EFFECT OF ACUTE AND CHRONIC LEAD NITRATE EXPOSURE ON SOME PHYSIOLOGICAL PARAMETERS IN EXPERIMENTAL MICE

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ABSTRACT

The research include the study of the effect of lead in the form of lead nitrate on some hematological parameters (Hb concentration, total WBC count , differential WBC count) and liver enzymes, glutamate-pyruvate transaminase (GPT) or (ALT) and glutamate-oxaloacetate transaminase (GOT) or (AST) levels during the acute and chronic exposure in experimental mice , the research performed by feeding the mice to two different concentration of lead nitrate 50 ppm/kg and 100 ppm/kg for two exposure periods the acute (10days) and chronic (20 days) . the results showed that the lead in 50 ppm/kg con. in chronic period showed much less decrease in hemoglobin concentration, neutrophils percentage (6.9 g/100ml , 12.25% respectively) than in the acute period (9 g/100ml , 18.5% respectively) and an increase in total WBC_s count, basophils, eosinophils and lymphocytes percentage (5.01×10^3,2.25% , 4% and 65.25% respectively) much than in the acute period(3.11×10^3 , 0.75% , 0.75% , 63.25% respectively) , AST level increase significantly in the acute period of 100 ppm/kg exposure where as ALT level increase significantly in the acute period of 50 ppm/kg exposure , we also found that females were more hematologically affected with lead compared with males while the AST and ALT levels were high in males than females for both periods .

INTRODUCTION

The environmental pollutant is one of many factors affect adversely on both animal and human organisms [(1)] , lead is one of these pollutant so lead pollution constitute a major health problem that had been intensively debated [(2)] . Lead is not an essential element needed in cellular functions . in environment it may derived from either natural or anthropogenic sources ; it's level in soil is about 20 mg/kg but in the atmosphere lead concentration reached 50 pg/m^3 [(3)] . The systemic toxic effect of lead in human had been well documented by many of researchers [(4 , 5 , 6)]. Lead absorbed into the body and distributed in three major compartments : blood , soft tissue and the largest compartment are bones , almost all (99%) blood's lead is associated with erythrocyte and (50%) of erythrocyte's lead bound to the hemoglobin . Exposure to lead can be investigated by measuring erythrocyte's protoporphyrin (EP) , which is in blood samples a part of red blood cell that known to increase when lead amount in

the blood is high [7] . It is known that the lethal acute and chronic concentration of lead alert the immunological parameters [8] associated with assessment of some morphophysiological changes [9] . Blood's lead level change rapidly with the exposure and it used as an indicator of recent exposure with a small fraction of lead in plasma and serum [7]. Numerous studies in both occupationally exposed subjects and the general population had tried to correlated blood's lead level and the changing in hematological parameters , all parameters examined appear to be the most sensitive indicator of lead exposure [7 , 10 , 11] as the effect of lead on hemoglobin had been known , the potential consequences of lead reducing the heme synthesis were summarized by [12] . Since lead is an element, its neurotoxic actions might be due to interactions with other essential elements, and compete with them , especially divalent and monovalent cations such as Ca^{2+}, Mg^{2+}, Na^{+}, and Fe^{2+} [13] . the exposition to lead can damage living functions and this can open a pathway in the knowledge of the pathological processes that this metal causes in the organism in different organs such as liver [14] . There are two enzymes considered to be the most known indicators of liver damage the first is Glutamic-pyruvic transaminase (GPT) - also known as alanine aminotransferase or (ALT) is a cytoplasmic hepatocellular enzyme, the second is Glutamic-oxaloacetic transaminase (GOT) - also known as aspartate aminotransferase or (AST), is an enzyme found in the mitochondrion and cytoplasm of all cells [15].

Because of that animal studies provided a limited evidence that lead may affect the liver, the aim of the present study is to evaluate the changes or consequences of AST and ALT which indicate liver damage and dysfunction and also some hematological parameters during the acute and the chronic lead exposure and the differences of these parameters in both genders in experimental mice.

MATERIALS AND METHODS

The experiment was performed with 24 Balb/c mice the mean weight was about 18 gm , they were bred in the animal house in the department of biology / college of science . The animals were divided into six groups each group included 4 mice (2 females and 2 males) Three of the six groups used for the acute period of the experiment (10 days) the others used for the chronic period (20 days) they were fed with two kinds of fodder the first one contain's 50 ppm/kg lead nitrate while the second contain's 100 ppm/kg . The groups were distributed as following :

1- The acute period (10 days after feeding)

A_1 = group fed with 50 ppm/kg lead nitrate containing fodder.

B_1 = group fed with 100 ppm/kg lead nitrate containing fodder.

C_1 = control group fed with standard fodder.

2- The chronic period (20 days after feeding)

A_2 = group fed with 50 ppm/kg lead nitrate containing fodder.

B_2 = group fed with 100 ppm/kg lead nitrate containing fodder.

C_2 = control group fed with standard fodder.

The animals were autopsied in each period after they were anesthetized by chloroform . The thoracic cage was opened by surgical scissor and direct aspiration of blood from the heart using 2 ml syringe , blood immediately transferred to an EDTA tube (AFMA-DISPO) and mixed gently . Then blood parameters were analysed according to [16] it was included (haemoglobin concentration , Total white blood cells count , Differential Leukocyte count **)** . Sahli method was used to measure haemoglobin concentration . The total white blood cells count had been performed by the haemocytometer and the diluent solution (Turk's solution). The Differential Leukocyte count was performed with Gimsa stain [17] .

plasma separation by centrifugation (3000 rpm for 10 min.) alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured as indicators of liver function.

The statical analysis was performed by Minitab program using ANOVA test under probability ($p< 0.05$) .

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RESULTS

It had been noticed from observing the animals during the experiment periods an indolence in all treated animals (A_1 , B_1 , A_2 , B_2) with a clear weakness and weight loss compared with the control groups (C_1 and C_2) , also it had been noticed a corrosion in the extremities of the body (hands , feet and tail) of two animals (male and female) in group A_2 .Fig.(1&2).

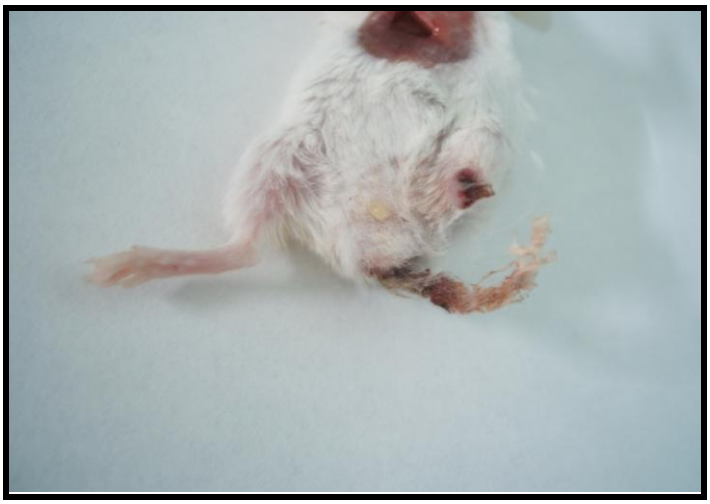

Fig. (1) : corrosion of foot and tail in male mouse in group A_2

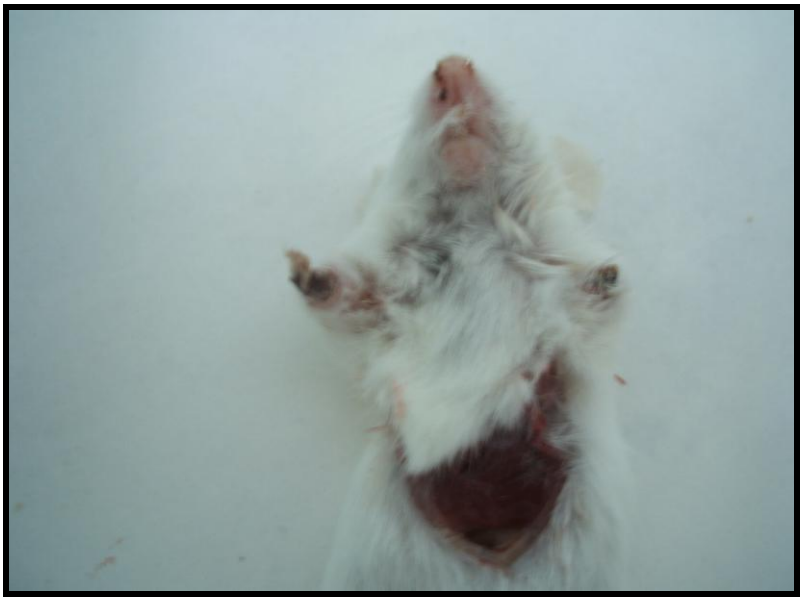

Fig. (2) : corrosion of hands in female mouse in group A_2

The results of blood parameters analyses in the acute period (table 1) showed a significant decrease in hemoglobin concentration in group B_1 compared with the control group where as there was a significant increase in total WBC_s count in group B_1 compared with the control group. The differential WBC_s count showed a significant decreased in neutrophils percentage (Neutropenia) in group B_1 compared with the control group. and a significant increase in lymphocytes percentage in both groups A_1 and B_1 compared with the control group .

Table (1): changes in some blood parameters in the acute period (10days)

groups	Hb con. g/100ml	Total WBC_s count $\times 10^3$	Differential WBC_s count				
			N.	B.	E.	M.	L.
A_1 N=4	9 ± 1.27	3.11 ± 0.79	18.5 ± 2.38	0.75 ± 0.5	0.75 ± 0.5	16.75 ± 2.75	63.25* ± 0.95
B_1 N=4	8.2* ± 1.64	5.52* ± 1.46	13.7* ± 3.86	0.75 ± 0.95	3.0 ± 1.82	19.0 ± 4.69	63.5* ± 6.75
C_1 N=4	11.7 ± 0.38	2.25 ± 0.19	34.25 ± 3.3	0.75 ± 0.5	1.5 ± 0.57	22.5 ± 1.91	43.0 ± 1.41

* Significant differences under probability ($p<0.05$)

The results of blood parameters analyses in the chronic period (table 2) showed a significant decrease in hemoglobin concentration in group A_2 compared with the control group. The differential WBC_s count showed significant decrease in neutrophils percentage (Neutropenia) in group A_2 compared with the control group , and a significant increase in lymphocytes percentage in both groups A_2 and B_2 compared with the control group .

Table (2): changes in some blood parameters in the chronic period (20days)

groups	Hb con. g/100ml	Total WBC_s count $\times 10^3$	Differential WBC_s count				
			N.	B.	E.	M.	L.
A_2 N=4	6.9* ± 2.02	5.01 ± 2.99	12.25* ± 7.13	2.25 ± 2.63	4.00 ± 4.69	16.25 ± 3.4	65.25* ± 11.84
B_2 N=4	8.6 ± 1.29	2.83 ± 1.16	13.25 ± 4.19	1.5 ± 1.0	1.5 ± 1.73	16.75 ± 2.7	67.0* ± 4.96
C_2 N=4	11.9 ± 1.17	2.88 ± 1.09	40.0 ± 3.36	0.25 ± 0.5	1.25 ± 0.95	14.5 ± 4.04	43.75 ± 7.13

* Significant differences under probability ($p<0.05$)

In AST, ALT analyses (table 3) showed a significant increase in AST and ALT in both concentration of lead nitrate (50 and/or 100 ppm/kg) and in both periods of exposure the acute and the chronic .

Table (3): AST and ALT levels in both concentration and both periods of the experiment

concentrations	periods	AST	ALT
50 ppm/kg Lead nitrate fodder	Acute	94.75 ± 24.58	77.0* ± 27.29
	Chronic	68.25 ± 13.05	31.25 ± 3.69
100 ppm/kg Lead nitrate fodder	Acute	98.00* ± 10.2	55.25 ± 10.24
	Chronic	58.5 ± 15.18	41.5 ± 13.63
control	Standard fodder	62.00 ± 15.77	33.5 ± 17.64

* Significant differences under probability ($p<0.05$)

Table 4 showed that the effect of lead on hematological parameters in the low concentration in females was higher than males in both periods where as hematological effect of lead in high concentration 100 ppm/kg appeare clearly in males animals more than females in both periods of the experiment . AST and ALT levels appeare to be higher in males animals than females.

Table (4) : differences in hematological values AST and ALT levels in males and females in both periods of the experiment

Con.	period	Gender	Hb con. g/100l	Total WBC count $\times 10^3$	Differential WBC count					AST U/L	ALT U/L
					N.	B.	E.	M.	L.		
50 ppm/kg	Acute	Female	8.2±0.56	2.67±0.38	20.5±0.7	1±0.00	0.5±0.7	14.5±0.7	63.5±0.7	82.5±30.41	58.5±13.44
		Male	9.8±1.41	3.55±0.98	16.5±0.7	0.5±0.7	1±0.00	19±1.41	63±1.41	107.0±16.97	95.5±26.16
	chronic	Female	6.6±1.98	6.27±4.49	7.0±1.41	1.5±0.7	1.5±0.7	18±4.24	72±4.24	75.5±3.54	29.0±2.82
		Male	7.2±3.25	3.75±0.63	17.5±6.36	3±4.24	6.5±6.36	14.5±2.12	58.5±14.85	61.0±16.97	33.5±3.53
100 ppm/kg	Acute	Female	9.6±0.56	6.2±2.05	11.0±1.41	0.5±0.7	3±2.82	16.5±0.7	69±2.82	91.0±4.24	48.5±2.12
		Male	6.8±0.00	4.85±0.63	16.5±3.53	1±0.00	3±1.41	21.5±6.36	58±2.82	105.0±9.9	62.0±11.31
	Chronic	Female	8.7±1.83	2.12±0.24	11.5±6.36	1±1.41	1.5±2.12	17.6±3.53	68.5±6.36	54.0±9.9	44.5±6.36
		Male	8.5±1.27	3.55±1.41	15.0±0.00	2±0.00	1.5±2.12	16±2.82	65.5±4.95	63.0±22.63	38.5±21.12

DISCUSSION

In this study the effect of acute and chronic lead exposure were detected by giving the experimental mice a diet containing lead nitrate .The corrosion of hands ,feet and tail in chronic exposed animal agreed with the fact that the largest fraction of lead retained in the body is found in the bone and it appear to specifically inhibit osteoblastogenesis in vivo in adult rats [7 , 18] .The effect on bones had been shown to be carcinogenic in rats and mice [3] and in a study of the effect of different concentration of lead acetate on bone, it had been found that after chronic exposure each mouse underwent a tibia fracture [19] .

In Haematology there was a significant decrease in hemoglobin concentration that may be resulted from the effect of lead on the haemopoietic system may cause anemia which may result from shortened life span of erythrocytes that due to increase of their membrane fragility, and in another hand, it may cause a decreased hemoglobin synthesis which may result from decreased levels of enzymes involved in heme synthesis [3 , 7] , the alteration in the heme biosynthesis pathway were demonstrated after the first week of exposure [20] .the mechanism seems that Lead inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Specifically, lead decreases heme biosynthesis by inhibiting δ-aminolevulinic acid dehydratase and ferrochelatase activity, this Ferrochelatase, which catalyzes the insertion of iron into protoporphyrin IX, is quite sensitive to lead. A decrease in the activity of this enzyme results in an increase of the substrate of erythrocyte protoporphyrin (EP), in the red blood cells [21] .

The high total WBC count in our result agreed with the results that found a high increase in total WBC count after acute and chronic exposure [8] , the increase in basophils , eosinophils and lymphocytes agreed with the investigation that found a high number of these types in a patient that had a high level of blood lead [22].

Acute high lead exposure can cause serious effects, including death or long-term damage to brain function and organ systems [21] .

The high levels of AST and ALT indicate a liver damage and dysfunction as a consequences of the lead ingestion, this hepatic dysfunction may due to metal accumulation in this organ [14] the differences in hematological results between males and females may explained by the physiological differences between the two genders and agreed with the results that found that lead acetate may stimulate leukocytosis in female mice [23]. The high levels of AST and ALT in males agreed with the results of researcher who found a high level of AST and ALT in males than females which may explained by that males may have a high blood lead level than females [24] .

تأثير التعرض الحاد و المزمن لنترات الرصاص على بعض المعايير الفسلجية في الفئران المختبرية

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الخلاصـــــة

تضمن البحث دراسة تأثير عنصر الرصاص و بشكل نترات الرصاص على بعض المعايير الدموية (تركيز خضاب الدم ، التعداد الكلي لخلايا الدم البيض ، العد التفريقي لخلايا الدم البيض) ومستوى انزيمي الكلوتاميت- بايروفيت ترانساميناز(ALT) و الكلوتاميت – اوكزالواستيت ترانساميناز (AST) و التي يحدثها التعرض الحاد و المزمن للعنصر في الفئران المختبرية إذ شمل البحث تعريض الفئران إلى تركيزين من نترات الرصاص هما 50 ppm/kg و 100 ppm/kg و لنوعين من التعرض الحاد (10 أيام) و المزمن (20 يوم) و قد أظهرت النتائج إن الرصاص بتركيز 50 ppm/kg و في التعرض المزمن يؤدي إلى انخفاض أكثر في كل من تركيز الهيموغلوبين و النسبة المئوية للخلايا العدلة يصل إلى (6.9 g/100ml ، 12.25% على التوالي) مقارنة مع التعرض الحاد والذي كانت قيمه (9 g/100ml ، 18.5% على التوالي) كما ظهرت زيادة في كل من العدد الكلي لخلايا الدم البيضاء و القعدات و الحمضات و الخلايا اللمفاوية(5.01×10^3 ، 2.25% ، 4% و 65.25% على التوالي) اكثر في التعرض المزمن مقارنة مع التعرض الحاد و الذي كانت قيمه (3.11×10^3 ، 0.75% ، 0.75% و 63.25% على التوالي). و ازدادت مستويات إنزيم AST و بشكل معنوي في التعرض الحاد لـ 100 ppm/kg في حين ارتفعت مستويات إنزيم ALT و بشكل معنوي في التعرض الحاد لـ 50 ppm/kg . كما اوضحت الدراسة ان التغيرات في المعايير الدموية تظهر بشكل اوضح في الاناث منها في الذكور في حين ان مستويات انزيمي AST و ALT ترتفع في الذكور منها في الاناث .

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