Role of Oxidative Stress in Assessment of Damage Induced by Lead Acetate in Mice Gastrocnemius Muscle

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ABSTRACT
Heavy metal deposition has increased due to the anthropogenic activities leading to heavily polluted areas worldwide. The objective of present study was to evaluate the effect of lead acetate [Pb(C₂H₃O₂)₂] on antioxidant enzyme activities in gastrocnemius muscle of mice. Lead is a toxic heavy metal widely distributed in the environment due to its role in modern industry. Normal 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 at 40 and 80 days stages. Lead acetate significantly decreases antioxidant enzymes and increase oxidative stress along with muscle tissue damage.

KEY WORDS: Lead acetate, gastrocnemius muscle, Antioxidant enzymes

INTRODUCTION
There are 35 metals that are of concern for us because of residential and occupational exposure. These heavy metals are commonly found in the environment and diet. In small amount they are required for maintaining good health but in large amount they can become toxic or dangerous. Increase in blood lead concentration also affects a person IQ (Taylor et al., 2012). Exposure to the heavy metals mainly lead occurs from automobile exhaust in those areas where leaded gasoline is still used and also from the drinking water in those regions where lead pipes are being used. Of all heavy metal that contaminate the environment and pose hazards to public health lead has been of major concern. Along with environmental pollutant, lead is a metabolic poison with a variety of toxic effects.

Recent studies proposed that one possible mechanism of lead toxicity is the disturbance of pre-oxidant and antioxidant balance by generation of reactive oxygen species (ROS) (Gurer and Ercal, 2000; Wang et al., 2000). At very high concentration reactive oxygen species may causes structural damage to cells, proteins, nucleic acid and lipids resulting in a stressed situation at cellular level (Mathew et al., 2011). It has been also reported that lead exposure has a dose dependant response relationship with changes in antioxidant enzyme levels and their activities (Adonaylo and Oteiza, 1999). The ionic mechanism of lead toxicity occurs mainly due to the ability of lead metal ions to replace other bivalent cations like Ca²⁺, Mg²⁺, Fe²⁺ and monovalent cations like Na⁺, which ultimately disturbs the biological metabolism of the cell (Flora et al, 2012).

Material and methods: All the experimental procedure was conducted after the approval of Institutional Animal Ethics Committee (IAEC/Bio/6-2011) of Himachal Pradesh University, Shimla, India.
Present study was conducted on gastrocnemius muscle of adult sexually mature Swiss albino mice weighing 20 – 30g. They were maintained in polypropylene cages under hygienic conditions. Animals were allowed to acclimatize to the laboratory conditions for 10 days. They were fed upon Hindustan lever pellets diet and water ad libitum.

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:
Normal healthy looking mice showing no sign of morbidity 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: The present study confirmed that exposure to lead acetate produced significant alterations in antioxidant enzyme activities i.e. Superoxide dismutase (SOD) and catalase (CAT). In gastrocnemius muscle, at day 1, after administration of lead acetate, the SOD and CAT activity showed a progressive increase which was due to increased oxidative stress. However at 40 days stage, significant decrease was seen in antioxidant levels after lead acetate treatment. Again at 80 days stage enzyme activity was found to be low when compared with normal ones, although percentage decline was less than that of 40 days stage.

Table and Fig. I: Change in superoxide dismutase specific activity (units/mg protein) in gastrocnemius muscle of normal and lead acetate treated mice 1-80 days period. Values are mean ± SEM; n = 3 (P* < 0.05)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>8.97±0.016</td>
</tr>
<tr>
<td>Lead acetate (10 mg/kg body weight)</td>
<td>9.23±0.012</td>
</tr>
<tr>
<td>% increase or decrease</td>
<td>2.89%</td>
</tr>
<tr>
<td>Lead acetate (150 mg/kg body weight)</td>
<td>10.32±0.017</td>
</tr>
<tr>
<td>% increase or decrease</td>
<td>15.05%</td>
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Fig. 1
The objective of this study was to see the effect of lead acetate on antioxidant enzymes. Experimental evidence generated during exercise and increase in response to oxidative stress. Antioxidants, present in the cell protect it from free radicals, however under the influence of lead the level of reactive oxygen species increases and the level of antioxidant decreases (Jaishankar, M. et al., 2014). The antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) are primary defense against reactive oxygen species generated during exercise and increase in response to exercise (Ji, 1995; Sen, 199).

The finding of present investigation explains the ability of lead acetate to cause disturbances in normal functioning of muscles. The antioxidant enzyme level in control group remains fairly constant throughout the experimental time period. After 24 hours of exposure it exhibited a significant increase when compared with control. SOD and CAT activities were decreased after 40 days treatment of lead acetate. However at 80 days stage, after the withdrawal of lead acetate at 40 days stage decline in antioxidant enzymes was less than the control mice at 80 days stage.

The overproduction of reactive oxygen species such as hydrogen peroxide \( (H_2O_2) \), superoxide anion \( (O_2^-) \) and hydroxyl radical \( (OH) \) in the tissue of organisms causes oxidative damage in organism (Bhanu, 2016). As in present study lead acetate is found to cause oxidative stress in muscle as the antioxidant system has been estimated to increase the production of various defensive enzymes. Our findings were in accordance with those from previous studies. Aina et al., (2012) who have shown that the oxidative stress...
would increase the level of defensive enzymes under the toxicity of pollutants. Saliu and Bawa Allah (2012) have found that the increased quantity of antioxidant enzymes would help the organisms to overcome the oxidative stress by regulative bioaccumulation of the metals to levels the body can tolerate. According to Begum and Sengupta (2014) increased activity level of antioxidant enzyme indicate the adaptational and protective response of animals under pollutant toxicity.

Conclusion:
Data from present study suggest that entire antioxidant system is stimulated to start increased production of antioxidant enzymes to detoxify the free radicals and to remove from the cells to overcome the oxidative stress caused by lead acetate.

References: