

STUDY ON HEMATOLOGICAL ALTERATIONS IN EXPERIMENTAL LEAD TOXICOSIS IN LONG EVANS RATS

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ABSTRACT. The study of alterations of some hematological parameters in a experimentally induced lead toxicosis were carried out on a total of 15 (15 days old) male weaning Long- Evans (ICDDR B strain) rats. The rats were randomly divided in to three equal groups, each consisting of five rats. Rats of group A were kept as control (without giving any treatment), group B received lead acetate alone @ 6 mg/ml drinking water and group C received lead acetate @ 6 mg/ml plus whole milk (Star ship®) 150 mg/ml drinking water. The result showed a most significantly ($p < 0.01$) decreased TEC, TLC and Hb% observed on day 56 in group B but in group C, these counts decreased significantly ($p < 0.05$) on days 56 of experiment. From the study, it was concluded that treatment with lead acetate at low doses has harmful effects on experimental animals including hematological alterations.

Keywords: lead acetate, whole milk, body weight, hematological parameters, rat.

INTRODUCTION

Lead is a dangerous heavy metal which is widely spread in the environment. Lead content in the air, food and tap water has increased several folds during recent years due to extensive use of this metal in petrol, paints, battery and other industries (Tuormaa, 1995). Despite attempts of reducing the exposure to this metal, there are still some reports of cases with severe lead toxicity (Hershko *et al*, 2005). In some cases, haematological parameters may provide an indication of lead intoxication. Among the major effects of lead poisoning is anemia, which results from inhibition of the heme synthesizing enzymes with concurrent elevation of protoporphyrin (Lee, 1981). This assertion was further buttressed by Osweiler (1996) who reported that lead slows down haemosynthesis through inhibition of enzymes. A hypochromic, regenerative anaemia was reported to occur in some affected birds poisoned with lead (McDonald,

1988). According to studies (Stohs and Bagchi, 1995; Mateo *et al.*, 2003), lead has a potential to induce oxidative stress and acts as a catalyst in the oxidative reactions of biological macromolecules. Hence, the toxicities associated with lead might be due to oxidative tissue damage (Gurer and Ercal, 2000; Ercal *et al.*, 2001). Chronic lead poisoning is a problem which threatens mankind's life and seems to be an unknown reason for some diseases during aging (Coyle *et al.*, 2005). The toxic effects of lead on blood indices are well known. A Significant decrease in RBC count, hematocrit (Hct) and hemoglobin (Hb) were seen in rats and human with high blood lead levels (Hofmann and Segewitz, 1975; Alexa *et al.*, 2002; Noori *et al.*, 2003; Toplan *et al.*, 2004). Lead is considered as pathogenic factor of atherosclerosis, arterial hypertension and may cause an anemia. Belacy *et al.*, (1996) reported that lead induce inhibition of renal and hepatic transaminase and alkaline phosphates. More than 99% of lead in whole blood is associated with erythrocyte. Almost 70% of total lead clearance occurs in the urine and the remainder is excreted in the feces and sweat, and may be accumulated in hair and nails. After a chronic exposure, lead removal usually follows a multicompartement kinetic model: a fast compartment in the blood and soft tissues with a half-life of 1-2 months. In Bangladesh there is no available data in this context, so this research work has been carried out to study the alterations of blood

parameters in in experimentally induced lead toxicosis in Long- Evans rats.

MATERIALS AND METHODS

Materials

Experimental Animals

Fifteen-day-old male weaned Long Evans rat (*Rattus norvegicus*) weighing between 182-294 g were purchased from ICDDR, Dhaka and brought to the Experimental Pharmacology and Toxicology laboratory at Bangladesh Agricultural University (BAU) for the present study. They were housed throughout the entire period of study in Perspex cages with aluminium grid on the bottom fixed on inch a part to facilitate fecal materials and urine in a room maintaining 23 ± 1 °C. After 6 days of acclimatization animals were segregated on the basis of their age and body weight without significant differences. The rats were fed on standard rat chow (15 g/rat/day) for 56 days formulated by ICDDR, Dhaka and supplied fresh water.

Experimental chemicals

Lead acetate 500 mg (BDH co.) from Hatkhola market, Dhaka and Whole milk (Starship) from local market were purchased and brought to the laboratory for this study.

Experimental design

A total of 15 (15 days old) male weaned Long Evans rats were used. These rats were randomly divided into 3 equal groups, and numbered them as group A, B and C. Out of 3 groups, rats of group A was kept as control without giving any treatment, rats of group B received lead acetate alone @ 6 mg/ml drinking water and group C received lead acetate @ 6 mg/ml plus whole milk (Star ship®) 150 mg/ml of drinking water. After administration of lead acetate with both drinking water and whole milk, all the rats were kept under close observation for a whole period of study and three blood parameters such as total erythrocyte count (TEC), total leukocyte count (TLC) and hemoglobin percentage (Hb%) were recorded at twenty-eight-day intervals.

Methods

Procedures for the collection of blood sample for measuring hematological parameters

Blood was collected just before treatment i.e. day 0, day 28 and day 56 of treatment directly from tip of the tail of ether-anesthetized rat. Immediately after collection, blood was transferred to sterile tubes containing anticoagulant (4% sodium citrate solution) at a ratio of 1:10 and used for different hematological parameters within two hours after collection.

Hematological parameters

Total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin content (gm %) were determined as per methods cited by Coffin (1955).

Determination of Total Erythrocyte Count (TEC)

The blood was sucked by the red pipette up to 0.5 mark of the pipette. Then the tip of the pipette was placed in to red cell diluting fluid and the pipette was filled with the fluid up to 101 mark. The contents of the pipette were mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. The counting chamber was placed with cover glass under microscope using low power (10×) objective and a small drop of fluid was placed properly on the counting chamber. The cells were counted from the recognized 80 small squares under high power objectives (40×). After completion of counting total cells, the number of RBC recorded from the supplied samples were expressed as, number of cells counted × 10,000 and the result was expressed in million/mm³.

Determination of Total Leukocyte Count (TLC)

Well mixed blood was drawn up to 0.5 mark of white blood cell pipette. The diluting fluid (N/10 HCl) was filled up to the 11 mark of the pipette and the contents were thoroughly mixed for 2 minutes.

The counting chamber was then placed properly and filled with one drop of fluid and examined under low power (10×) objective. The leukocytes in the four large squares (each 1 square mm) of the counting chamber were counted. The number of WBC was calculated as follows: No. of WBC = No. of cell counted × 50. The result was expressed in thousand /mm³.

Determination of Hemoglobin Content (gm %)

The N/10 HCl solution was taken in a graduated diluting tube up to 2 marks with the help of a dropper. Citrated, well homogenized blood, was then drawn into Sahli pipette up to 20 µl mark. The blood of the pipette was immediately transferred into the diluting tube containing HCl solution. This blood and acid were thoroughly mixed by a glass stirrer in to the diluting tube so that acid hematin mixture is formed. The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes and distilled water was added drop by drop. The solution was mixed well with a stirrer until the color of mixture resembled the standard color of the comparator. The result was read in daylight observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in gm %.

Statistical Analysis

The data of the total erythrocyte count (TEC), total leukocyte count (TLC) and hemoglobin (gm %) were analyzed statistically using T- test.

RESULTS

Effect on Hematological Parameters

Total Erythrocyte Count (TEC) decreased significantly ($p < 0.05$) in group B on day 28 and in group C on day 56 but most significant reduction ($p < 0.01$) was recorded in group B on day 56 (Table 1). The oral administration of lead acetate in drinking water in group B, TEC count most significantly decreased on day 56 but in group C, TEC count decreased significantly on day 56 of experiment. Total leukocyte count (TLC) decreased significantly ($p < 0.05$) in rats of both group B and C but most significant reduction ($p < 0.01$) was recorded in group B on day 56 (Table 1). The hemoglobin level was most significantly decreased ($p < 0.01$) on day 56 in group B but comparatively less significant ($p < 0.05$) was recorded on day 28 of dosing in group B. In group C, Hb % gradually reduced. At day 56 of experiment of group C, Hb % significantly ($p < 0.05$) reduced (Table 1). The administration of lead acetate caused the significant reduction of hemoglobin percentage. The Hb percentage significantly decreased on day 28 of dosing of group B but most significant decrease value was seen on day

Table 1. Effect of oral administration of lead acetate on some haematological parameters in experimentally induced lead toxicosis in rats

| Parameters | Group | Chemicals with dose | Pre-treatment | Post treatment | |
|------------------------------|-------|--|---------------|----------------|---------------|
| | | | Day 0 | Day 28 | Day 56 |
| Total erythrocyte count(TEC) | A | Control | 10.13 ±0.09 | 10.00± 0.18 | 9.75± 0.02 |
| | B | lead acetate @6 mg/ml drinking water | 9.26± 0.05 | 7.11± 0.09* | 6.97± 0.02** |
| | C | lead acetate @ 6 mg/ml plus whole milk (star ship) 150 mg/ml of drinking water | 9.39± 0.30 | 8.54 ±0.17 | 7.18± 0.50* |
| Total leukocyte count (TLC) | A | Control | 10.48 ±1.72 | 10.39± 0.14 | 10.43± 0.14 |
| | B | lead acetate @6 mg/ml drinking water | 10.11± 0.27 | 10.26±0.14 | 8.12± 0.12** |
| | C | lead acetate @ 6 mg/ml plus whole milk (star ship) 150 mg/ml of drinking water | 9.62± 0.11 | 8.78 ±0.24* | 8.98± 0.15 |
| Hemoglobin (Hb) | A | Control | 13.52 ±0.11 | 13.29± 0.13 | 13.17± 0.10 |
| | B | lead acetate @6 mg/ml drinking water | 12.09± 0.58 | 11.91±0.32* | 10.25± 0.53** |
| | C | lead acetate @ 6 mg/ml plus whole milk (star ship) 150 mg/ml of drinking water | 11.30± 0.10 | 11.15 ±0.16 | 10.94± 0.05* |

Values above represent the mean ± SE of 5 rats. * Indicates significant values ($p<0.05$). ** Indicates highly significant values ($p<0.01$)

56. In case of group C, hemoglobin level decreased significantly at day 56 of the experiment but the percentage was less in group B.

DISCUSSIONS

In the case of total erythrocyte count (TEC), the toxic effect in group C was found somewhat less than the rats of group B. This might be due to the positive effect of whole milk supplementation along with lead acetate. The results are similar to the previous reports of works (Purser *et al.*, 1983; Wilson *et al.*, 1979; Mugahi *et al.*, 2003; Toplan *et al.*, 2004 and Alwaleedi 2015). The inhibitory effect of lead acetate on conversion of coproporphyrinogen III to protoporphyrin IX resulting in shortening

erythrocyte life span and decrease the production of haemoglobin (Klassen, 2001). The reduction of hematological values might be attributed to binding of lead to red blood cells which increase membrane fragility and RBCs destruction (Rous, 2000). Total leukocyte count (TLC) decreased significantly ($p<0.05$) in rats of both groups B and C but most significant reduction ($p<0.01$) was recorded in group B on day 56 (Table 2). The TLC reduced most significantly on day 56 in lead acetate in group B due to lead deposit in the bone marrow, but in group C, decreased TLC was less probably because milk slightly minimized the toxic effect of the lead acetate. This result is dissimilar to the result reported by Mugahi *et al.*, 2003; Karamala *et al.*, 2011 and Alwaleedi,

2015 where increased TLC was observed in chronic lead acetate intoxicated adult male rat. Berney *et al.*, (1994) observed that TLC count were higher also in dog and cat with higher blood lead concentrations. The possible causes might be due to toxic effects of lead on the hemopoietic organs of the body. The exact mechanism of highly significant decrease of TLC could not be fully explained because of the lack of work in this field. It has been reported that lead induced inflammation led to increase in white blood cells (Yagminas *et al.*, 1990) which does not support this study. The reasons were not clear. The reasons might be animal management practices, age, body weight of the experimental animals and the quality and dosages of the chemical used. The hemoglobin level was most significantly decreased ($p < 0.01$) on day 56 in group B but comparatively less significant ($p < 0.05$) was recorded on day 28 of dosing in group B. In group C, Hb % gradually reduced. At day 56 of experiment of group C, Hb % significantly ($p < 0.05$) reduced (Table 1). The administration of lead acetate caused the significant reduction of hemoglobin percentage. The Hb percentage significantly decreased on day 28 of dosing of group B but most significant decrease value was seen on day 56. In the case of group C, hemoglobin level decreased significantly at day 56 of the experiment but the percentage was less in group B. In accordance with present findings, Lassen and Buck (1979) observed the moderate decrease in blood Hb levels and mean corpuscular hemoglobin

levels. Similarly Szymezak *et al.*, (1983) reported that blood hemoglobin levels were reduced after intoxicated with lead which was similar to the findings of this work. Purser *et al.*, (1983) also observed a progressive decrease in blood hemoglobin concentration. The result of this study was in agreement with the earlier reports of several workers (Dobryczycka *et al.*, 1984; Berny *et al.*, 1994; Toplan *et al.*, 2004; Karamala *et al.*, 2011; Alwaleedi 2015). Continuous exposure to lead might adversely affect the heme biosynthesis in the body due to the inhibition of cytoplasmic and mitochondrial enzymes (ATSDR, 1993). The depressing effects of lead acetate on the activity of the major enzymes in heme biosynthesis process might be referred to imperfection of iron metabolism (Chmielnika *et al.*, 1994; Yagminas *et al.*, 1990). Soils and water may become polluted by the accumulation of heavy metals and metalloids like lead through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition which might be inhaled or ingested by the animals at a lower dosages. These metals can accumulate in animal body which in turn may cause human health hazards. For detailed explanation of the matter, an extensive study is essential. Besides, the findings of this study with

others such as history of intoxication, factors increase toxic exposure together, may be an important tool to diagnose acute or chronic lead toxicosis .

CONCLUSION

Treatment with lead acetate at low doses has harmful effects on experimental animals including hematological alterations. Therefore, whole milk (star ship) might be helpful to reduce the body burden of lead toxicities.

REFERENCES

- Alwaleedi S. A. (2015). Haemato-biochemical changes induced by lead intoxication in male and female albino mice. *Intl. J. Rec. Sci. Res.* **6(4)**: 3999-4004.
- Alexa I. D., Mihalache I.L., Panaghiu L and Palade F. (2002). Chronic lead poisoning – a “forgotten” cause of anemia. *Rev. Med. Chir. Soc. Med. Nat. Iasi.*, **106(4)**: 825-8.
- ATSDR (1993). *Toxicological profile for lead. Update*. Prepared by Clement International Corporation under contact no. 205-88-060 for ATSDR, U.S. Public Health Services, Atlanta, GA.
- Belacy N., Shaha H., Edrees G. and EL-Arbagy A.R. (1996). The contribution of heavy metal in kidney dysfunction. *J. Union. Arab. Biol.* **5 (A) Zoology**: 147-155.
- Berny P.J., Cote L.M. and Buck W.B. (1994). Low blood lead concentration associated with various biomarkers in household pets. *American Journal of Veterinary Research* **55(1)**: 55-62
- Coffin D.I. (1955). *Manual Veterinary clinical Pathology* 3rd ed. Comstock Publishing Associates. Inc. Newwork pp 116-157
- Coyle P., Kosnett M.J. and Hipkins K. (2005). Severe lead poisoning in the plastic industry: a report of three cases. *Am. J. Ind. Med.*, **47(2)**: 172-5.
- Chmielnika J., Zareba G. and Nasiadek M. (1994). Combined effect of tin and lead on heme biosynthesis in rats. *Ecotox. Environm. Safety* **29**: 165-173.
- Dobryazycka W., Sawicki G. and Gasiorowski K. (1984). Effect of acute intoxication of rats with Pb²⁺ ions on hemoglobin glycosylation *in vivo* and *in vitro*. *Bromatol. Chem. Toksykol* **17(1)**: 11-16.
- Ercal N., Gurer-Orhan H., and Aykin-Burns N., (2001). Toxic metals and oxidative stress. Part I: Mechanisms involved in metals-induced oxidative damage. *Current Topics Med. Chem.*, **1**: 529-539.
- Gurer H. and Ercal N., (2000). Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radic. Biol. Med.*, **29**: 927-945.
- Hershko C., (2005). Lead poisoning by contaminated flour: an unfinished story. *Harefuah.*, **144(7)**: 458-528.
- Hofmann U. and Segewitz G. (1975). Influence of chelation therapy on acute lead intoxication in rats. *Arch. Toxicol.* **34(3)**: 213-25.
- Karamala S.K., Sri Latha C., Anjaneyulu Y., Rao C.S.T.S., Vasulu S.D. and Pidugu A.P. (2011). Hematobiochemical changes of lead Poisoning and amelioration with *Ocimum sanctum* in wistar albino rats. *Vet. world* **4(6)**: 260-263.
- Klassen C.D. (2001). *Casarett and Doull's Toxicology: The basic science of poisons*. 6th edn. McGraw-Hill Medical Publishing Division. pp 812-841.
- Lassen E.D. and Buck W.B. (1979). Experimental lead toxicosis in swine. *American J. Vet. Res.* **40(10)**: 1539-1564.
- Lee W.R. (1981). What happens in lead poisoning? *Journal of the Royal College of Physicians of London*, **15**: 48-54.
- Mugahi M.N., Heidari Z., Sagheb H.M. and Barbarestani M., (2003). Effects of chronic lead acetate intoxication on blood indices of male adult rat. *DARU* **11(4)**: 147-151.
- Mateo R., Beyer W.N., Spann J.W., Hoffman D.J. and Ramis A., (2003). Relationship between oxidative stress, pathology and behavioural signs of lead poisoning in mallards. *J. Toxi. Environ. Health A*, **66**: 1371.
- Noori M.M., Heidari Z., Sagheb H.M. and Barbarestani M. (2003). Effects of chronic lead acetate intoxication on blood indices of male adult rat. *Daru.Pharm. J.*, **11(4)**: 147-51,
- Osweiler G.D., (1996). *Toxicology*. Wilians and Wilkins, Philadelphia, pp 191-197.
- Purser D.A., Berrill K.R. and Majeed S.K. (1983). Effects of lead exposure on peripheral nerve in the cynomolgus monkey. *Brazilian J. Int. Med.* **40**: 402-412.
- Rous P. (2000). The effect of heavy metals boundary contaminated soil on haematological and selected biochemical parameters in blood plasma of rabbits. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis.* **48**: 93-99.
- Stohs S.J. and Bagchi D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radicals Biology Chemistry*, **18 (2)**: 321.
- Szymezak J., Zechalko A. and Biernat A. (1983). Effect of fodder fat type on blood plasma lipids in rats intoxicated with lead. *Bromatol. Chem. Toksykol.* **16**: 98-94.
- Toplan S., Ozcelik D., Gulyasar T. and Akyolcu M.C. (2004). Changes in hemorheological parameters due to lead exposure in female rats. *J Trace Elem Med Biol.* **18(2)**:179-82.

27. Tuormaa T.E. (1995). The adverse effects of lead. *J. Orthomol. Med.*, **10(3-4)**: 149-64.
28. Wilson G.I., Osweiler G.D. and Garner H.E. (1979). Experimental induction and review of lead toxicity in a shetland pony. *Journal of Equine Medicine and Surgery* **3(8)**: 386- 390.
29. Yagminas A.P., Franklin C.A., Villeneuve D.C., Gilman A.P., Little P.B. and Valli V.E. (1990). Subchronic oral toxicity of triethyl lead in the male weanling rat. Clinical, biochemical, hematological, and histopathological effects. *Fundam. Appl. Toxicol.* **15**: 580-596.