A study of Haemato toxicity of lead on Swiss mice

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ABSTRACT

Lead is a major heavy metallic pollutant of human environment; lead affects the health of people drinking water transported through PVC pipes and also, the people working in paint industries. For these reasons, a study of lead was carried out in a murine model. Adult swiss mice were intraperitoneally injected with the lead nitrate solution at doses equivalent to $1/10^{th}$ of the respective LD_{50} values, for five days in a week and for tenure of three weeks. Due to lead treatment, blood hemoglobin level and erythrocyte count were significantly reduced, as compared to the controls. A fair proportion of the erythrocytes were transformed into echinocytes for the lead treatment. Cytochemical tests (acid ferrocyanide reaction and crystal violet staining) revealed that lead does not cause any oxidative denaturation of hemoglobin or affects iron utilization for hemoglobin biosynthesis. Anemia caused by the heavy metal is, therefore, lightly to arise only due to rupture of erythrocytes' membranes following echinocytic transformation. The blood leukocyte count was also significantly reduced in the treated animals. However, marked neutropenia was observed. The study indicates the necessity of periodic monitoring of blood picture in human subjects chronically exposed to lead in the environment.

Key Words: Lead (lead nitrate), Swiss mice, LD_{50} value of lead nitrate, neutropenia

INTRODUCTION

Occupational and environmental exposures to lead (Pb), one of the toxic metal pollutants, is of global concern. It is considered as a common occupational and environmental hazard throughout the world. Lead adversely affects the nervous, hematopoietic, endocrine, renal and reproductive systems of the body. Health risks are increasingly associated with environmental exposures to Pb emissions from, for example, the widespread use of PVC pipes for drinking water transportation, ceramic glazes, cosmetic, food can soldering, leaded gasoline in developing countries. Exposure occurs mainly through the respiratory and gastrointestinal systems, and then ingested and absorbed Pb is stored primarily in soft tissues and bone (Pande and Flora, 2001). Multimedia exposure to Pb through occupational and environmental settings is of major concern in developing countries, such as India. Leaching of Pb from cooking and storage vessels containing Pb into the water and from lead-containing cooking vessels with acidic food conditions contribute to the potential Pb exposure through ingestion. In India, elevated Pb Concentrations in drinking water from the massive use of PVC pipes as well as in beer (10 mg/l) and the use of Pb arsenate pesticides also contribute to increased Pb exposure through ingestion. Hence, an evaluation of Pb exposure through the ingestion route is critical in assessing its health effects including the morbidity associated with its extra pulmonary effects such as those that occur in the blood cells, gastrointestinal and hepatic systems. Here mainly heamatotoxicity of lead is studied.

AIMS AND OBJECTIVES

Kolkata is in the top position regarding lead toxicity as per the report published in Lead Action News very recently. After seeing this kind of report to observe how lead actually contaminates our blood cells and destroy our immune system. Thus the best mammalian model is a murine model to pursue this observation.

MATERIAL AND METHODS

- **Chemicals:** Lead nitrate was purchased from Central Drug House (India). All other chemicals used in the study were of analytical reagent and obtained from Sisco Research Laboratories (India). Qualigens (India/Germany), SD fine chemicals (India), HIMEDIA (India) and Central Drug House (India).
- **Materials required:** (I) Distilled water, (II) Tri – sodium citrate solution (anticoagulant), (III) Methanol, (IV) Ethanol, (V) Giemsa stain, (VI) 0.1M phosphate buffer of 7pH, (VII) Acid ferrocyanide, (VIII) Crystal violet, (IX) N/10 hydrochloric (HCl) acid, (X) Cotton.
- **Glassware used:** (I) Beaker (100ml, 200ml, 500ml), (II) Measuring cylinder (100ml), (III) Pipette (1ml, 2ml, 5ml), (IV) Conical flask (250 ml capacity), (V) Stoppered reagent bottle (250ml capacity), (VI) Petridis, (VII) Glass slides.
- **Instruments:** (I) Scissors, (II) Forceps, (III) Petit balance, (IV) Haemoglobinometer, (V) Zeiss microscope.
- **Experimental animal:** Swiss mice (*Mus musculus*) were obtained for experimental purpose. The Animal Ethics Committee of Presidency University, Kolkata, India has approved experimental protocol. All experiments were conducted on adult Swiss mice (*Mus musculus*) weighing 25-30g (3-4 months old).
- **Animal maintenance:** They were housed in polypropylene cages (Figure 1) in an air-conditioned room with a temperature maintained at $25^{o}C \pm 30^{o}C$, relative humidity of 50% ± 5% and 12h alternating light and dark cycles. The mice were provided with a nutritionally adequate food and drinking water throughout the study.
- **Experimental design:** In the present study 10 Swiss mice (Mus musculus) weighing 25-30g (3-4 months old) were used for haematological study. For this these mice were placed as two groups.

  Group-1: Consisted of four mice served as control.
  Group-2: Consisted of six mice and they received lead nitrate solution.
  - **Injection time:** In a 24h interval lead nitrate solution was injected for five days in a week and for tenure of three weeks.
  - **Injection site:** These adult Swiss Mice were intraperitoneally (Figure 2) injected with the lead nitrate solution.
  - **LD50:** LD50 of lead nitrate is 74mg/kg (IPR - MUS).
  - **Doses:** The doses were injected equivalent to $1/10^{th}$ of the respective LD_{50} values.
  - **Autopsy:** Autopsy was made on the 20^{th} day of studies both in the control mice and treated mice.
  - **Fixation of tissue:** Blood films were fixed with methanol then stained with Giemsa's stain (pH - 6.8).
  - **Light Microscopy:** All the prepared slides were observed under bright field of a Zeiss microscope at various magnifications.

METHODS OF ANALYSIS

At first lead nitrate solution was prepared by mixing the 15mg of lead nitrate powder with 200ml of distilled water. This stock solution was stored in a stoppered reagent bottle that was thoroughly cleaned and sterilized by autoclave and placed in the refrigerator at a controlled temperature to avoid contamination and to better use in further.

All total 10 adult female mice were taken for this study. 4 mice were reared in one polypropylene cage and remaining 6 mice in the other polypropylene cage. The four mice were taken to control and the remaining were taken for lead nitrate study. These mice were provided with nutritionally adequate food materials and drinking water.

The first injection containing 1ml of lead nitrate solution was injected intraperitonially. 1ml Insulin syringes were used as injecting device. The time was around 1.30pm. All of the six mice received the first dose of lead nitrate solution. After 1 hour their movement was observed and no abnormal movement was seen.

This schedule was repeated up to the 6^{th} day of study except no injection was injected on the 6^{th} day. Thus a two day break was there in the first week of treatment and the treatment was carried on as the aforesaid process up to the third week of study. During treatment no mice show any abnormality in their behavior and intake of giving food and water was normal. Some physiological changes occurred that is mentioned in the observation tab.

On the first day of the 4th week autopsy was done of two treated mice and two controlled mice. The blood sample was collected on the glass slides and thin blood films were made on each side by a narrow edge of the other glass slide. Then the blood films were fixed by methanol (6 times). Next the slides were air dried. A few air dried slides were then stained with Giemsa's stain of pH 6.8 (approx) and washed with distilled water. And in the remaining air dried slides, a few were taken to perform cytochemical tests by acid ferrocyanide reaction and crystal violet staining. Then prepared slides were air dried and ready for observation under bright field of a Zesiss microscope and also the slides were studied under a Scanning Electron Microscope.

OBSERVATIONS AND RESULTS

After conducting the above mentioned experiment the following data were observed and are given in the tabular form and also in the charts.

- **Blood leukocyte count:**

Group	Leukocyte count (percentages)			
	Neutrophil	Lymphocyte	Eosinophil	Monocyte
Control	22.4 ± 1.6	72.6 ± 0.8	2.0 ± 0.5	3.0 ± 0.5
$Pb(NO_3)_2$ treated	12.6 ± 0.4	82.4 ± 1.2	2.5 ± 0.5	2.5 ± 0.5

- **Haemoglobin level:**

Group	Haemoglobin level
Control	12.5g/100ml
$Pb(NO_3)_2$ treated	10.2g/100ml

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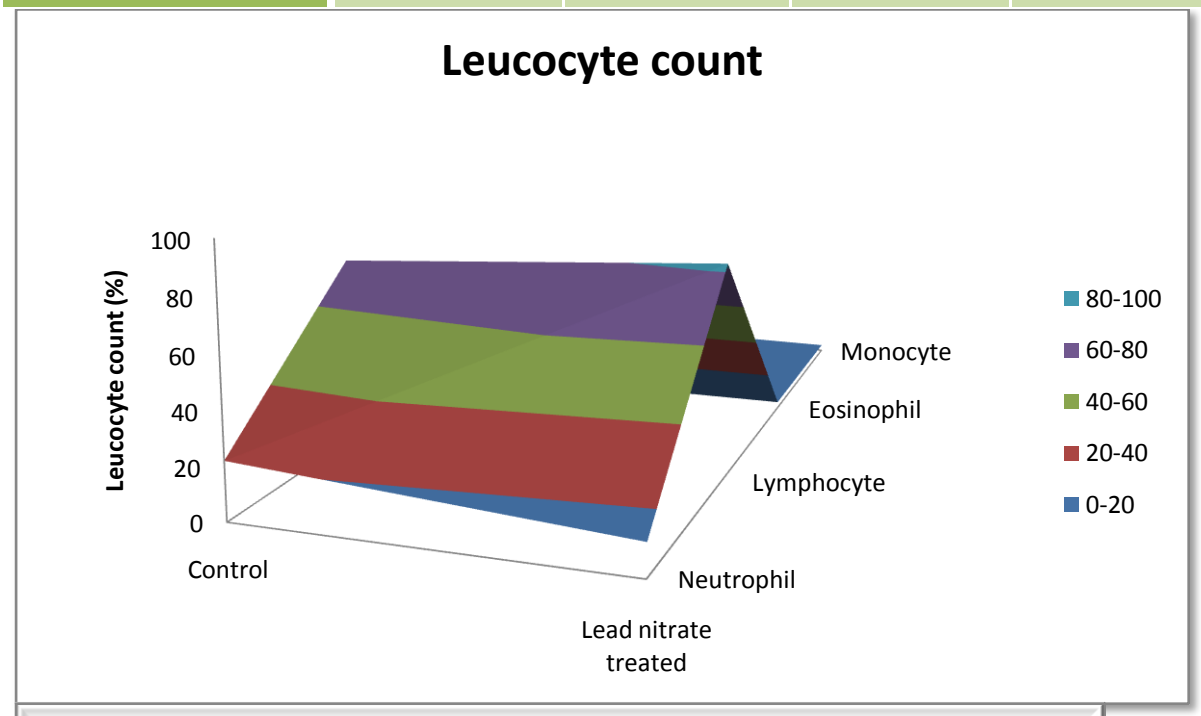
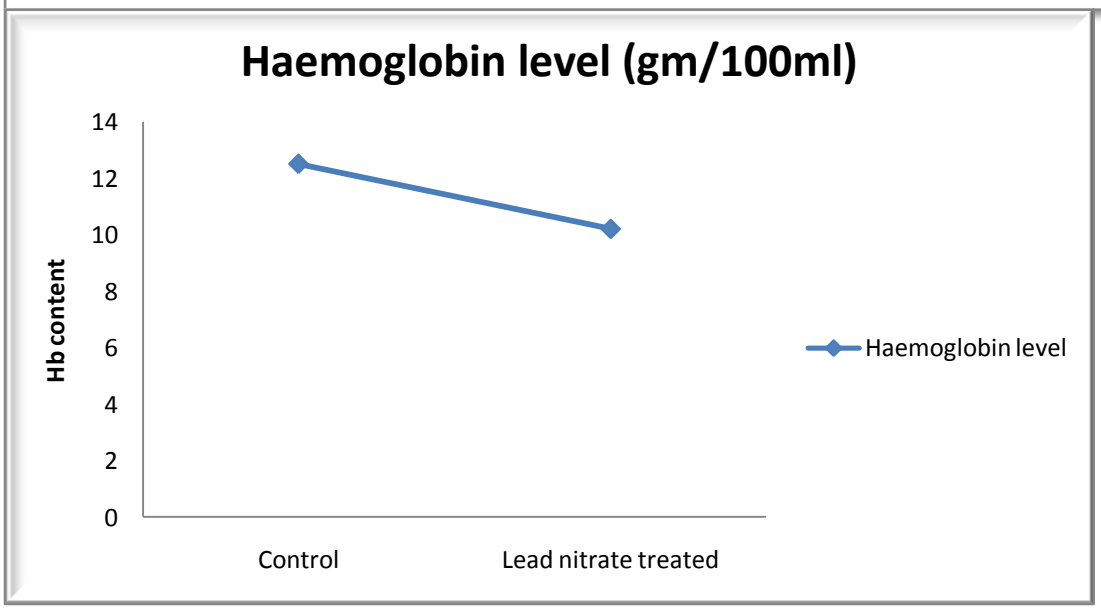
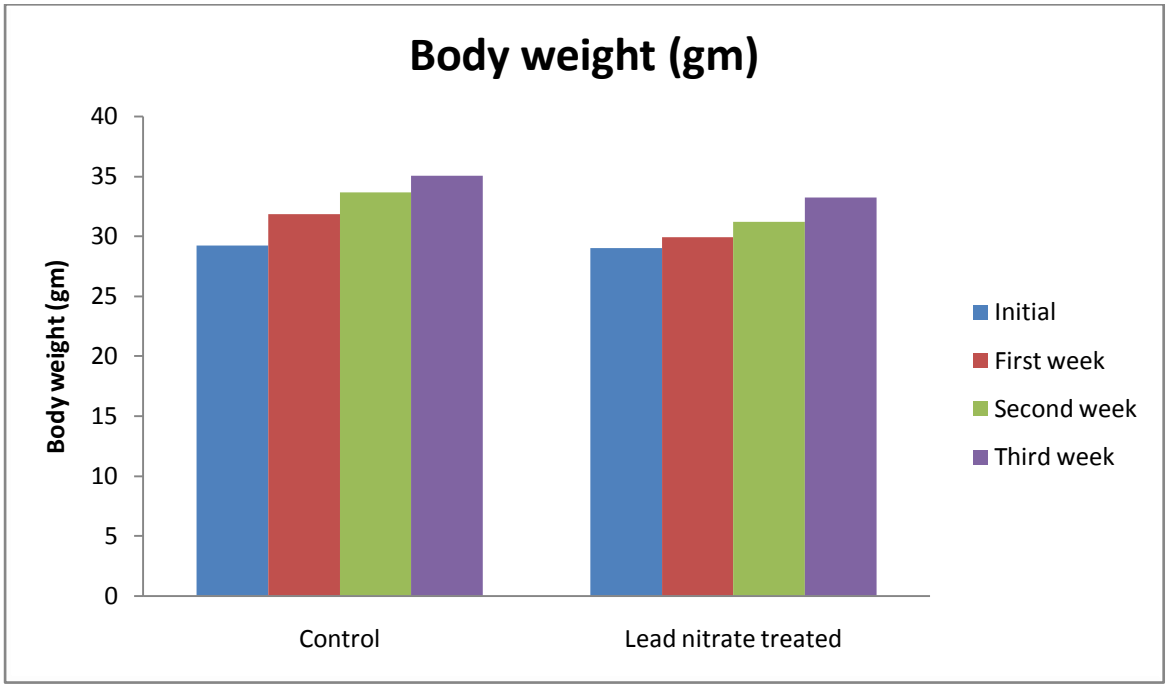
➢ **Body weight:**

Group	Body weight of mice during the study			
	Initial	1st week	2nd week	3rd week
Control	29.23 ± 0.809	31.85 ± 1.12	33.65 ± 1.50	35.05 ± 1.70
$Pb(NO_3)_2$ treated	28.90 ± 0.37	29.90 ± 0.920	31.20 ± 1.65	33.20 ± 1.82







From the above observations, it was observed that the body weight gradually decreased from control to treat group (Control > Lead nitrate treated). The blood leukocyte count was significantly reduced in the treated mice. Haemoglobin content of the blood was also estimated by the Haemoglobinometer . By this study it was revealed that Blood haemoglobin level and erythrocyte count of treated mice were significantly reduced, as compared to the controls (Figure 1, Figure 2). A fair proportion of the erythrocytes were transformed into echinocytes (Figure 3,4) of treated mice. Cytochemical tests (acid ferrocyanide reaction and crystal violet staining) revealed that lead did not cause any oxidative denaturation of haemoglobin or affected iron utilization for haemoglobin biosynthesis. A tumor like tissue growth also observed just above the right kidney of all treated mice.

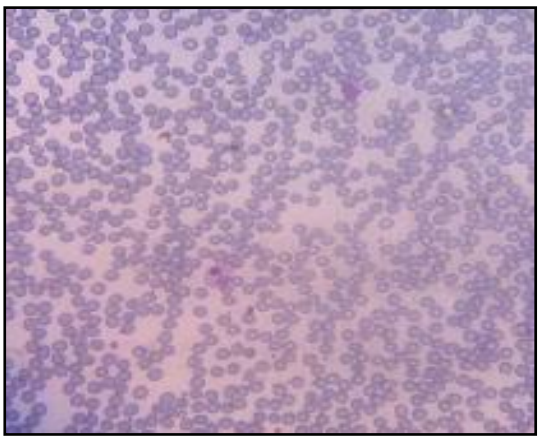


Fig 1: Control taken by Light Microscope

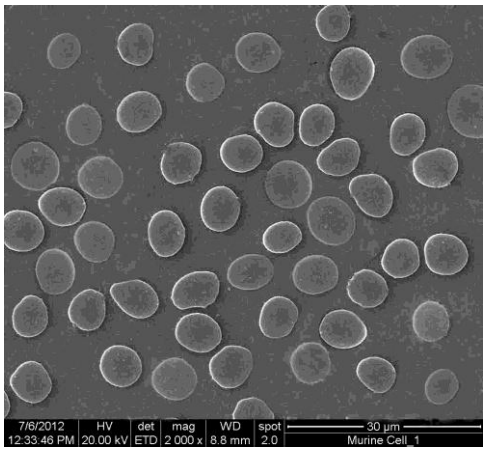


Fig 2: Control taken by SEM Study

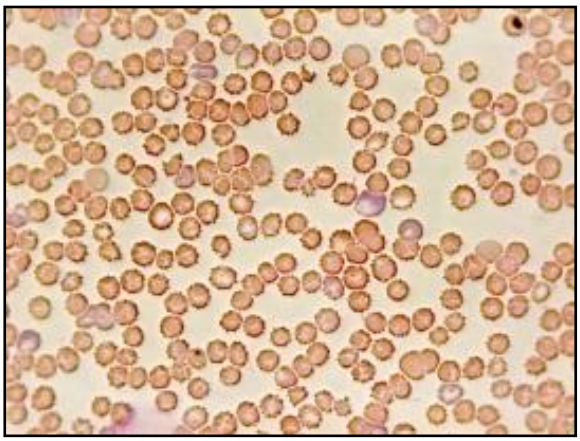


Fig 3: Lead Nitrate Treated taken by Light microscopes

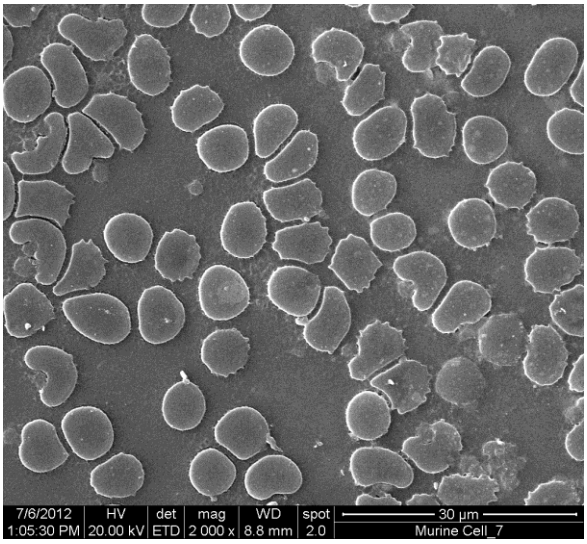


Fig 4: Lead Nitrate Treated taken by SEM

DISCUSSION

After the microscopic study, it is concluded that severe Anemia caused the heavy metal lead is, therefore, likely to arise due to rupture of erythrocyte membranes following echinocytic transformation. Due to reduction of blood leukocytes, marked neutropenia was observed as well as reduction of body weight to some extent was also seen. The study indicates the necessity of periodic monitoring of blood picture in human subjects chronically exposed to lead in the environment.

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