EFFECTS OF CHRONIC LEAD INTAKE VIA DRINKING WATER ON SOME HEMATOLOGIC PARAMETERS IN MICE
Farelerde İçme Suyu ile Kronik Kurşun Alımının Bazı Hematolojik Parametreler Üzerine Etkisi

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Summary: *The aim of this study was to investigate the effects of chronic lead intake by drinking water at the dosages of 250 and 1000 ppm of lead (as lead acetate) on some hematologic parameter in albino mice. A total of 120 six-month-old 120 white male albino mice, weighing 35- 40 g fed with same pellet feed and water were used in this study. Animals were equally divided into three groups of 40 animals each. Group I served as a control. Group II and group III received 250 ppm and 1000 ppm lead acetate via drinking water during 120 days, respectively. Blood samples were taken by cardiac puncture on days 60, 90 and 120 of study from animals. On days 60 and 90 there were no statistical difference in hematological parameters between groups. On day 120, mice in group III received 1000 ppm lead ecetate had significantly lower erythrocyte count, hematocrit and hemoglobin, MCV and MCH values than the control mice. Additionally, platelet counts, total leukocyte counts and lymphocyte ratios were significantly lower in lead-treated mice than the control animals. On the other hand, neutrophil ratio in lead treated mice were significantly elevated compared with the control mice. In conclusion, it was observed that lead acetate at the dose of 250 ppm does not affect the hematologic parameters while lead acetate at the dose of 1000 ppm leads to hematological disorders.*

Key words: *Mouse, chronic lead intake, hematologic parameter*

Özet : *Bu çalışma, 250 ve 1000 ppm dozunda kurşun asetat içeren içme sularının farelere kronik uygulanmasının bazı hematolojik parametrelere-lere etkisini incelemek amacıyla yapılmıştır. Çalışmada 6 aylık, 35-40 g ağırlığında 120 erkek beyaz fare kullanıldı. Hayvanlar her bir grupta 40 fare olacak şekilde üç eşit gruba ayrıldı. I.grup control grubu olarak tutulurken II. ve III. gruptaki farelere 250 ve 1000 ppm kurşun asetat içeren su 120 gün süreyle verildi. Hayvanlardan 60., 90. ve 120. günlerde kalpten kan örnekleri alınarak incelendi. İncelenen hematolojik parametrelerde 60. ve 90. günlerde her hangi bir farklılık belirlenemedi. 1000 ppm dozda kurşun alan III. gruptaki hayvanların kontrol gurubuna göre eritrosit, lökosit, platelet sayısı ve lenfosit oranı ile ile hemoglobin, hematokrit, MCV ve MCH değerlerinde önemli bir azalma gözlenirken nötrofil oranında ise önemli bir artma tespit edilmiştir.*

Sonuç olarak, 250 ppm dozda kronik olarak kurşun alımının hematalojik parametreleri etkilemediği buna karşılık 1000 ppm dozda kurşun asetatın alımının ise hematolojik bozukluklara yol açtığı gözlenmiştir.

Anahtar kelimeler: *Fare, kronik kurşun alımı, hematolojik parametre*

Lead is a common cause of poisoning of domestic animals throughout the world. Lead level in air, water and soils have been increasing during the last years, both in urban and petiurban areas. In terms of potential adverse effects on animal and human health, lead is among the substance that has caused most concern. Lead is considered to be one of the most important environmental pollutants and has been accused as a cause of accidental poisoning in domestic animals more than any other hazardous

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substance. Cattle, sheep and horse are the most susceptible animals. However, lead poisoning can occur in all domestic animals such as poultry and dogs (1). Some of the potentially lead most contaminating activities are related to lead mining, lead smelting and battery treatment. Areas near lead industrial establishments may be enriched by aerial deposition of particles. As a consequence, soil pollution and quality deterioration of edible portions of vegetation can be produced due to metal enrichment. Animals eating this vegetation can accumulate enough lead to produce clinical signs of lead poisoning. It induces physiological, biochemical and behavioral dysfunctions (2).

The aim of this study was to investigate the effects of the chronic lead intake via drinking water at the dosages of 250 and 1000 ppm of lead (as lead acetate) on the some hematologic parameters in male albino mice.

MATERIALS AND METHODS

In this study, 120 white male albino mice, weight of 35 and 40 g, and 6 months old were used. Animals were equally divided into three groups of 40 animals each. Group I served as a control. Group II and group III received 250 ppm and 1000 ppm lead acetate via drinking water during 120 days, respectively. The animals were kept in cages, fed with pellet feed and given *ad libitum* drinking water including lead acetate. The animals were kept at 22- 24 ^{0}C with 12 h light/dark cycle.

For the hematologic analysis, blood samples were taken by cardiac puncture on days 60, 90 and 120 of study from animals.

Hematologic parameters were analyzed with an automated hematology cell counter (Symex SE-9000) and included total erythrocyte (RBC), leukocyte (WBC) and platelet counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), hematocrit value, and percentage of neutrophil, lymphocyte, monocyte, eosinophil and basophil.

Data were analyzed by SPSS 10.0 version for windows. The differences between groups were determined with variance analysis (ANOVA). When the differences were significant, Duncan's multiple range test was performed. Means were considered significantly different at $p< 0.05$. Data were expressed as means ± SD.

RESULTS

Results concerning hematologic parameters are given in Table I.

On days 60 and 90 there were no statistical difference in hematological parameters between groups.

On day 120, mice consumed 1000 ppm dose of lead acetate showed a significant decrease ($p<0.05$) in leukocyte, erythrocyte, platelet counts, lymphocyte ratio and in hemoglobin, hematocrit, MCV and MCH values while neutrophil ratio increased.

Table I. The effect of the chronic lead intake at various dosages on the blood parameters of mice in control group (n= 40) and experimental groups (n= 80)

Parameter	Groups	Day 60	Day 90	Day 120
Erythrocyte	Control	10.6 ± 1.4	10.4 ± 1.2	10.3 ± 1.3[a]
($x10^6/mm^3$)	250 ppm	9.9 ± 1.7	10.1 ± 1.2	10.2 ± 1.3[a]
	1000 ppm	9.8 ± 1.9	9.6 ± 1.8	7.5 ± 1.6[b]
Leukocyte	Control	7.6 ± 1.3	6.8 ± 1.5	6.2 ± 1.2[a]
($x10^3/mm^3$)	250 ppm	6.9 ± 1.4	5.8 ± 1.2	6.1 ± 1.3[a]
	1000 ppm	7.2 ± 1.3	6.6 ± 1.4	4.1 ± 1.2[b]
Hemoglobin	Control	15.9 ± 2.3	15.2 ± 2.5	14.9 ± 1.7[a]
(g/ dl)	250 ppm	14.9 ± 2.1	15.3 ± 2.4	14.8 ± 1.9[a]
	1000 ppm	14.7 ± 1.3	14.9 ± 1.7	12.3 ± 1.1[b]
Hematocrit	Control	45.2 ± 5.7	44.3 ± 5.3	42.8 ± 4.9[a]
(%)	250 ppm	44.9 ± 4.8	45.8 ± 4.1	43.2 ± 4.0[a]
	1000 ppm	43.3 ± 6.1	44.9 ± 5.9	37.7 ± 5.3[b]
MCV	Control	43.7 ± 2.4	42.6 ± 2.1	42.3 ± 1.9[a]
(fl)	250 ppm	44.5 ± 2.5	43.1 ± 2.1	42.9 ± 1.9[a]
	1000 ppm	45.7 ± 3.1	44.9 ± 2.1	38.1 ± 1.8[b]
MCH	Control	18.9 ± 1.1	17.2 ± 1.3	16.9 ± 1.4[a]
(pg)	250 ppm	18.1 ± 1.2	17.8 ± 1.9	16.3 ± 1.8[a]
	1000 ppm	19.0 ± 1.3	17.7 ± 2.1	13.1 ± 1.3[b]
MCHC	Control	32.3 ± 2.2	31.9 ± 2.1	33.2 ± 2.8
(g/dl)	250 ppm	33.2 ± 2.1	31.4 ± 2.7	32.8 ± 2.4
	1000 ppm	34.1 ± 2.8	33.6 ± 2.1	33.2 ± 1.7
Neutrophil	Control	24.9 ± 2.1	25.3 ± 2.3	27.0 ± 2.4[a]
(%)	250 ppm	25.3 ± 2.5	28.3 ± 2.6	30.2 ± 3.2[a]
	1000 ppm	26.2 ± 2.3	29.2 ± 3.1	38.3 ± 2.4[b]
Lymphocyte	Control	72.3± 4.3	72.0 ± 4.7	69.7 ± 3.8[a]
(%)	250 ppm	71.1 ± 3.9	68.6 ± 4.1	67.0 ± 4.3[a]
	1000 ppm	69.6 ± 3.7	67.7 ± 4.3	58.9 ± 3.1[b]
Monocyte	Control	2.5 ± 0.7	2.1 ± 0.3	2.6 ± 0.9
(%)	250 ppm	3.1 ± 0.6	2.3 ± 0.2	2.1 ± 0.7
	1000 ppm	3.5 ± 0.8	2.4 ± 0.4	2.3 ± 0.5
Eosinophil	Control	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.1
(%)	250 ppm	0.3 ± 0.1	0.5 ± 0.3	0.5 ± 0.1
	1000 ppm	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.2
Basophil	Control	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
(%)	250 ppm	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
	1000 ppm	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
Platelets	Control	7.5 ± 0.8	7.2 ± 0.4	7.9 ± 0.2[a]
($x10^5/mm^3$)	250 ppm	7.1 ± 1.2	7.5 ± 1.1	7.3 ± 1.2[a]
	1000 ppm	8.0 ± 0.4	7.7 ± 0.6	5.1 ± 0.3[b]

[a,b]: Mean values with different superscripts in the column are significantly different ($p< 0,05$).
Data are the mean ± SD.

DISCUSSION

The values obtained in the current study for hematological parameters are within the normal physiological ranges (3).

Lead impacts many organ systems, but this study will focus on hematological effects.

In our study, on day 120, mice in group III had significantly lower erythrocyte count, hematocrit and hemoglobin, MCV and MCH values than the control mice. These findings are similar to those previously reported by earlier workers (4, 5). However, in this study, the decrease in the MCV and MCH values were also observed, which were not reported by Othman et al.(4). In one study (6), lead poisoning combined with cadmium in sheep and horses has been reported to reduce Hb concentration, hematocrit, MCV and MCH values. In this study, a important finding of chronic lead intake is anemia. The nature of anemia formation in lead poisoning is still unknown. (7). In another study, it was emphasized that the most important effect of acute lead intake was on the level of hemoglobin (8). This difference may depend on duration of intake and dose of lead. It was suggested that lead induced inhibition of haem synthesis and acceleration of erythrocyte deformation (9). Lead is known to have some toxic effects on membrane structure and function (10). The effects of erythrocyte membranes in particular, are analyzed because erythrocytes have a high affinity for lead, and are more susceptible to oxidative damage than other cells (11, 12). Osmotic susceptibilities of erythrocyte were reported to be increase in lead toxicity accompanied by decreased deformability and a shortened life span (13).

In contrast to our findings, Altinsaat et al. (8) reported that total white blood cell counts were not affected. In agreement with Othman et al. (5), we also found that platelet counts, total leukocyte counts and lymphocyte ratios were significantly lower in lead-treated mice than the control animals. On the other hand, neutrophil ratio in lead treated mice were significantly elevated compared with the control mice. Similarly, the increase in the percentage of neutrophils in guinea pigs was also reported by Altinsaat et al. (8). It was found that T-cell subpopulation was significantly lower and B-cell absolute counts were significantly higher in lead workers compared to the control workers (14). Individual differences in the susceptibility to the harmful effect of lead were reported by Van Den Heuvel (15) among human and murine hematopoietic progenitor cells. These different findings reported in the peripheral blood cell in probably result from effect of lead on progenitor cells.

In this study, it was observed that there was a significant difference between the experimental groups according to increase in the dose of lead except the MCHC, monocyte, eosinophile and basophile. Similarly, Jacob et al (4) found that hematocrit and hemoglobin levels increase with blood lead levels. The same outhor reported that a negative association between blood lead levels and MCV and MCH (4). On the other hand, Peng et al. (16) found that there was not any positive correlation between hematologic parameters and dose of lead except red cell distribution width (RDW). All of these findings suggest that the effect of lead on hematologic parameters might not be linear.

In conclusion, it was observed that lead intake via drinking water at the dose of 250 ppm does not affect the hematologic parameters while lead acetate at the dose of 1000 ppm causes some hematological disorders.

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