Int J Pharm Bio Sci 2013 Jan; 4(1): (B) 22 - 30

Research Article **Pathology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

LEAD (Pb) INDUCED ANAEMIA IN SWISS MICE – LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDIES

RINA RANI RAY AND NIRMAL KUMAR SARKAR*

Department of Zoology, Molecular Biology and Genetics, Presidency University, Kolkata, India.

ABSTRACT

Marked reductions in blood haemoglobin level, haematocrit value and erythrocyte count were observed after 1 and 2 weeks of intraperitoneal treatment of Swiss mice with lead nitrate (8 mg/Kg for 5 consecutive days per week), as compared to the respective controls. Echinocytic transformation of many erythrocytes and the formation of a few microcytes, as revealed by both light and scanning electron microscopy, indicated the anaemia to be anisocytic and haemolytic in nature. Moreover, leucopenia accompanied by a decrease of lymphocyte count but rise of neutrophil count was apparent after 2 weeks but not 1 week of treatment. However, cytochemical tests (acid-ferrocyanide reaction and crystal violet staining) indicated that lead treatment did not interfere with utilization of iron for haemoglobin synthesis and also, did not cause denaturation of haemoglobin in already formed erythrocytes. The study hints to the necessity of periodic monitoring of blood picture in workers of different lead-based industries.

KEYWORDS: *Lead nitrate, Echinocyte, Anaemia, Leucopenia.*

NIRMAL KUMAR SARKAR
Department of Zoology, Molecular Biology and Genetics,
Presidency University, Kolkata, India.

INTRODUCTION

At present, environmental and occupational exposures to lead (Pb), one of the heavy metallic pollutants of our environment, are of global concern. A recent report holds that 33% of over 370 water samples from the top 26 cities of India tested positive for harmful content of lead (over 10 parts per billion); this lead-contamination of water is held to result mainly from PVC pipes[1]. Lead also enters into human body through inhalation of air polluted by automobile exhaust[2]. Again, occupational exposure to lead is common in workers of lead-paint industries and battery producing or recycling factories as well as construction labourers using different lead-paints[3]. Different lead compounds find access into human body through ingestion or inhalation, and gradually produce anaemia[4], hepatotoxicity[5], neurotoxicity[6], nephrotoxicity[7] behavioral disorder[8], proneness to allergy [9] and even, male hypogonadism[10]. Some reports on induction of anaemia by lead compounds in animal models have been published in the last few years. Marked reduction of blood haemoglobin level, haematocrit value and erythrocyte count were reported in mice, following exposure to lead acetate in diet in concentrations between 2-13 µg/100 g for 3 weeks[11]. Mice treated with a single dose of lead nitrate (20 or 30 or 40 µg/g body weight) showed marked decrease of blood haemoglobin level and erythrocyte count after 5 and 10 days[12]. Wister rats treated with lead acetate in drinking water (1g/100 ml) for 4 weeks showed significant decrease in blood haemoglobin level, haematocrit value as well as erythrocyte and leucocyte counts, as compared to the controls[13]. Similar findings were recorded in Swiss mice treated intraperitoneally with 5 mg of lead nitrate/Kg body weight for 1 month[14]. However, the precise nature and mechanism of lead-induced anaemia have remained poorly explored as yet. It remains to be confirmed whether lead produces microcytic or macrocytic or anisocytic anaemia. Whether chronic exposure to lead brings about any change in the surface topography of erythrocytes needs to be ascertained. Further, it remains to be explored if lead causes any denaturation of haemoglobin in already formed erythrocytes and if lead interferes with utilization of iron for haemoglobin synthesis. The present study involves a thorough examination of the blood picture of a murine model chronically treated with lead nitrate, in order to add to the existing information on induction of anaemia by lead. Blood haemoglobin level, haematocrit value, total counts of erythrocytes and leucocytes as well as differential counts of leucocytes have been compared between the experimental and the control groups of mice. Moreover, light and scanning electron microscopy have been exploited to study lead-induced changes in erythrocyte surface topography. Cytochemical staining techniques have been used to determine if lead causes denaturation of haemoglobin and interferes with utilization of iron for haemoglobin synthesis.

MATERIALS AND METHODS

The study was carried out in adult (10 weeks old) inbred and healthy, male, albino mice of the Swiss strain. The mice were divided into control and experimental groups. Only male mice were taken in view of the fact that the workers of different lead-based industries of our state are generally males. The animals were maintained in polypropylene cages with sawdust bedding in a well-ventilated and well-lighted animal room under strict vigil of an Institutional Ethical Committee, which in turn is registered with the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Registration No. 796/03/ac/CPCSEA). The mice were daily provided with fresh food pellets and filtered tap water ad libitum. The experimental mice

were intraperitoneally injected with an aqueous solution of lead nitrate [$Pb(NO_3)_2$] (HiMedia, India; purity: 99·5%) at a dose of 8 mg/Kg body weight for 5 consecutive days per week, for a total period of 2 weeks. The selected dose of $Pb(NO_3)_2$ was nearly one-tenth of its LD_{50} value (74 mg/Kg) for mice[15]. The control mice were injected with physiological saline (0·9%) for a similar tenure.

After 1 and 2 weeks of $Pb(NO_3)_2$ treatment, 5 experimental and 5 control mice were anaesthetized under ether vapour and blood samples were collected in heparinized vials by cardiac puncture. Blood haemoglobin (Hb) level was determined by the acid-haematin method; total counts (TC) of erythrocytes and leucocytes were determined by refined visual methods using a haemocytometer having an improved Neubauer counting chamber while haematocrit values of blood samples were determined by the capillary method[16]. Differential counts (DC) of leucocytes were determined from microscopic examination of blood smears fixed in methanol and stained with 10% Giemsa's stain buffered to pH 7·0 with 0·1 M phosphate buffer[16]. Erythrocyte morphology was examined under the oil-immersion objective of a Zeiss Axiolab microscope and the abnormal morphs of erythrocytes, whenever observed were named as per an international system of nomenclature of erythrocytes[17]. Some of the methanol-fixed but unstained blood smears were subjected to acid-ferrocyanide reaction for microscopic detection of non-haem iron granules within erythrocytes[16] while others were subjected to crystal violet staining for detection of denatured haemoglobin (Heinz bodies) within erythrocytes[18].

For scanning electron microscopic study of erythrocyte surface topography, thin smears of heparinized blood samples were drawn over clean cover-slips and fixed in 2% glutaraldehyde solution in 0·1 M phosphate buffer at pH 7·0 at 4°C for 4 h, followed by a thorough washing in the buffer alone[19]. The cover-slips were air-dried, cemented on metal stubs with a silver-adhesive, coated with a 100 Å thick layer of gold by the help of an ion-coater and observed under a Hitachi S-520 Scanning electron microscope at an accelerating voltage of 15 kV. All reagents used in the study were of analytical grade. All experiments were performed in triplicate and significant differences between the mean values of different blood parameters observed in the experimental and the control groups of mice (results of all 3 sets of experiments were considered together) were statistically determined by Fisher's two-tailed t-test[20].

RESULTS

After the first week of intraperitoneal $Pb(NO_3)_2$ treatment, blood Hb level, Ht value and TC of erythrocytes were observed to be considerably decreased in the experimental mice, as compared to those in the controls ($p < 0·001$ in all cases; Table 1). Moreover, a conspicuous change was noticed in the morphology of many of the erythrocytes, which transformed into the so-called 'echinocytes' with crenated cell membranes (Fig 1). The erythrocytes of the control mice were almost exclusively round and discoid 'normocytes' having smooth cell membranes (Fig 2). Scanning electron microscopic examination of erythrocytes under high magnification (2,000X̃) revealed that the echinocytes had 10-16 short projections from their cell membranes (Fig 3) while the erythrocytes of the control mice were simply biconcave discs (Fig 4). Such echinocytes with short projections were designated as 'type I' echinocytes at par with the international system of nomenclature of erythrocytes[17]. Moreover, both light and scanning electron microscopic studies revealed the transformation of a small percentage of the erythrocytes into the so-called 'poikilocytes' looking like tennis rackets (Figs 1 and 3). Moreover, a small number of deeply stained, basophilic reticulocytes were

observed (Fig 1). However, at the end of the first week, TC and DC of leucocytes did not differ significantly between the experimental and the control groups of mice ($p > 0{\cdot}05$ in either case; Table 2).

At the end of the second week of $Pb(NO_3)_2$ treatment, further reductions in blood Hb level, Ht value and TC of erythrocytes were observed and the percentages of echinocytes, poikilocytes and reticulocytes had increased considerably, as compared to those observed after the first week of treatment (Table 1). Moreover, anisocytosis or heterogeneity of erythrocyte size was quite apparent; some microcytes (4-5 µm in diameter) were scattered among normocytes (7-8 µm in diameter) in blood smears (Fig 1). Besides, marked changes in TC and DC of leucocytes were apparent at this stage. TC of leucocytes was considerably decreased along with a fall in the DC of lymphocytes but a rise in the DC of neutrophils, as compared to the control ($p < 0{\cdot}001$ in all cases; Table 2). Interestingly, cytochemical studies (acid-ferrocyanide reaction and crystal violet staining) did not reveal the presence of either non-haem iron granules or denatured haemoglobin (Heinz bodies) in erythrocytes of the experimental mice after both 1 and 2 weeks of $Pb(NO_3)_2$ treatment.

Table 1
Erythrocytic indices in lead-treated and control Swiss mice (15 mice per group)

Parameter under study	Groups of mice		Groups of mice	
	1 week after treatment	Respective controls	2 weeks after treatment	Respective controls
Blood Hb (mg/dL)	10·56 ± 0·36*	12·47 ± 0·48	10·16 ± 0·42*	12·51 ± 0·36
Erythrocyte (million/c.mm)	4·97 ± 0·32*	5·58 ± 0·29	4·72 ± 0·26*	5·64 ± 0·27
Ht value	0·42 ± 0·01*	0·46 ± 0·01	0·38 ± 0·01*	0·46 ± 0·01
Echinocyte (%)	21·47 ± 3·26*	0·28 ± 0·06	27·85 ± 4·23*	0·23 ± 0·07
Poikilocyte (%)	3·44 ± 1·05*	0	4·55 ± 1·09*	0
Microcyte (%)	5·74 ± 2·22*	2·33 ± 0·63	8·27 ± 2·35*	2·57 ± 0·72
Reticulocyte (%)	2·90 ± 0·75*	0·28 ± 0·05	3·50 ± 1·02*	0·25 ± 0·07
Crystal violet staining	–ve	–ve	–ve	–ve
Acid-ferrocyanide reaction	~ve	–ve	–ve	–ve

Statistically significantly different from respective controls ($p < 0{\cdot}001$)

Table 2
Leucocyte profile in lead-treated and control Swiss mice (15 mice per group)

Parameter under study	Groups of mice		Groups of mice	
	1 week after treatment	Respective controls	2 weeks after treatment	Respective controls
TC of leucocytes (thousand/c.mm)	6·41 ± 0·42	6·52 ± 0·48	4·97 ± 0·29*	6·47 ± 0·43
Lymphocyte (%)	74·83 ± 2·12	73·50 ± 2·98	65·83 ± 2·41*	74·33 ± 2·86
Neutrophil (%)	19·67 ± 2·43	21·67 ± 2·86	28·67 ± 2·86*	20·67 ± 2·43
Monocyte (%)	3·67 ± 0·74	3·50 ± 0·50	3·67 ± 0·74	3·67 ± 0·74
Eosinophil (%)	1·83 ± 0·68	1·33 ± 0·47	1·83 ± 0·68	1·33 ± 0·47

Statistically significantly different from respective controls (p < 0·001)

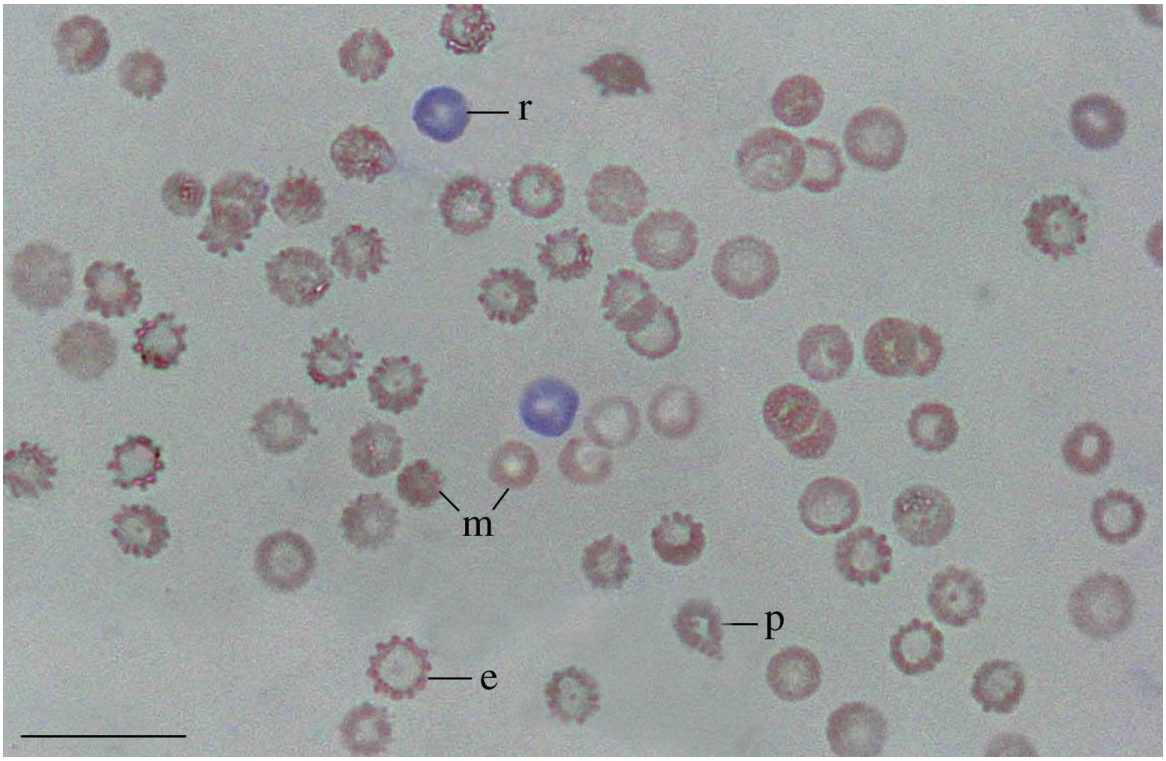

Figure 1
Echinocyte (e), poikilocyte (p), microcyte (m) and reticulocyte (r) in blood smear of a lead nitrate-treated Swiss mouse (X 1,000, Bar = 20 µm)

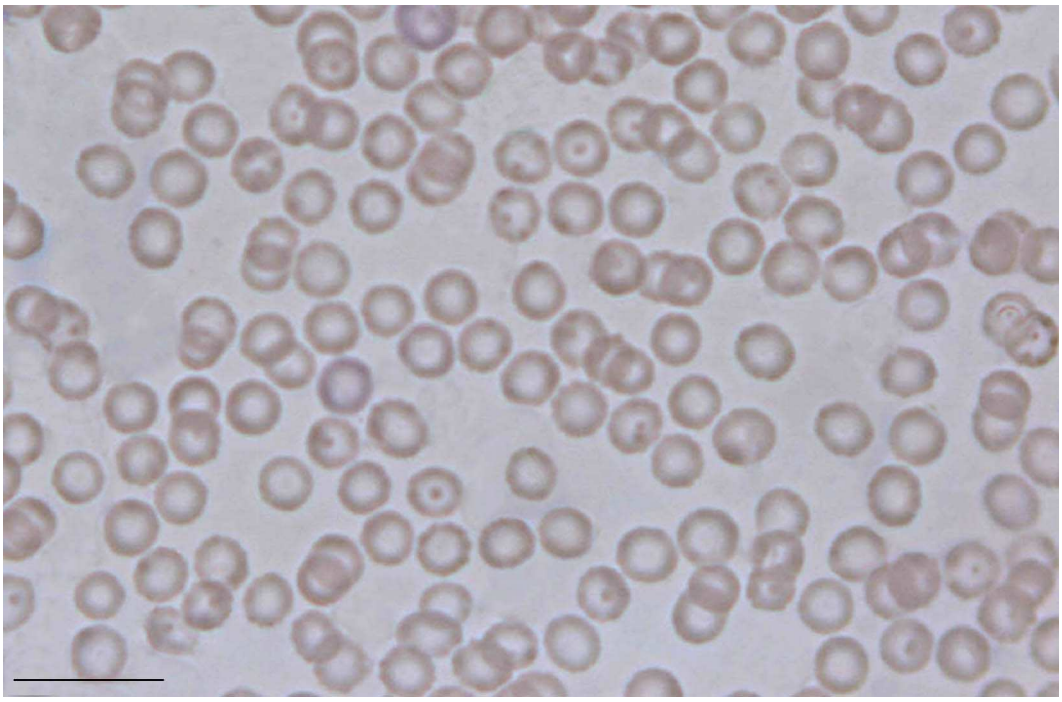

Figure 2
Normocytes in blood smear of a control Swiss mouse (X 1,000, Bar = 20 µm)

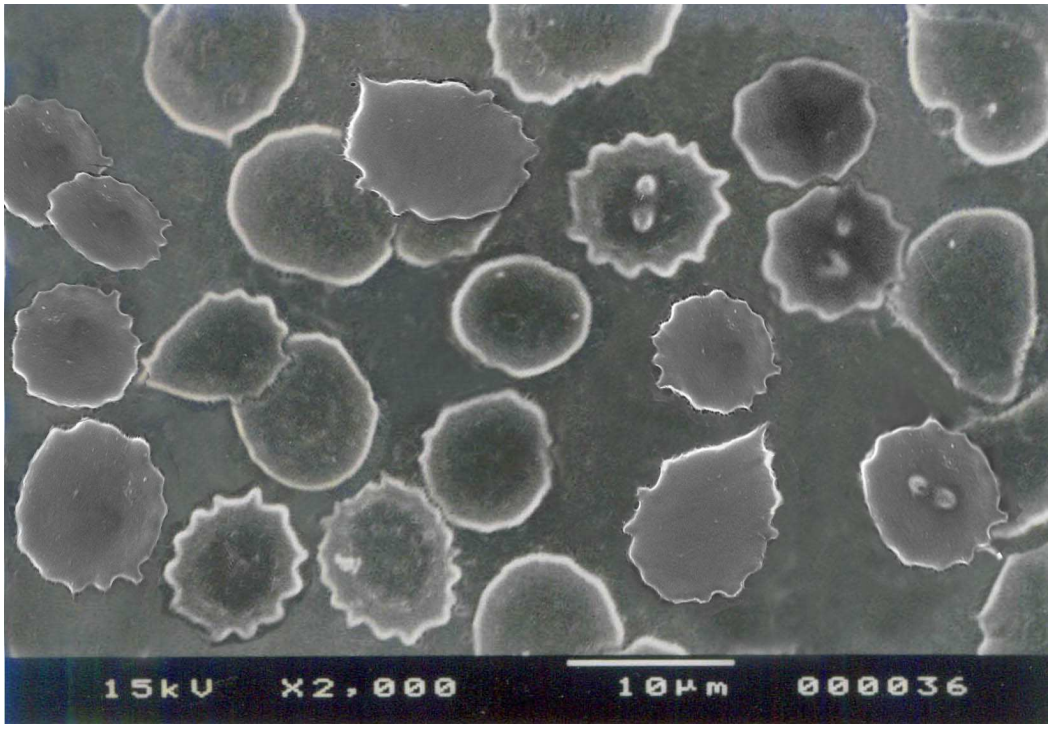

Figure 3
Echinocyte, poikilocyte and microcyte (under scanning electron microscope) in blood smear of a lead nitrate-treated Swiss mouse (X2,000, Bar = 10 µm)

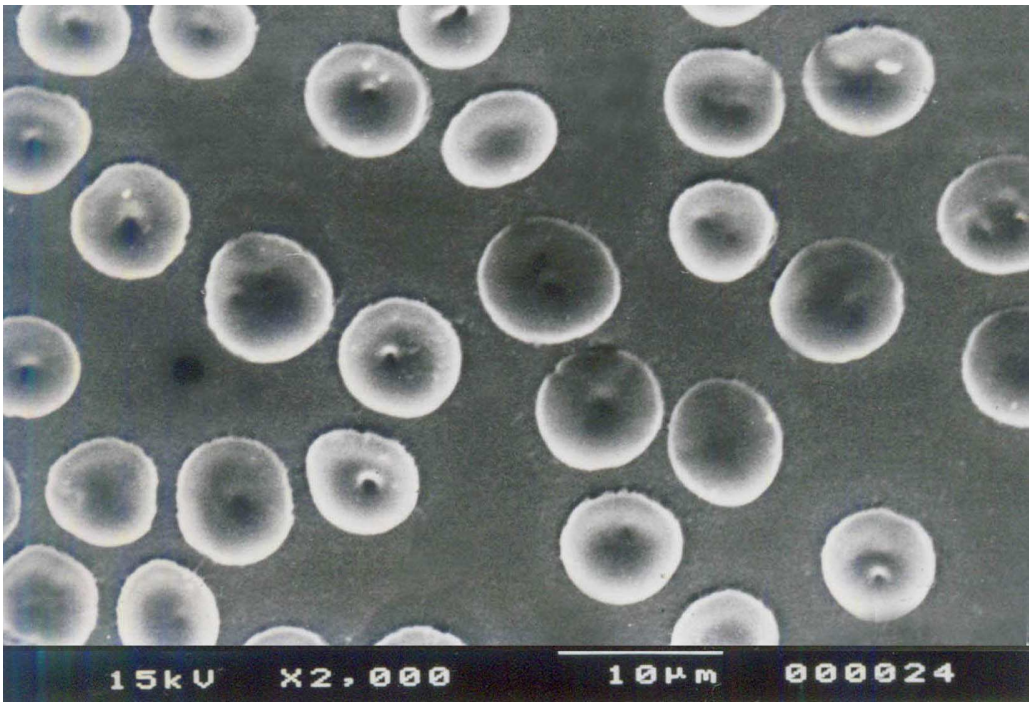

Figure 4
Normocytes (under scanning electron microscope) in blood smear of a control Swiss mouse (X 2,000, Bar = 10 µm)

DISCUSSION

Reductions in blood Hb level, Ht value and TC of erythrocytes after 1 and 2 weeks of $Pb(NO_3)_2$ treatment clearly indicate that lead induces anaemia in the murine model under study. Echinocytic transformation of a fair proportion of erythrocytes along with the finding of a few poikilocytes indicates that lead may induce deformation and fragility of erythrocytic membranes, resulting in haemolysis; echinocytosis is considered by modern haematologists to be an indication of haemolysis to follow[21]. Further, the appearance of a few reticulocytes along with some microcytes in blood smears indicates that the treated mice might have attempted to compensate for the loss of erythrocytes by enhancing the rate of erythropoiesis, but did not have time to synthesize sufficient quantities of Hb. This possibly resulted in the formation of some microcytes instead of normocytes. The negative result obtained with crystal violet staining of blood smears indicates that lead does not cause any denaturation of haemoglobin in already formed erythrocytes. Again, the negative result obtained with another cytochemical technique (acid-ferrocyanide reaction) indicates that lead does not directly interfere with utilization of iron for haemoglobin synthesis during the 2 week tenure of intraperitoneal treatment of the murine model. The present study further indicates that chronic lead treatment may gradually exert a cytotoxic action on both lymphopoietic and myelopoietic tissues, resulting in a marked leucopenia in the treated mice. However, the lymphopoietic tissues may be more sensitive than the bone marrow to the cytotoxic action of lead, resulting in a marked decrease in the DC of lymphocytes along with an apparent but not absolute rise in the DC of neutrophils.

CONCLUSION

The present study involving both light and scanning electron microscopic examinations of blood reveals that chronic lead treatment induces anisocytic and mild haemolytic anaemia along with marked leucopenia in a murine model and the findings strongly hint to the necessity of periodic monitoring of blood picture in the workers of different lead-based industries.

REFERENCES

1. Multani A, Lead contamination of drinking water in India due to PVC pipes, Lead Action News, 10(1): 12-15, (2010).
2. Chelani AB and Devotta S, Air quality assessment in Delhi: Before and after CNG as fuel, Environ Monit Assess, 125: 257-263, (2007).
3. Pal TK, Chatterjee N, Darbar S and Chattaraj TK, Occupational lead (Pb) exposure of construction workers engaged in buildings construction of South Kolkata, J Pharma Res, 4(8): 2455-2457, (2011).
4. Jain NB, Laden F, Guller U, Shankar A, Kazani S and Garshick E, Relation between blood lead levels and childhood anemia in India, American J Epidemiol, 161(10): 968-973, (2005).
5. Mudipalli, A, Lead hepatotoxicity and potential health effects, Indian J Med Res, 126: 518-527, (2007).
6. Lidsky TI and Schneider JS, Lead neurotoxicity in children: basic mechanism and clinical correlates, Brain, 126(1): 15-19, (2003).
7. Lyn P, Lead toxicity, a review of the literature. Part I: exposure, evaluation and treatment, Altern Med Rev, 11: 2-22, (2006).
8. Yadav S and Sharma, V, Lead induced encephalopathy: an overview, Int. J. Pharma .Bio. Sci, 2 (1) : 70-86, (2011).
9. Hegazy RM, Hamdy R and Kamel HF, Modulation of IgE Levels in lead exposed children by parental smoking, Qualyobia Gonernate, Egypt. Int. J. Pharma . Bio. Sci, 2 (2) : 372-385, (2011).
10. Naha N and Manna B, Mechanism of lead induced effects on human spermatozoa after occupational exposure, Kathmandu Univ Med J, 5(1): 85-94, (2007).
11. Iavicoli I, Carelli G, Stanek EJ, Castellino N and Calabrose EJ, Effects of low doses of dietary lead on red blood cell production in male and female mice, Toxicol Lett, 137(3): 193-199, (2003).
12. Goenarso D, Fitri LL and Musthapia I, The effect of lead nitrate on growth rate and blood characteristics of adult male mice, Proc 9th Int Conf Environ Sci Technol, Rhodes Island, Greece, pp. B274-B279, (2005).
13. Wahab AA, Mabrouk MA, Joro JM, Oluwatobi SE, Bauchi ZM and John AA, Ethanolic extract of *Phoenix dactylifera* L. prevents lead induced haematotoxicity in rats, Contin J Biomed Sci, 4: 10-15, (2010).
14. Sharma V and Pandey D, Beneficial effects of *Tinospora cordifolia* on blood profiles in male mice exposed to lead, Toxicol Int, 17(1): 8-11, (2010).
15. Anachemia MSDS Canada/4 (08.06.2007), Lead Nitrate (Material Safety Data Sheet).http://www.chemistry.mcgill.ca/msds/msds/10099-74-8.pdf
16. John V Dacie and Shirley M Lewis. Practical Haematology, 6th Edn., Churchill Livingstone: 22-83, (1984).
17. M. Bessis, L. S. Lessin and E. Beutler. Morphology of the erythron. In: W. J. Williams, E. Beutler, A. J. Erslev and M. A. Lichtman (eds.), *Hematology,* 3rd Edn,

McGraw-Hill, New York, 1986, pp. 257-280.
18. E. Beutler. Heinz body staining: blood, marrow and urine iron staining. In: W. J. Williams, E. Beutler, A. J. Erslev and M. A. Lichtman (eds.), *Hematology*, 3rd Edn, McGraw-Hill, New York, 1986, pp. 1603-1604.
19. M. A. Hayat. Introduction to Biological Scanning Electron Microscopy, Park Press: 23-46, (1978).
20. Atindra M Goon, Milan K Gupta and Bhagabat Dasgupta. Basic Statistics, 3rd Edn, The World Press: 306-310, (1981).21. Frank Firkin, Colin Chesterman, David G. Penington and Bryan Rush. De Gruchy's Clinical Haematology in Medical Practice, 5th Edn, Oxford University Press: 152-153, (1990).