Evaluation of oxidative stress, liver functions and anemia in lead intoxicated Sprague Dawley rats

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Objective: To evaluate quantitative effect of lead exposure on the oxidative stress, liver functions and hematological profile.

Methodology: Seventy male healthy Sprague Dawley rats were randomly divided into two groups with thirty five rats in each group. Rats of lead intoxicated group were given weekly intraperitoneal injection of lead acetate 10 mg/kg body weight. After 6 weeks, intracardiac sampling was done and blood was used to determine hemoglobin concentration, erythrocyte count, red cell indices, serum MDA, serum ALT and AST levels.

Results: Lead intoxication of rats for 6 weeks revealed that serum MDA levels were increased to 7.8±0.48 µmol/l (control=3.2±0.27µmol/l), ALT levels to 76.26±5.88 IU/l (control=44.7±2.96) and AST levels to 258.06±13.30 IU/l (control =156.8±5.04). Hematological parameters of lead intoxicated group reveals lowered levels of hemoglobin, RBC count, MCHC and MCH while MCV remained statistically unchanged; manifesting hypochromic normocytic anemia.

Conclusion: Lead intoxication for 6 weeks induced oxidative stress, hepatotoxicity and hypochromic normocytic anemia. (Rawal Med J 2013;38: 181-183).

Key words: Lead poisoning, erythrocyte indices, malondialdehyde, alanine transaminase, aspartate aminotransferase.

INTRODUCTION

Lead is the most common heavy metal toxin for humans that has been used widely because of its durability, malleability, low melting point, and ability to form compounds. Its toxic effects including anemia and colics are recognized for more than 2,000 years. As per WHO estimates, over 120 million people have blood lead levels above the maximum acceptable limit (10 g /dl). The proposed mechanism of intoxication advocates the generation of reactive oxygen species (ROS) by lead. ROS cause lipid peroxidation of the cell membrane, which becomes less flexible and can be ruptured easily and lipid peroxidation of polyunsaturated fatty acids releases malondialdehyde (MDA) as a byproduct which is used as a marker of oxidative stress. Both the excessive generation of ROS as well as the depletion of the antioxidant reserves, cause accumulation of ROS in hepatocytes and cause hepatotoxicity. Serum ALT and AST levels are normally used as indicators of hepatotoxicity. Erythrocytes are the most vulnerable cells to the oxidative stress of lead as they have very limited reservoirs of antioxidant enzymes to defend against ROS. In addition, they are unable to replenish antioxidant enzymes because of lack of rough endoplasmic reticulum and become more prone to the damage by ROS. Red cell indices are used anemia produced by lead intoxication. Mechanism of lead intoxication and possible role of reactive oxygen species in its pathogenesis is being studied by various researchers however quantitative estimation of effects of lead exposure on the oxidative stress, liver functions and hematological profile needs elucidation. The aim of this study was designed to evaluate oxidative stress, liver functions and hematological profile in lead intoxicated Sprague Dawley rats.

METHODOLOGY

Seventy male Sprague Dawley healthy rats of 90-120 days age and 200-250 grams weight were purchased from National Institute of Health (NIH), Islamabad. They were randomly divided into two groups with thirty five rats in each group. Rats of the control group were fed normal standard rat's diet without any supplementation and were given
weekly intraperitoneal injections of sodium acetate 10 mg/kg body weight. Rats of lead intoxicated group were given weekly intraperitoneal injection of lead acetate 10 mg/kg body weight for 6 weeks and were fed standard diet.\textsuperscript{5}

After 6 weeks, intracardiac sampling is done at the animal laboratory of NIH. Estimation of serum malondialdehyde (MDA) levels was done by thiobarbituric acid reactive substances (TBARS) assay kit, on spectrophotometer. Estimation of serum ALT and AST was carried out by using commercial kits on Merck Microlab 200.\textsuperscript{9}

Hemoglobin concentration, erythrocyte count and red cell indices were determined by Hematology Analyzer Sysmex KX-21. Statistical analysis of the differences between means of all the parameters was assessed by students t-test.

**DISCUSSION**

Our study demonstrated that lead intoxication produced statistically significant changes in MDA, ALT, AST, hemoglobin concentration, RBC Count, hematocrit, MCH and MCHC in Sprague Dawley rats. Serum MDA levels in the control group was consistent with the published data of different studies.\textsuperscript{10,11} MDA is produced as bi-product of lipid peroxidation of the membranes of cell and subcellular organelles. Intraperitoneal injections of lead acetate increased serum MDA level (7.8±0.48 \( \mu \)mol/l) which is consistent with the published data on lead intoxication.\textsuperscript{12}

The levels of serum ALT and AST in the control group of this study (ALT 44.7±2.96 IU/l and AST 156.8±5.04 IU/l) are consistent with the levels in healthy Sprague Dawley rats as documented in other international studies.\textsuperscript{8,13} Quantitative estimation of damage to the hepatocytes has been documented by serum ALT and AST levels which were raised from 24.85±0.60 IU to 32.19±0.35 IU and from 90.50±3.0 IU respectively, after the administration of lead acetate.\textsuperscript{13}

Lead is believed to cause damage to the cell membrane of hepatocytes and various cytoplasmic proteins which increase vulnerability of hepatocytes to ROS. Lead causes damage to the DNA of hepatocytes which contributed to their increased damage.\textsuperscript{14,15} It is also known to activate the cascade of apoptosis in the hepatocytes.\textsuperscript{16} Lead induced apoptosis also occurs in many other tissues of the body including germ cells of seminiferous tubules.\textsuperscript{17} Thus, apoptosis following lead intoxication may be contribute in hepatotoxicity by lead.

In present study, hematological parameters of the control group were consistent with published data of RBC indices in this species of rat.\textsuperscript{18,19} Intraperitoneal lead acetate administration caused a decrease in hemoglobin, hematocrit and RBC count, while MCV remained statistically unaltered. Anemia in lead toxicity can be attributed to the decreased RBC

**RESULTS**

Ages of rats of control and lead groups were 91±3.2 and 93±6.4 days, while the weights of the two groups were 227±5.2 and 229±7.7 grams, respectively (p=NS). All rats remained alive and healthy throughout the study. After 6 weeks, the weight of rats was recorded to compare the change in body weight in different groups. Weight of the control group (255 ± 7.2 g) was higher than the lead intoxicated group (238 ± 4.7 g) (p<0.01).

<table>
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<tr>
<th>Table 1: Comparison of serum MDA, ALT, AST levels and hematological parameters between control and lead intoxicated groups.</th>
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<tbody>
<tr>
<td><strong>Variable</strong></td>
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<tr>
<td>MDA (( \mu )mol/l)</td>
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<td>ALT (IU/l)</td>
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<td>AST (IU/l)</td>
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<td>Hb (g/dl)</td>
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<td>Hct (%)</td>
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<td>RBC Count (10(^6)/( \mu )l)</td>
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<td>MCH (pg)</td>
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<td>MCV (fl)</td>
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<td>MCHC (g/dl)</td>
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All values have been expressed as Mean ± SD.
survival because of the increased membrane fragility caused by the loss of its fluidity. In addition, inability of the erythrocytes to resynthesize antioxidant enzymes because of the lack of ribosomes in them, also makes erythrocytes more vulnerable to oxidative stress. Hemolysis results in anemia, owing to the decrease in the RBC count and fall in the hematocrit level.

**CONCLUSION**

Lead exposure enhanced oxidative stress and hepatotoxicity which is manifested by increase in serum MDA levels, serum ALT and AST levels. The anemia was characterized by low Hb, RBC count, MCH and MCHC whereas MCV did not change on lead administration.

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**REFERENCES**


