Lack of Reversal of Oxidative Damage in Renal Tissues of Lead Acetate-Treated Rats

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ABSTRACT: Removal of lead from the environment of man or otherwise, the movement of man from lead-contaminated areas has been employed as a means of abatement of the toxic effects of lead. Whether toxic effects in already-exposed individuals subside after lead withdrawal remains unanswered. To understand the reversibility of nephrotoxicity induced by lead acetate, male Wistar rats were orally exposed to 0.25, 0.5, and 1.0 mg/mL of lead acetate for 6 weeks. Activities of glutathione-s-transferase, catalase (CAT), superoxide dismutase (SOD) and the concentrations of hydrogen peroxide (H_2O_2), and malondialdehyde increased significantly ($p < 0.05$) in a dose-dependent manner, whereas reduced glutathione (GSH) level and glutathione peroxidase activity were significantly reduced. The pattern of alterations in most of the oxidative stress and antioxidant parameters remained similar in rats from the withdrawal period, although CAT and SOD activities reduced, in contrast to their elevation during the exposure period. Serum creatinine levels were significantly elevated in both exposure and withdrawal experiments whereas serum blood urea nitrogen levels were not significantly different from the control in both exposure and withdrawal periods. The histological damage observed include multifocal areas of inflammation, disseminated tubular necrosis, and fatty infiltration of the kidney tubules both at exposure and withdrawal periods. The results suggest that lead acetate-induced nephrotoxicity by induction of oxidative stress and disruption of antioxidant. The aforementioned alterations were not reversed in the rats left to recover within the time course of study. © 2014 Wiley Periodicals, Inc. Environ Toxicol 30: 1235–1243, 2015.

Keywords: lead acetate; nephrotoxicity; exposure; withdrawal; oxidative stress

INTRODUCTION

Lead is considered to be a pervasive and persistent toxic metal with high potential for environmental and occupational health hazards (Hernberg, 2000; Zbakh and Abbassi, 2012). The Centers for Disease Control has highlighted lead poisoning as the foremost health threat to children in the United States (Patrick, 2006). Also, recent outbreaks of lead poisoning in some developing countries including Nigeria, in particular, outline the significance of this metal in environmental pollution issues (Attina and Trasande, 2013). Lead poisoning is also important in farm animals, especially those grazing in vegetation close to mining sites. Young animals are usually poisoned when they lick painted pens (Sujatha et al., 2011).

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Routes of exposure to lead have been principally from contact with lead in paints, fertilizers, cosmetics, automobiles, and batteries (Nevin, 2007). Indiscriminate mining activities and uncontrolled dispersal of items containing lead in the environment of developing countries have further served to increase the risk of exposure in these parts of the world (Galadima et al., 2012; Lin et al., 2004). Lead absorbed into the bloodstream via the respiratory or gastrointestinal tracts is eventually distributed into the bones and soft tissues. Significant amounts may be retained in the body largely in the bones, liver, kidneys, and other tissues (Patrick, 2006). Several reports have documented the toxicity of lead to different tissues including the liver, heart, kidneys, brain, and the hematopoietic system (Patra et al., 2001; Sivaprasad et al., 2004; Bellinger, 2008; Rastogi, 2008).

Generally, the toxic effects of lead are believed to be due to the generation of reactive oxygen species (ROS) and the inhibition of antioxidant enzyme activities in various tissues (Patra et al., 2001; Franco et al., 2009; Sharma et al., 2010). Effects produced by free radicals and ROS include the stimulation of lipid peroxidation and depletion of antioxidant status (Sandhir and Gill, 1995; Bolin et al., 2006; Patrick, 2006). Lead can bind to sulfhydryl groups of antioxidant enzymes and thereby cause their inactivation (Patrick, 2006). Lead-induced oxidative stress is of immense importance in tissues where lead accumulates after exposure (Mestek et al., 1998).

The renal tissues represent one of the major tissues in the body where higher contents of lead have been detected in intoxicated animals (Sujatha et al., 2011). The kidneys are particularly exposed to the toxic effects of lead being the major route of excretion of the metal from the body, and are thought to be one of the major target organs of inorganic lead (Gonick et al., 2008). Lead is known to have a propensity to settle in the proximal tubules of the nephron (Pentschen and Garro, 1966; Gonick, 2008).

Abatement of lead poisoning by elimination of exposure to lead-based substances or removal or encapsulation of lead-containing substances by thorough clean-up procedures has been recommended as a control measure for lead toxicity. However, lead is a persistent metal and despite efforts to remove its sources, it is still present in the environment. The diagnosis of lead toxicity has been based primarily on the detection of significantly elevated levels of lead in blood after exposure to the metal. It is now known that significant accumulation of lead may occur in other tissues of the body even when blood levels are quite low (Patrick, 2006). Blood levels may reduce when exposures to lead are withdrawn. However, the accumulated portions present in other tissues may still exert significant impairment of the structural integrity and the functioning of those tissues (Mestek et al., 1998).

This study was carried out to determine whether the nephrotoxicity that arises as a result of lead acetate exposure would be reversed following the withdrawal of lead acetate-treatment from Wistar rats.

MATERIALS AND METHODS

Chemicals

Epinephrine, glutathione, 5, 5′-dithiobis-2-nitrobenzoic acid, hydrogen peroxide, thiobarbituric acid, and trichloroacetic acid, were purchased from Sigma Chemical (St. Louis, MO). All other reagents used were of analytical grade and were obtained from British Drug houses.

Experimental Animals and Design

This study utilized 64 healthy adult male Wistar rats (200–250 g) obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. They were randomly assigned into four groups comprising control group (group I), and other groups II, III, and IV, exposed to 0.25, 0.5, and 1.0 mg/mL lead-acetate (PbA), respectively (Lynda et al., 2011). Each group consisted of 14 animals. The animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow *ad libitum* and liberally supplied with water. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. One half of the population of the rats in each group was sacrificed at the end of 6 weeks. Lead acetate (PbA) was withdrawn from the remaining rats for another 6 weeks. At the end of each period, blood was collected from seven overnight-fasted animals, which were later sacrificed by cervical dislocation, and the kidneys were removed.

Sample Preparation and Collection

The organs were rinsed and homogenized using 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl. The homogenate was subjected to cold centrifugation at 4°C using a speed of 10 000 × *g* for 15 min. The post-mitochondrial fraction obtained after centrifugation of kidney homogenates was used for biochemical assays. About 3 mL of blood was collected from the retro-orbital venous plexus of the rats into plain tubes and was allowed to clot. The clotted blood was then centrifuged at 4000 revolution per minute (rpm) for 10 min. Clear serum was separated with Pasteur pipette into another plain tube and then stored at 4°C until the time of analysis. Serum processed was used for the determination of blood urea nitrogen (BUN) and creatinine. One of the pair of kidneys from some of the animals

TABLE I. Body and organ weights of rats following exposure and withdrawal of lead acetate

		Group I	Group II	Group III	Group IV
Body weight (g)	Exposure	179.17 ± 30.37	174.54 ± 10.97	177.31 ± 20.88	165.83 ± 32.18
	Withdrawal	225.00 ± 34.35	223.33 ± 6.05	218.17 ± 20.61	220.00 ± 21.21
Organ weights (g)	Exposure	1.43 ± 0.19	1.44 ± 0.14	1.52 ± 0.23	1.53 ± 0.09
	Withdrawal	1.49 ± 0.54	0.79 ± 0.51[a]	1.22 ± 0.24	1.38 ± 0.03

[a]Indicates significant difference at $p < 0.05$.
Values are presented as mean ± standard deviation.

in each group was fixed in 10% formalin and processed for histological assessment.

Biochemical Assays

Protein concentration was determined by the Biuret method of Gornal et al. (1949). Hydrogen peroxide generation was assessed by the method of Wolff (1994). Malondialdehyde (MDA) concentration as an index of lipid peroxidation was quantified according to the method described by Farombi et al. (2000). Reduced glutathione concentration was determined using the method of Jollow et al. (1974). Catalase (CAT) activity using hydrogen peroxide as substrate was measured by the method of Claiborne (1995). Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich (1972), with slight modification in our laboratory. Briefly, 50 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. Thirty microliters of kidney extract was added to 2.5 mL 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300 µL of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 s for 150 s. Glutathione peroxidase (GPX) activity was measured by the method of Rotruck et al. (1973). Glutathione-S-transferase (GST) was estimated by the method of Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene as substrate. The

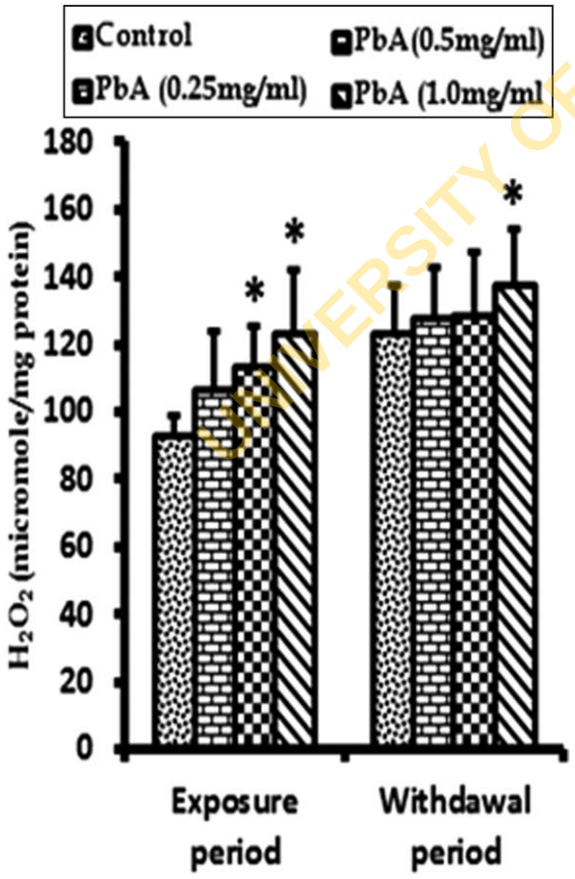

Fig. 1. Effect of Lead acetate on Hydrogen peroxide (H_2O_2) generation in the kidneys of exposed and recovering rats.

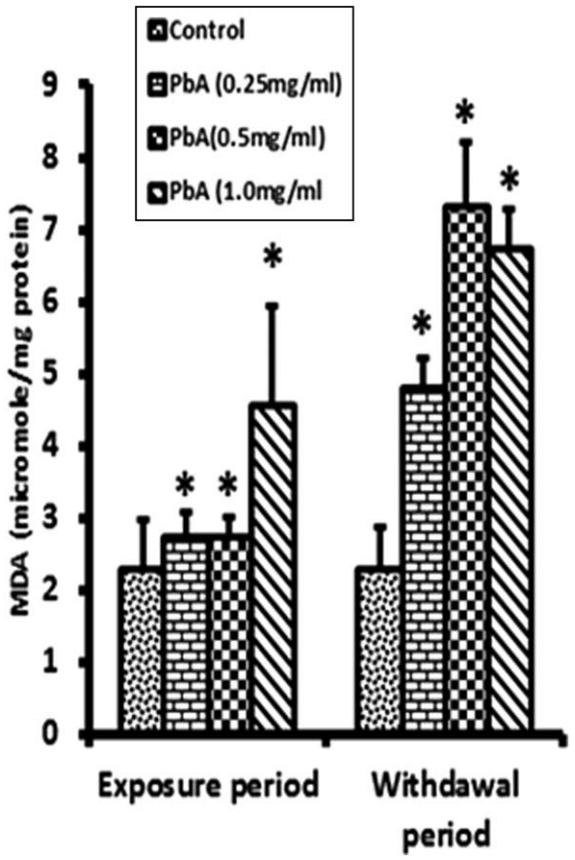

Fig. 2. Effect of lead acetate on malondialdehyde (MDA) levels in the kidneys of exposed and recovering rats.

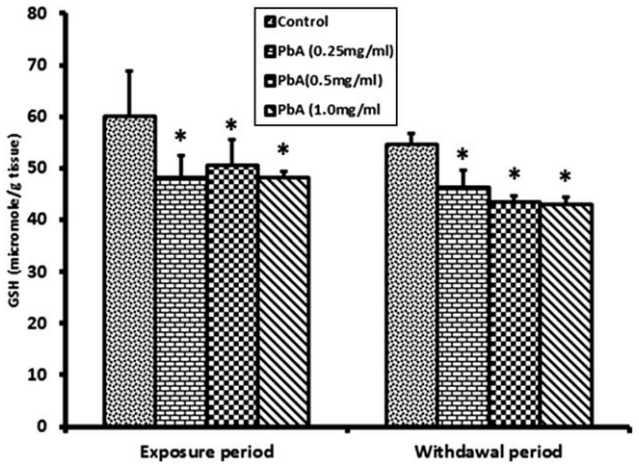

Fig. 3. Effect of lead acetate on reduced glutathione (GSH) in the kidneys of exposed and recovering rats.

concentrations of BUN and creatinine were determined using the Randox Kit (Random Laboratories Limited, UK).

Histopathology

Small pieces of kidney tissues were fixed in 10% formal saline buffer, embedded in paraffin wax, and sections of 5–6 μm in thickness were made and thereafter stained with hematoxylin and eosin for histopathological examination according to Drury et al. (1976). The sections were examined with light microscopy.

Statistical Analysis

All values are expressed as mean ± S.D. The test of significance between two groups was estimated by Student's *t* test. "One-way ANOVA with Dunnett's post-test was also performed using GraphPad Prism version 4.00.

RESULTS

Effects of Lead-Acetate Exposure and Withdrawal on Body and Kidney Weights in Rats

The effects of treatment of rats for 6 weeks with lead-acetate and withdrawal for another 6 weeks on body and kidney weights are presented in Table I. Body weight gain in the rats was unaffected in both exposure and withdrawal periods across the different groups. Kidney weights also remained unaltered throughout the exposure period. However, there was a significant drop in the weights of the kidneys in rats that received the smallest dose of PbA at the end of the withdrawal period.

Effects of Lead Acetate Exposure and Withdrawal on Antioxidant Systems in Rats

Lead-acetate exposure caused significant dose-dependent increase in H_2O_2 and MDA compared to the controls. The same effect was obtained in animals sacrificed at the end of the withdrawal period (Figs. 1 and 2). However, there were dose-dependent reductions in GSH at the end of both the exposure and withdrawal time (Fig. 3).

As presented in Table II, lead-acetate exposure caused significant increases in GST, CAT, and SOD activities compared to the controls, while the activity of GPX was reduced in the same time interval. However, lead acetate withdrawal resulted in significant decreases in CAT, SOD, and GPX activities, compared to the control, while GST activity still witnessed significant increases compared to the control (Table III).

Effects of Lead Acetate Exposure and Withdrawal on Serum Creatinine and BUN Levels

Lead acetate increased the blood creatinine level significantly without affecting the BUN level during the treatment and withdraw periods (Figs. 4 and 5).

Microscopy

Figures 6 and 7 show the representative photomicrographs of different parts of the kidneys of rats at the end of the exposure and withdrawal periods. With light microscopy, it was observed that the kidneys from control animals appeared largely normal. The kidney cortex was observed to possess adequate number of glomeruli showing well preserved morphology with mesangia and capillaries appearing normal. On

TABLE II. Renal antioxidant enzyme status in rats after 42 days of lead acetate treatment

	Control	PbA (0.25 mg/mL)	PbA (0.5 mg/mL)	PbA (1.0 mg/mL)
GPX	30.71 ± 2.45	28.23 ± 0.19[a]	27.17 ± 4.89	27.18 ± 2.27[a]
GST	0.30 ± 0.001	0.60 ± 0.06[a]	0.63 ± 0.02[a]	0.64 ± 0.08[a]
CAT	97.45 ± 7.14	102.12 ± 13.37	110.77 ± 8.39[a]	118.48 ± 2.46[a]
SOD	5.13 ± 0.31	5.75 ± 0.87	5.39 ± 0.98	5.72 ± 0.36[a]

[a]Indicates significant difference at $p < 0.05$.
Values are presented as mean ± standard deviation.
GPX activity (units/mg protein); GST activity (mmole1-chloro-2, 4-nitrobenzene GSH omplex formed/min/mg protein); CAT activity (mmoleH_2O_2 consumed/min/ mg protein); SOD activity (units/mg protein).

TABLE III. Renal antioxidant enzyme status in rats after 42 days of lead acetate withdrawal

	Control	PbA (0.25mg/mL)	PbA (0.5mg/mL)	PbA (1.0mg/mL)
GPX	30.60 ± 5.58	24.25 ± 3.98[a]	25.70 ± 3.41[a]	23.39 ± 1.43[a]
GST	0.40 ± 0.02	0.50 ± 0.02[a]	0.70 ± 0.01[a]	0.70 ± 0.01[a]
CAT	76.33 ± 3.90	74.70 ± 7.63	70.29 ± 3.06[a]	70.55 ± 1.81[a]
SOD	5.46 ± 0.67	3.94 ± 0.19[a]	5.18 ± 0.59	4.15 ± 0.21[a]

[a]Indicates significant difference at $p < 0.05$. GPX (units/mg protein); GST (mmole1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); CAT (mmoleH_2O_2 consumed/min/ mg protein); SOD (units/mg protein).

Values are presented as mean ± standard deviation.

the other hand, kidneys from rats exposed to 0.25, 0.5, and 1.0 mg/mL of lead acetate showed significant pathologies including multifocal areas of inflammatory cell infiltration; disseminated tubular necrosis; moderate to severe congestion of interstitial vessels; few hemorrhagic lesions and presence of vesicular nuclei in the tubular epithelial cells.

DISCUSSION

Lead toxicity manifests predominantly in oxidative damage as a result of the generation of ROS and the direct depletion of antioxidant defenses (Patrick, 2006; Jurczuk et al., 2007; Franco et al., 2009). In this study, administration of lead acetate (PbA) for 6 weeks resulted in dose-dependent increases in H_2O_2 and MDA, with dose-dependent reduction in GSH. This clearly indicates an induction of oxidative stress during the period of lead exposure. Elevated MDA levels point to enhanced lipid peroxidation probably due to the production of superoxide, peroxyl, and hydroxyl radicals (Abdel-Wahhab et al., 2008). Increased peroxidation of membrane lipids is one principal consequence of oxidative damage produced by lead. Generation of peroxyl radicals following intoxication by lead stimulates lipid peroxidation by production of endoperoxides through cyclization reactions (Sharma et al., 2010).

H_2O_2 is produced in cells by a dismutation of superoxide radicals generated in the oxidative process by SOD (Dixit et al., 2012). H_2O_2 can produce cytotoxicity in endothelial cells of different organs. However, the presence of GSH and antioxidant enzymes such as CAT, GPX, and GST, should normally ensure the removal of H_2O_2 (Sharma et al., 2010).

GSH is a multifunctional thiol-containing intracellular non-enzymatic antioxidant. It occurs intracellularly and is considered to be the redox buffer of the cell as it helps to protect cells from ROS, while it is converted to its oxidized form, GSSG, in the process (Jones, 2002). Lead is known to have a strong and irreversible affinity to thiol groups, causing depletion of antioxidant enzymes and GSH levels (Ercal et al., 2001). Results from this study suggest a depletion of

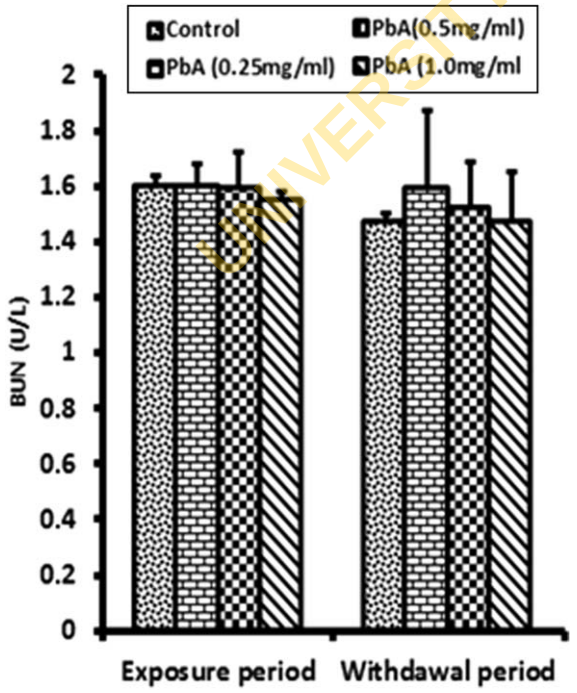

Fig. 4. Effect of lead acetate on blood urea nitrogen (BUN) levels in the kidneys of exposed and recovering rats.

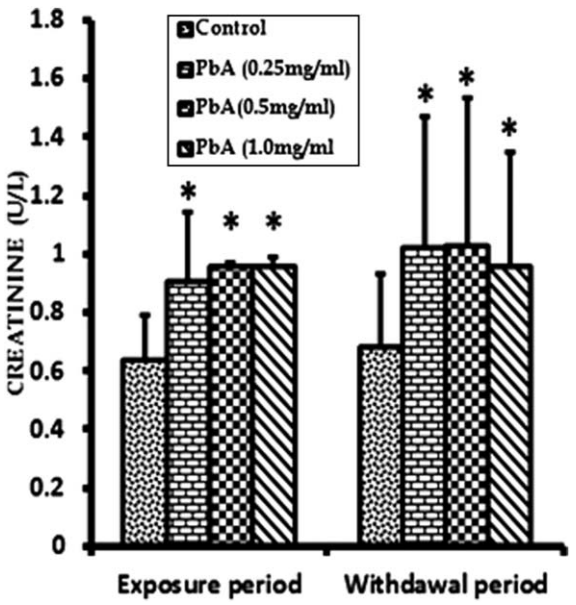

Fig. 5. Effect of lead acetate on creatinine levels in the Kidneys of exposed and recovering rats.

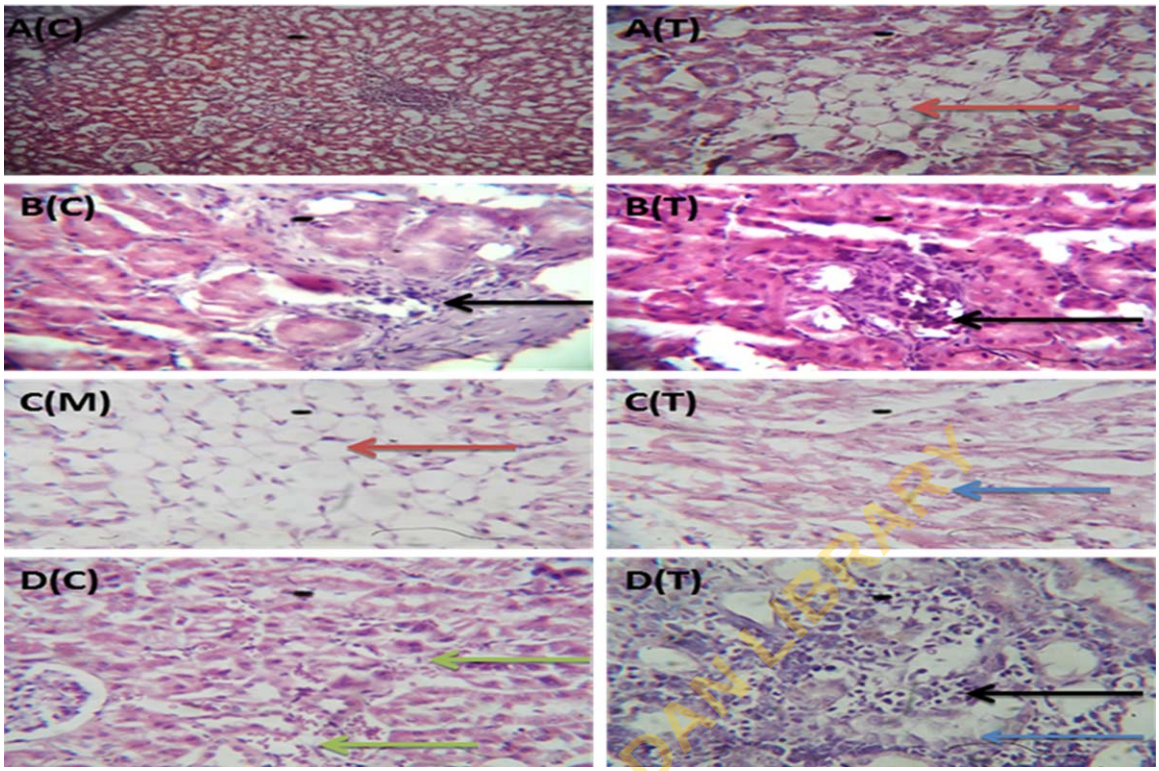

Fig. 6. Plates show the histological appearance of the kidneys of rats exposed to lead acetate (PbA) for six weeks. A. Control; B. 0.25mg/ml PbA; C. 0.5mg/ml PbA and D.I.Omg/ml PbA. (C), (M) and (T) represent Kidney Cortex, Medulla and Tubules, respectively. Red arrows indicate areas of fatty infiltration; Blue arrows indicate areas of tubular necrosis, while Black arrows represent areas of inflammatory cell infiltration (Mag. × 100 and × 400) with H & E stains. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

GSH levels and elevation of H_2O_2 which could produce cytotoxicity to cells of the renal tissues. Withdrawal of lead for 6 weeks, however, revealed that these effects were not recovered as the rats showed continued elevation in H_2O_2 and MDA levels and depletion of GSH levels.

GPX and GST are key enzymes that take part in maintaining GSH homeostasis in the tissues. GPX and GST as antioxidant enzymes work together with GSH in the decomposition of H_2O_2 and other organic hydroperoxides (Chen et al., 2009), and as such constitute a major part of the defense system against free radical mediated tissue or cellular damage after lead exposure (Arai et al., 1999; Neal et al., 1999; Newairy and Abdou, 2009). CAT is a key enzyme, which catalyzes the decomposition of H_2O_2 to H_2O and O_2 (Gutteridge, 1995). At the end of the exposure period to lead in this study, there were significant increases in GST, CAT, and SOD, compared to controls, while the activity of GPX was reduced in the same time interval. However, lead acetate withdrawal resulted in significant decreases in CAT, SOD, and GPX activities. Increase in antioxidant enzyme activities on toxicant exposure has been suggested to serve as a protective adaptation to the toxicant exposure (Okamoto et al., 2001). Thus, the increased activities of CAT, SOD, and GST during the exposure of rats to lead in this study may serve a compensatory role to protect tissues against the oxidative damage.

It has been reported that chronic administration of inorganic lead during development or acute exposure produced cell-type specific increases in particular isoforms of GST in the rat kidney (Oberley et al., 1995).

During exposure to Lead, the heavy metal usually distributes into the hard tissues like bone and teeth, where it accumulates, only to result in a sustained release and maintenance of unacceptable blood lead levels, long after the exposure period (Pounds et al., 1991). The fresh release of lead following its withdrawal from the rats in this study from body stores may be responsible for the decreases in the antioxidant enzyme activities observed after the withdrawal period (Haleagrahara et al., 2011). The catalytic function of GPX is dependent on the selenium-containing functional group at the active site of the enzyme. Lead, however, is capable of displacing the selenocysteine group from the active site, there rendering the enzyme inactive (Mudipall, 2007). Similarly, Lead binds irreversibly with sulfhydryl groups on proteins and may thereby render the antioxidant enzymes, GST or CAT inactive (Hsu and Guo, 2002). Several workers have reported the reduction in antioxidant enzyme activities as a result of lead administration (Flora et al., 2007; Newairy and Abdou, 2009).

Whereas BUN levels were unaltered through the period of this study, serum creatinine levels were significantly

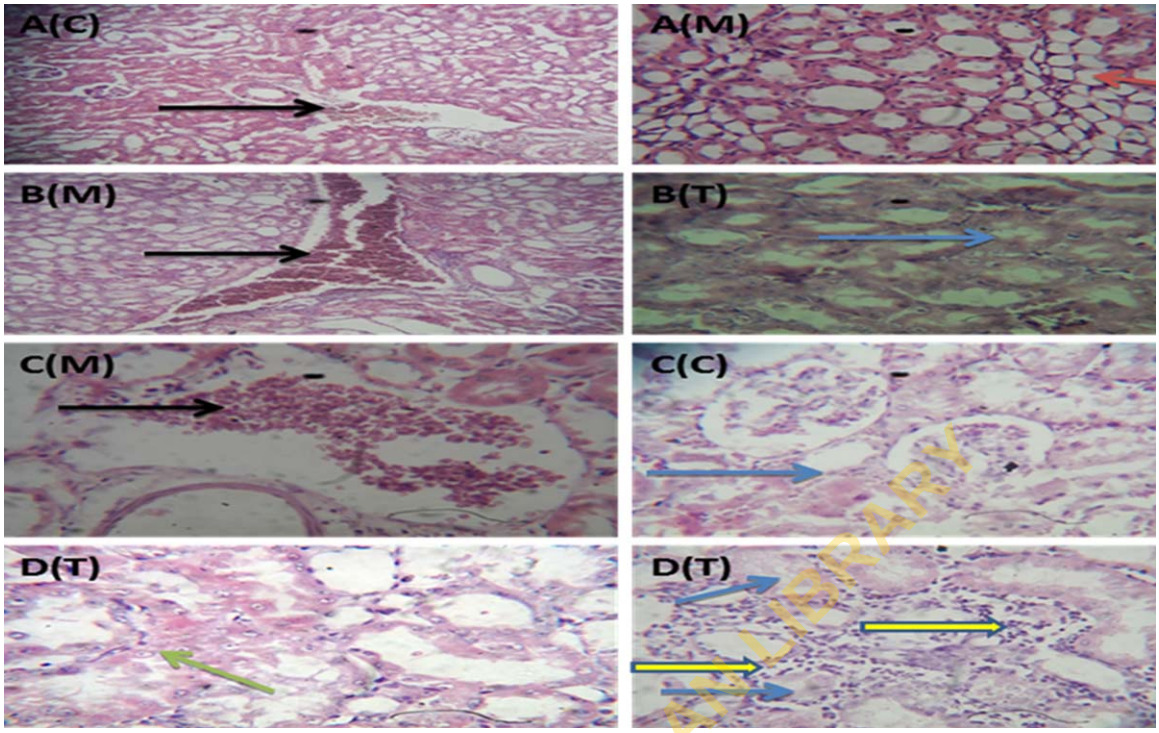

Fig. 7. Plates show the histological appearance of the kidneys of rats after withdrawal of lead acetate (PbA) for six weeks. A. Control; B. 0.25mg/ml PbA; C. 0.5mg/ml PbA and D.l.Omg/ml PbA. (C), (M) and (T) represent Kidney Cortex, Medulb and Tubules, respectively. Red arrows indicate areas of fatty infiltration; Blue arrows indicate areas of tubular necrosis, Black arrows represent areas of vessel congestion yellow arrows indicate presence of vesicular nuclei in tubular epithelial cells (Mag. × 100 and × 400) with H & E stains. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

elevated in both the exposure and withdrawal periods of this investigation. Lead intoxication is known to cause significant increases in blood urea and serum creatinine in rats (Ghorbe et al., 2001; Farrag et al., 2007). Urea is an end-product of protein metabolism. Increased serum level of urea may result with increased protein catabolism or reduced glomerular filtration rate (Salmean et al., 2013). It has been reported that ROS may produce damage to kidney tubular epithelial cells, causing necrosis, with increased tubular permeability and a diffusion and back-leak of the glomerular filtrate across the tubular basement membrane back to the interstitium and then circulation (Anetor, 2002). This results in increased retention of nitrogenous waste in the serum. Although, BUN levels were not significantly altered in this study, there were significant dose-dependent increases in serum creatinine. Creatinine is a product of muscle metabolism resulting from an irreversible non enzymatic conversion of creatine to creatinine. Damage to tubular epithelial cells may also reduce the rate of creatinine clearance from the kidneys and similarly its retention in the blood circulation (Gowrisri et al., 2012).

Histologically, the kidneys of lead-exposed rats showed significant pathology indicated by areas of infiltration by inflammatory cells, disseminated tubular necrosis, fatty infiltrations, and hemorrhagic lesions. The pathological picture was further illustrated in the withdrawal period by congestion of interstitial vessels, perivascular inflammation, and inflammation of the interstitial tissue. There were vesicular nuclei of the cells of the tubules as well as areas of tubular necrosis. There were also areas of architectural anarchy in some cases. However, the majority of the kidneys from control rats showed cortex with adequate number of glomeruli showing a well preserved morphology with the mesangia and the capillaries appearing normal. The pathological findings observed at histology further confirms the nephrotoxicity of lead at the end of both the exposure and withdrawal periods. Cytotoxicity by ROS may be responsible for the tubular necrosis and hence a reduction in renal clearance of creatinine.

CONCLUSIONS

In conclusion, lead acetate in this study caused induction of oxidative stress and produced significant pathology in the renal tissues of rats. The different assessments of lead toxicity carried out in this study indicate that the alterations produced by the administration of lead to rats were not reversed by mere withdrawal of lead.

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