Global Veterinaria 4 (5): 532-535, 2010
ISSN 1992-6197

Investigation on Subchronic Lead Intoxication on Blood Indices of Male Rats

Okediran B. Samuel, Ajibola E. Solomon, Thomas F. Clara, Abam O. Esther, Biobaku K. Talha, Olaniyi M. Olajire and S. Rahaman

Department of Veterinary Physiology and Pharmacology,
University of Agriculture, Abeokuta, Nigeria

Abstract: Lead is an environmental contaminant that affect every living organism. The present study investigated the haematological changes following intraperitoneal administration of varying doses of lead acetate in adult male rats for a period of fourteen days. Blood lead concentrations, haematological analyses and differential blood cell counts were investigated. At the end of fourteen days of lead acetate administration, there was significant increase ($p<0.05$) in blood lead concentration, this was accompanied with decreased packed cell volume (PCV), haemoglobin concentration and red blood cell count. In addition there was leukocytosis, monocytosis and neutrophilia which were prominent at higher dosage of lead acetate administration. It was concluded that the anaemia indued by lead was microcytic hypochromic.

Key words: Anaemia · Haemoglobin concentration · Intraperitoneal · Lead · Packed cell volume

INTRODUCTION

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the environment [1]. Lead is a major environmental pollutant and its toxicity continues to be a major public health problems and there is a growing consensus that lead cause toxic injury to human at a level of exposure that was considered to be safe only a decade ago [2]. As the chronic exposure to lead, even at low levels, can result in slow progressive (in most of time), irreversible damage to haematopoietic, nervous and renal systems [3].

Exposure to lead may occur during the manufacture of batteries, painting, pottery glazing and lead smelting processes. Exposure may also occur during the construction of tanks linings, piping and other equipments that carries corrosive gases and liquids, superconductors and fiber optic technologies [4, 5]. All sources of lead contribute to an increased in permissible exposure limit for metallic lead, lead oxide and lead salts and soaps that has set by world health organization and other health organizations [6].

Lead is absorbed through digestive, respiratory tracts and skin. After absorption into the blood, 99% of lead is bound to erythrocytes and the remaining 1% stay in the plasma to be carried to other tissues [7]. Lead has long been known to alter the haematological system by inhibiting the activities of several enzymes involved in haem biosynthesis. Once absorbed, it is distributed particularly to the liver, kidney, heart, male gonads as well as it affects the immune system [8].

In recent years, research efforts are directed towards quantification of the impact of lead exposure on human health, particularly from environment, regarding the effects of lead on the haematological parameters.

The present study was conducted to investigate the blood cell count and morphology in adult male rats following subchronic lead intoxication in a fourteen days period.

MATERIALS AND METHODS

Animals: Twenty five adult male Wistar rats were randomly selected and transferred to an animal housing having standard conditions at a temperature of 23-25°C. The average weight of the rats was 150±4.60g. Food and drinking water were available *ad libitum*. A week after adaptation to new environment, animals were divided into five groups (A, B, C, D and E) of five rats per group.

Corresponding Author: Okediran Babatunde Samuel, Department of Veterinary Physiology and Pharmacology University of Agriculture, Abeokuta, Nigeria, Tel: +234 8034241610, E-mail: bokediran@yahoo.co.uk

Group A served as the control while groups B, C, D and E were administered graded doses (0.5, 1.0, 1.5 and 2.0mg Pb^{2+}/kg body weight respectively) of lead as lead acetate intraperitoneally for a period of fourteen days, while the control group (group A) received a daily dose of sterile distilled water.

At the end of the exposure time, animals of all groups were anaesthesized with ether and blood samples were obtained from their heart.

Analyses

Blood Lead Concentration: Concentration of blood lead was determined using atomic absorption spectrophotometer (GBC Avanta; GBC Scientific Equipment PTY. LTD; Dandenos Victoria, Australia) after digestion with a mixture of nitric acid and perchloric acid (6:1 v/v).

Blood Analysis: A cell counter was used to analyze haematological indices. The packed cell volume, platelets, red and white blood cell counts were determined as described by Schalm *et al.* [9]. Blood smears were also prepared from these blood specimens for manual differential cell counts. Haemoglobin concentration were determined as described by Van Kamper [10] using haemoglobin kit supplied by Cypress Diagnostics; Langdorp-Belgium.

Statistical Protocol: Data are expressed as mean ± S.D. and $p<0.05$ was considered statistically significant. The significance of the differences was assessed by one-way of analysis of variance (ANOVA). When significant effects were found among the groups, Tukey's test was used to assess which of the groups were significantly different from each other.

RESULTS

In the present study, the blood lead concentrations of the rats exposed to different doses of lead as lead acetate was significantly higher ($p<0.05$) as compared with the control group. The highest blood lead was found in group E which was about six times that of the control group (Table 1).

The haematological parameters of rats exposed to varying doses of lead as lead acetate are shown in Table 2. There was significant reduction in the packed cell volume and haemoglobin concentration of rats exposed to graded doses of lead. The reduction in haemoglobin concentration was more prominent in group E which was about 53.34% reduction compared to the control group. There was also significant reduction in the red blood cells count which decrease as the dosage of lead exposure also increases. The decrease was about 43% in group E compared to the control group.

There was significant reduction in both the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) as exposure to varying doses of lead increases. This decrease was in the range of 19.63 to 42.53% compared to the control group.

Table 1: Blood lead concentration of rats exposed to varying doses of lead

Groups	Mean blood lead concentration (µg/ml)
A	0.12 ± 0.01^{a}
B	0.16 ± 0.01^{b}
C	0.21 ± 0.00^{c}
D	0.25 ± 0.01^{d}
E	0.67 ± 0.02^{e}

Values are mean ± S.D. Values in a column having no (a-e) in common are significantly different from each other ($p<0.05$)

Table 2: Haematological parameters of rats exposed to varying doses of lead as lead acetate

GROUPS	PCV (%)	Haemoglobin (g/dl)	RBC count 1 x 10^{6}	MCV (pg)	MCHC (g/dl)	Howell jolly bodies	Basophilic stippling
A	49.75 ± 2.06^{b}	16.12 ± 4.53^{b}	7.23 ± 0.57^{a}	66.72 ± 2.11^{a}	32.71 ± 0.56^{a}	_	_
B	42.25 ± 2.63^{a}	13.82 ± 0.51^{a}	7.08 ± 0.52^{b}	58.57 ± 1.80^{b}	32.40 ± 0.60^{a}	_	_
C	41.25 ± 3.40^{a}	13.00 ± 2.65^{a}	7.10 ± 1.28^{b}	56.10 ± 1.77^{b}	31.61 ± 0.63^{a}	+	_
D	41.05 ± 1.89^{a}	12.34 ± 2.75^{c}	7.00 ± 0.39^{b}	55.60 ± 1.63^{b}	30.06 ± 0.57^{c}	+	+
E	40.00 ± 3.46^{a}	7.52 ± 2.21^{d}	6.80 ± 0.57^{c}	53.62 ± 1.54^{b}	18.80 ± 0.65^{b}	+	+

Values are mean ± S.D. Values in a column having no (a-d) in common are significantly different from each other ($p<0.05$)

PCV= Packed cell volume, RBC= Red blood cell, MCV=Mean corpuscular volume,

MCHC= Mean corpuscular haemoglobin concentration

not observed (-) and, moderately observed (+)

Table 3: White blood cell count and differentials of rats exposed to varying doses of lead.

Groups	Lymphocyte count(1 x10^3/ml)	Monocyte count(1 x10^3/ml)	Eosinophil count(1 x10^3/ml)	Neutrophil count(1 x10^3/ml)	Platelet count(1 x10^3/ml)	Leukocyte count(1 x10^3/ml)
A	7.00 ± 0.81^a	0.39 ± 0.13^a	0.40 ± 0.03^a	5.21 ± 0.12^a	718 ± 50^a	13.51 ± 0.60^a
B	7.10 ± 0.21^a	0.34 ± 0.10^a	0.41 ± 0.01^a	5.12 ± 0.11^a	721 ± 48^a	12.94 ± 0.45^a
C	7.30 ± 0.17^a	0.33 ± 0.14^a	0.30 ± 0.02^b	5.01 ± 0.10^a	739 ± 53^a	12.97 ± 0.52^a
D	8.11 ± 0.12^b	0.52 ± 0.13^b	0.21 ± 0.03^b	5.91 ± 0.13^b	756 ± 43^b	14.75 ± 0.47^b
E	9.00 ± 0.11^b	0.61 ± 0.11^b	0.19 ± 0.11^b	6.13 ± 0.20^b	762 ± 48^b	15.94 ± 0.61^b

Values are mean ± S.D. Values in a column having no (a-b) in common are significantly different from each other ($p<0.05$)

Evidence of Howell jolly body and basophilic stippling were seen most, especially in groups C to E. The basophilic stippling was more pronounced in group E.

Table 3 shows the white blood cell count and differentials of rats exposed to varying doses of lead. There were no significant changes in the total leukocyte counts in rats exposed to low doses of lead; however at higher dosage (groups D and E) there was significant increase in leukocyte counts in comparison with the control group, which is an indication of lead induced leucocytosis.

Study of the different kinds of leukocytes revealed significant increase in lymphocyte count most especially in groups D and E that were exposed to higher dosage of lead, while those exposed to lower dosage were not statistically different compared to the control groups.

There was no significant difference in the monocyte count at lower dosage of lead compared with the control group, however at higher dosage there was significant increase in the monocyte count which was about 56.41% (group E) compared with the control group.

There was significant decrease in the eosinophil count of rats exposed to varying dosage of lead in comparison with the control group. The neutrophils and the platelets count show significant increase most especially at higher dosage of lead exposure.

DISCUSSION

Lead is a toxic agent with multiple target systems or organs such as haematopoietic, immune, nervous and the kidney. After absorption into the blood 99% of lead is bound to erythrocytes and the remaining 1% stay in the plasma to be carried to other tissues. Serum lead half life is around twenty five days [11]. In the present study, the increase in blood lead concentration following intraperitoneal administration might as a result of rapid absorption by the blood and it strong affinity for the red blood cells.

Developments of basophilic stippling and Howell-jolly bodies are features of anaemia due to lead intoxication [12, 13]. This is consistent with our findings. The presence of nucleated red blood cells has also been reported in some cases [12]. Anaemia, reticulocytosis and basophilic stippling have been recognized in lead poisoning; this might be due to lead binding avidly to the red cells with the abnormalities being confined to the erythroid series with ineffective erythroid hyperplasia being prominent [13]. The basophilic stippling has been attributed to the accumulated pyrimidine nucleotides which inhibits ribonucleic acid breakdown, resulting in aggregates of undegraded and partially degraded ribosomes which causes basophilic stippling [14].

Lowered red blood cell count, decrease haemoglobin concentration, decrease mean corpuscular volume and mean corpuscular haemoglobin concentration in this study is suggestive of lead induced anaemia. The anaemia is microcytic hypochromic. This might be due to lead altering the properties of haemoglobin by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable, which probably results in cell swelling, deformation and damage. Yagminas *et al.* [15], reported decrease in red blood cell count, mean corpuscular volume and mean corpuscular haemoglobin in rats exposed to lead acetate, while Vutkuru [16] and Shalaby [17] reported significant decrease in red blood cell count, haemoglobin concentration and packed cell volume of fresh water fish exposed to lead.

Lead induced inhibitory effects on the erythrocyte enzymes galactose-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase have already been reported [18]. Interaction of lead with haem biosynthesis has been related to the inhibition of cytoplasmic and mitochondrial enzyme [5] and a decrease in the activity of the main enzymes in haem biosynthesis due to defect in iron metabolism [5, 15, 18].

The increase in the total leukocyte count observed in this study was contributed majorly by the neutrophils and secondly by the lymphocytes. The leukocytosis observed could be attributed to lead induced inflammation.

Hogan and Adams [19] reported a three fold increase in neutrophil and monocyte count along with severe leukocytosis in the young rats that were exposed to lead.

There was also lead induced eosinopenia which was similar to the observation of Xintaras [20]. There was also lead induced thrombocytosis most especially at higher dosage.

In conclusion, this study has further confirmed haematotoxic effect of lead, most especially on the erythroid cell lineage and the leukocytes following exposure to lead. The haematological parameters observed in this study could be used as a biomakerof lead toxicity most especially as environmental lead pollution cannot be underestimated in our major cities in the developing countries.

REFERENCES

1. Yang, H. and N.L. Rose, 2003. Distribution of Mercury in the Lake Sediments across the United Kingdom. Science Total Environ., 304: 391-404.
2. Okediran, B.S., E. Abam, O.O. Odukoya, I. Adamson and O. Ademuyiwa, 2009. Membrane Intracellular, Plasma and Urinary Sodium and Potassium in Occupational Lead Exposure: Effect of Vitamin C Supplementation. Trace Element Electrolytes, 26: 49-59.
3. Silbergeld, E.K., 1997. Preventing Lead Poisoning in Children. Annual Review of Public Health, 18: 801-810.
4. Ghorbe, F., M. Boujelbene, F. Makni-Ajadi, F. Guermazi, A .Kammoum and J.C. Murat, 2001. Effect of Chronic Lead Exposure on Kidney Function in Male and Female Rats: Determination of a Lead Exposure Biomarker. Archive of Physiological Biochemis., 109: 457-463.
5. A.T.S.D.R. (Agency for Toxic Substances and Diseases Registry) 1993. Toxicological Profile for Lead. Prepared by Clement International Corporation Under Contract no. 205-88-060 for ATSDR, U.S.A. Public Health Services, G.A. Atlanta,
6. Harbison, R.D., 1998. Lead. In: Harbison, R.D (ed). Hamilton and Hardy's industrial Toxicology. 5th edition. Mosby, Philadelphia, pp: 70-76.
7. Howard, D., D.V.M. Stowe, R.A. Goyer and M. Krigman 1973. Experimental Oral Lead Toxicity in Young Dogs. Archive of Pathol., 95: 106-116.
8. A.T.S.D.R. (Agency for Toxic Substances and Diseases Registry) 2005. Draft Toxicological Profile for Lead. U.S.A. Department of Health and Human Services. Atlanta Georgia, pp:102-225.
9. Schalm, O.W., N.C. Jain and E.J. Carrot, 1975. Veterinary Haematology. 3rd edition, Eds. Lea and Febiger, Philadelphia, pp: 20-28.
10. Van Kamper, 1961. Standardization of Haemoglobinometry. Clinical. Chemis., 6: 438-544.
11. Zook, B.C., 1972. Lead Poisoning in Dogs. American J. Veterinary Res., 33: 902-981.
12. Falke, H.E. and W.C.M. Zwennis, 1990. Toxicity of Lead Acetate to Female Rabbits after Chronic Subcutaneous Administration: Biochemical and Clinical Effects. Archive of Toxicol., 64: 522-529.
13. Albahary, C., 1972. Lead and Haemopoiesis. American J. Medic., 52: 367-378.
14. Goldberg, A., 1972. Lead Poisoning and Haem Biosynthesis. Britain J. Haematol., 23: 521-524.
15. Yagminas, A.P., C.A. Franklin, D.C. Villeneuve, A.P. Gilman, P.B. Little and V.E. Valli, 1990. Subchronic Oral Toxicity of Triethyllead in the Male Weanling Rat. Clinical, Biochemical, Haematological and Histopathological Effects. Fundamamental Applied Toxicol., 15: 580-596.
16. Vutkuru, S.S., 2005. Acute Effects of Hexavalent Chromium on Survival, Oxygen Consumption, Haematological Parameters and some Biochemical Profiles of the Indian Major carp, Labeo rohita. International J. Environmental Research Public Health, 2: 456-462.
17. Shalaby, A.M., 2001. Protective Effect of Ascorbic acid against Mercury Intoxication in Nile Tilapia, (*Oreochromis niloticus*). J. Egypt Academy for Society Environmental Development. CD-Environmental Stud., 2(3): 79-97.
18. Calderon-Salinas, V., C. Hernandez-Luna, M. Maldonado and D. Saenz, 1993. Mechanisms of the Toxic Effects of Lead. I. Free Lead in Erythrocytes. J. Exposure Analytical Environmental Epidemiol., 3(1): 153-164.
19. Hogan, G.R. and D.P. Adams, 1979. Lead Induced Leukocytosis in Female Mice. Archive of Toxicol., 41: 295-300.
20. Xintaras, C. , 1992. Impact of Lead Contaminated Soil on Public Health. Analysis Paper, U.S.A. Department of Health and Human Services. Public Health Service., Agency for toxic substances and Disease Registry.