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

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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(C₂H₃O₂)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.

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. Many mechanisms are proposed for lead–induced hypertension, including alteration in calcium (Ca²⁺) flux, lowering the calcium binding capacity in intracellular Ca²⁺ concentration, inhibition of sodium pump, increased activity of renin-angiotensin system, altered kallikrein-kinin system causing decreased plasma levels of bradikinin and increased cardiovascular sensitivity to endogeneous substances such as catecholamines. Lead exposure results in oxidative stress and inflammation which in turn lower bioavailability of NO and promote hypertension, endothelial injury/ dysfunction and cardiovascular disease.

Keywords: Oxidative stress, Heart, Free radicals, SOD, CAT

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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. 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.

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

Lead is known to cause oxidative damage in various tissues by bringing about imbalance in the generation and removal of reactive oxygen species. 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$_2$O$_2$). Catalase is responsible for conversion of H$_2$O$_2$ to water. Deficiency of catalse results in accumulation of H$_2$O$_2$ that contribute to inflammation and cardiovascular remodeling.

In addition H$_2$O$_2$ is substrate for production of hydroxyl radical (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$_2$O$_2$ generation by cultured human coronary endothelial cells.

**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). Superoxide dismutase was done as per the method of Mishra and Fridovich (1972) 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)

<table>
<thead>
<tr>
<th>Superoxide dismutase (SOD) (units/mg protein)</th>
<th>Group</th>
<th>1</th>
<th>40</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.84 ± 0.006</td>
<td>10.92 ± 0.009</td>
<td>10.99 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>Lead acetate (10 mg/kg body weight)</td>
<td>11.63 ± 0.012</td>
<td>8.76 ± 0.003*</td>
<td>9.32 ± 0.007</td>
<td></td>
</tr>
<tr>
<td>Lead acetate (150 mg/kg body weight)</td>
<td>12.62 ± 0.036</td>
<td>8.31 ± 0.011</td>
<td>9.04 ± 0.009*</td>
<td></td>
</tr>
</tbody>
</table>

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)

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

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 food. 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. 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. 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.

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 dehydrate), resulting in defective heme synthesis and anaemia. It is reported that low blood lead level (about 15μg/dl) is sufficient to inhibit the activity of this enzyme.

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.

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:

13. Prentice RC and Kopp SJ: Cardiotoxicity of lead at various perfusate calcium concentrations: functional and metabolic...

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