# Effects of the Zinc on Activity of Immune System in Male Albino Mice

**Z. Hussein**
**Department of Biology, Collage of Education Ibn Al-Haitham , University of Baghdad**

## Abstract

Effect of zinc chloride on the immune functions was studied in male albino mice aged 6-7 weeks. It was administrated orally (1ml) in three concentrations (0.5ppm, 1ppm, 2ppm) for 9 days.

The results showed that the first concentration was not effective comparing with control while the second concentration increased the enhancement of immune system and the cell third one killed the mice 6 hours post administration, so we can conclude that the high dose of $ZnCl_2$ could be harmful for all metabolism.

## Introduction

Zinc, an essential trace element, is rich in animal products such as meat (especially beef), poultry, sea food (especially oysters) and dairy foods, which are the best food sources of zinc [1].Cereals, grain products, fruits and vegetables also contain zinc although in smaller amount than in animal products. In addition, zinc, is more readily absorbed from animal than from vegetable sources [2]. It is present in all organs, tissues, fluids, and secretions in the body [3].More than 100 specific enzymes require zinc for their catalytic function.Moreover, under physiological conditions, zinc is not subjected to oxido-reductive reactions, so it is relatively non-toxic.These properties make zinc an ideal element to participate in catalytic, structural, and cellular regulatory functions[4].Also it has a variety of effects on the immune and nervous system in vivo and in vitro and these effects mainly depend on the zinc concentration [5].Zinc also plays an important role in specific immune defenses such as humoral and cell- mediated immunity. In mice fed, a low zinc diet for 30 days, 30% to 80% losses in immune defense capacity occurred [6].Zinc has other properties that could contribute to its role in lymphocyte functions. It is an antioxidant, protecting cell from the damaging effects of oxygen radicals [7].Besides all these, zinc is requied as a co-factor for numerous metalloenzymes involved in the continuous production of the immune system cells[8].Many biological studies indicate that zinc deficiency decreases resistance to infections diseases.Zinc deficient animals have suppressed immune responses and are more susceptible to a diverse range of infections agents including *herpes simplex* virus [9], bacteria such as *salmonella enteritidis* [10] and *mycobacterium tuberculosis* [11],and the protozoan parasites *typanosoma cruzi* [12], *fasciola hepatica* [13],and *schistsoma mansoni* [14].Zinc supplements also have beneficial effects when administered during infections, and zinc lozenges were shown to decrease the duration of the common cold [15], and this may be due to the increased zinc concentration in the nasal mucosa, which may alter the conformation of the binding site between the virus and ICAM-1[16]. At last, the very high zinc intakes in adults and children can result in copper deficiency, anemia, growth retardation and immunodepression[17].Thus too much zinc can be as bad as too little, as in most cases; abdominal pain, anemia, fever and bleeding in the stomach can result[18].

## Material and Methods

A. Animals: Albino mice were brought from the centre for infertility in al_kadimiya and, animals were divided into four groups, each group involved 6 mice. The first was a

control group and administrated orally (1ml) with distilled water, while the other three groups were administrated orally (1ml) with $ZnCl_2$ in three concentrations (0.5ppm, 1ppm, 2ppm). One dose per day for 9 days.

B. Prepration of $ZnCl_2$: $ZnCl_2$ solutions were prepared depending on human needs of zinc (15mg/60kg)[18], and accordingly 4 mg of zinc was dissolved in 100ml of distilled water to yield a final concentration of 2ppm.

C. Tests: 1. White blood cell count: blood taken from mouse tail and examined according to the method of [19].

2. Diffrential count: blood smear, stained with leishman stain and examined the samples under the light microscope by using the oily lens and counted (100) random cells from each slide then find the average percentige of each animal according to number of each type of cell.

3. Phagocytic index: phagocytes were isolated from peritone and examined according to the method of [20].

4. Mitotic index: examined according to the method of [21], isolation of cells (bone marrow and spleen) in the method of [22]

5. Arthurs reaction: Examinted according to the method of [23] then
examination of delayed type hypersensitivity 24 hour.

6. Adenosine deaminase activity in serum: The activity of ADA was determind according to the method of Giusti [24].

# Results

The results in tables 1,2,3,4 and 5 showed that the first concentration of $ZnCl_2$ (0.5ppm) was associated with normal values and did not show any effect on mice, while the second concentration (1ppm) showed that zinc chloride enhanced immune system in all tests, but it made mice suffered from lassitude and diarrhea. In the third concentration (2ppm), symptoms of lassitude, diarrhea, loss of appetite, depression, extremities palsied and death appeared 6 hour post treatment.

# Discussion

The parameters investigated in tables 1,2,3,4 and 5 showed increased levels with the increase of concentration compared with control. Table 1 showed that increased intake of ($ZnCl_2$) stimulated white blood cell count [ total and differential] especially at second concentration, this is may be due to zinc effect on nonspecific immunity by its affecting neutrophil and natural killer cells activity [25].Many studies showed that very high zinc concentrations could express a microbicidal activity [26].When $ZnCl_2$ was used in concentrations of 10(-4);10(-5);10(-6)M in PFC it was found that the numbers of anti-SRBC antibody-producing cells in mice injected with zinc were greater than in the control. This enhancement of PFC was proportional to concentration of zinc. It was determined that $ZnCl_2$ in concentration 10(-4)M activated mouse lymphocytes for migaration inhibitory factors production. This prove that zinc may enhance the effectiveness of anti-infections immunity [27].Thus more zinc addition in vitro alters the expression, function, or both, of lymphocyte surface molecules governing cell-cell interactions [28]. The results in table 2 showed that the increased intake of ($ZnCl_2$) enhanced phagocytosis .One important group of leucocytes is the phagocytic cells. These cells bind to microorganisms, neutralize them and then kill them [29].Many studies indicated that when zinc deficient animals infected with pathogenic parasites, the function of macrophage were impaired however, phagocytosis can be restored to normal when the animal receive sufficient zinc (6, 30), Roitt [29] indicates that there were cooperation between T-lymphocyte and phagocytic cell to kill microbes. Other studies showed that a very high concentration of zinc in vitro inhibited macrophage activity, mobility, phagocytosis and oxygen consumption[31].There is much speculation regarding the role of zinc in killing pathogens by oxygen radicals produed by macrophages [32].

Thus zinc was not affected on absolute numbers of peripheral polymorphonuclear leukocytes, but chemotactic response was impaired and was reversible by in vitro addition of zinc [33].In table 3 the results showed increased levels of mitotic activity compared with control .This may be due to carcinogenesis effect of zinc on cell growth [34].Zinc was demonstrated to inhibit the mutagenic action of some genotoxic carcinogens [35].Furthermore zinc chloride did not induce mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells [36]

The parameter in table 4 showed increased levels in Arthus reaction and delayed type hypersensitivity with the increased level of concentrations in comparison with control. In Arthus reaction, the animal had appreciable levels of serum antibody, following the injection of antigen reaction develops at the reaction site [29], and zinc plays an important role in specific immune response [5], this explains why there is an increase in thickness pad of injected mice. Our results were similar to the finding reported by[37]

Adenosine Deaminase (ADA) is a cytosolic enzyme. ADA participates in the purine metabolism where it degrades either adenosine or 2'-deoxyadenosine producing inosine or 2'-deoxyinosine, respectively [38]. The results in table 5 showed the effect of $ZnCl_2$ on ADA activity in animal showed a dose dependent manner because the activity of ADA was elevated when we use a high dose of $ZnCl_2$ in the administration mice. Zinc is a structural constituent of a great number of proteins, including enzymes belonging to cellular signaling pathways and transcription factors, and it is essential for biological activity [39].

At last this result suggests that $ZnCl_2$ has enhancement immune system but it mightbe deadly impact when administrated with high concentration since results showed that third concentration fatal for animals may be due to zinc chloride toxicity. It can produce significant lung damage in rate when instilled directly into the lung [40]. Thus oral exposure to zinc chloride causes reduced growth rates, reduced body weight and anemia in numbers of rat, the mouse and a sheep studies, following high oral or dietary intake of zinc [31, 41]. Thus, exposure to high doses of zinc was associated with pancreatic atrophy and histological changes in kidneys, accompanied by changes in kidney function in rats, mice and sheep [31].Changes in liver, including decreased activities of cytochrome p450 and liver catalase [42].

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**Table (1): Effects of $ZnCl_2$ on white blood cell count [Total and Differential] in male albino mice**

White blood cells /$Mm^3$ blood and differential count × $10^3$

| groups | Dose/ml | eosinophils | monocytes | neutrophils | lymphocytes | w.b.c count |
|---|---|---|---|---|---|---|
| 1 | D.W | 0.01± 0.08 | 0.01± 0.25 | 0.06± 2.56 | 0.02± 4.65 | 0.50±7.55 |
| 2 | 0.5 | 0.01± 0.09 | 0.01± 0.26A | 0.10± 2.62 | 0.06± 4.66B | 0.10±7.65A |
| 3 | 1 | 0.03± 0.11 | 0.04± 0.32A | 0.57± 3.26 | 0.99± 6.22B | 1.60±9.91A |
| 4 | 2 | – | – | – | - | – |

(A) Significant (a<0.05) (B) Significant (a<0.01) (C) Significant (a<0.001)

**Table (2): Effects of $ZnCl_2$ on phagocytic index in male albino mice**

| groups | dose | Phagocytic index (%) |
|---|---|---|
| 1 | D.W | 0.03 ±10.23 |
| 2 | 0.5 | 0.38 ±10.51**C** |
| 3 | 1 | 1.24 ±15.18**C** |
| 4 | 2 | – |

(C) Significant (p<0.001)

**Table (3): Effects of $ZnCl_2$ on mitotic index of bone marrow and spleen cells**

| groups | Dose/ml | Spleen (%) | Bone marrow (%) |
|---|---|---|---|
| 1 | D.W | ± 11.50 1.29 | ± 17.00 1.15 |
| 2 | 0.5 | ± 12.36 0.96 | 18.90 ± 2.33 |
| 3 | 1 | 14.77**A** ± 2.31 | ± 23.53**A** 1.75 |
| 4 | 2 | – | – |

(A) Significant (p<0.05)

**Table (4): Effects of $ZnCl_2$ on hypersensitivity reactions (Arthus and delayed type)**

| groups | Dose/ml | Delayed type(Mm) | Arthus(Mm) |
|---|---|---|---|
| 1 | D.W | 0.02 ± 0.62 | 0.03 ± 0.54 |
| 2 | 0.5 | 1.63 **B** ± 1.11 | 0.88 **A** ± 0.25 |
| 3 | 1 | 1.88 **A** ± 0.95 | 1.13 **B** ± 0.47 |
| 4 | 2 | – | – |

(A) Significant ($p<0.05$) (B) Significant ($p<0.01$)

**Table (5): Effect of $ZnCl_2$ on adenosine deaminase activity in serum**

| groups | Dose/ml | ADA activity/ U/mg of protein |
|---|---|---|
| 1 | D.W | 0.01 ± 1.54 |
| 2 | 0.5 | ± 1.68 **A** 0.17 |
| 3 | 1 | ± 1.86 **B** 0.29 |
| 4 | 2 | - |

(A) Significant ($P<0.05$) (B) Significant ($P<0.01$)

# تأثير عنصر الزنك في فعالية الجهاز المناعي في ذكور الفئران البيض

**زهراء حسين**
**قسم علوم الحياة ، كلية التربية –ابن الهيثم، جامعة بغداد**

## الخلاصة

تمت دراسة تأثير كلوريد الزنك $ZnCl_2$ في الفعالية المناعية على ذكورالفئران البيض بعمر6–7 اسابيع. استعملت ثلاثة تراكيز من مادة $ZnCl_2$ وهي (2ppm,1ppm, 0.5ppm) وبواقع جرعة واحدة في اليوم (1ml ) مدة 9 ايام. اظهرت النتائج ان التركيز الاول لم يؤثر تاثيرا معنويا، بينما التركيز الثاني اظهر زيادة معنوية مقارنة بالسيطرة والجرعة الثالثة كانت مميتة اذ قتلت الحيوانات بعد مرور 6 ساعات على التجريع الاول، لذا نستنتج ان الجرع العالية من $ZnCl_2$ يكون ضارا للفعاليات الايضية كافة داخل الجسم.