International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Effects of Acute and Chronic Lead Exposure on Kidney Lipid Peroxidation and Antioxidant Enzyme Activities in BALB-C Mice (*Mus Musculus*)

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Abstract: ***Extensive and unmonitored use of lead in developing and industrialised countries is posing a serious threat to human health. Prolonged exposure of a sub-lethal dose of this toxicant is closely related to its accumulation in various soft tissues and its interference with bio elements that hamper several physiological processes. Present study was carried out to investigate the toxic effects of lead acetate on biochemical parameters in the kidney of Swiss-albino mice with a trial to evaluate their self recovery from this toxicant in respective parameters without any supplement. Therefore eighteen normal mice showing no sign of morbidity were divided into three groups of 6 animals each. Lead acetate was given orally to mice for 40 days. First group served as control and was given normal saline solution as vehicle. Mice of second group were given daily 10mg/kg bw of lead. Whereas third group was given daily 150mg/kg bw of lead. The mice of both the groups were sacrificed by cervical dislocation after day 1, 40 and 80 along with control animals. The findings indicated that exposure for 40 days resulted in significant increase ($p<0.005$) in lipid peroxidation and decrease ($p<0.005$) in kidney total antioxidant enzymes (SOD and CAT). However the mice which were left for 80 days showed some improvements in lipid peroxidation as well as restoration of total antioxidants.***

Keywords: Lead acetate, oxidative stress, kidney.

1. Introduction

Lead is a naturally occurring bluish grey heavy metal found in earth's crust. It became popular because of its dense, ductile, malleable and corrosion resistant properties. These have made lead useful in paints and protective coatings, pigments to glaze ceramics, water pipes, storage batteries and gasoline additives (9, 12). Due to its wide usage and applications, human exposure to lead and its derivatives in day to day life is unavoidable. There is a considerable variation between species in their susceptibility to lead and the compounds containing lead, which influence its toxicity. Toxicity also varies with the chemical form of lead. Lead acetate is very soluble and more toxic than insoluble lead oxides or solid lead sheeting.

Although atmospheric lead pollution due to tetraethyl lead from gasoline has been improved over the last decades, humans are still exposed to lead via contaminated foods and water through industrial activities. Lead is also one of the most frequently reported cause of poisoning in farm animals especially in cattles (3). Source of lead poisoning in farm animals are due to suckling lead paints or lead toys and drinking water contaminated with petroleum industries. The major source of soil contamination is waste, combustion of leaded gasoline and use of lead arsenate pesticides (13).

The concentration of lead residues in various animal tissues depend upon the route of entering and period of exposure to environmental pollutants. Where inhalation of polluted dust is more dangerous than oral ingestion of polluted water and contaminated foods for long period (13). Initially orally ingested lead is deposited in the skeleton until a possible threshold is reached then it deposited in other tissues especially liver and kidney. The absorbed lead is conjugated in liver and passed to kidney, where a small quantity is excreted in urine and rest accumulates in various body organs and interferes with their functions (10, 18, 20). Early stages of lead exposure are manifested by loss of appetite, weight loss, constipation, occasional vomiting, lead lines of gums and anaemia. However prolonged exposure of a sub-lethal dose of this toxicant produces wide range of biological and physiological dysfunctions. One of the major mechanisms behind heavy metal toxicity is attributed to oxidative stress. There is experimental evidence to indicate that cellular damage mediated by reactive oxygen species can be involved in lead induced oxidative stress (19). Lead is also known to cause oxidative damage in several tissues by bringing about imbalance in the generation and removal of reactive oxygen species (21, 17). Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage. A growing amount of data provide evidence that metals are capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules. As kidney is a common target organ for injury from exposure to a broad range of chemicals and drugs, the present study is an attempt to characterize biochemical alterations induced by lead in the kidney of Swiss- albino mice with a trial to evaluate their self recovery from this toxicant without any supplement.

2. Materials and Methods

2.1 Laboratory mice

Eighteen sexually mature Swiss-albino mice of Balb-C strain weighing 20-24g were utilized in the present study. The mice were procured from the Central Research Institute (CRI), Kasauli, Himachal Pradesh (India). They were kept for two weeks in a pathogen free, well ventilated room in the departmental animal house in order to enable them to acclimatize to their environment. During the period of experiment, the animals were supplied with food pellets and

drinking water on daily basis and their beddings were changed, discarded and disinfected. All experiments were conducted after the approval of Institutional Animals Ethics Committee, Himachal Pradesh University (IAEC/BIO/7-2011), Shimla (India).

2.2 Chemicals

Lead acetate [$(CH_3COO)_2$ $Pb.3H_2O$] was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used in the experiment were of analytical grade.

2.3 Experimental Design

Adult Swiss-albino mice were divided into three groups of 6 mice each. Group1 served as control, received normal saline solution. Group 2 and group 3 were given lead acetate at a dose of 10 mg/kg bw and 150 mg/kg bw respectively by oral gavage once daily for 40 days. Two mice from each group were sacrificed after day 1, 40 and 80 under light chloroform anaesthesia. Kidneys were excised, weighed and homogenised in ice cold buffer for various biochemical parameters.

2.4 Biochemical Analysis

Kidney was minced and homogenised (10% w/v) in ice cold buffers (pH 7.4). The homogenate was centrifuged at 10000 rpm for 15-20 min at 4^0C to get enzyme fraction. The resultant supernatant was used for various biochemical assays. Lipid peroxidation (LPO) was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids (Dhindsa *et al*., 1981). Superoxide dismutase (SOD) activity was assayed according to the method of Mishra and Fridovich (1972). Catalase (CAT) activity was assayed following the method of Aebi (1984).

3. Results

Table 1 showed that different doses of lead acetate induced a significant percentage of lipid peroxidation in mice renal tissue after 1, 40 and 80 days stages. This percentage increase with increasing dose and with longer duration of treatment. It reached a maximum of 68.65% after repeated daily oral treatments for 40 days with highest test dose (150 mg/kg bw) as compared to control group. The results in table1 also demonstrate that the percentage of lipid peroxidation in renal tissue was significantly reduced in the treated mice, which were left for regeneration without any supplement for 80 days after withdrawal of both doses of lead acetate at 40 days stage. This percentage decrease reached a maximum of 29.13% in mice group which was treated with highest dose of lead acetate compared with 16.15% for low dose treated mice group. The level of enzymatic antioxidant system in the kidney tissue of mice was significantly higher ($p<0.005$) in lead treated animal groups as compared to control group after 1 day stage. The activity of antioxidant enzymes SOD and CAT in renal homogenate of mice were decreased by -24.42% and -27.85% respectively with highest test dose of lead acetate (150 mg/kg bw) after 40 days of treatment. However the treated mice which were left for 80 days showed improvements in the restoration of total antioxidants.

Table 1

Groups	Days		
	1	40	80
Control	28.34±1.11	30.91±1.02	31.23±1.38
Lead acetate (10 mg/kg body weight)	29.61±0.83*	39.86±3.11*	36.37±3.89*
% increase or decrease	4.48%	28.95%	16.15%
Lead acetate (150 mg/kg body weight)	31.63±1.18*	52.13±2.62*	40.33±1.31*
% increase or decrease	11.60%	68.65%	29.13%

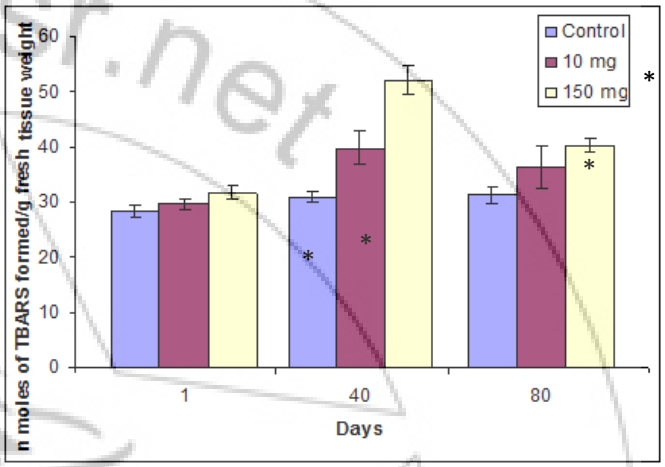

Figure 1

Table & Figure 1: Lipid peroxides (n moles of TBARS formed/g of fresh tissue weight) in kidney of normal and lead acetate treated mice during 1-80 days period. Values are mean ± SEM; n = 3 (P* < 0.05)

Table 2

Groups	*Days*		
	1	*40*	*80*
Control	11.30±0.020	12.61±0.07	12.60±0.003
Lead acetate (10 mg/kg body weight)	12.79±0.019*	10.25±0.006	11.67±0.005*
% increase or decrease	13.18%	-18.17%	-7.38%
Lead acetate (150 mg/kg body weight)	12.81±0.064	09.53±0.018*	10.89±0.009
% increase or decrease	13.36%	-24.42%	-13.57%

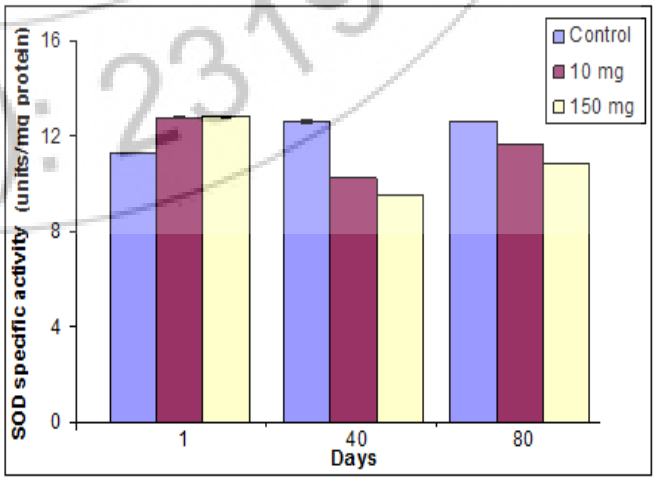

Figure 2

Table & Fig. II: Changes in SOD specific activity (units/mg protein) in kidney of normal and lead acetate treated mice during 1-80 days period. Values are mean ± SEM; n = 3 (P* < 0.05)

Table 3

Groups	Days		
	1	40	80
Control	13.37±0.03	13.68±0.13	13.98±0.094
Lead acetate (10 mg/kg body weight)	14.01±0.012*	11.43±0.06	11.90±0.018*
% increase or decrease	4.78%	-16.44%	-14.87%
Lead acetate (150 mg/kg body weight)	16.89±0.12*	9.87±0.07	10.68±0.03
% increase or decrease	26.32%	-27.85%	-23.60%

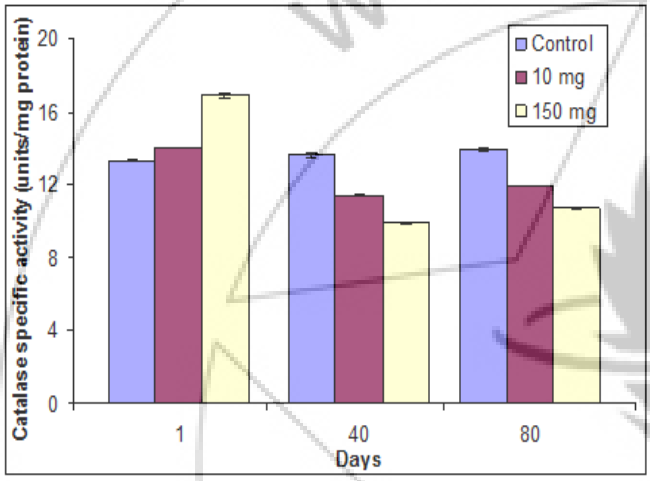

Figure 3

Table & Figure 3: Catalase specific activity (units/mg protein) in kidney of normal and lead acetate treated mice during 1-80 days period. Values are mean ± SEM; n = 3 (P* < 0.05)

4. Discussion

Lead is known to cause oxidative damage in various soft tissues by bringing about imbalance in the generation and removal of reactive oxygen species (21, 17). The results of the present study showed that lead acetate exposure in drinking water for 40 days resulted in severe increase in renal lipid hydroperoxides and decrease in antioxidant enzymes like SOD and CAT, with both doses of lead acetate tested.

Even though the exact molecular mechanism of lead toxicity on various tissues has still not been convincingly explained, evidence is accumulating to support the role of free radicals in the pathophysiology of lead toxicity. Oxidative stress appears to be a possible mode of the molecular mechanism for lead toxicity. This toxic metal induces disturbance in the physiological and biochemical state in different tissues, resulting in an alteration of the enzyme homeostasis and distortion in cell organelle function (5, 4).

The observed increases in lipid hydroperoxides in the present study are consistent with the lead induced peroxidation of membrane lipid in the bone marrow (5, 14, 22). The generation of reactive oxygen species such as superoxide ions, hydrogen peroxides and hydroxyl radicals or by products of lipid peroxidation such as lipid hydroperoxides and lipid aldehyde (1, 5) have been implicated in lead toxicity. Our study has confirmed that chronic lead exposure induces similar effects in the renal tissue that could result in severe oxidative stress. The cell membrane is the most important target of the free radical damage by xenobiotics (6). Lead acetate generation of free radicals may attack not only DNA in the cell, but also the polyunsaturated fatty acid residues of phospholipids in other organelles that are sensitive to oxidation (17). Lead is also known to cause oxidative damage in various peripheral organs by enhancing lipid peroxidation (11, 7). Lipid hydroperoxides are formed due to oxidation of lipids and cholesterol containing cellular molecules like cell membrane phospholipids, lipoproteins, glycolipids and other lipid containing structures (15). The oxidation is usually caused by ROS like oxy radicals, peroxy radicals and hydroxyl radicals. The balance between the production of oxidants and their scavenging by antioxidants determines the extent of lipid peroxidation (8). Increased lipid hydroperoxides could be explained by lead-induced inhibition of free radicals scavenging enzymes, leading to the accumulation of ROS to accumulate and cause increased oxidation in various body organs (16). The reason for LPO increase could also be due to the combined inhibitoty effects of the various antioxidants enzymes (SOD and CAT) as observed in our results.

In our results, the levels of kidney SOD and CAT were reduced by lead acetate exposure for 40 days, thus rendering the tissue susceptible to the peroxidative damage. These antioxidant enzymes rely on essential trace elements and prosthetic groups for proper molecular organisation and their enzymatic reaction (5). Because lead being itself bivelently charged in its ionic form, is also known to displace bivalent ions such as Zn^{2+}, Cu^{2+} and Fe^{2+} . Since these transitional metal ions have variable oxidation states, this characteristic allows them to switch between oxidised and reduced states easily (2). Hence, they are important players in redox reactions involving, neutralizing oxidative stress in renal tissue. SOD and CAT are metalloproteins and complete their antioxidant functions by detoxifying the free radicals. The present study also confirmed that exposure to lead acetate in drinking water for 40 days, produced significant alterations in kidney antioxidant enzymes and an increase in kidney lipid peroxidation in Swiss-albino mice. The association of significant kidney oxidative stress with lead exposure suggests that an antioxidant may enhance the efficacy of therapeutic agents used in the treatment of lead toxicity. Hence, an ideal treatment for lead intoxication or lead-induced organ pathology should include both lead-chelating and antioxidant actions.

5. Conclusion

Present study confirmed that exposure to lead acetate in drinking water for 40 days, produced significant alterations in renal antioxidant enzymes and an increase in renal lipid peroxidation in Swiss-albino mice.

6. Conflict of Interest

The authors confirmed that this article content has no conflicts of interest.

7. Acknowledgment

One of the authors duly acknowledged and thanks the Council of Scientific and Industrial Research, New Delhi for the award of Junior Research Fellowship (JRF).

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