

## EFFECTS OF CHRONIC LEAD ACETATE INTOXICATION ON BLOOD INDICES OF MALE ADULT RAT

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### ABSTRACT

Lead as one of the environmental pollutants can threaten the life of living creatures in many ways. In this study, hematological effects of chronic toxicity of the lead acetate in adult male rats through measurement of the lead concentration in the blood of animal's heart by atomic absorption as well as hematological analyses and differential cell count were investigated. Results showed that lead concentration in the treatment group was significantly higher than that of the control groups ( $P < 0.001$ ), and basophilic stippling, Howell-Jolly bodies, decreased RBC count (anemia), increased leukocyte count (leukocytosis), monocytosis, eosinopenia, neutrophilia, and thrombocytosis were observed in the test group ( $P < 0.001$ ). It is concluded that microcytic hypochromic anemia can be attributed to the interaction of lead with iron and copper metabolism and increased leukocyte count may be linked to the inflammatory effects of lead on lymphatic organs.

**Key words:** Lead, Hematology, Erythrocyte, Leukocyte, Rat.

### INTRODUCTION

Due to the industrial processes and smokes from petrol vehicle (1) lead is considered as one of the major environmental pollutants (2). Although lead is eliminated from petrol in many countries, but it may have other origins such as industrial pollution (2,3). Occupational lead exposure may occur during the manufacture of batteries, painting, printing, pottery glazing, and lead smelting processes. Exposure may also occur during the construction of tank linings, piping and other equipments that carries corrosive gases and liquids, superconductors, and fiber optic technologies (3-5), during magnetic resonance imaging, and nuclear medicine (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 WHO and other health organizations (4,6).

There are evidences, which show that lead is a toxic agent with multiple target organs such as hematopoietic system, immune system, kidneys, and nervous system (5). There are some controversies over the influence of lead on hematological parameters.

Lead is absorbed through digestive and respiratory tracts, and skin. After absorption into

the blood, 99% of lead is bound to erythrocytes and the remaining 1 percentage stay in plasma to be carried to other tissues. Serum lead half-life is around 25 days (7). In a study performed on young dogs, basophilic stippling of the RBCs, nucleated RBCs, and proteinuria were observed (8). Also development of anemia, leukocytosis, monocytopenia, polychromatophilia, glycosuria, increased serum urobilinogen, and hematuria has been reported (8). In more advanced cases of lead toxicity, absolute neutrophilia, leukocytosis (with shifting to left), eosinopenia, and monocytopenia have been reported (9). In another study, lead was shown to induce microcytic hypochromic anemia that was due to interference with iron and copper metabolism (10). Furthermore, decreased hematocrit and hemoglobin levels might arise from reduction in serum copper as well as reduced iron metabolism and consumption induced by lead (5,10). Lead suppresses bone marrow hematopoiesis, probably through its interaction with the enteric iron absorption (10,11). Investigation of the toxicity of triethyl lead on some hematological indices has revealed a significant decrease in the MCH, MCV, and RBC count, and an increase in monocyte count, and platelets in comparison with the control

group (12). In a study on rats, 62 days after administration of lead, reticulocytosis and thrombocytopenia and after 92 days thrombocytosis were observed (13).

Administration of high doses of lead in female rabbits has caused mild anemia, reduced MCH, MCV, and MCHC; low ALAD enzyme activity in erythrocytes; and development of stippled RBCs, all of which disappeared when acute intoxication resolved (14). Investigation of effects of lead in amphibians demonstrated no changes in the normal range of hematologic parameters (15). There was only a significant increase in reticulocyte count that was considered as an early response to lead intoxication (15). Long-term administration of lead in monkeys did not show any significant changes in hematologic indices, blood biochemistry, and growth and development curves (16).

Taking into account existing controversies regarding the effects of lead acetate on the hematological parameters, present study was conducted to investigate the blood cell count and morphology in adult male rats following chronic lead intoxication in a 12-weeks period.

## MATERIAL AND METHODS

### Chemicals

All chemical materials that used in this study including lead acetate, glacial acetic acid, and ether were produced by Merck (Germany).

### Animals

Forty-five adult male Wistar rats were randomly selected and transferred to an animal house having standard conditions, at a temperature of 18-24°C and 12 hours light and darkness. Food & drinking water were available *ad libito*. A week after adaptation to the new environment, animals were divided into three groups of 15 rats. Rats in the test, negative, and positive control groups were given 1% lead acetate in 0.4% acetic acid, distilled water, and 0.4% acetic acid solution respectively in their daily water supply for 12 weeks. All animals were then anesthetized by ether and blood samples were prepared from their hearts.

### Assay by atomic absorption

For the assay of lead by this method, 2.5ml of blood was drawn into a capped test tube, and after treatment with 1ml of sodium ammonium pyrolidin dithio carbamate test tubes were capped and shaken tightly for 2 minutes. Following addition of 2.5ml of the normal butyl acetate to tube, they were shaken again fully. After high-spin centrifugation for 10 minutes,

the upper transparent layer was separated and transferred to a new and entirely clean-capped container and the plasma level of lead in ppm were calculated from the following formula

$$C = (K.A) * 20 + B$$

Where *C* is the concentration of lead (ppm) in plasma; *K* and *B*, are constant numbers equal to 89.628 and 3.3781 respectively; *A* is the amount of absorbed lead; and 20 is the dilution ratio of plasma for the atomic absorption apparatus.

### Blood analysis

A cell counter was used to analyze hematological indices including hemoglobin level, MCV, MCHC, RBC, and WBC counts, and the amount of platelets. Blood smears were also prepared from these blood specimens for manual differential cell counts.

### Statistical analysis

One-way analysis of variance (ANOVA) and Tuckey HSD statistical tests were used to compare these parameters in the study groups. Results were displayed as means  $\pm$  standard deviation and in testing among p-values, those less than 0.05 were assumed significant.

## RESULTS

Serum level of the lead in three groups of this study was significantly higher in the test group in comparison with negative and positive control groups ( $p < 0.05$ ). However, no significant difference in lead concentration was found between control groups ( $p > 0.05$ ) (table-1). While hematological study in the group, which was given lead acetate, showed basophilic stippling of the cells together with the appearance of Howell-Jolly bodies, such changes were not observed in the negative and positive control groups.

**Table 1.** Calculated blood lead concentration by the atomic absorption apparatus in negative control, positive control, and lead acetate administration groups. Data are presented as mean  $\pm$  SD

Group	Blood lead conc. (mg/dl)	Blood lead conc. (ppm)
Negative control	13 $\pm$ 1.2	2 $\pm$ 0.4
Positive control	17 $\pm$ 3.1	2 $\pm$ 0.3
Lead acetate	85 $\pm$ 4 *	12 $\pm$ 0.8 *

mg/dl = milligram per deciliter, ppm = part per million, \* $p < 0.001$

RBC counts in the test group showed a 22.4% statistically significant decrease ( $p < 0.05$ ) compared to the control groups. Rats in this test group also demonstrated 4.8% and 3.2% decreases in the hemoglobin and hematocrit

**Table 2.** Hematological determinants in negative control, positive control, and lead acetate administration groups. Data are presented as mean  $\pm$  SD

Group	RBC count $1 \times 10^6$	Hemoglobin (g/dl)	Hematocrit (%)	MCH (pg)	MCV (fl)	Howell-Jolly bodies	Basophilic stippling
Negative control	$7.6 \pm 0.7$	$14.5 \pm 3$	$41 \pm 1$	$17.7 \pm 0.7$	$50.3 \pm 2.1$	–	–
Positive control	$7.9 \pm 0.3$	$14.4 \pm 8$	$41.1 \pm 2$	$17.9 \pm 0.7$	$51.2 \pm 1.9$	–	–
Lead acetate	$5.9 \pm 0.4$	$13.8 \pm 4$	$39.7 \pm 2$	$16.7 \pm 0.5^*$	$48.1 \pm 1.9^*$	+	++

g/dl = gram per deciliter, MCH = mean corpuscular hemoglobin, pg = Picogram, MCV = mean corpuscular volume, fl = femtoliter, the sign (–) means: did not observed, the sign (+) means: observed moderately, and the sign (++) means: observed numerously. \* $p < 0.05$

**Table 3.** The mean of hematological indices of leukocytes and platelets in negative control, positive control, and lead acetate administration groups. Data are presented as mean  $\pm$  SD

Group	Lymphocyte count $1 \times 10^3$ /ml	Monocyte count $1 \times 10^3$ /ml	Eosinophil count $1 \times 10^3$ /ml	Neutrophil count $1 \times 10^3$ /ml	Platelet count $1 \times 10^3$ /ml	Leukocyte count $1 \times 10^3$ /ml
Negative control	$9.5 \pm 0.8$	$0.47 \pm 0.09$	$0.42 \pm 0.04$	$5.2 \pm 0.2$	$917 \pm 55$	$15.6 \pm 0.8$
Positive control	$9.5 \pm 0.9$	$0.48 \pm 0.09$	$0.47 \pm 0.08$	$5.2 \pm 0.2$	$923 \pm 72$	$15.7 \pm 0.12$
Lead acetate	$10 \pm 0.7$	$0.80 \pm 0.03^{**}$	$0.13 \pm 0.04^{**}$	$6.3 \pm 0.2^{**}$	$1378 \pm 151^{**}$	$17.3 \pm 0.7^*$

ml = milliliter, \* $p < 0.05$ , \*\* $p < 0.001$

levels respectively in comparison with the control groups, which were not significant ( $p > 0.05$ ). MCH and MCV were also decreased by 5.6% and 4.4% respectively ( $p < 0.05$ ) which were significant (table-2). Leukocyte counts in the group, which were administrated lead acetate, increased significantly by 11%, compared to the control groups ( $p < 0.05$ ), which indicate leukocytosis (table-3). Study of the different kinds of leukocytes revealed a 5.3% increase of lymphocyte count in the test group in comparison with the control groups, which was not statistically significant ( $p > 0.05$ ). However, neutrophils and monocytes increased 21.2% and 70.2% respectively, which were statistically significant ( $p < 0.001$ ) and indicated lead-induced neutrophilia and monocytosis. A decrease of 69% in the eosinophil count of the test group was significantly different in comparison with the control groups ( $p < 0.001$ ), which suggest eosinophilia following lead intoxication in rats (table-3). Platelets in the lead administered group increased by 50.3% compared to the control, which was again significant ( $p < 0.001$ ) and suggest lead induced thrombocytosis (table-3). No significant differences were observed when hematological parameters between negative and positive control groups were compared ( $p > 0.05$ ).

## DISCUSSION

In the present study, significant increase in the blood and serum lead levels were found in the test group compared to the negative and positive control groups ( $p < 0.05$ ). Development of basophilic stippling and Howel-Jolly bodies, which are features of anemia due to lead intoxication, has been previously reported (8,14). The presence of nucleated RBCs has also been reported in some cases (8). Although not statistically significant, hemoglobin and hematocrit level also decreased in the test group ( $p > 0.05$ ), which in some reports has been attributed to decrease in copper metabolism and iron consumption (5,10,17).

Lowered RBC count, decreased MCH and MCV are other concordant hematological change were found in the group which lead acetate was administrated (12,14). 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, and interaction with reactions in which calcium is their secondary mediator. Lead induced inhibitory effects on the erythrocyte enzymes GA3PD and G6PD have already been proved (18). Interaction of lead with heme biosynthesis has been related to the inhibition of cytoplasmic and mitochondrial enzymes (5), and a decrease in

the activity of the main enzymes in heme biosynthesis due to defects in iron metabolism has also been reported (5,11,12,18).

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 (5,19,20).

The use of lead acetate in the erythroid tissue culture medium has shown that lead nearly inhibits the proliferation of erythroid lineage, and perturbs cell development and hemoglobin synthesis (21). In the present study, total leukocyte count had increased mainly due to an increase in neutrophil and monocyte count. There was also an increase in lymphocyte count, which was not statistically significant ( $p>0.05$ ). In some reports, leukocytosis has been attributed to the lead-induced inflammation (12). A three-fold increase in neutrophil and monocyte count along with severe leukocytosis in the young rats that were exposed to lead has also been reported (21). Controversies exist about monocytes; since in some studies lead-induced monocytopenia (8,9) and in others significant increases in monocyte count have been reported (12,21). The reason for such difference is probably due to the extent of lead-induced inflammation. Consistent with other reports severe eosinopenia were observed in this study (9). Platelet count showed considerable increase compared to the control

groups ( $p<0.001$ ). In the previous studies, some cases of thrombocytopenia after lead intoxication (13) followed by thrombocytosis have been reported (12,13), which is consistent with the findings of this study which was conducted over a long period of 12 weeks.

This investigation shows additional hematotoxic effects of lead on the erythroid cell lineage and leukocytes following long-term exposure in rats. Severe changes in blood indices by lead that were found in the present investigation and other studies indicate the necessity of even more concerns about the bio-environment pollution of lead. Designation and provision of the health programs to limit causal exposure to this toxic element is highly important for our health.

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