The supernatant was transferred for standard and test, and absorbance was measured at 530nm.

Chloride assessment

Serum chloride was estimated by colorimetric method in which 1000 µl of chloride reagent, 10 µl of standard and 10 µl serum were mixed well and allowed to incubate for 1min. Comparisons were done between the measurement of absorbance of standard and sample against the reagent blank.

Tissue Processing

The tissues were collected and stored in 10% formal saline and processed at the Histopathology Department of Ahmadu Bello University Teaching Hospital Shika for bothhistological preparations usingHaematoxylin and Eosin (H&E) and Periodic Acid Schiff Reagent (PAS) stains.

Haematoxylin and Eosin Staining Method

The method of H&E technique was carried out by de-waxing the tissue in xylene for three (3) minute each, hydrated by passing them through descending grades of alcohol (100%, 95%, 90%, and 70%) for three minute each, and stained in Harris' haematoxylin for ten minute, and washed in tap water to remove excess stain. The slides were then flooded with acid alcohol for few seconds for differentiation and then washed in tap water again. The slides were then blued in Scott's tap water for five minute and counter stained with Eosin for three minutes. The sections were rinsed in tap water, and then dehydrated in ascending grades of alcohol, cleared in xylene. Tissues were then mounted on the slides andcover slipped using a mounting media. Sections of the tissues were viewed under light microscope and photomicrographs were taken using digital Amscope 900 photomicroscope.

Statistical Analysis

Data were analyzed using statistical package for social sciences (SPSS) version 20. One-way analysis of variance (ANOVA) was used to find the mean differences between groups and Tukey's Post hoc used to compare the mean differences. Results were presented as mean ± standard error of mean and *p*-value ≤ 0.05 was considered as significant.

RESULTS

Result of Renal Electrolytes

The result of renal biochemical parameters shows a dose dependent decrease in sodium ion concentration across the treated groups. The highest serum sodium ion concentration was observed in the control group, followed by the group that received 60 mg/kg bwt. Group III treated with 120 mg/kg bwt and IV treated with 180 mg/kg bwt exhibited almost same serum sodium concentration, with group III being slightly higher. The serum sodium concentration between group III and the control group is significant. A similar pattern was observed for chloride ions, as there was decrease in concentration in dose-dependent manner with control having the highest followed by the group treated with 60 mg/kg bwt, 120 mg/kg bwt and 180 mg/kg bwt. The difference between the control group and the group treated with 120 mg/kg bwt of lead was statistically significant. Potassium ion increased across the group with group III being the highest followed by group IV, II, and then the control group. On the other hand, carbonate and calcium ions decreased across the groups with the Control having the highest concentration followed by groups II, III and IV in a dose-dependent manner. These variations are also not significant statistically. A unique phenomenon occurred for total serum protein, though the control group exhibited the highest value, the treated groups showed a dose-dependent decrease which was not significant as shown in Table 2 below:

Table 2: Serum electrolytes across the group in adult Wistar rats following oral lead administration

Group	Sodium (mmol/L)	Potassium (m/Eq/L)	Chloride (m/Eq/L)	Carbonate (m/Eq/L)	Calcium (mg/dl)	Iron (µmol/L)
I	140 ± 0.82	4.38 ± 0.17	100.50 ± 0.87	23.00 ± 1.29	2.39 ± 0.06	66.00 ± 1.87
II	139.6 ± 1.44	4.46 ± 0.27	99.2 ± 0.97	22.00 ± 1.67	2.27 ± 0.05	78.6 ± 6.01
III	134.6 ± 1.50*	4.82 ± 0.27	94.4 ± 1.12*	18.60 ± 1.33	2.26 ± 0.03	124.20 ± 15.02[c]
IV	135.6 ± 0.75	4.48 ± 0.14	97.6 ± 0.93	21.20 ± 1.02	2.32 ± 0.02	123.80 ± 15.71[c]

Data are presented as mean ±standard error of mean and same sign or alphabets on the same column signifies significance at pvalue ≤ 0.05

Results of Renal Proteins

The result of creatinine, showed increased in the concentration of creatinine in dose dependent manner with group IV which received 180 mg/kg bwt of lead showing the highest level of serum creatinine followed by other treated groups i.e. group III, treated with 120 mg/kg bwt has higher concentration than group II, treated with 60 mg/kg bwt which was statistically significant in comparison to the control.

Serum urea showed similar pattern to creatinine, with a significant difference between the high lead treated group and the control group. For other treated groups no statistically significant difference was observed.

The highest concentration of serum albumin was observed in the control group, 39.25 ± 1.75 g/L, followed by group III with a mean concentration of 36.40 ± 1.6 g/L concentrations and then group 1V, with a mean value of 36.40 ±1.6 g/L as shown in Table 3 below:

Table 3: Result of serum proteins

Group	Urea (mmol/L)	Creatinine (µmol/L)	Albumin (g/L)
I	3.45 ± 0.23	44.5 ± 1.04	39.25 ± 1.75
II	3.46 ± 0.12	47.6 ± 1.36	32.60 ± 0.93[d]
III	4.08 ± 0.43	61.80 ± 1.46*[b]	36.40 ± 1.6
IV	4.92 ± 0.24*	53.6 ± 1.44*	34.80 ± 0.97

Data are presented as mean ±standard error of mean and same sign or alphabets on the same column signifies significance at p value ≤ 0.05

Histological of observation

The control group revealed normal kidney histology i.e. normal glomerulus, glomerular basemen membrane, mesangial cells, and podocytes with no sign of inflammation or fibrosis. While the treated groups showed histopathological distortions in a dose-dependent manner.

Figures 1A and 2A were sections of the kidney of the control group showing normal histomorphology i.e. normal glomerulus, uriniferous tubule, glomerular basement membrane, mesangial cells and capillaries, macular denser cell, interstitial is scanty with no inflammatory cells seen for both H&E and PAS stains.

Figures 1B and 2B represent photomicrographs of group II (60mg/kg bwt) showing mild distortion of the histomorphology, with proximal convoluted tubule enlargement, normal glomerular membrane, uriniferous tubule, and podocyte and macular denser cells. The tubular epithelial cells showed mild cytoplasmic eosinophilia and vacuolation with blurring of cellular membrane. The nuclei and blood vessels appeared unremarkable and the interstitium was scanty.

Figures 1C and 2C represent the histological tissues of group III (120 kg/bwt) showing distorted histomorphology, fibrosis of the mesangial cells of the glomerulus, thin glomerular basement membrane, podocyte, macular denser, proximal convoluted tubule. The tubules showed less cellular boundary and marked cytoplasm eosinophilia. The interstitium is mildly increased with mild infiltration by mononuclear, inflammatory cellular infiltration and area of haemorrhage and fibrosis. Figures 1D and 2D showed histological feature of rats treated with 180 mg/kg dose. The glomerulus revealed mild mesangial hyper cellularity with congestion of mesangial blood vessels. The tubules showed detached tubular epithelium occluding and forming cast in some areas. The nuclei are reactive but pyknosis cells were also seen. The interstitium showed areas of vascular congestion, haemorrhage, fibrosis, and infiltration by mononuclear cells present.

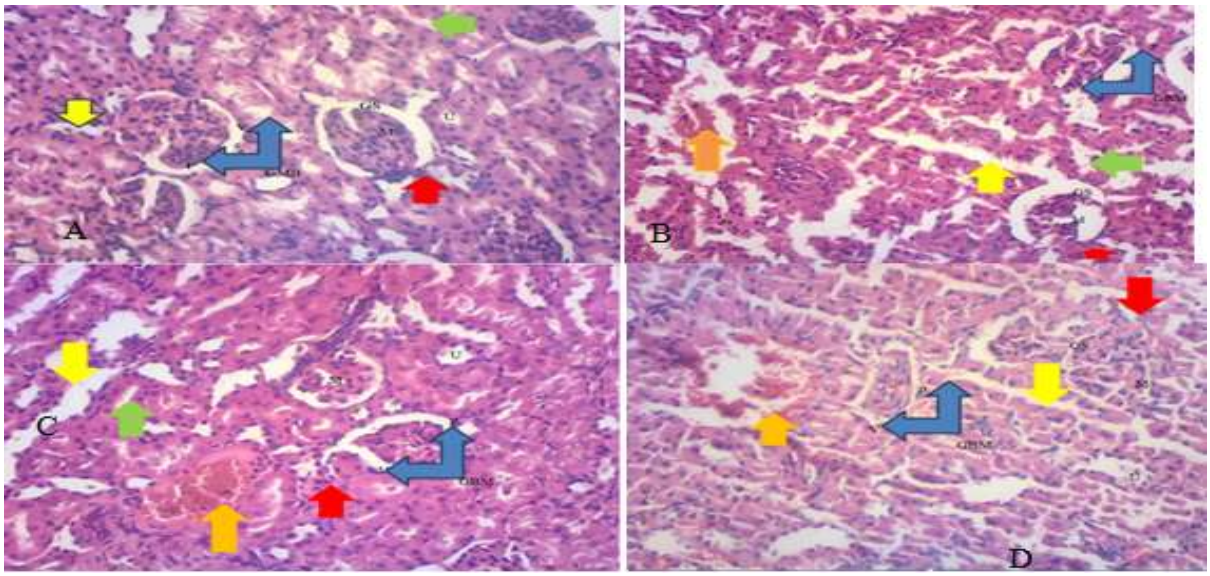

Figure 1: Sections of rat renal tissue, **A:** controlled group (I), showing normal glomerular membrane GMB, V-viscera and P-parietal, GS-glomerular space, M-mesangial cells, U-uriniferous tubule, macular densa-red arrow, proximal convoluted tubule-yellow arrow, distal convoluted tubule-green arrow. **B, C and D** section of renal tissue of group (II), 60 mg/kg bwt, group (II), 120 mg/kg bwt, group (II), 180 mg/kg bwt showing distorted glomerular membrane GMB, V-viscera and P-parietal, GS-glomerular space, M-mesangial cells, U-uriniferous tubule, macular densa-red arrow, proximal convoluted tubule-yellow arrow, distal convoluted tubule-green arrow and mild inflammatory site-orange arrow.

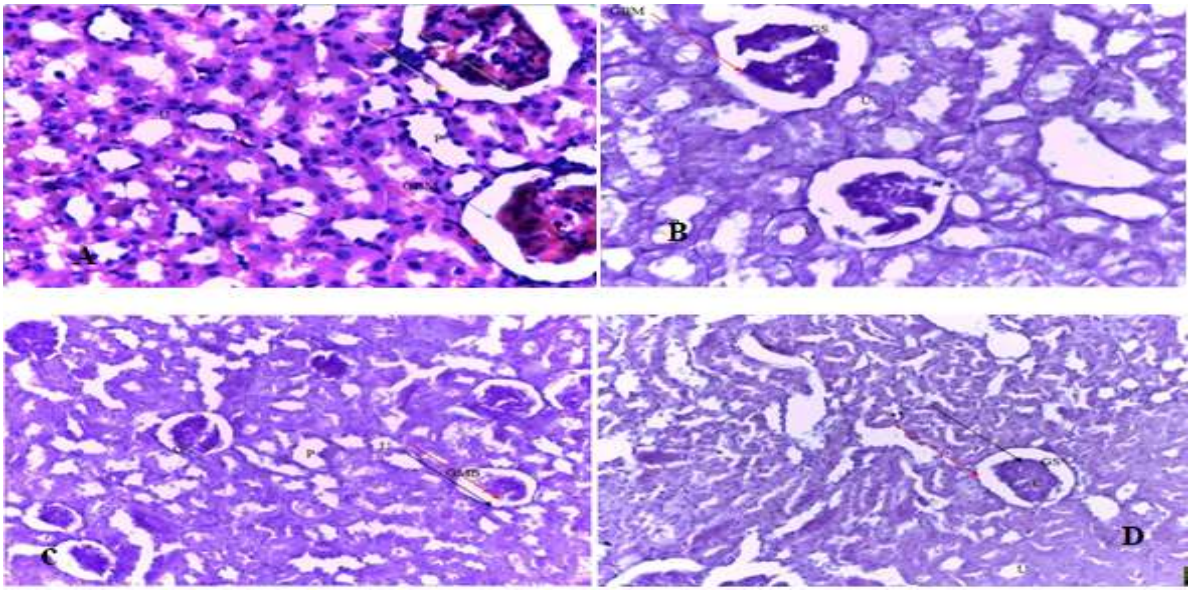

Figure 2: **A**: Section of kidney tissue of controlled rats that received normal saline, showing normal histomorphology of P- proximal convoluted tubule, U- uriniferous tubule, GBM- glomerular basemen membrane, Blue arrow- viscera & red arrow- parietal membrane. **B**: section of kidney tissue of group 2 (60 mg/kg bwt) showing mild distortion of the glomerular membrane (red arrow viscera & black viscera), U- uriniferous tubule, GS- glomerular space, P- proximal convoluted tubule. **C**: section of kidney tissue of group III animal (120 mg/kg bwt): Showing abnormal; U- uriniferous tubule, M- mesangial cells, C- Capillaries, GS- glomerular space, red arrow parietal membrane, black arrow viscera membrane. **D**: section of kidney tissue of group IV animals (180 mg/kg bwt): Showing abnormal histology of; U- uriniferous tubules, P-proximal convoluted tubule, PD- podocytes and mesangium (PAS X 250).

DISCUSSION

The decreased in concentration of both sodium and chloride ion from this study as compared to the control, could be as result of deleterious effect of lead on the renal tubule as a result of released of reactive oxygen species[10]. This also shows that lead effect is less on the renal tubule compared to the glomerulus, since the tubule deals with electrolyte absorption [19]. The difference in carbonate concentration in this study was statistically insignificant which could further ascertain the fact that effect of lead toxicity on the kidney is less on the renal tubule and compared to the glomerulus. This is in accordance to the work of Gonick [13], which shows that lead exposed workers have no serum carbonate rise. Also, calcium absorption is inversely proportional to lead absorption, hence the exhibition of highest concentration in the control group. Serum iron rise observed in this study in the lead treated groups is likely due to the fact that lead and iron both compete for binding sites, thereby making more iron to be in the blood.

Serum urea increases across the group in dose dependent manner for the treated groups, the least serum concentration was observed in the controlled group, followed by the group treated with low, medium and high concentration of lead. This coincides with the work of Liu et al[16], and Zhou et al., [17] where lead expose group showed a rise in serum urea than the controlled group. The serum urea concentration difference between the control and the highest dose group was statistically significant. This agrees with the work of Baki et al, [18], where lead exposed workers and animals exposed to mercury shows a significant rise in serum urea level.

The rise in serum creatinine observed in this study was as result of increase reactive oxygen species by the lead[10], this increase was observed in dose dependent manner, with the group treated with normal saline exhibiting the lowest creatinine concentration followed by the groups treated with low, medium and high lead concentration. There was statistically significant difference between group three and four and the control group. This agrees with earlier reports [17], where workers exposed to lead showed a higher serum creatinine than control group. Serum calcium and total protein showed a sporadic trend with no statistically significant feature, this could be as result of sporadic nature of urine

The normal glomerulus, uriniferous tubule, glomerular basement membrane, mesangial cells and capillaries, macular denser cell, interstitial is scanty with no inflammatory cells seen in the control group for both Hematoxylin and Eosin stain and the Periodic Acid Schiff Reagent Stain was due the fact that the amount of lead in the distilled water is not harmful, as there is a generally acceptable level of lead in the environment [13]. This corresponds to the work of Karimfa et al., [7] where normalcy was observed in the histoarchitecture of rabbit expose the lead.

The mild distortion of the histomorphology observed in the group treated with 60 mg/kg bwt was due to increased concentration of lead as result of additional lead toxicity to synergize the effects of the ingested lead with the environmental lead, thereby causing increase in oxidative stress markers as result of lead toxicity; also causing more effects directly on tissues [4]. This is in accordance to WHO, fact report sheet which states that, lead is a cumulative toxicant that affect multiple system [6]. Hence, the more the concentration of lead in the body, the more the deleterious effects it will exert on the body tissues. This is evidence in the groups treated with 120 mg/kg bwt and 180 mg/kg bwt.

Mild mesangial hypercellularity with congestion of mesangial blood vessels observed in the medium and high dose group were as a result of necrosis of mesangial cells due to direct effect of lead on tissues also due to release of free radical that tend to damage tissues. This agrees with the work of Aziz et al. [9] and Ahmad and Siddiqque [10] where increase in oxidative stress and direct effect of lead leads to damage is renal tissues. A similar finding was observed by Karinfa et al., [7] in the renal tubules of rabbit exposed to lead acetate which shows similar distortions in the histomorphology. Reactive oxygen species (ROS, e.g. superoxide, hydrogen peroxide and hydroxyl radical) are intermediary metabolites that are normally produced in the course of oxygen metabolism. ROS can avidly react with and denature proteins, lipids, nucleic acids, carbohydrates and other molecules, and cause inflammation, apoptosis, fibrosis and cell proliferation [14]. However, under normal conditions, ROS play a critical role as signal molecules, and ROS produced by activated leukocytes and macrophages are essential for defense against invading microorganisms. ROS are generated by mitochondrial cytochrome oxidase, nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, lipooxygenase, cyclooxygenase, hemeoxygenase, cytochrome P-450 enzymes, nitric oxide synthase (in the presence of cofactor/ substrate depletion or uncoupled state) and various other oxidase enzymes [15].

CONCLUSION

Sub-acute oral lead administration causes dose-dependent alterations of renal histomorphology and biochemistry.

REFERENCES

1. Abadin H, Ashizawa A, Stevens YW, LIados F., Diamond, G. Sage, G, et al. Toxicological profile for lead. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US), Chemical and Physical Information. Available from: https://www.ncbi.nlm.nih.gov/books/NBK158769
2. Khan, MSH., Mostafa, M., Jahan MS, Sayed MA, & Hossain, MA. Effects of garlic and vitamin-B complex in lead acetate induced toxicity in mice. Bang. J Vet Med 2008; 6 (2): 203-210
3. Shalan, MG, Mostafa, MS., Hassouna, M.M. Hassab elnabi, S.E and El-refaie, A. Amelioration of lead toxicity on rat liver with Vitamin C and silymarin supplements. Tox. 2005, 206(1): 1-15
4. Abdalla M. Essential and toxic trace elements and vitamins in human health. Chapter13,2020;181-191
5. Tiwari, S, Tripathi, IP and Tiwari, HI. Effect of Lead on Environment. J. of Emerg. Res. in Man. and Tech. 2013; 2(6): 1-6
6. World Health Organisation (WHO). Lead poisoning and health: Fact sheet. 2016; Geneva: World Health Organization; 2016
7. Karimfar, MH, Bargahi, A, Moshtaghi, D, Farzadinia, P. Long-time exposure of lead acetate on rabbit renal tissue. Iran Red Cres. Journ.2016; 2(18), 20-29
8. Godwill EA, Jane IC, Scholastica IU, Marcellus U, Eugene AL, Gloria OA. Determination of some soft drink constituents and contamination by some heavy metals in Nigeria. Tox. Rep 2015;384-390
9. Aziz FM, Maulood MI, Chawsheen MAH. Effects of Melatonin, Vitamin C and E alone or in Combination on Lead-induced Injury in Liver and Kidney Organs of Rats. IOSR J. of Pharm 2012; 2(5): 13-18.
10. Ahamad M, Siddiqui MKJ. Low level lead exposure and oxidative stress: current opinion. Clinica Chimita Acta 2007; 383: 57-64
11. World Health Organisation (WHO). Lead poisoning and health. Key facts; Review. 2018; Geneva, World Health Organisation
12. Iliyasu MO, Ibegbu AO, Sambo JS, Musa SA, Akpulu PS. Histopathological changes on the hippocampus of adult Wistar rats exposed to lead acetate and aqueous extract of *Psidiumguajava* leaves. African Journal of Cellular Pathology. 2015;31, 26–31.
13. Gonick, HC. Nephrotoxicity of Cadmium and Lead. *Ind. J. Med.Res.*,2008;128 ,335 - 352.
14. Vaziri, ND. Roles of oxidative stress and antioxidant therapy in chronic kidney disease and hypertension. Lippincott Williams & Wilkins. 2004: 1062-4821
15. Odenigbo, CU, Oquejiofor, OC, Onwubuya EL, and Onwukwe, CH. Prevalence of kidney diseases in apparently healthy retired subject in Asaba Nigeria. An. of Med. and Health Sci. Res. 2014: 2-7
16. Liu C, Sun YZ, Sun JM, Ma JQ, Cheng C. Protective role of quercetin against lead-induced inflammatory response in rat kidney through the ROS-mediated MAPKs and NF-κBpathway; Elsevier Biochemica et Biophysica Acta, 2012 (1820) , 1693 - 1703.
17. Zhou R, Xu Y, Shen J, Han L, Chen X, Feng X, et al. Urinary KIM-1; a novel biomarker for evaluation of occupational exposure to lead. Scientific Reports. 2016
18. Baki AE, Ekiz T, Ozturk GT, Tutkun E, Yilmaz H, Yildizgoren M. Effect of lead exposure on serum uric acid and hyperurecemia in young adult workers; A cross Section Study. Arc of Rheumatol.2016, 31(1): 71-75
19. Hall, Jennifer. Guyton and Hall Textbook of Medical Physiology. Elsevier, 2015.
20. Janssen JW, Helbing AR, Arsenazo III an improvement of the routine calcium determination in serum. Eur J Clin Chem Clin Biochem, 29(3): 197-201.