IJPSR (2017), Vol. 8, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 September, 2016; received in revised form, 13 October, 2016; accepted, 19 October, 2016; published 01 March, 2017

BIOCHEMICAL STUDIES ON THE MICE HEART REGARDING LEAD ACETATE INDUCED OXIDATIVE STRESS

Sushma Sharma and Anita Thakur *

Department of Biosciences, Himachal Pradesh University, Summer Hill, Shimla - 171005, Himachal Pradesh, India.

Keywords:

Oxidative stress, Heart, Free radicals, SOD, CAT

Correspondence to Author:
Anita Thakur

Ph. D. Scholar, Department of Biosciences, Himachal Pradesh University, Summer Hill, Shimla – 171005, India.

E -mail: thakuranitabiosci21@gmail.com

ABSTRACT: Lead is important heavy metal and due to widespread use in industry, it has become an important pollutant that exerts toxic effects on human health. Lifestyle factors (e.g. cigarette smoking), proximity to industrial areas, lead mines, lead based paints and leaded gasoline significantly contribute to lead pollution of the air, food, water and soil. Several antioxidant enzymes and molecules have been used to evaluate lead–induced oxidative damage. Present study aimed to evaluate the lead acetate induced cardio-toxicity. Lead acetate is known to induce changes in free radical scavenging enzymes like superoxide dismutase (SOD) and catalase (CAT). Healthy looking mice showing no sign of morbidity were divided into three groups. Group I was designated as control whereas group II and group III received lead acetate having doses 10 mg/kg body weight of lead acetate, daily and 150 mg/kg body weight of lead acetate, weekly respectively. Study was performed after 24 hours, 40 and 80 days stages. Lead acetate significantly decrease antioxidant enzymes and increase oxidative stress along with cardiac tissue damage.

INTRODUCTION: Lead acetate ($[Pb(C_2H_3O_2)_2]$) is a toxic white crystalline chemical compound made by treating lead {II} oxide with acetic acid. Lead has been shown to cause damage by inducing oxidative stress. Lead causes high protein carbonyl content (PCC) which is indicative of oxidative damage and low antioxidant level. According to Neman, most of lipsticks contain lead. Lead poisoning due to occupational exposure is very common in adults leading to reversible changes in mood and personality [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(3).1388-92

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (3).1388-92

The effects on physiology, histomorphology development and biomarkers have been observed on different organ of animals and humans. In most of the previous studies, the harmful effects of lead were noted [2, 3, 4]. Many mechanisms are proposed for lead– induced hypertension, including alteration in calcium (Ca^{2+}) flux, lowering the calcium binding capacity in intracellular Ca^{2+} concentration [5, 6], inhibition of sodium pump [7], increased activity of renin-angiotensin system [8], altered kallikrein-kinin system causing decreased plasma levels of bradikinin [9, 10] and increased cardiovascular sensitivity to endogeneous substances such as catecholamines [11]. Lead exposure results in oxidative stress and inflammation which in turn lower bioavailability of NO and promote hypertension, endothelial injury/ dysfunction and cardiovascular disease.

Pathogenesis of lead-induced hypertension could be explained by increase in rate and contractility of the heart. Findings include direct effects on the excitability and contractility of the heart [12, 13]. Myocarditis, electrocardiographic abnormalities, altered heart rate activity, slowed ventricular systole, and vascular degeneration have all been among the reported cardiovascular aberrations detected in human chronically and acutely exposed to toxic lead levels. Environmental and occupational lead exposures that raise blood lead levels above 100 µg % and 60 µg % in adults and children respectively are frequently with transient as well as permanent cardiac and vascular lesions and functional disturbances [14, 15, 16, 17].

Oral administration of lead (5µg/ml drinking water) for 15 and 20 months has been reported to be associated with a significant slowing of aterioventricular conduction [18]. However there is not much data on cardiovascular effects of either low or moderate amounts of lead exposure [19].

Lead is known to cause oxidative damage in various tissues by bringing about imbalance in the generation and removal of reactive oxygen species [20, 21]. Oxidative stress is an imbalance between free radical generation and antioxidant defense system. CAT and SOD are metalloproteins and complete their action by detoxifying the free radicals. SOD is a group of enzyme which catalyses the conversion of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2). Catalase is responsible for conversion of H_2O_2 to water. Deficiency of catalse results in accumulation of H_2O_2 that contribute to inflammation and cardiovascular remodeling.

In addition H_2O_2 is substrate for production of hydroxyl radical ($^{\cdot}OH$), which is highly reactive free radical and as such can cause oxidative injury. Studies showed that addition of lead acetate to culture medium results in transient rise in O_2^- production followed by a sustained increase in H_2O_2 generation by cultured human coronary endothelial cells [22].

MATERIAL AND METHODS: The present investigation was carried out on heart of adult sexually mature Swiss albino mice weighing 20 – 30g. They were maintained in polypropylene cages under hygienic conditions with proper temperature and light. Mice were fed upon Hindustan lever pellets diet and water *ad libitum.* All experimental procedure was conducted after approval of Institutional Animal Ethics Committee (IAEC/Bio/6-2011) of H. P. University, Shimla.

Chemicals: All reagents used were of highest grade. Lead acetate used for this study was obtained from Sigma Chemicals, St. Louis, MO, USA.

Grouping of Animals and Dose Administration:

Mice were divided into three groups:-

Group I served as control

Group II received oral administration of lead acetate (10 mg/kg body weight) daily

Group III administered lead acetate (150 mg/kg body weight), weekly

Lead acetate was given for 40 days and mice were sacrificed at 1, 40 and 80 days period by cervical dislocation.

Biochemical studies: Total protein was measured as per the method of Lowry *et al*., (1951) [23]. Superoxide dismutase was done as per the method of Mishra and Fridovich (1972) [24] and catalase activity was determined by monitoring the decomposition of hydrogen peroxide by measuring the changes in absorbance at 240 nm. The enzyme activity was calculated in units' mg^{-1} protein.

RESULTS: After 1 day of lead acetate treatment level of antioxidant enzymes was found to be increased however, at later stage there was significant decrease in heart antioxidants after lead acetate treatment. The decrease was much pronounced after 40 days stage. At 80 days, after the withdrawal of lead acetate at 40 days, the enzyme activity was found to be low in comparison to control, although the decrease was less than 40 days stage.

TABLE 1: CHANGES IN SUPEROXIDE DISMUTASE SPECIFIC ACTIVITY (UNITS mg $^{-1}$) IN HEART OF NORMAL AND LEAD ACETATE TREATED MICE DURING 1 – 80 DAYS PERIOD. VALUES ARE MEAN ± SEM; N = 3 (P* < 0.05)

	Group	1	40	80
Superdioxide dismutase (SOD) (units/mg protein)	Control	10.84 ± 0.006	10.92 ± 0.009	10.99 ± 0.010
	Lead acetate (10 mg/kg body weight)	11.63 ± 0.012	8.76 ± 0.003*	9.32 ± 0.007
	Lead acetate (150 mg/kg body weight)	12.62 ± 0.036	8.31 ± 0.011	9.04 ± 0.009*

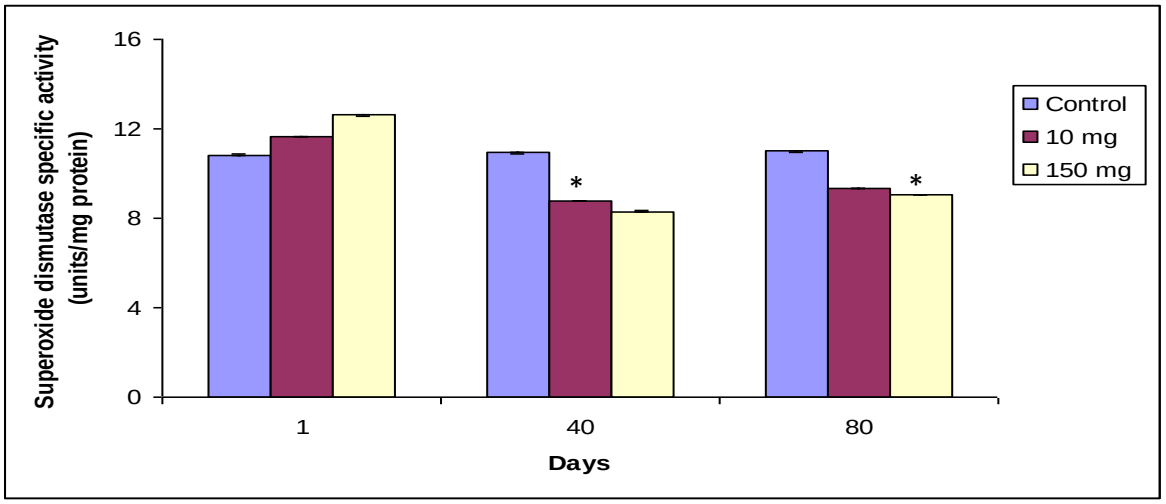

FIG. 1: CHANGES IN SUPEROXIDE DISMUTASE SPECIFIC ACTIVITY (UNITS mg $^{-1}$) IN HEART OF NORMAL AND LEAD ACETATE TREATED MICE DURING 1 – 80 DAYS PERIOD. VALUES ARE MEAN ± SEM; N = 3 (P* < 0.05)

TABLE 2: CHANGES IN CATALASE SPECIFIC ACTIVITY (UNITS/MG PROTEIN) IN HEART OF NORMAL AND LEAD ACETATE TREATED MICE DURING 1 – 80 DAYS PERIOD. VALUES ARE MEAN ± SEM; N = 3 (P* < 0.05)

	Group	1	40	80
Catalase (units/mg protein)	Control	11.20 ± 0.012	11.66 ± 0.009	11.40 ± 0.019
	Lead acetate (10 mg/kg body weight)	12.11 ± 0.08	10.20 ± 0.09	10.97 ± 0.08
	Lead acetate (150 mg/kg body weight)	14.88 ± 0.013*	9.87 ± 0.005*	10.20 ± 0.14

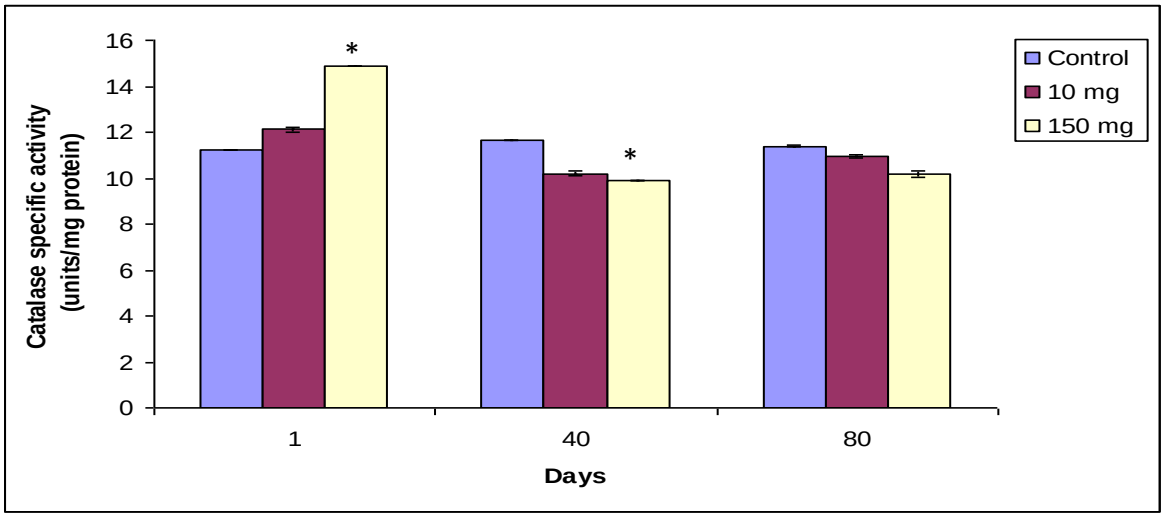

FIG. 2: CHANGES IN CATALASE SPECIFIC ACTIVITY (UNITS/MG PROTEIN) IN HEART OF NORMAL AND LEAD ACETATE TREATED MICE DURING 1 – 80 DAYS PERIOD. VALUES ARE MEAN ± SEM; N = 3 (P* < 0.05)

DISCUSSION: lead toxicity has been among the most studied health problem in the recent years. Lead is toxic in most of its chemical forms, whether it is inhaled or ingested via water or feed. The extent to which orally administered lead absorption is small, however due to its slow rate of elimination, harmful level of lead can accumulate in tissues after prolonged exposure in low quantities [25, 26]. The toxicant chemicals induce perturbations in the physiological and biochemical state, which affect the enzyme activity. It then causes distortions in the cell organelles which may lead to alterations in various enzyme concentrations [27]. The quantitative importance of oxygen – derived free radicals can be realized by the fact that above 250 gm of oxygen is consumed every day by humans. Of this, 2–5% would be converted to the superoxide [28].

No significant differences were found in tissue SOD and CAT activity among control group in the present study. The lead induced toxicity stimulated the oxidative stress and the antioxidant enzymes level were increased as a defence mechanism. In our findings, the antioxidant enzymes (SOD) and (CAT) showed progressive increase in comparison to control after 1 day. The increase was more pronounced in heart of mice administered with 150 mg/kg body weight of lead acetate than in the heart of mice given 10 mg/kg body weight of lead acetate. Thus, our findings supported the involvement of oxidative stress in the pathophysiology of lead toxicity in tissues.

The activities of SOD and CAT antioxidants were reduced after 40 days and tissue get susceptible to the peroxidation damage. The oxidative stress is reduced after lead acetate withdrawal at 40 days and decrease in antioxidant enzymes was slight less than the control mice at 80 days stage. Lead might affect the production of haemoglobin by interfering the enzymes necessary for the biosynthesis of heme (e.g. δ–aminolevulinic acid dehydrase), resulting in defective heme synthesis and anaemia [29]. It is reported that low blood lead level (about 15µg/dl) is sufficient to inhibit the activity of this enzyme [30].

Although the oxidative stress induced by pre – oxidant effect of lead is not well established, some authors suggested that autoxidation of excessively accumulated δ–aminolevulinic acid resulting from the inhibition of δ–aminolevulinic acid dehydrase might result in the formation of intermediary oxygen radicals and non significant changes in the activity of antioxidant enzymes [31].

CONCLUSION: Thus, the strong association was observed between heart and lead levels with oxidative biomarkers and it is suggested that lead–induced oxidative stress should be considered as important, if not the primary cause of pathogenesis of lead related pathologies.

REFERENCES:

1. Dhir V and Dhand P: Toxicological approach in chronic exposure to lead on reproductive functions in female rats. Toxicol. Int. 2010; 17 (1): 1-7.
2. Ait HN Slimani M Merad BB and Zaoiu C: Reproductive toxicity of lead acetate in adult male rats. American J. of Scientific Research 2009; 3: 38-50.
3. Eugenia D Alexandra T Diana A and Cristina RT: The consequences in utero exposure to lead acetate on exposure and integrity biomarkers of reproductive system in female rats. Medicina Veterinara 2009; (2): 295-300.
4. Yousif WH and Adbullah ST: Reproductive efficiency of rats whose mother treated with lead acetate during lactation: role of vitamin E. Iraqi J. of Veterninary Sciences, 2010; 24 (1): 27-34.
5. Goldstein GW. Evidence that lead acts as a calcium substitute in second messenger. Metabolism, 1993; 14: 97-101.
6. Schanne FA Long GJ and Rosen JF: Lead induced rise in intracellular free calcium is mediated through activation of protein kinase C in osteoblastic bone cells. Biomechemica Biophysica Acta, 1997; 1360: 247-254.
7. Weiler E Khalil-Manesh F and Gonick H: Effects of lead and natriuretic hormone on kinetics of sodium-potassium activated adenosine triphosphatase. Environ. Health Prospect., 1998; 78: 113-115.
8. Sharifi AM Darabi R Akbarloo N Larijiani B and Khoshbaten A: Investigation of circulatory and tissue ACE activity during development of lead induced hypertension. Toxicol., 2004; 153: 233-238.
9. Carmignani M Voipe AR Boscolo P Poma A and Boscolo P: Kininergic system and arterial hypertension following chronic exposure to inorganic lead. Immunopharmacol., 1999; 44: 105-110.
10. Karimi GR Khashbaten A Abdollahi M Sharifzadeh M Namiranian K and Dehpour AR: Effects of sub-acute lead acetate administration on nitric oxide and cyclooxigenase pathways in rat isolated aortic ring. Pharmacol. Res. 2002; 46 (1): 31-37.
11. Heydari A Khoshbaten A Asgari AR Naroozzadeh A Ghasemi A Najafi S and Badalzadeh R: Effects of short term sub chronic lead poisoning on nitric oxide metabolites and vascular responsiveness in rat. Toxicol., 2006; 166 (1): 88-94.
12. Kopp SJ and Barany M: Influence of isoproterenol and calcium on cadmium or lead-induced negative intropy related to cardiac myofibrillar protein phosphorylation in perfused rat heart. Toxicol. & Appl. Pharmacol., 1980; 55:8-17.
13. Prentice RC and Kopp SJ: Cardiotoxicity of lead at various perfusate calcium concentrations: functional and metabolic

responses of the perfused rat heart. Toxicol. Appl. Pharmacol., 1985; 81: 491-501.
14. Bertal O Buhler FR and Ott J: Lead-induced hypertension: blunted beta-adrenoreceptors mediated functions. Br. Med. J., 1978; 4: 551-558.
15. Jhaveri RC Lavorgna L Dube SK Glass L Khan F and Evans HE: Relationship of blood pressure to blood lead concentrations in small children. Pediatrics. 1979; 63:674-676.
16. Boscolo P Galli G Iannaccone A Martino F Porcelli G and Troncone L: Plasma rennin activity and urinary kallikrein excretion in lead-exposed workers as related to hypertension and nephropathy. Life Sci., 1981; 28:175-184.
17. Kirkby H and Gyntelberg: Blood pressure and other cardiovascular risk factors of long-term exposure to lead. Scand. J. Work Environ. Health, 1985; 11: 15-19.
18. Kopp SJ Perry HM Glonek T Erlanger M Perry EF Barany M and D' Agrosa LS: Cardiac physiologic-metabolic changes after chronic low level heavy metal feeding. Am. J. Physiol., 1980; 239: H22-H30.
19. Evis MJ Kane KA Moore MR and Parratt JA: The effects of chronic low lead treatment and hypertension on the severity of cardiac arrhythmias induced by coronary artery ligation in anesthetized rats. Toxicol. & Appl. Pharmacol., 1985; 80: 235-242.
20. Gurer H and Ercal N: Can antioxidants be beneficial in the treatment of lead poisoning. Free Radio. Biol. Med., 2000; 29: 927-945.
21. Hamadouche NA Slimani M Merad-Boudia B and Zaoui C: Reproductive toxicity of lead acetate in adult male rats. Am. J. Sci. Res., 2009; 3: 38-51.
22. Ni Z Hou S Barton CH and Vaziri ND: Lead exposure raises superoxide and hydrogen peroxide in human endothelial and vascular smooth muscle cells. Kidney Int., 2004; 66: 2329-2336.
23. Lowry O H Rosenbrough NJ Farr AL and Randall RJ: Protein measurements with the Folin – phenol reagent. J. Biol. Chem., 1951; 193: 265-275.
24. Mishra HP and Fridovich I: The role of superoxide anion in the auto – oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 1972; 247: 3170-3175.
25. Ercal N Gurer-Orhan H and Aykin-Burns N: Toxic metals and oxidative stress part I: Mechanism involved in metal-induced oxidative damage. Top. Med. Chem., 2001; 6: 529-539.
26. Madhavi D Rudrama DK Kesava Rao K and Reddy PP: Modulating effect of phyllanthus fruit extract against lead genotoxicity in germ cells of mice. J. Environ. Biol., 2007; 28:115-117.
27. Vinodhini R and Narayanan M: Biochemical changes of antioxidant enzymes in common Carp after heavy metal exposure. Turk. J. Vet. Anim. Sci., 2009; 33: 1-6.
28. Rice-Evans CA: Formation of free radicasls and mechanisms of action in normal biochemical processes and pathological states. In: Free radical damage and its control. (Eds. C.A. Evans, R.H. Burdone), Elsevier Science, 1994; pp. 131-153.
29. Gurer H Ozgunes H Neal R Spitz DR and Ercal N: Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicol., 1998; 128: 181-189.
30. Zhao Y Wang L Shen HB Wang ZX Wei QY and Chen F: Association between delta-aminolevulinic acid dehydrase (ALAD) polymorphism and blood lead levels: a meta-regression analysis. J. Toxicol. Environ. Health, 2007; 70: 1986-1994.
31. Monterio HP Abdallo DSP Faljoni-Alario A and Bechara EJH: Generation of active oxygen species during coupled auto-oxidation of oxyhaemoglobin and gamma-aminolevulinic acid. Biochem. Biophys. Acta, 1986; 881: 100-106.

How to cite this article:
Sharma S and Thakur A: Biochemical studies on the mice heart regarding lead acetate induced oxidative stress. Int J Pharm Sci Res 2017; 8(3): 1388-92.doi: 10.13040/IJPSR.0975-8232.8(3).1388-92.