# EXPERIMENTAL CLINICOPATHOLOGICAL STUDIES ON ZINC TOXICITY IN RATS

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## ABSTRACT

This work aimed to study the effect of increasing zinc on hematological, biochemical and immunological parameters in albino rats. This study performed on 75 male albino rats, which divided into 3 equal groups (each one contains 25 rats).The first group (control group), received no treatment. The second group received low dose of zinc, 100mg of zinc acetate/liter in drinking water for 3 months. The third group received high dose of zinc, 200mg of zinc acetate/liter in drinking water for 3 months. Collection of blood samples and separation of serum samples occurred at the end of the 1st, 2nd and 3rd months of experiment for hematological, serum biochemical and immunological analysis. Administration of high dose of zinc (200mg) at the third group resulted in, presence of anemia which was macrocytic hypochromic, also we recorded decrease in total leukocyte count. Regarding serum biochemical analysis we recorded, significant elevation in ALT, AST, ALP and total bilirubin. On the other hand there were significant decrease in total protein and albumin. High zinc level group showed significant increase in MDA, SOD and catalase activity. IL 1β and lysozyme were significantly decreased. It concluded that high doses of zinc revealed regenerative anemia together with significant undesirable changes in serum biochemical and immunological parameters.

## INTRODUCTION:

Zinc is an environmental pollutant and omnipresent in the environment **(Weltje, 1998)**. Millions of tons of zinc metal are used commercially, principally to galvanize iron and to manufacture brass. It is also used widely in preservative treatment, fungicidal action and medicine, etc. **(Barceloux, 1999)**. Natural water supplies usually contain only trace amounts of zinc, but the concentration may be increased if the water flows

through galvanized, copper or plastic pipes (**Llobet et al., 1988**). It is well known that zinc is an essential trace element and has important biological functions that control many cell processes including DNA synthesis, normal growth, brain development, behaveioral response, reproduction, fetal development, bone formation, and wound healing (**Barceloux, 1999**). Zinc deficiency results in growth retardation, testicular atrophy, skin changes, and suppressed appetite, etc. (**Prasad, 1991**). Misunderstanding the availability of zinc supplements, lack of knowledge about zinc toxicity and the ease with which many preparations of zinc salts can be obtained over the counter in drug stores and in health food stores, have led to zinc supplements being widely used by the public as self-medication at unknown dosages (**Sandstead, 1995**). Some cases of intoxication following ingestion of elemental zinc in an attempt to promote wound healing or control anger were reported (**Lewis and Kokan, 1998**). Studies on rats have shown that excessive dietary zinc in these animals induces deficiencies of copper and iron, retard growth and anemia (**Llobet et al., 1988**). These findings indicate that excessive intake of zinc supplements is also a potential risk to humans. The present study was designed to investigate the toxic effects of zinc on the hematopoietic system, biochemistry and immune system function in rats.

## 2. MATERIALS AND METHODS:

### 2.1- Experimental design:

**Experimental animals**

Seventy five male albino rats of Wistar strain (130±10 g, 10 to 12 weeks old) purchased from Laboratory Animal institute in Hellwan were used for the study. Animals were fed with comm.-ercially available standard and balanced rat ration and water was provided ad libitum. All animals were acclimatized for 2 weeks before experimentation.

Animals were randomly divided into 3 equal groups (each 25 rat). The first group was control group, the second group received zinc as zinc acetate at dose of 100mg/liter in drinking water for 3 months (Loba Chemie, India. Batch No. O 050404) and the third group received zinc acetate at dose of 200mg/liter in drinking water for 3 months.

Blood samples were collected in heparinized tubes for the estimation of RBCs count, hemoglobin content, PCV value, TLC count and DLC of rats of different groups after 1$^{st}$, 2nd and 3$^{rd}$ month of the experiment. Blood samples were also collected in separate

tubes and serum was separated for biochemical and immunological investtingations.

**2.2. Hematological studies:**

Erythrocytes, Hb, PCV, MCV, MCH, MCHC, TLC and differential leucocytic count were performed by manual method according to **Jain (1986)**.

**2.3. Biochemical studies:**

Both ALT and AST activeities were assayed according to **Reitman and Frankel (1957)** using kits obtained from Quimica Clinical Aplicada (Spain). Determination of ALP according to **Tietz (1976)** using kites provided by Scalvo diagnostic Italy. Amylase activity was assayed according to **Caraway et al., (1959)** using kits obtained from Scalvo diagnostic Italy. Total protein was measured according to **Young (2001)** by kits provided by Scalvo diagnostic Italy. Albumin was assayed according to **Dumas and Biggs (1972)** using kits obtained from Scalvo diagnostic Italy. Serum uric acid was determined according **Caraway (1963)** using kits obtained from Bio Analytics USA. Creatinine was measured according to **Henry (1974)** using kits provided by Bio Analytics USA. Total bilirubin determined according to **Wahlefeld et al (1972)** using kits obtained from Bio Analytics USA. Calcium and phosphorus were measured according to **Tiez (1976)** using kits obtained from Bio Analytics USA.

**2.5. Oxidative stress studies:**

MDA (Malondialdehyde), catalase and SOD (super oxide dismutase) activity were assayed according to **Sharma and Wadhwa (1983)**; **Cohen et al., (1970)** and **Maral et al., (1977)** respectively using kits provided by Biodiagnostic Egypt.

**2.4. Immunological studies:**

Measurement of level of Interleukin-I beta according to **Chan and Perlstein (1987)** by kits purchased from Pierce Biotechnology, USA. Serum lysozyme activity was assayed according to **Parry et al, (1965)** using kits obtained from Sigma Aldrich, USA.

**2.5. VI -Statistical Analysis:**

Hematological, serum biochemical and immunological parameters were analyzed by analysis of variance (**ANOVA**) using SPSS 16 for window. Two groups were significantly different if P was statistically lower than 0.05 Statistical analysis was carried out with one way NOVA test **(Snedecor and Cochran, 1982).**

**3. RESULTS:**

**3.1. Hematological results:**

Administration of zinc caused no changes in hematological parameters after the 1st month, while after 2nd and the 3rd month there were significant reduction in RBCs, Hb and PCV

in the 3rd group in compare with control group. On the other hand there was significant increase in MCV value together with decrease in MCHC value in the 3rd group in compare with control group. Total and differential leukocytic count revealed no significant change after the 1st month in all treated groups, but after the 2nd and 3rd month there were significant decrease in WBCs, also showed significant decrease in lymphocytes and significant increase in neutrophils in the 3rd group compared with control group **tables (1-4)**.

**Table (1):** Erythrogram in control, low zinc and high zinc groups (Mean ± S.E.) after two months.

| Parametes & groups | RBC $10^6/\mu L$ | Hb g/dL | PCV % | MCV fl | MCH pg | MCHC % |
|---|---|---|---|---|---|---|
| control | 7.84± 0.17a | 13.87± 0.13a | 40.7± 0.67a | 51.90± 1.04c | 17.7± 0.22a | 34.11± 50.48a |
| The 2nd group | 7.77± 0.56a | 13.74± 0.26a | 39.67± 0.88a | 51.79± 4.99c | 17.9± 1.5a | 34.69± 1.12a |
| The 3rd group | 4.17± 0.17bc | 7.63± 0.32b | 35.33± 0.67b | 85.0± 2.89a | 18.35± 0.82a | 21.58± 0.50b |

The same Column not followed by the same letter differ significantly ($P<0.05$)

**Table (2):** Erythrogram in control, low zinc and high zinc groups (Mean ± S.E.) after three months.

| Parameters & groups | RBC $10^6/\mu L$ | Hb g/dL | PCV % | MCV fl | MCH pg | MCHC % |
|---|---|---|---|---|---|---|
| control | 8.27± 0.16a | 13.53± 0.42a | 41± 0.58a | 49.58± 0.88d | 16.37± 0.61a | 33.05± 1.44a |
| The 2nd group | 8.2± 0.31a | 13.47± 0.29a | 40.0± 0.58a | 48.95± 2.31d | 16.48± 0.86a | 33.66± 0.25a |
| The 3rd group | 3.93± 0.7c | 6.83± 0.17b | 33.67± 0.67b | 85.7± 3.2b | 17.39± 0.63a | 20.3± 0.46b |

The same Column not followed by the same letter differ significantly ($P<0.05$).

**Table (3):** Leukogram in control, low zinc and high zinc groups (Mean ± S.E) after two months.

| Parameters & groups | TLC $10^3$ / µL | Neutroph. $10^3$ / µL | Lymph. $10^3$/µL | Monocyt. $10^3$/µL | Eosino. $10^3$ / µL | Basophil. $10^3$ / µL |
|---|---|---|---|---|---|---|
| control | 10.04± 0.03a | 3.69± 0.23b | 6.62± 0.2a | 0.06± 0.02aa | 0.01± 0.01a | 0.00± 0.00a |
| The 2nd group | 9.87± 0.13a | 3.27± 0.27b | 6.55± 0.23a | 0.04± 0.01a | 0.01± 0.01a | 0.00± 0.00a |
| The 3rd group | 8.72± 0.19b | 4.71± 0.16a | 3.93± 0.07b | 0.06± 0.01a | 0.01± 0.01a | 0.00± 0.00a |

**Table (4):** Leukogram in control, low zinc and high zinc groups (Mean ± S.E) after three months.

| Parameters & groups | TLC $10^3$ / µL | Neutroph. $10^3$ / µL | Lymph. $10^3$/µL | Monocyt. $10^3$/µL | Eosino. $10^3$ / µL | Basophil. $10^3$ / µL |
|---|---|---|---|---|---|---|
| control | 9.67± 0.67a | 3.17± 0.12b | 6.75± 0.46a | 0.05± 0.01a | 0.04± 0.01a | 0.00± 0.00a |
| The 2nd group | 10.0± 0.29a | 3.03± 0.03b | 6.88± 0.32a | 0.06± 0.02a | 0.03± 0.01a | 0.00± 0.00a |
| The 3rd group | 7.63± 0.15c | 5.14± 0.09a | 3.1± 0.19b | 0.06± 0.01a | 0.02± 0.00a | 0.00± 0.00a |

### 3.2. Biochemical results:

There were no significant changes in ALT, AST, ALP, amylase, TP, Albumin, UA, creatinine, total bilirubin, Ca and Ph all groups after the 1st month. While after the 2nd and 3rd months group Zn2 showed significant elevation of ALT, AST, ALP, amylase, creatinine, uric acid and total bilirubin together with significant decrease in total protein, albumin, Ca and Ph compared with control group **tables (5 and 6)**.

**Table (5):** Some serum biochemical parameters profile in control, low zinc and high zinc groups (Mean ± S.E) after two months.

| Prameters & goups | ALT (U/L) | AST (U/L) | ALP (U/L) | Amylase (U/L) | TP (gm/dl) | Albumin (gm/dl) | Creatin (mg/dl) | Uric acid (mg/dl) | T. Bili. (mg/dl) | Ca (mg/dl) | Ph (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| control | 33.67 ± 10.22c | 42.67 ± 1.76c | 165 ± 12.56b | 862 ± 56.01b | 6.17 ± 0.13a | 3.50 ± 0.13a | 0.48 ± 0.08b | 2.63 ± 0.09c | 0.22 ±0.04c | 8.98 ± 0.69a | 6.9 ± 0.65a |
| The 2nd group | 33 ± 1.67c | 41.67 ± 0.88c | 180.33 ± 4.91b | 753.33 ± 86.67b | 6.38 ± 0.16a | 3.27 ± 0.15a | 0.49 ± 0.06b | 2.63 ± 0.89c | 0.2 ± 0.01c | 8.57 ± 0.0.27a | 7.87 ± 0.33a |
| The 3rd group | 61.67 ± 2.08a | 91.33 ± 0.67a | 251 ± 17.03a | 1443.67 ± 23.45a | 3.23 ± 0.146b | 1.5 ± 0.15b | 0.7 3± 0.09a | 3.69 ± 0.15a | 1.4 ± 0.21b | 6.47 ± 0.26b | 5.47 ± 0.29b |

The same Column not followed by the same letter differ significantly (P<0.05).

**Table (6):** Some serum biochemical parameters profile in control, low zinc and high zinc groups (Mean ± S.E) after three months.

| Prameters & goups | ALT (U/L) | AST (U/L) | ALP (U/L) | Amylase (U/L) | Total p. (gm/dl) | Albumin (gm/dl) | Creatin (mg/dl) | Uric acid (mg/dl) | T. Bili. (mg/dl) | Ca (mg/dl) | Ph (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| control | 33.33 ± 2.73b | 39 ± 0.58c | 168.33 ± 4.41 | 591.33 ± 39.60c | 6.07 ± 0.06a | 3.93 ± 0.45a | 0.51 ± 0.03c | 1.83 ± 0.22c | 0.21 ± 0.03c | 8.96 ± 0.54a | 8.09 ± 0.56a |
| The 2nd group | 33 ± 1.73b | 37.33 ± 0.88c | 162.0 ± 7b | 558.33 ± 35.31c | 6.21 ± 0.31a | 3.30 ± 0.25a | 0.54 ± 0.08c | 2.08 ± 0.22c | 0.22 ± 0.03c | 8.2 ± 0.41a | 7.28 ± 0.31a |
| The 3rd group | 48.33± 5.61a | 81.67± 3.33a | 282.67 ± 12.67a | 1300 ± 57.74a | 3.33± 0.18b | 1.67± 0.09b | 1.13± 0.07a | 3.75± 0.178a | 2.17± 0.17b | 5.87± 0.09b | 5.3± 0.35b |

The same Column not followed by the same letter differ significantly (P<0.05)

### 3.2. Oxidative stress results:

There were no significant changes in MDA, SOD and catalase in all treated groups. After 2 & 3 months the 3rd group revealed significant increase in MDA, Catalase and SOD values compared with control **tables (7 and 8).**

**Table (7):** Some oxidative stress parameters in control, low zinc and high zinc groups (Mean ± S.E) after two months.

| Parameters & groups | MDA (nmol/g) | Catalase (U/L) | SOD (U/ml) |
|---|---|---|---|
| control | 7.67± 1.2b | 587.78± 6.19c | 282.82± 14.0c |
| The 2nd group | 8.0± 0.58b | 546.67± 27.28c | 261.34± 4.42c |
| The 3rd group | 17.33± 2.4a | 876± 13.01a | 528.33± 19.22a |

The same Column not followed by the same letter differ significantly ($P<0.05$).

**Table (8):** Some oxidative stress parameters in control, low zinc and high zinc groups (Mean ± S.E) after three months.

| Parameters & groups | MDA (nmol/g) | Catalase (U/L) | SOD (U/ml) |
|---|---|---|---|
| control | 7.33± 0.88b | 509.11± 8.78c | 281.33± 10.44c |
| The 2nd group | 8.33± 0.58b | 505± 20.21c | 290.93± 4.65c |
| The 3rd group | 15.67± 1.53a | 672± 14.74a | 450± 17.32a |

The same Column not followed by the same letter differ significantly ($P<0.05$).

### 3.3. Immunological results:

There were no significant changes in IL 1β and serum lysozyme after one month. After 2 and 3 months group the 3rd showed significant decrease in IL 1β value and serum lysozyme activity in compare with control **tables (9 and 10).**

**Table (9):** IL 1 β value in control, low, low zinc and high zinc groups (Mean ± S.E) after one, two and three months.

| Time & groups | Control group | 2nd group | 3rd group |
|---|---|---|---|
| One month | 59.68± 2.5a | 59.25± 0.25a | 53.94± 3.17a |
| Two months | 49.15± 1.37b | 54.98± 2.64b | 38.33± 0.88c |
| Three months | 50.40± 0.77b | 50.60± 1.25b | 36.67± 1.20c |

**Table (10):** Serum lysozyme value in control, low zinc and high zinc groups (Mean ± S.E) after one, two and three months

| Time & groups | Control group | 2nd group | 3rd group |
|---|---|---|---|
| One month | 2.15± 0.18a | 2.20± 0.20a | 2.51± 0.11a |
| Two months | 2.73± 0.13a | 2.71± 0.18a | 2.15± 0.28b |
| Three months | 1.96± 0.07a | 1.84± 0.12a | 1.47± 0.09b |

The same Column not followed by the same letter differ significantly ($P<0.05$).

## 4. DISCUSSION:

The hematological change was macrocytic hypochromic anemia (regenerative), it appeared at 2nd and 3rd months post zinc administration at high zinc dose. our result in agreement with **Meurs et al., (1991)** who reported that clinicopathological findings result from oxidative damage include the formation of eccentrocytes and nucleated red blood cells, Heinz bodies, basophilic stippling and target cells. Spherocytosis has also been reported in the few previous cases of zinc toxicity **(Breitschwerdt et al., 1986b)**. Erythrocytes containing Heinz bodies are rigid, have decreased deformability, and therefore may lyse or be removed prematurely from the circulation by macrophages resulting in anaemia **(Desnoyers, 2000)**. Meanwhile, anemia induced by zinc was reported by other researcher **Liobet et al., (1988); Latimer et al., (1989)** was microcytic hypocromic). Leukogram showed that zinc dosed rats at high dose had decrease in total leukocytic count our results in line with **Khan et al., (2007)** who recorded reduction of total leukocytic count. Neutrophilia and lymphopenia. The result agrees with that of **Tvedten and Weiss, (2000)** they mentioned that, neutrophilia and lymphopenia. were

present as result of stress response or an inflammatory or infectious process. Regarding lymphopenia our histopathological findings showed severe congestion and degeneration of lymphocytes, this is in line with **Osatiashtiani et al., (1998) and Fraker et al., (1995)** who observed thymic atrophy in vivo, which resulted in an impaired T cell development and decreased T cell counts. Concerning biochemical changes, ALT is often tested along with AST, ALP to evaluate whether liver damaged or diseased. When the liver is dysfunction, the levels of above enzymes will rise **(Kellermen et al., 1995)**. Therefore, in this study the significantly elevated levels of ALT, AST and ALP serum measurements due to high zinc level indicated occurrence of liver damage. This is matched with other previous studies which reported liver dam-ages by high zinc salt administration **(Paio et al., 2003)**. The significantly increased levels of creatinine and uric acid may be attributed to renal disease. Our result in agreement with **Llobet et al., (1988)** who reported that the concentrations of creatinine and uric acid significantly increased after exposure to high dose zinc acetate in drinking water. Hyperuricema may found as a result of renal damage of decreased rate of tubular excretion **(Coles, 1986)**.

The present study showed slight hypoproteinemia and hypoalbuminemia which may be due to liver damage or due to that zinc is initially bound to plasma albumin. Also most zinc is deposited in the liver, kidney and pancreas **(Osweiler, 1996)**. These results are in harmony with the finding of **(Levengood et al., 2000)**.

Hypocalcaemia was characteristic in high zinc dosed animals which, associated with renal disorders **(Coles, 1986)**. Also may due to impaired absorption that high levels of zinc compete calcium absorption **(Osweiler, 1996)**. Our results consistent with **Levengood et al., (2000)** who reported hypocalcaemia in Zn dosed groups and they concluded that diets high in protein with other organic matter and calcium did not prevent or substantially alleviate zinc toxicosis. **Stewart and Magee (1964)** mentioned that zinc toxicity affect on calcium and phosphorus and interfere with the normal absorption and increase the fecal excretion of these minerals in the young rat. Slight decrease in phosphorus level in the serum at high zinc dosed rats is recorded in our study. We suggest that the Hypophosphatemia may due to increase phosphorus excretion **(Kaneko et al., 1997)**. Our biochemical

findings revealed presence of hyperbilirubinemia which may be due to the intravascular hemolysis. This results in line with (**Angie et al., 2003**). Other biochemical findings include hyperamylasemia may attributed to pancreatic inflameemation, this result in agree with (**Angie et al., 2003**).

The result of the present study showed that treatment with zinc acetate caused a general significant increase of catalase, MDA and SOD activity after 2 and 3 months post administration. Antioxidant enzymes have been shown to work in a cooperative or synergistic way to protect against oxidative stress **Bagnyukova et al., (2006)** Oxidative stress due to the existence of the toxic metals can be demonstrated by MDA content, which is considered to be a general indicator of lipid peroxidation (**Chaoui *et al.*, 1997**). These results matches with **Alia et al., (1995)** who reported that excess of zinc promoted MDA production due to increased lipid peroxidation through excessive generation of free radicals. CAT, a primary antioxidant defense component, protects fish from oxidative stress by converting the hydrogen peroxide to oxygen and water (**Atli G and Canli M 2007**). Our results in accordance with **Ozgur and Ferit (2010)** who mentioned that the increase in the activity of CAT following metals treatment suggests increased production of $H_2O_2$ and the protective role of this enzyme against metal-induced oxidative stress. Another explanation for increasing level of MDA provided by **Prasad** and **Pardha (1995)** who suggesting that higher concentrations of zinc promote free radical generation and hence, lipid peroxidation. The increase in free radical production could be due to interference of zinc with normal functioning of electron transport chains of mitochondria. Heavy metals including zinc have been reported to suppress electron transport associated with this organelle (**Van and Clijsters 1986**). Our results also matches with **Li et al., (2008)** who reported a rapid increase in MDA content and electrolyte leakage in case of elevated zinc concentrations in *Sedum alfredii* Hance. Significant enhancement in SOD activity was observed in seedlings exposed to toxic levels of zinc. Furthermore, the activity of SOD in the enzyme extract was insensitive to $H_2O_2$, suggesting that Mn-SOD must be playing an important role in detoxification of superoxide radicals in *B. juncea* under zinc toxicity (**Van and Clijsters 1990**). **Li et al., (2008)** attributed the increase in CAT and SOD to the increased ROS production or

increased expression of genes encoding CAT and SOD.

In our study we found that high concentration of zinc led to reduction in the level of IL 1β, this result agree with **Verena et al., (2005)** who investigated whether zinc interferes with monokine production via modulation of cyclic nucleotide signaling. A dual effect of zinc on PDE (Phosphodiesterase) activity was found in Mono Mac1 cells. First, the enzymatic activity of PDE is directly and reversibly inhibited by zinc ions. Second, zinc inhibits the gene expression of several PDE subtypes and can also block the LPS-stimulated increase in PDE-4B expression. We found that high concentration of zinc resulted in reduction of lysozyme activity. This is in accordance with **Stabili and Pagliara (2009)** who said that zinc might act directly on the enzyme and suppress the lysozyme. Anyway the suppression of lysozymelike activity, by increasing the susceptibility of seastars to bacteria, may portend a decrease in immunocompetence and pathological manifestations.

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## الملخص العربي

## دراسات باثولوجية إكلينيكية تجريبية على التسمم بالزنك في الفئران

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استهدف هذا البحث دراسة تأثيرات الزنك على خصائص الدم و المؤشرات البيوكيميائية بالإضافة إلى الاختلافات في المؤشرات المناعية و مضادات الأكسدة التي تحدث نتيجة تركيزات غذائية مختلفة من الزنك في الفئران. أجريت هذه الدراسة على عدد خمسة وسبعون من ذكور الفئران البيضاء قسمت إلى ثلاث مجموعات متساوية.المجموعة الأولى:استخدمت هذه المجموعة كضابطة و لم تعطى اى زنك. المجموعة الثانية: : أعطيت الزنك بجرعة مائة مج في مياه الشرب لمدة ثلاثة أشهر على هيئة اسيتات الزنك. المجموعة الثالثة: أعطيت الزنك بجرعة مئتان مج في مياه الشرب لمدة ثلاثة أشهر على هيئة اسيتات الزنك. تم فحص عينات الدم و السيرم عند نهاية الشهر الأول و الثاني و الثالث. أدى إعطاء الزنك بجرعة عالية إلى حدوث أنيميا مع نقص في العدد الكلى لخلايا الدم البيضاء. تم حدوث زيادة انزيمات الكبد و حمض البوليك والكرياتينين والصفراء. على النقيض من هذا حدث انخفاض ملحوظ في مستوي البروتين الكلى و الزلال و الكالسيوم و الفسفور بالإضافة إلى زيادة انزيمات الأكسدة مثل الكتالاز و المالونای الدهيد و السوبر اوكسيد دسميوتاز. كان من الملاحظ أيضا حدوث نقص في الانترلوكين 1 بيتا و الليسوزيم.