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ORIGINAL ARTICLE

Lead acetate induced toxicities and antitoxic effect of Vitamin E and selenium in mice

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Abstract

Background: The experiment was conducted to evaluate the effect of different dosages of lead in the hematological parameters of mice and to observe the antitoxic role of Vitamin E and Selenium in induced lead toxicities. Moreover, the toxic effect of lead in the reproduction of female mice was also examined.

Methods: A total of 72 (48 male and 24 female) Swiss albino mice were used in the experiment. After adaptation, 42 male mice were selected for hematological studies and divided into seven groups (n=6) where Group A represented healthy control mice and Group B, C, and D were treated with lead acetate at the rate of 0.5mg/kg, 1mg/kg and 2mg/kg respectively. Similarly, three other groups B+, C+, and D+ were treated with lead acetate plus Vitamin E and Selenium at the rate of 2ml per liter drinking water. A total of 24 female mice were divided into four groups (n=6), group E represented control mice and Group F, G, and H were treated with lead acetate at the rate of 0.5mg/kg, 1mg/kg, and 2mg/kg for three weeks followed by matting and treatment was continued for another one week of gestation. Blood sample was analyzed from the hematological study group.

Result: The lead treatment caused a dose-dependent decrease in the value of Hb and PCV significantly whereas the value of TEC and TLC were significantly decreased in Group C and D in relation to Control. The value of ESR increased significantly in a dose-dependent manner in Group D in relation to Control whereas MCV and MCH values were significantly decreased than that of control. The value of TEC, Hb, PCV, ESR, and TLC improved in the Lead plus Vitamin E- Selenium treated group as compared to the Lead treated group but, only Group C+ showed significant improvement as compared to Group C. The value of neutrophil and monocyte were significantly decreased were as lymphocyte and eosinophil were significantly increased relative to control. There was a dose-dependent effect of lead in pregnancy of female mice with the highest effect (premature delivery and infant mortality) on high dose treated mice.

Conclusion: It can be concluded that lead has a great impact on hematological parameters and has an effect on various systems of the body. Premature birth and abortion are major effects of lead toxicity. Our results suggest that hemolysis of RBC and or impairment of erythropoiesis may be caused by lead toxicity and the hematological values can be restored by the use of Vitamin E plus Selenium.

Keywords: Hemolysis, Hematological parameters, Abortion, Infant mortality, Pregnancy.

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Introduction

Excessive amount of pollutants such as heavy metals in animal feed and feed stuffs are often due to human actions, resulting from either agricultural or industrial production or accidental or deliberate misuse (Aboul-Enein et al.,2010). Heavy metals like Fe, Zn, Ca and Mg have been reported to be of bio-importance to man and their daily medicinal and dietary allowances had been recommended but, some others like As, Cd,Pb, and methylated forms of Hg have been reported to have no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic (Ferner, 2001; European Union, 2002; Nolan, 2003;Young, 2005).

Lead is one of the most ubiquitous toxic materials, oldest occupational and environmental diseases in the world. At present lead is commonly used for commercial purposes and many household products, lead containing paints on walls and woodwork or weather lead paint dust and flakes leaching from the exterior of residential and commercial structures into adjacent soil and dust (WHO, 2006). The important routes of absorption of lead are ingestion and inhalation. Ingestion occur primarily form dietary contamination. Lead poisoning may be acute (from intense exposure of short duration) or chronic (from repeat low-level exposure over a prolonged period), but the latter is much more common (Needleman, 2004).

A not-ably serious effect of lead toxicity is its teratogenic effect. Lead poisoning also causes inhibition of the synthesis of haemoglobin; dysfunctions in the kidneys, joints and reproductive systems, cardiovascular system and acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS) (Ogwuebgu and Muhanga, 2005). Other effects include damage to the gastrointestinal tract (GIT) and urinary tract resulting in bloody urine, neurological disorder and can cause severe and permanent brain damage. While inorganic forms of lead, typically affect the CNS, PNS, GIT and other biosystems, organic forms predominantly affect the CNS (McCluggage, 1991; INECAR, 2000; Ferner, 2001; Lenntech, 2004).

Lead toxicity in children lead to the poor development of the grey matter of the brain, thereby resulting in poor intelligence quotient (IQ) (Udedi, 2003). Its absorption in the body is enhanced by Ca and Zn deficiencies. Acute and chronic effects of lead result in psychosis. According to the report by UNICEF and Pure Earth (2020), around 35.5 million children of Bangladesh have blood lead level ≥5 μg/dL, which makes Bangladesh fourth most-seriously affected in world in terms of number of children affected. According to UNICEF (2021), in Bangladesh, about 1.7 billion children are involved in child labor and about 25% of them are of age group 6 to 11 and they are mainly involved in hazardous and risky condition like welding, car repair, lead melting, etc. Lead poisoning can occur in all domestic animals including cattle, buffalo, horses, birds/poultry and dogs.

Vitamin E is one of the fat-soluble vitamins and acts as a major chain breaking antioxidant in the membrane (Packer, 1997). It is the first line of defense against lipid peroxidation and plays a very important function of red blood cells (RBC) flexibility as they make their way through the arterial network. Vitamin E administration offered protection to the cell from expansion or abnormalities in their structural features. It is reported that vitamin E not only confers protection against lead toxicity but it can also perform therapeutic role against toxicity (Bhattacharjee et al., 2003).

Trace amount of selenium is necessary for cellular function in many systems, in all animals. Selenium is a component of the antioxidant enzyme glutathione peroxidase and thioredoxinreductase which indirectly reduce certain oxidized molecules in animals and some plants. Glutathione is a substance produced in the liver

and most important substance in the animal/human body. When glutathione production is low, detoxification in the liver is seriously impaired. Thus, this function of selenium is also a critical one (Matthes et al., 2002). Selenium supplementation has been shown to have a protective effect when given prior to lead exposure in animals (Patrick, 2006). During lead exposure selenium binds to lead and form selenium-lead complexes, which have been proposed as a mechanism for selenium protective effect in lead toxicity (Nedkova et al., 2000; Matthes et al., 2002).

Only a few researches have been done in this aspect in Bangladesh. Therefore, the aims of our study were to evaluate the effect of different dose of lead on hematological parameter and observe the antitoxic role of vitamin E and selenium in lead induced toxicities. In addition, the toxic effects of lead in gestation period of mice also evaluated.

Materials and methods

A total of 72 (28 day old, 48 male and 24 female) Swiss albino mice of body weight (23±2) were purchased from Experimental Animal House, (icddr,b) Mohakhali, Dhaka. These animals were housed in the laboratory animal room of the Department of Physiology, Bangladesh Agricultural University, Mymensingh, Bangladesh. All mice were kept under normal laboratory conditions and adapted for two weeks. They were allowed free access to tap water and fed on standard pellet diet. Out of 48 male mice, 42 mice were selected for hematological studies and divided into seven groups (n=6) as A, B, C, D, B+, C+, D+. Group A was considered as control group and was treated I/P with 0.5ml normal saline. Remaining groups were treated with lead acetate solution (lead acetate doses were dissolved in 0.5ml normal saline) at one day interval at equal volume during the experimental period of 4 weeks. The mice of Group B, C and D were treated with lead acetate at the rate of 0.5mg/kg, 1mg/kg and 2mg/kg body weight respectively. Similarly, mice of Group B+ was treated with lead acetate at the rate of 0.5mg/kg body weight plus vitamin E and selenium at the rate of 2ml per liter drinking water, Group C+ with lead acetate at the rate of 1mg/kg body weight plus vitamin E and selenium at the rate of 2ml per liter drinking water and Group D+ with lead acetate at the rate of 2mg/kg body weight plus vitamin E and selenium (E-Sel®, Square Pharmaceutical company Ltd.) at the rate of 2ml per liter drinking water. The female mice were divided into four groups (6 mice each) and were treated with lead acetate for the period of 3 weeks except the first group which acted as control (dose and procedure are same as described previously). Then male mice were added for successful production of progeny and treatment was continued another weeks of the gestation.

At the end of the experimental period (4 weeks, hematological study groups), animals were killed by decapitation. Blood samples were collected by cardiac puncture with the help of disposable syringe and needle and immediately after collection; one drop of blood was kept in a clean slide for Differential Leukocyte Count (DLC). 2ml of blood was transferred in sterile tube containing anticoagulant at a ratio of 1:10 for the hematological examination like Total Erythrocyte Count (TEC), Hemoglobin concentration (Hb), Packed cell volume (PCV), Erythrocyte Sediment rate (ESR), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Total Leukocyte Count (TLC). The hematological parameters were determined using established methods (Lamberg and Rothstein, 1977).

All the values were expressed as mean±SE for each group. The data were calculated and analyzed using graph pad prism by one-way repeated measure ANOVA followed by Turkey's multiple comparisons test. The criterion for statistical significance was set at $P<0.05$.

Results

Effect on hematological values

The Table 1 and Figure 1 showed the dose dependent effect of lead acetate toxicity on different blood picture. On the final day of the experiment, the values of Hb and PCV decreased significantly in dose dependent manner by lead acetate treatment. This trend was also observed for RBC and leukocyte count. However, the TEC and TLC count decreased significantly in group C and D in comparison to control. The mean values of TEC, HB, PCV and TLC were reduced to (98%, 72%, 76% and 97%), (93%, 68%, 68% and 92%), (85%, 62%, 65% and 87%), respectively by ingestion of 0.5mg/kg, 1mg/kg and 2mg/kg of lead acetate relative to the healthy normal control. The Figure-1 showed that ESR values were affected by lead acetate ingestion. There was an increasing trend of the ESR values in dose dependent manner and it was increased significantly ($p< 0.05$) in group D than that of control. The values of MCH (pg) and MCV (cumm) in group A, B, C and D were (2.16 and 80.36), (1.62 and 62.77), (1.60 and 58.7) and (1.59 and 61.63) respectively. Intoxication of mice with lead acetate caused a significant ($p<0.01$) reduction of MCV and MCH values ($p< 0.001$) than that of control.

The impact of chelation therapy of vitamin E and selenium against lead toxicities was shown in Figure 2. The values of TEC, Hb, PCV, TLC and ESR in Group B+, C+ and D+ (combined treatment of lead acetate plus Vit-E and selenium) were (5.10±0.06 million/mm3, 8.40±0.50 gm/dl, 32.40±1.50%, 10.27±0.16 thousand/mm3, 3.0±0.31 mm in first hour), (4.79± 0.09 mm3, 7.96±0.21gm/dl, 30.80±1.49%, 9.77±0.32 thousand/mm3, 3.2±0.48 mm in first hour) and (4.69±0.10 mm3, 7.04±0.38 gm/dl, 27.80±1.24% , 9.44±0.08 thousand/mm3, 3.04±0.40 mm in first hour)respectively. The values of TEC, Hb, PCV and TLC were gradually improving in Group B+, C+ and D+ (Lead+E-Sel treated) as compared to the Group B, C, and D (Lead induced). However, this improvement was statistically significant ($P>0.001$) in group C+ in comparison to group C. ESR levels were improved by ingestion of vitamin E and selenium but the changes were not statistically significant in comparison to lead acetate alone ingested groups.

The effect of lead acetate toxicity and role of vitamin E and selenium in lead induced toxicity on Differential Leukocyte Count (DLC) was presented in Table 2. The neutrophil, eosinophil, lymphocyte and monocyte percentage in Group A, B, C and D were (31.00±0.70, 0.90±0.33, 65.06±0.20 and 3.11±0.30),(25.42±0.10, 2.40±0.43, 71.00±0.44 and 1.20±0.34), (26.46±0.33, 2.20±0.58, 70.00±0.63 and 1.40±0.50) and (24.40±0.74, 2.00±0.22, 72.30±0.37 and 1.80±0.37) respectively. The neutrophil and monocyte counts decreased significantly whereas lymphocyte and eosinophil counts increased significantly to lead acetate intoxication relative to control. Study of the different kinds of leukocytes revealed a 9%, 7% and 11% increase of lymphocyte count in the test group respectively in comparison with the control groups, which were statistically significant ($P\leq 0.05$).

On the other hand, neutrophils and monocytes decreased (18% and 61%), (15% and 55%) and (21% and 42%) respectively, which were statistically significant ($p<0.001$) and indicated lead-induced neutropenia and monocytopenia. The values of neutrophil (%), eosinophil (%), lymphocyte (%), monocyte (%) in Group B+, C+ and D+ were (30.0±0.30, 1.80±0.48, 65.00±0.65 and 3.20±0.12), (29.10±0.31, 2.0±0.20, 65.10±0.71 and 3.50±0.31) and (29.08±0.41, 1.50±0.22, 65.00±0.54 and 3.80±0.33)respectively. The values of four different types of leukocyte changed significantly by the treatment of vitamin E and selenium and the values restored to those in healthy control group. The mean value of neutrophil and monocyte in all treated (Lead + E-Sel) group were significantly higher than the non-treated group. The mean value of lymphocytes in the treated groups reduced significantly than lead induced group (Table 2).

Toxic effect of Lead in mice during gestation and lactation period

The effects of lead acetate in mice during the gestation and lactation period were shown in Table 3. In the Control Group (E) the parturition of the pups was normal and parturition occurs at usual date. The three doses of lead acetate ingestion exhibited some problem in gestation period of mice, the lower doses group (Group F, 0.5mg/kg BW lead) of mice delivered pups normally at usual date but all pups died within two days of birth. Similarly in Group G and H where the mice were treated with 1mg/kg BW and 2mg/kg BW respectively, late and early abortion occurred respectively.

Table 1. Analysis of mean values due to lead induced toxicities along with standard error of PCV and MCV corresponding to the different groups

Parameters	Group-A (Mean±SE)	Group-B (Mean±SE)	Group-C (Mean±SE)	Group-D (Mean±SE)
PCV (%)	41.60±0.92	31.60±1.72***	28.20±0.48***	27.20±1.30***
MCV (cumm)	80.36±1.95	62.77±3.79**	58.70±1.42***	61.64±3.32**

Group A= Control, Group B=0.5mg/kg lead,Group C=1mg/kg lead and Group D=2mg/kg lead treated
** indicates significant at P<0.01, *** indicates significant at P<0.001

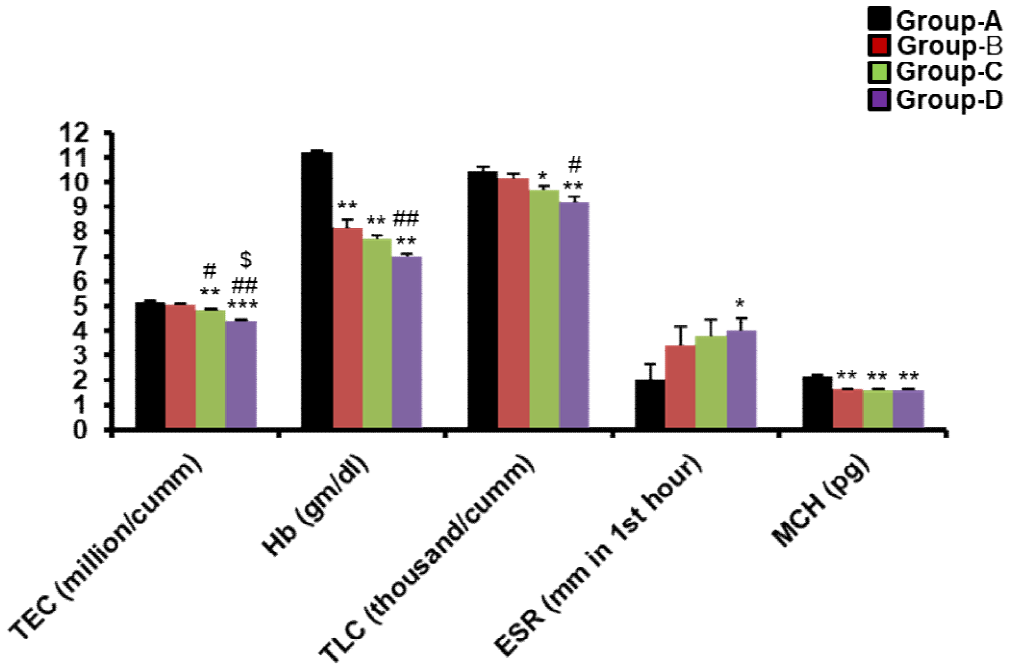

Figure 1.The dose dependent effect of lead acetate on several blood parameters of mice. (*Control vs Treated group, # Group B vs other treated groups and $ Group C vs Group D).

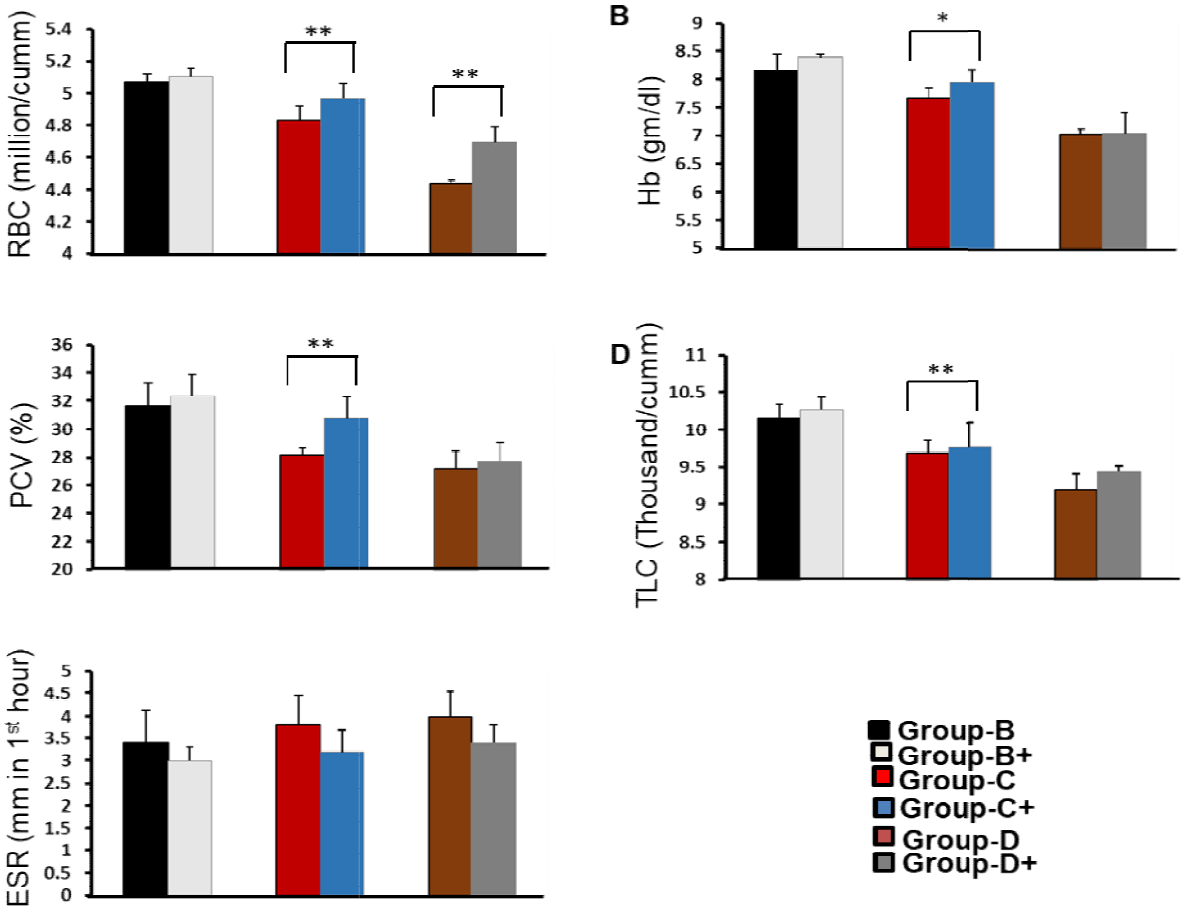

Figure 2.The effect of vitamin E and selenium to ameliorate the lead induced toxicities on mice blood parameters.

Table 2.The neutrophils, eosinophils, lymphocytes and monocytes percentage in different group treated with lead and vitamin E and selenium

Name of cell	A	B	B+	C	C+	D	D+
Neutrophil (%)	31.00±0.70	25.42±0.10	30.00±0.31***	26.46±0.33	29.10±0.31***	24.40±0.74	29.08±0.41*
Eosinophil (%)	0.90±0.33	2.40±0.43	1.80±0.48	2.20±0.58	2.00±0.20	2.00±0.54	1.50±0.22
Lymphocyte(%)	65.06±0.20	71.00±0.44	65.00±0.65***	70.00±0.63	65.40±0.71***	72.30±0.37	65.00±0.54***
Monocyte (%)	3.11±0.30	1.20±0.34	3.20±0.12*	1.40±0.50	3.50±0.31**	1.80±0.37	3.80±0.33***

Group A= Control, Group B=0.5mg/kg lead,Group B+=0.5mg/kg lead+ E-Sel,Group C=1mg/kg lead, Group C+=1mg/kg lead +E-Sel and Group D=2mg/kg lead, Group D+=2mg/kg lead +E-Sel.
*indicates P<0.05, ** indicates P<0.01*** indicates P<0.001

Table 3.Effect of lead acetate during gestation and lactation period

Group	No.of pups	Day of Parturition		
		19-21(day)	16-18 (day)	13-15 (day)
Group E	32	32 (Alive)		
Group F	34	34(Died within two days after birth)		
Group G	30		Aborted	
Group H	31			Aborted

Discussion

We evaluated the effect of lead on different hematological parameter and also assessed the role of vitamin E and selenium to ameliorate the lead induced toxicities in mice. Our results suggest that hemolysis of RBC and or impairment of erythropoiesis may be caused by lead toxicity and the hematological values can be restored by the use of vitamin E plus selenium.

Exposure to lead presents a major concern because of its toxic effect on the urinary, nervous, hematopoetic, reproductive and gastrointestinal systems (Durgut et al., 2008).The hematopoietic system is also known to be highly sensitive (De Silva, 1981). All the blood parameters such as TEC, Hb, PCV, TLC of lead intoxicated mice was significantly lower than that of healthy normal group. This harmful effect of lead on blood parameters were elevated collaterally with the increase lead acetate doses. The severe effect was found in the mice injected with 2mg/kg bw lead acetate. Excessive lead exposure inhibits heme synthesis, leading to anemia and erythrocytes degeneration (National Occupational Exposure Survey, 1988; Lockitch, 1993). Lead is toxic to the proximal tubules of kidneys, causing interstitial changes, which possibly culminated anemia in the present study. Dose dependent reduction in RBC, hemoglobin and PCV levels were also detected in treatment groups.

Previous study suggested that lead affects the hematological system by (a) changes the morphology and survival, and (b) inhibiting the heme and hemoglobin synthesis (Klaassen et al., 1990). Intoxication of lead inhibits the activity of enzymes d-aminolevulinic acid dehydratase (ALAD) and ferrochelatase which are very essential for heme synthesis. In addition, lead reduces the absorption of iron in the gastrointestinal tractand inhibits the heme biosynthesis (Lubran, 1980). Similar to our results, the mean values of TEC, PCV and Hb were reported to be reduced significantly in lead feed rats when compared to control (Jassim and Hassan, 2010). These results in reduced blood parameters may be caused by the toxic ions could be associated with several mechanisms such as decreased life span of erythrocyte and increased fragility of erythrocyte leading to the risk for hemolysis and inhibitory effect of lead on erythrocyte enzyme (GA3PD and G6PD) which is required for production of RBC (Surdakar et al., 2009).Lead acetate ingestion induced alteration in redox status as indicated by a decrease in glutathione levels, an increase in lipid peroxidation end product-4-hydroxynonenal level which may be produced by damage in RBCs membrane and increased LDH in plasma (Seddik et al., 2010). The reduction of Hb confirmed the decreased RBCs which may be attributed to the toxicity of lead acetate. It is in agreement with the

previous finding of elevated level of plasma bilirubin by lead ingestion which could be due to induction of hemeoxygenase. The catabolism of

hemeoxygenase is an enzyme which can convert heme to bilirubin (Ibrahin, 2012). It can be assumed that lead toxic effect on hematopoietic system may be responsible for changing the hematological parameters. In accordance to the present finding the decrease WBC count following lead administration at the dose rate of 0.5mg/kg, 1mg/kg and 2mg/kg body weight was also observed (Fartosi, 2008).

In addition to the lowered RBC count and hemoglobin concentration, decreased MCH and MCV are other concordant hematological change found in lead acetate treated groups. Anemia was in the form of microcytic and hypochromic. This might be due to effects of lead in cell metabolism, alteration of the enzyme activity. Lead induced inhibitory effects on the erythrocyte enzymes GA3PD and G6PD (Calderon-Salinas et al., 1993).

Interaction of lead with heme biosynthesis has been related to the inhibition of cytoplasmic and mitochondrial enzymes (A.T.S.D.R., 1993) and a decrease in the activity of the main enzymes in heme biosynthesis due to defects in iron metabolism has also been reported (A.T.S.D.R., 1993; Chmielnika, 1994; Yagminaset al., 1990; Calderon-Salinas et al., 1993). Development of anemia in lead toxicity may be attributed to the decreased red blood cell survival because of the increased membrane fragility, reduced RBC count, decreased hemoglobin production, or summation of all these factors (A.T.S.D.R., 1993; Rediget.al., 1991; Rio et. al., 2001)

Previous result suggested that vitamin E is a chain breaking free radical trapping antioxidant and it has protective action in membrane stability and prevents membrane lipoprotein from oxidation damages.Alpha-tochopherol prevents RBC membrane in lead toxicity by lowering lipid peroxidase levels and increasing superoxide dismutase (SOD) and catalase activity. Moreover, vitamin E is the first line of defense against lipid heme proteins is carried out in the microsomal fraction of cells by a complex enzyme system and

peroxidation and plays a very important function in lending red blood cells flexibility as they make their way through the arterial network by stable their structural features (Bhattacharjee et al., 2003). Selenium is a required mineral for the metallo-enzyme glutathione peroxidase (Gpx) which plays a key role in recycling glutathione and is effective in reducing free radical damage in specific disease states. Selenium supplementation has been shown to have a protective effect when given prior to lead exposure to animal by increasing the level of SOD and glutathione reductase in both liver and kidney tissues. Selenium can binds to lead and form highly bonded selenium-lead complex, which have been proposed mechanism for selenium protective effect in lead toxicity. Similar findings have also been reported by other authors (Anguelov et al., 2002; Chaurasia and Kar, 1997; Jassim and Hassan,2010).

In the present study, total leukocyte count had decreased mainly due to decrease in neutrophil and monocyte count. There was also an increase in lymphocyte count, which was statistically significant ($P\leq 0.05$). In some reports, leukocytosis has been attributed to the lead-induced inflammation (Yagminas et al., 1990). Controversies exist about different type of leukocytic cell count in some studies (Yagminas et al., 1990;NooriMugahi et al., 2003). The reason for such difference is probably due to the extent of lead-induced inflammation. Consistent with other reports neutropenia and lymphocytosis were observed in this study (Jassim and Hasan, 2011). Treatment of lead acetate induced mice with vitamin E and selenium concomitantly produced significant increase ($P<0.05$) in the percentage of neutrophils and monocytes, with significant decrease in the percentage of lymphocyte in mice compared with lead acetate group. The Vitamin E and selenium acts as chelating agents via rebalancing the prooxidant or antioxidant ratio (Pande et al., 2003).

Lead is one of the most significant reproductive toxicants causing infertility and abortion. Exposure to lead has been associated with reduced sperm count, morphology and function in male and in female, lead act as a potential cause of spontaneous abortion and some normal infants get the infection through milk and develop the physical and mental problems (Borja-Aburto et al., 1999). The harmful effect of lead we observedon gestation period, spontaneous abortion and death rate of pups were elevated with the increase of lead acetate doses. The severe effect such as spontaneous abortion within 13-15th day of gestation period was found in the mice injected with 2mg/bw lead acetate. The obtained results are in agreement with the finding of previous study (Tang and Zhu, 2003). One study reported increase rate of premature delivery and infant mortality in lead acetate intoxicated female Swiss albino mice (Sharma et al., 2012). Our result suggests that lead crosses the placenta by diffusion, reaches and accumulates within the tissues and body fluids of fetus at different period of gestation and produce the harmful effect.

Conclusion

It can be concluded that lead affects different organs and systems of the body like bone, kidney, liver and placenta. Premature delivery and abortion are major effect of lead toxicity. Our results suggest that hemolysis of RBC and or impairment of erythropoiesis may be caused by lead toxicity and the hematological values can be restored by the use of Vitamin E plus selenium. Further studies are needed to explore the complete antitoxic effect of Vitamin E and Selenium which will be beneficial for animals as well as humans.

Conflict of Interest

The authors declared no conflict of interest.

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