Effect of zinc toxicity on the blood parameters of the laboratory mice

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Abstract:

The present search conducted at the college of Veterinary Medicine – University of Basrah to investigate the effect of zinc toxicity on the physiological parameters, histopathological changes and reproductive efficiency in the laboratory mice.

In this search, 32 mice were used, they were divided into two groups, each one consisted of four equal subgroups with 4 mice in each. The first three subgroups of each group were dosed via intraperitoneal injection with 60, 80 and 100 mg/kg zinc in the form of $ZnSO_4$ for 15 and 30 days. An equivalent volume of saline (0.9 %N.S) was administered to the fourth subgroup which was set as the control group. The investigation of blood parameters included RBC count, Hb concentration, PCV concentration, total and differential WBC count. The results here showed a significant decrease ($P \leq 0.05$) of RBC count, Hb concentration, PCV value, and neutrophil count, whereas there was a significant increase ($P \leq 0.05$) of total WBC count, lymphocyte count, monocyte count, and there were no significant differences in the acidophil count and basophil count.

تأثير التسمم بالزنك على المعايير الدموية في الفئران المختبرية

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الخلاصة:

قسم ستين فأر مختبري إلى أربعة مجاميع، في كل مجموعه أربعة فئران ذكور،وجرعت المجاميع الثلاثة الأولى بمادة كبريتات الزنك بجرعة (60 mg/kg) للمجموعة الأولى ،(80 mg/kg) للمجموعة الثانية، و(100 mg/kg) للمجموعة الثالثة بطريقة الحقن داخل البريتون، بينما اعتبرت ألمجموعه الرابعة كمجموعة سيطرة وحقنت بالماء المقطر (0.9 % N.S) فقط.

درست التغيرات النسيجية لكبد وكليتي جميع الفئران وكانت النتائج ظهور العديد من التغيرات المرضية للمجاميع الثلاثة الأولى مثل حدوث تنخر، تورم، احتقان لخلايا الكبد والكليتين للحيوانات المعاملة بكبريتات الزنك مع حدوث اختفاء معالم الخلايا وحدودها، التهاب ، تجمع خلايا التهابيه وغيرها بالمقارنة مع كبد وكليتي حيوانات مجموعة السيطرة.

Introduction:

Excessive zinc can cause as many problems in the body as deficiency. The first signs of zinc toxicity include reduced feed intake, reduced weight gain, bone resorption. As the animal receives higher levels of zinc or toxic amount for long periods of time the animal will suffer from diarrhea, internal hemorrhage when mice take a low – dose zinc, the Hb and erythrocyte levels increase significantly (1,2). Whereas Matia *et al*. (1981) observed the high dose of zinc has decreased the Hb, erythrocyte and hematocrit levels significantly in both male and female. Leukocyte levels were also observed to decrease in the high – dose male mice, the study did not show any adverse clinical signs or increases in mortality, and the study has shown also that the body weight gain decreased in the high – dose male rats. Several significant alterations in serum clinical chemistry parameters were observed in the high – dosed rats (3) these included a decrease in serum total protein, cholesterol and calcium levels.

Groups of 13 males and 16 females Wister rats were exposed to (0 and 0.12 mg zinc/ml as zinc chloride) in the drinking water for four weeks. The study estimated the daily drinking water dose should not exceed 11.66 mg zinc/kg – day in the water in males and 12.75 mg zinc/kg – day in females. Although significant decreases in food and water intake were observed, the body weight gain was not significantly different from the control group. Significant alterations were observed in several hematological end points including a decrease in erythrocyte and Hb level and an increase in total white blood cell (WBC) and differential number (neurophils and lymphocytes) (4).

Patients with zinc toxicity had neutropenia and anemia of either the microcytic or sideroblastic variety (5). There was a significant decline in serum ferritin and hematocrit values at ten weeks of 50 mg supplemental zinc for one day or 0.23 mg/kg supplemental zinc for one day given to 18 healthy women (6).

High doses of zinc inhibit the hematopoiesis in both human and rabbits bone marrow and cause hepatospleenomegaly (7).

According to Matia *et al*. (1981), there was an alteration in serum clinical chemistry parameters which were observed in high – dose mice, including a decrease in total protein, glucose and cholesterol and an increase in alkaline phosphatase and urea nitrogen (3).

Chronic zinc toxicity causes copper deficiency, leucopenia, neutropenia, decreased erythrocyte superoxide dismutase (ESOD), decreased ceruloplasmin and decreased glucose (7 and 8).

Materials and methods:

The experiment was conducted at the animal house of the Veterinary Medicine College – University of Basrah, where 32 males mice sexually mature 12 weeks age and 20-25 grams weight were used. The experiment conditions were unified for all animals, where the room temperature was set between 20-25 ℃ by the use of air conditioner, and the light period was 12 hours daily, by the use of two

fluorescent lamps, and the humidity rate was about 50%. Food and water were provided daily (*ad libitum*).

Experimental design

LD_{50} of zinc was 116 (9). The experiment was divided into two parts:

The experiment was divided into two groups with 16 male mice each. The two groups were:

- ***First group***: consisted of the following subgroups and the hematology tests were done after 15 days of injection.

Treated 1 (T1) group: This group consisted of 4 male mice which were injected intraperitoneally (I.P) with 60 mg/kg of zinc as zinc sulphate ($ZnSO_4$) daily.

Treated 2 (T2) group: This group consisted of 4male mice which were injected I.P with 80 mg/kg $ZnSO_4$ daily.

Treated 3 (T3) group: In this group, 4 male mice were injected I.P with 100 mg/kg $ZnSO_4$ daily.

Control group: In this group, 4 male mice were injected I.P with 0.9% normal saline (N.S) daily.

- ***Second group***: consisted of the following subgroups and the hematology tests were done after 30 days of injection.

Treated 1 (T1) group: In this group, 4 male mice were injected I.P with 60 mg/kg $ZnSO_4$ daily.

Treated 2 (T2) group: In this group, 4 male mice were injected I.P with 80 mg/kg $ZnSO_4$ daily.

Treated 3 (T3) group: In which, 4 male mice were injected I.P with 100 mg/kg $ZnSO_4$ daily.

Control group: In this group, 4 male mice were injected I.P with 0.9% normal saline (N.S) daily.

Results:

1. Hematological results

1.1 Red blood cells count (RBC)

It seems from table (1) that the injection of zinc with 60 mg/kg,80 mg/kg and 100 mg/kg decreased the RBC significantly on ($p \leq 0.05$) after 15 days of the injection period T1 (6.437 cell/mm^3), T2 (6.363 cell/mm^3) and T3 (6.292 cell/mm^3) compared with the control one (6.510 cell/mm^3). It seems also that the T3 treatment decreased significantly more than the other two treatments compared with the control.

Table (1) shows also that when the zinc injection continued throughout 30 days the RBC was decreased significantly on ($p \leq 0.05$). The results indicated that the zinc toxicity with 100 mg/kg has the capability to decrease the RBC significantly (6.174 cell/mm^3) more than the other treatments T1 and T2 (6.372 cell/mm^3) and (6.297 cell/mm^3) respectively, whereas the T2 (80 mg/kg) decreased the RBC significantly more than T1 compared with the control (6.510 cell/mm^3).

The overall mean of the RBC results indicated that the zinc toxicity decreased the RBC significantly in T1, T2 and T3 (6.405 cell/mm^3), (6.330 cell/mm^3) and (6.233 cell/mm^3) respectively compared with the control. The findings in table (1) indicate that there were significant differences in all treated groups between 15 and 30 days periods.

TABLE (1): The effect of zinc toxicity on the red blood cells count.

parameters / Treatment	Red blood cell $\times 10^6$ cell/mm^3	
	15 days	30 days
Control 0.9% N.S	6.510 ± 0.01 A a	6.510 ± 0.01 A a
T1 60mg/kg	6.437 ± 0.02 B a	6.372 ± 0.02 B b
T2 80mg/kg	6.363 ± 0.02 C a	6.297 ± 0.02 C b
T3 100mg/kg	6.292 ± 0.04 D a	6.174 ± 0.01 D b
LSD	0.007	

- $(M \pm SD)$.
- ***N= 4.***
- The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Large letters refer to vertical comparable and small letters refer to horizontal comparable.

1.2 Hb concentration

The results of Hb concentration in the treated and control treatments are represented in table (2). The Hb results after 15 days of injection shows a significant decrease ($p \leq 0.05$) in T1 (12.138 g/dl), T2 (10. 825 g/dl) and T3 (9.450 g/dl) compared with the control one (13.438 g/dl). It seems that the Hb in T3 group which received (100 mg/kg) zinc decreased significantly more than T2 group which received (80 mg/kg) zinc and T1 group which received (60 mg/kg) compared with control one. However, it seems that Hb in T1 group was less significant than T2.

The overall mean of the Hb results indicated that the Hb were affected by zinc toxicity significantly when (60 mg/kg), (80 mg/kg) and (100 mg/kg) doses of zinc were used in 15 and 30 days periods of injection and the T3 was much more significant than the other two treatments in 15 days and 30 days respectively. The findings in table (2) indicate that there were significant differences in all treated groups between 15 and 30 days periods.

TABLE (2): The effect of zinc toxicity on the Hb concentration

Parameter / *Treatment*	*Hb concentration g/dl*	
	15 days	*30 days*
Control 0.9% N.S	13.438 ± 0.45 A a	13.438 ± 0.45 A a
T1 60mg/kg	12.138 ± 0.87 B a	10.225 ± 0.58 B b
T2 80mg/kg	10.825 ± 0.94 C a	9.100 ± 0.30 C b
T3 100mg/kg	9.450 ± 0.55 D a	7.438 ± 1.16 D b
LSD	0.577	

- ***(M±SD).***
- ***N= 4.***
- The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Large letters refer to vertical comparable and small letters refer to horizontal comparable.

1.3 PCV value

Depending on the results clarified in table (3), it seems that there is a significant decrease ($p \leq 0.05$) of PCV in treatment groups T1 (24.500), T2 (20. 375) and T3 (17.625) compared with the control one (26.875) of the 15 days of injection. The result of PCV in T3 showed that the use of (100 mg/kg) dose affects the PCV much more significantly than T1 and T2. It shows also that T2 group was affected more than T1. Table (3) shows that when the injection continues throughout 30 days, the PCV result decreased significantly in T3 (14.000) compared with the control (26.875) and the other treatments T1 (19.650) and T2 (17.813) and the T1 was less significant than T2.

The overall mean indicated that the zinc toxicity affects negatively the PCV values in T1, T2 and T3, and it seems that the T3 was much affected by the zinc toxicity than the other treatments. The findings in table (3) indicate that there were significant differences in all treated groups between 15 and 30 days periods.

TABLE (3): The effect of zinc toxicity on the PCV concentration.

parameters / *Treatment*	*PCV %*	
	15 days	*30 days*
Control 0.9% N.S	26.875 ± 2.23 A a	26.875 ± 2.23 A a
T1 60mg/kg	24.500 ± 0.92 B a	19.650 ± 0.83 B b
T2 80mg/kg	20.37 ± 1.84 C a	17.81 ± 0.57 C b
T3 100mg/kg	17.625 ± 1.18 D a	14.000 ± 0.82 D b
LSD	1.421	

- ***(M±SD).***
- ***N= 4.***
- The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Large letters refer to vertical comparable and small letters refer to horizontal comparable.

1.4 *White blood cell count (WBC) cell/mm^3*

The effect of zinc toxicity on WBC in both periods 15 and 30 days is displayed in table (4). Zinc toxicity in T1, T2 and T3 increased significantly ($p \leq 0.05$) the WBC (3.767 cell/mm^3), (3.951 cell/mm^3) and (4.019 cell/mm^3), respectively in 15 days and (3.767 cell/mm^3), (3.951 cell/mm^3) and (4.019 cell/mm^3) in 30 days respectively compared with the control group (3.555 cell/mm^3). It seems that the T3 had a higher effect on WBC compared with the other two groups, and T2 was affected more than T1.

The overall mean of zinc toxicity indicated that the high dose of the zinc led to an increase in WBC than the other treatments, and the T1 was less affected than T3 by zinc injection. The findings in table (4) indicate that there were significant differences in all treated groups between 15 and 30 days periods.

TABLE (4): The effect of zinc toxicity on the white blood cells count.

parameters / Treatment	$WBC \times 10^3$ cell/mm^3	
	15 days	*30 days*
Control 0.9% N.S	3.555 D a ± 0.77	3.555 D a ± 0.7
T1 60mg/kg	3.767 C b ± 0.06	3.893 C a ± 0.04
T2 80mg/kg	3.951 B b ± 0.02	4.084 B a ± 0.01
T3 100mg/kg	4.019 A b ± 0.02	4.184 A a ± 0.02
LSD	0.014	

- ***(M±SD).***
- ***N= 4.***
- The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Large letters refer to vertical comparable and small letters refer to horizontal comparable.

1.5 Neutrophil %

Table (5) indicates that the impact of zinc toxicity could affect the neutrophil count. It appears from the results in table (5) that the neutrophil count decreased significantly ($p \leq 0.05$) in T1 treated group (41.375 %) compared with control one (57.000 %). However, T2 and T3 treated mice groups, showed a significant decrease in the neutrophil count (31.875 %) and (27.500 %) respectively compared with the control one. There were no significant differences between them.

When the injection continues during 30 days the result showed a very high significant decrease in T3 treated group (20.750 %) compared with the control one (57.000 %). On the other hand, the neutrophil count in T1 and T2 were affected by zinc toxicity and both groups showed a significant decrease in the neutrophil count (33.000 %), and (26.125 %) compared with the control but they were not any significant difference between both treatment groups.

The overall mean indicated a high significant reduction in the neutrophil count in T3 treated group compared with the control and other treatments. The T2 treated group was affected by the zinc, and the neutrophil was fallen down (29.000 %) compared with the control, and it shows a significant

difference from T1 treated group results (37.188 %), which showed a significant reduction in the neutrophil count compared with the control. The findings in table (5) indicate that there were significant differences in all treated groups between 15 and 30 days periods.

TABLE (5): The effect of zinc toxicity on the neutrophil %.

parameter / *Treatment*	*Neutrophil %*	
	15 days	*30 days*
Control 0.9% N.S	57.000 ± 1.25 A a	57.000 ± 1.25 A a
T1 60mg/kg	41.375 ± 1.28 B a	33.000 ± 1.50 B b
T2 80mg/kg	31.875 ± 2.19 C a	26.125 ± 0.06 C b
T3 100mg/kg	27.500 ± 0.75 D a	20.750 ± 0.56 D b
LSD	1.59	

- $(M \pm SD)$.
- ***N= 4.***
- The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Large letters refer to vertical comparable and small letters refer to horizontal comparable.

1.6 *Acidophil %*

Table (6) shows that the injection of zinc for 15 and 30 days had no effect on the acidophil count, but it seems that the overall mean result showed a decrease in acidophil count in treated groups (3.063%), (2.625%) and (2.938%) compared with the control one (3.750%).

1.7 *Basophil %*

Table (7) shows the effect of zinc on the basophile count after 15 and 30 days. The results showed that there was not any effect of zinc on the basophile count after 15 and 30 days of treatment period, though a little insignificant increase in T3 treated group after 15 and 30 days of treatment was seen. The overall means of treated animals of the 15 and 30 days show some insignificant values as well.

TABLE (6):The effect of zinc toxicity on the acidophil %.

parameters / *Treatment*	*Acidophil %*	
	15 days	*30 days*
Control 0.9 % N.S	3.750 ± 0.63	3.750 ± 0.63
T1 60 mg/kg	3.250 ± 0.38	2.875 ± 0.22
T2 80 mg/kg	2.500 ± 0.50	2.750 ± 0.38
T3 100 mg/kg	2.875 ± 0.43	3.000 ± 0.50
LSD	Not significant	

- ***(M±SD).***
- ***N= 4.***
- There are no significant differences among groups at level of ($p \leq 0.05$).

TABLE (7): The effect of zinc toxicity on the basophil %.

parameters / *Treatment*	*Basophil %*	
	15 days	*30 days*
Control 0.9% N.S	3.750 ± 0.63	3.750 ± 0.63
T1 60mg/kg	3.250 ± 0.38	2.875 ± 0.22
T2 80mg/kg	2.500 ± 0.50	2.750 ± 0.38
T3 100mg/kg	2.875 ± 0.43	3.000 ± 0.50
LSD	Not significant	

- ***(M±SD).***
- ***N= 4.***

❖ There are no significant differences among groups at level of ($p \leq 0.05$).

1.8 Lymphocyte %

According to the results indicated in table 8, the zinc toxicity has a high significant effect ($p \leq 0.05$) on the lymphocyte count. It seems that the zinc toxicity in the T3 treated group increased significantly (53.625%) compared with T2 (50.250%) and T1 (54.000%) after 15 days of injection, and the table shows also that the T2 treated group was affected significantly more than T1 group after 15 days of injection. Whereas when the injection was continued till 30 days, the results showed an increase in lymphocyte in treated groups T1 (51.000%), T2 (54.500%) and T3 (59.375%) compared with the control one (33.500%).

It seems from the result of the 30 days that there was a high increase in T3 compared with the other treatments T1 (51.000%), T2 (54.500%) and the control (33.500%). It seems also that the T2 treated group was more increased significantly than T1. The findings in table (8) indicate that there were significant decreases in all treated groups between 15 and 30 days periods.

TABLE (8): The effect of zinc toxicity on the lymphocyte %.

parameters / *Treatment*	*Lymphocyte %*	
	15 days	***30 days***
Control 0.9% N.S	33.500 ± 1.25 D a	33.500 ± 1.25 D a
T1 60mg/kg	45.000 ± 1.00 C b	51.000 ± 1.00 C a
T2 80mg/kg	50.250 ± 1.25 B b	54.500 ± 0.50 B a
T3 100mg/kg	53.625 ± 0.88 A b	59.375 ± 0.88 A a
LSD	2.153	

❖ ***(M±SD).***

❖ ***N= 4.***

❖ The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Large letters refer to vertical comparable and small letters refer to horizontal comparable.

1.9 Monocyte %

Effect of zinc on the monocyte count is exhibited in table (9).

The treatment groups were affected and increased significantly ($p \leq 0.05$) T1 (6.625%), T2 (8.625%) and T3 (10.750%) compared with the control one (4.000%) during of the15 days of injection. It seems also the T3 was affected much more than the other treatments. Table (9) shows the effect of zinc on the monocyte after 30 days of treatment. It appears that there was a significant increase ($p \leq 0.05$) in the treatment groups T1 (8.625%), T2 (8.250%) and T3 (8.875%) compared with the control one (4.000%), but without any significant differences among the treated animals.

The overall mean indicated a significant increase in the treated animals T1(7.625%), T2 (8.438%) and T3 (9.813%) compared with the control group. The monocyte in treatments groups with T3 (9.813%) showed a high significant increase compared with the other treatment groups.

The findings in table (8) indicated that there were significant decreases in all treated groups between 15 and 30 days periods except in T2 group there was not any significant differences between 15 and 30 days period.

TABLE (9): The effect of zinc toxicity on the monocyte %.

parameters / *Treatment*	*Monocyte %*	
	15 days	*30 days*
Control 0.9% N.S	4.000 ± 0.50 D a	4.000 ± 0.50 B a
T1 60mg/kg	6.625 ± 0.47 C a	8.625 ± 0.47 A a
T2 80mg/kg	8.625 ± 0.63 B a	8.250 ± 0.56 A a
T3 100mg/kg	10.750 ± 0.56 A a	8.875 ± 0.66 A b
LSD	0.648	

❖ $(M \pm SD)$.

❖ *N= 4.*

❖ The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Large letters refer to vertical comparable and small letters refer to horizontal comparable.

Discussion:

Effect of zinc toxicity on the blood parameters

The results of the present search indicate that the treated mice with zinc toxicity in doses 60, 80 and 100 mg/kg daily for 15 and 30 days periods have shown a significant decrease ($P \leq 0.05$) in the blood parameters which included RBC count, Hb concentration and PCV concentration as represented in tables (1), (2) and (3) respectively compared with the control group which treated with 0.9 % N.S, and these results referred to anemia which is regarded as one of the toxicological signs with zinc element.

The above results corresponded with (4,6,10). It has been known that the causes of anemia may be due to copper deficiency which leads to antagonistic effect of zinc on copper uptake from the gastro – intestinal tract (GIT) (10), and that process made erythrocyte – superoxide dismutase (E – SOD) activity to decrease, this caused the protection against ROS within RBC reduces and then the damage of RBC occurs (7). Anemia could result from a decrease in the ceruloplasmin secondary to the decrease of copper which is a component of ceruloplasmin (11,7).

Zn – induced Cu deficiency can lead to a reduce in ceruloplasmin activity, which may result in trapping of iron within reticuloendothelial system. Consequently, iron is made unavailable for erythropoiesis and anemia ensue (2), or anemia occurs due to the competition between zinc and iron absorption, also zinc may affects iron metabolism by increasing iron turnover and decreasing the life span of erythrocytes (12).

Also anemia was probably associated with a defect of erythropoietin formation which is one of the important growth factors that regulate the production and maturation of red blood cell. It has been reported that 85 – 90 % of this hormone synthesis in the proximal tubules in the kidney, and these tubules were affected with zinc toxicity as shown in this study, that leads to a disturbance in the erythropoietin hormone and causes a decrease in the erythropoiesis process in the bone marrow and anemia will occur, in addition to, 10 – 15 % of erythropoietin product from the hepatic cells, zinc toxicity also causes a defect of the hepatic cells, that cause a reduction of erythropiotin hormone (13).

According to Julie *et al.*'s (2003) suggestion, the zinc toxicity causes an extensive vacculation of erythrocyte precursors in the bone marrow and that causes a defect of erythrocyte production which leads to anemia(10). The toxicological effect of zinc could be explained by accumulating the free radical of zinc ions (not connected with metallothionine) in the cells

especially fatty cells caused lipid acids which are present in the RBC membranes and that increases the osmotic fragility and the easy destruction of RBC, which caused anemia (14). Also the causes of anemia could be secondary to gastro – intestinal hemorrhage which may complicate significant zinc sulfate ingestion (15).

It seems that the mice in T3 group were more affected for the RBC count, Hb concentration and PCV concentration than the other two treatments T1 and T2 of each parameters, that is due to the increase of zinc concentration in the plasma which leads to an increase toxicity, the results showed also that there were more effects of the zinc toxicity on the 30 days period than 15 days period because of the accumulation of the zinc in the tissues especially liver, kidney and bone marrow tissues.

Effect of zinc toxicity on the total and differential WBC count

The effect of zinc toxicity on the total and differential WBC count in the T1, T2 and groups which were treated with 60, 80 and 100 mg/kg zinc respectively for the 15 and 30 days periods indicated a significant increase ($P \leq 0.05$) in the total WBC count, lymphocyte count and monocyte count, whereas the effect of zinc toxicity on the neutrophil count has shown a significant decrease ($P \leq 0.05$). The results showed also peroxidation mainly unsaturated fatty that there were not any significant differences between the acidophil and basophil count. These results agreed with(4,10,5), but disagree with the study of De Oliveira *et al*.,2001 in the neutrophil result only (he did not mention the cause)(4). Disagreed results obtained also with(3,11).

Leukemia was observed in the present study may be considered as a defense mechanism against the inflammatory processes in the body especially in the liver and kidneys, the inflammation will stimulate the bone marrow to produce a large number of WBC. Zinc toxicity causes hepatospleenomegaly (4), which leads to the production of large numbers of the lymphocytes resulting in the increase of the total WCB count, therefore, the neutrophil count decreased. The increasing of the monocyte count could be due to the long period of the toxicity.

It seems that the mice in T3 group were more affected than the other two treatments (T1 and T2) in the total and differential WBC count due to the increase of zinc concentration in the plasma which leads to zinc toxicity, the results showed also that there were more effects of the zinc toxicity on the 30 days period than 15 days period because of the accumulation of the zinc in the tissues especially liver and spleen tissues.

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