Tropical Journal of Pharmaceutical Research July 2023; 22 (7): 1403-1409
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i7.7

Original Research Article

Effect of zinc on weight gain, hematological parameters and tissue structure of the liver, kidney and spleen in albino mice

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Sent for review: 20 March 2023 *Revised accepted: 17 June 2023*

Abstract

Purpose: *To investigate the effect of varying zinc concentrations on weight gain, hematological parameters, and tissue structure of the liver, kidney, and spleen in albino mice.*
Methods: *The mice were administered daily zinc at 50, 70, and 90 mg/kg body weight. Weight gain was measured every two weeks. Hematological parameters were evaluated after 4 and 8 weeks, while biomolecular parameters were assessed after 8 weeks. Liver, kidney, and spleen were also assessed after 8 weeks using H & E staining procedures.*
Results: *Albino mice exhibited reduced weight gain, and altered hematological parameters after 8 weeks of exposure to varying zinc concentrations, with the highest dose of 90 mg/kg body weight having the most significant impact. Hematological parameters including blood cells, hemoglobin, hematocrit, blood clotting time, aspartate aminotransferase, and alanine transaminase decreased, while bilirubin, urea, and mean corpuscular hemoglobin concentration increased. Liver and kidney tissues exhibited widespread destruction such as necrosis and hemorrhage, while red pulp invaded white pulp activity in the spleen.*
Conclusion: *High levels of zinc from zinc chloride (90 mg/kg) affect body weight, and hematological parameters, as well as liver and kidney histology in albino mice. The most significant negative effects include the potential toxicity and adverse health consequences of excessive zinc intake. Consequently, this study provides the groundwork for investigating natural compounds that inhibit zinc toxicity.*

Keywords: *Hematology, Kidney, Liver, Spleen, Zinc*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

There has been an increasing focus on limiting zinc pollution due to its widespread use, which has led to a proportional increase in daily zinc exposure for both humans and living organisms, making it a matter of concern. Zinc has the ability to accumulate in organisms and humans through various pathways, such as respiration and digestion, potentially leading to toxicity in specific organ systems like the digestive, respiratory, nervous, and blood systems. This is especially true when excessive accumulation occurs [1-3]. Therefore, understanding the optimal intake of

zinc and its potential effects on the body is critical for maintaining good health.

Recent studies have shown that zinc has significant effects on body weight, hematological parameters, and the function of various organs, such as the liver, kidney, and spleen in mice or rats. Zinc impedes the body's absorption of iron and copper, which can elevate the risk of developing microcytic or macrocytic anemia when it accumulates in the body [2,4]. Therefore, it can cause a decrease in the content of hemoglobin (Hb) and a significant drop in the levels of red and white blood cells as well as hematocrit. Besides, biochemistry parameters may also be affected by zinc toxicity and can result in a number of severe consequences, including internal bleeding, diarrhea, and damage to the liver and spleen [2]. The liver, kidney, and spleen are vital organs involved in metabolic processes and immune function, making them important targets for studying the effects of zinc. Due to their crucial roles in metabolism and immune function, the hematological parameters and organs such as the liver, kidney, and spleen are significant targets for investigating the effects of zinc. In this study, we aimed to investigate the effects of zinc on weight gain, hematological parameters, and histology of the liver, kidney, and spleen in albino mice. This research provides a better understanding of the potential benefits and risks associated with zinc intake and its impact on overall health.

METHODS

Chemicals

Zinc chloride (Merck); Na_2SO_4, NaCl, acid acetic, Magnesium sulfate, KH_2PO_4, and Na_2HPO_4 were obtained from Scharlab SL Spain, formalin (China). All chemicals used in this study were provided by the Laboratory of Human and Animal Anatomy Physiology - Ho Chi Minh City University of Education, Vietnam.

Animals and treatments

Forty-eight (48) male mice, 4 weeks old and weighing between 14 - 16 g, were purchased from Pasteur Institute in Ho Chi Minh City, Vietnam. The animals were acclimatized for two weeks at a temperature 27 - 28 ^oC and a light-to-dark cycle of 10 : 14. The animals were housed in glass cages (30 x 19 x 19 cm^3), with 6 mice per cage. They were provided with specialized mice food pellets and drinking water. The animals were weighed and divided randomly into four groups (12 mice/group), and treated for eight weeks. Group 1 (Control), were provided with water ad libitum; group 2 (Zn50), group 3 (Zn70) and group 4 (Zn90) were administered zinc from $ZnCl_2$ doses of 50, 70 and 90 mg/kg body weight (BW), respectively, every other day for 8 weeks. Zinc chloride ($ZnCl_2$) was dissolved in distilled water was administered to mice through the esophagus in doses of 50, 70 and 90 mg/kg, respectively, every other day for 8 weeks with a 1 mL syringe and 8 G needle. Body weight of each mouse in the treatment groups were recorded in the morning before feeding every 2 weeks for a period of 8 weeks. The weight gain of mouse was then calculated by subtracting the weight before treatment from the weight after treatment. Approval for this study has been granted by the Animal Care and Use of Committee of University of Science, VNUHCM (ACUCUS; approval no. 230B/KHTN-ACUCUS).

Haematological evaluation

Blood samples were collected from the tail-vein to evaluate hematological parameters such as red blood cells (RBCs), white blood cells (WBCs), platelets, Hemoglobin (Hb), hematocrit (HTC), mean corpuscular hemoglobin concentration (MCHC) and clotting time at 1^{st}, 4^{th} and 8^{th} week [5].

Biochemistry and histological evaluation

At the end of the experiment, the mice were anaesthetized by ketamine and xylazine (0.1 mL/10 g BW) in the morning. Blood samples were collected from retro-orbital sinus at the media canthus of eye using micro-hematocrit tubes. The samples were then centrifuged at 3200 rpm for 15 min. Collected serum was stored at –20 ^oC for subsequent biochemical analyses (bilirubin, aspartate amino transferase (AST), alanine amino transferase (ALT), urea and creatinine). The animals were sacrificed by cervical dislocation, and their liver, kidney and spleen tissues were fixed in 10 % formalin, embedded in paraffin blocks, and cut into 5 – 6 µm thick sections, after that, the sections were stained with hematoxylin–eosin. The degree of histological damage was evaluated by visually examining the images using a Nikon microscope (TiU, Nikon).

Statistical analysis

Data were analyzed using the Minitab 18 software. The results obtained were presented as mean ± standard deviation (SD). Data were statistically analyzed by one-way ANOVA and $p < 0.05$ was considered significant.

RESULTS

Weight gain

There was a gradual decrease in weight gain in all treatment groups. During the same period, the rate of weight gain in the zinc-supplemented group was slower than control group. This difference was significant from 4th to 6th week in groups Zn70 and Zn90 ($p < 0.01$). After the 8th week, among the three zinc treated groups, treatment Zn90 showed slower weight gain compared to other groups (Table 1).

Haematological parameters

Zinc treated groups showed declining RBCs, platelets count, Hb and HCT indices. Zn90 group showed significantly lowered haematological parameters than control and other groups (Zn50 and Zn70) ($p < 0.01$). At the 8th week, there was a significant decrease in WBC count compared to both the 4-week group and the control. Mean corpuscular hemoglobin concentration (MCHC) index in Zn50 and Zn70 groups significantly increased compared to week 4 group and the control ($p < 0.01$).

Clotting time decreased gradually in the zinc treated groups over 4 weeks, with the Zn90 group having the shortest clotting time, significantly different from other groups ($p < 0.01$) (Table 2). Hematological parameters were most affected at a concentration of 90 mg/kg BW compared to other concentrations and the control.

Table 1: Weight gain of mice (g) every 2 weeks (mean ± SD)

Group	Weeks				
	W0	W2	W4	W6	W8
Control	0^{Aa}	$7.51±0.46^{Aa}$	$4.21±0.66^{Ba}$	$2.31±0.63^{Ca}$	$1.04±0.13^{Db}$
Zn50	0^{Aa}	$7.27±0.52^{Aab}$	$3.64±0.70^{Ba}$	$1.11±0.21^{Cc}$	$1.35±0.19^{Cab}$
Zn70	0^{Aa}	$6.75±0.61^{Ab}$	$2.54±0.30^{Bb}$	$1.76±0.38^{Cb}$	$1.56±0.39^{Ca}$
Zn90	0^{Aa}	$7.03±0.41^{Aab}$	$2.06±0.35^{Bb}$	$1.82±0.33^{Bb}$	$1.25±0.40^{Cab}$

A, B, C and D represents the row difference at the 95 % confidence level; a, b and c represents the column difference at the 95% confidence level; W: week

Table 2: Hematological parameters at 4th and 8th week

Hematological parameter	Group	Survey timeline		
		W0	W4	W8
RBCs ($x10^6$ TB/mm^3)	ĐC	$8.47±0.28^{Aa}$	$8.93±0.72^{Aa}$	$8.76±0.61^{Aa}$
	Zn50	$8.31±0.18^{Aa}$	$8.22±0.64^{Aa}$	$7.71±0,69^{Bb}$
	Zn70	$8.40±0.30^{Aa}$	$7.52±0.66^{Bb}$	$7.13±0.74^{Bb}$
	Zn90	$8.35±0.28^{Aa}$	$7.36±0.70^{Bb}$	$6.88±0.51^{Bc}$
WBCs ($x10^3$ TB/mm^3)	ĐC	$6.27±0.72^{Aa}$	$6.47±0.69^{Aa}$	$6.59±0.44^{Aa}$
	Zn50	$6.5±0.67^{Aa}$	$7.01±0.21^{Aa}$	$4.69±0.56^{Bb}$
	Zn70	$6.5±0.62^{Aa}$	$6.98±0.71^{Aa}$	$3.67±0.71^{Bc}$
	Zn90	$6.18±0.88^{Aa}$	$7.22±0.7^{Bb}$	$4.29±0.55^{Cb}$
Platelets ($x10^5$ TB/mm^3)	ĐC	$9.08±0.33^{Aa}$	$9.04±0.34^{Aa}$	$8.82±0.28^{Aa}$
	Zn50	$8.84±0.38^{Aa}$	$8.42±0.39^{Bb}$	$7.11±0.26^{Cb}$
	Zn70	$8.83±0.26^{Aa}$	$7.78±0.45^{Bc}$	$6.82±0.5^{Cb}$
	Zn90	$8.86±0.32^{Aa}$	$7.55±0.6^{Bc}$	$6.61±0.54^{Cc}$
Hb (g/dL)	ĐC	$12.73±0.53^{Aa}$	$13.03±0.22^{Aa}$	$12.82±0.25^{Aa}$
	Zn50	$12.67±0.41^{Aa}$	$12.28±0.64^{Ab}$	$12.02±0.25^{Bb}$
	Zn70	$12.58±0.39^{Aa}$	$12.02±0.34^{Bb}$	$11.72±0.32^{Bb}$
	Zn90	$12.67±0.41^{Aa}$	$11.9±0.42^{Bb}$	$11.25±0.37^{Cc}$
Haematocrit (%)	ĐC	$47.33±3.75^{Aa}$	$47.58±4.12^{Aa}$	$49.00±4.05^{Aa}$
	Zn50	$48.25±3.60^{Aa}$	$45.5±2.39^{Aab}$	$41.50±2.68^{Bb}$
	Zn70	$49.00±2.92^{Aa}$	$42.92±2.07^{Bb}$	$40.67±4.58^{Bb}$
	Zn90	$47.00±3.84^{Aa}$	$42.33±2.84^{Bb}$	$39.00±2.49^{Cb}$
MCHC (g/dL)	ĐC	$27.04±2.18^{Aa}$	$27.56±2.23^{Aa}$	$26.33±2.30^{Aa}$
	Zn50	$26.38±2.10^{Aa}$	$27.02±1.24^{Aa}$	$29.07±2.08^{Bb}$
	Zn70	$25.78±2.00^{Aa}$	$28.05±1.33^{ABa}$	$29.15±3.37^{Bb}$
	Zn90	$27.10±2.18^{Aa}$	$28.24±2.33^{Aa}$	$28.95±2.04^{Aab}$
Blood clotting time (min)	ĐC	$1.74±0.16^{Aa}$	$1.69±0.21^{Aa}$	$1.76±0.11^{Aa}$
	Zn50	$1.72±0.16^{Aa}$	$1.47±0.23^{Bb}$	$1.40±0.10^{Bb}$
	Zn70	$1.73±0.13^{Aa}$	$1.28±0.12^{Cb}$	$1.18±0.21^{Bc}$
	Zn90	$1.75±0.15^{Aa}$	$1.21±0.18^{Cc}$	$1.10±0.24^{Cb}$

A, B, C, D represents the row difference at the 95% confidence level; a, b, c represents the column difference at the 95% confidence level; W: week

Table 3: Biochemical parameters after 8 weeks

Biochemical parameter	Group			
	Control	***Zn50***	***Zn70***	***Zn90***
Bilirubin T[2] (mg/dL)	0.14±0.005[a]	0.19±0.005[bc]	0.20±0.01[c]	0.18±0.005[b]
Bilirubin D[2] (mg/dL)	0.05±0.01[a]	0.06±0.01[ab]	0.07±0.01[ab]	0.08±0.01[b]
Bilirubin I (mg/dL)	0.09±0.01[a]	0.13±0.015[c]	0.13±0.01[bc]	0.11±0.02[ab]
AST (U/L)	345.57±8.16[a]	381.7±8.15[b]	226.8±9.98[c]	99.26±8.16[d]
ALT (U/L)	132.17±2.31[a]	95.45±2.31[b]	86.31±2.82[c]	65,.44±2.30[d]
Urea (mg/dL)	28.27±0.68[a]	30.70±0.68[b]	35.89±0.83[c]	35.71±0.68[c]
Creatinine (mg/dL)	0.37±0.02[a]	0.38±0.015[a]	0.39±0.02[a]	0.36±0.01[a]

a, b, c, d represents the row difference at the 95% confidence level

Zinc supplementation led to significant changes in biochemical indicators in mice. Total bilirubin and urea levels increased, while ALT and AST levels significantly decreased ($p < 0.01$). Creatinine levels did not show a significant difference. The Zn90 group had the most significant impact, with the highest direct bilirubin/total bilirubin ratio (44.44 %, $p < 0.01$) and the lowest ALT and AST levels (Table 3).

Effects of zinc on liver histology

The control group had normal liver tissue (Figure 1 A). Zinc treated groups exhibited varying degrees of cellular damage. The Zn50 group had fading cell walls, lymphocyte infiltration, and some abnormal cells (Figure 1 B). The Zn70 group showed deformed nuclei, degeneration, and necrosis (Figure 1 C). The Zn90 group had severe damage with necrotic areas and loss of cell structure (Figure 1 D). The Zn90 group had the highest level of damage, consistent with the direct to total bilirubin ratio. Thus, zinc treatment induced liver damage, with Zn90 mg/kg causing the most cellular damage.

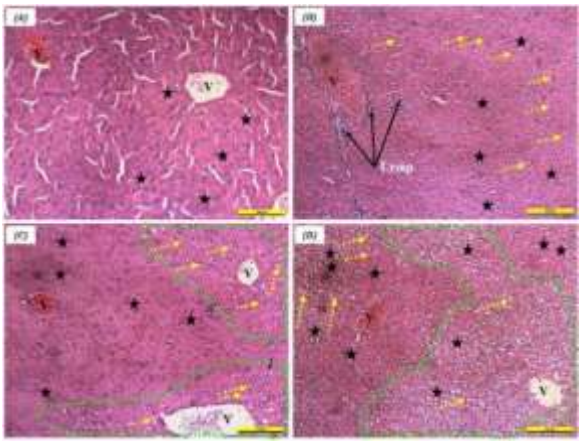

Figure 1: Effects of zinc on the appearance of mouse liver tissue**.** Control group (A); Zn50 group (B); Zn70 group (C); Zn90 group (D); **Key:** Vein (V); polynuclear leukocytes (star); lymphocyte cells (Lymp); nuclear shrinkage (dashed arrow); necrotic area (dashed area); scale bar 100 µm

Effects of zinc on kidney histology

Renal tissue of the control group had normal histology with minimal damage (Figure 2 A). In contrast, mice treated with zinc (Zn50, Zn70, Zn90 in Figure 2 B, C and D, respectively) showed varying degrees of damage including, lymphocyte infiltration, glomerular destruction, hemorrhage, and tubular damage. The highest concentration (Zn90) caused the most severe damage, leading to constricted or destroyed glomeruli, damaged tubular structure, and loss of normal structures. The concentration of 90 mg/kg was most damaging to mice kidney tissue compared to other concentrations.

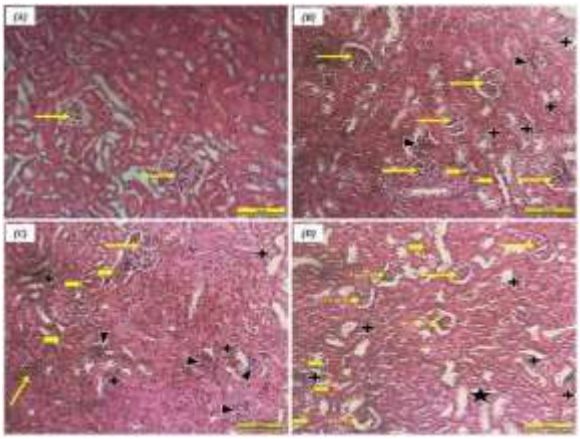

Figure 2: Effects of zinc on the appearance of mouse kidney tissue. Control group (A); Zn50 group (B); Zn70 group (C); Zn90 group (D); **Key:** The glomerular (long thin arrows); lymphocyte cells (isosceles triangles); glomeruli are destroyed (cross); glomeruli shrinkage (dashed thin arrows); renal tubular epithelial cells have severe hemorrhage (short thick arrows); scale bar 100 µm

Effects of zinc on spleen histology

There was no damage to the spleen tissue when exposed to zinc. Spleen tissue in the zinc treated groups (Figure 3 B and 3 C) and the control group (Figure 3 A) had a clear structure with consistent white pulp bundles and comparable proportions of red and white marrow. However, in the Zn90 group (Figure 3 D), there was a distinct

invasion of red pulp into white pulp, and the white pulp bundle was smaller compared to other groups.

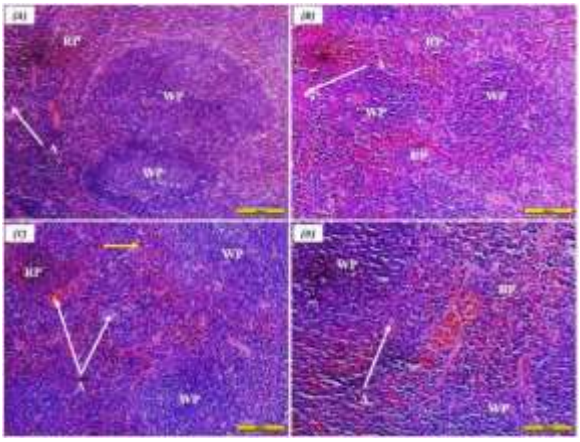

Figure 3: Effects of zinc on the appearance of mouse spleen tissue. Control group (A); Zn50 group (B); Zn70 group (C); Zn90 group (D). **Key:** White pulp (WP); red pulp (RP); artery (A); scale bar 100 µm

DISCUSSION

Zinc supplementation has been implicated in various metabolic processes thus contributing to altered biochemical parameters [6]. Mice initially gained weight rapidly, followed by a slower increase, which is typical for their development. Zinc-supplemented mice gained weight slower than the control group at 4 – 6 weeks, possibly due to zinc's role in regulating metabolic processes [6]. It can impair metabolic function and lead to increased fat storage [7]. Morever, high levels of zinc may negatively affect nutrient absorption and metabolism [7]. High dose of zinc (90 mg/kg) resulted in slower weight gain compared to other zinc supplementation groups. This may be due to a more pronounced effect on metabolic processes related to weight gain. However, further research is needed to understand the exact mechanism of zinc supplementation on weight gain in mice.

Zinc significantly affects hematological parameters, reducing RBCs, platelets, Hb, MCHC, HCT, and clotting time [4,8,9]. This is as a result of zinc interference with iron metabolism and erythropoietin production resulting in bone marrow defects and anemia. Zinc toxicity leads to accumulation of lipid acids in RBCs, making them more prone to destruction. This lowers RBC count and induces anaemia [4]. Furthermore, zinc increases total bilirubin levels, correlating with direct and indirect bilirubin levels. Higher zinc concentrations cause greater RBC destruction and higher bilirubin levels. Hemoglobin, hematocrit, or MCHC are more parameters to evaluate anemia. Our findings suggest that mice infected with zinc (90 mg/kg) experienced mild anemia after 8 weeks. WBCs increased for first four weeks and decreased after eight weeks with zinc treatment. A high daily intake of zinc could lead to long-term consequences on the immune system [10], decrease in B lymphocytes count [11]. Mice platelet counts decreased in the zinc groups at 90 mg/kg BW, consistent with previous studies [8,9]. Clotting time went down instead of increasing, possibly due to increased plasma fibrinogen content influenced by zinc activation of the coagulation system [12]. Further research is needed to fully comprehend role of plasma fibrinogen in inflammation or coagulation.

Excessive intake can be toxic to the liver, increasing liver enzymes AST and ALT [11,13]. Higher urea and creatinine levels indicate kidney disorder. Mice consuming zinc had elevated bilirubin levels in plasma and this indicates potential liver damaged or anemic [14]. Histological imaging confirmed previous findings of zinc-induced damage in the liver and kidney, including increase in kupffer cells, congestion, hepatitis, chromatin condensation, glomerular cell proliferation, interstitial inflammation, and glomerular obstruction, liver cell necrosis, glycogen accumulation, epithelial cell necrosis in the proximal tubule and swelling [15]. Zinc accumulates in these organs causes inflammation, degeneration, hemorrhage, necrosis, and destruction of glomeruli in the liver. High levels of zinc are toxic to cells, causing inflammation, necrosis, and oxidative stress in the liver and kidney. It disrupts sugar and fat metabolism, leads to glycogen reabsorption in the kidney, causing necrosis and swelling of the proximal tubule. Zinc toxicity can also damage cell membranes, causing cell death and tissue damage in the liver and impaired kidney function [15]. Studies related to toxicity caused by heavy metals, especially zinc, only focus on the liver and kidney, with no previous research on the histological structure of the spleen. Consequently, the experimental results indicated that zinc at the three different concentrations investigated did not appear to have a significant impact on the histological structure of the spleen after the eight weeks of treatment.

Accumulation of zinc in the liver and kidney can cause a range of cellular and tissue changes that can lead to inflammation, degeneration, hemorrhage, necrosis, and destruction of glomeruli in the liver. Damage to the spleen has not been recorded. However, further research is

necessary to comprehensively investigate the effect of zinc toxicity on the spleen.

CONCLUSION

Administering varying zinc concentrations to albino mice for 8 weeks results in harmful effect on weight gain, hematological parameters, and organ structures. The group receiving the highest dose has the most severe negative effect, including a reduction in weight gain, abnormal hematological parameters, lower liver enzyme levels, and damage to the liver and kidney with the spleen unaffected. High levels of zinc are toxic and may have adverse consequences on mammalian health. However, further studies are needed to evaluate this level of toxicity. Exploring natural compounds to counteract zinc toxicity is a promising area of research.

DECLARATIONS

Acknowledgements

Financial support was from the Ho Chi Minh City University of Education, Vietnam (Project Code CS.2021.19.39).

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Tri Truong Van: carried the study out, acquisition of data, analysis and interpretation of data, drafting the manuscript, final approval; Nhi Ho Linh Kieu: Analysis and interpretation of data, drafting the manuscript, final approval; Huyen Nguyen Thi Thuong: Analysis and interpretation of data, reviewed the draft of the manuscript, final approval. All authors read and approved the final version of the manuscript.

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