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Original Research Article

Effect of high dose of Zinc on increasing IFN-γ levels

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ABSTRACT

Zinc is administered in the management of diarrhea as a daily supplement at a maintenance dose. However, the dosage of this trace element remains unclear in allergic conditions. Allergy requires strengthening the pattern of Th1 (IFN-γ) and Treg cell cytokines (TGF-β) to suppress the production of Th2 (IL-4). The effect of different zinc dose must be identified on the balance between pro-inflammatory Th1 (IFN-γ) and anti-inflammatory cytokines Treg (TGF-β). Therefore, this research aims to examine the effect of zinc sulfate supplementation from maintenance to high dose on IFN-γ and TGF-β levels before and after supplementation. An experimental analysis, "Pre-Post Control Group Design," was conducted on 30 two-week-old male *Rattus norvegicus* rats. This research included an oral supplementation group receiving zinc sulfate solution at maintenance (0.05 mg and 0.1 mg) and high (0.15 mg and 0.35 mg) dose for 14 days, with one control group. The results showed that IFN-γ levels increased significantly with high zinc dose, while TGF-β levels had a tendency to decrease ($p<0.05$). TGF-β levels were higher at a dose of 0.15 mg compared to 0.35 mg or 0.1 mg. The optimal dose was 0.15 mg, which increased IFN-γ levels and maintained optimal TGF-β levels. High-dose zinc supplementation of 0.15 mg/day was most effective in increasing IFN-γ (Th1) cytokines. Meanwhile, high-dose zinc sulfate supplementation increased IFN-γ and decreased TGF-β levels.

Keywords: High Dose Zinc, Interferron-γ, Transforming Growth Factor-β, T helper cell immune response

Introduction

Naive CD4+ (Th0) cells are differentiated into distinct subsets under the influence of T-cell receptor stimulation by peptides presented on Major Histocompatibility Complex (MHC) class II molecules of antigen-presenting cells (APCs). This subset differentiation can be Th1, Th2, T17, Th9, and Treg. Tregulators play an important role in preventing the presence of excessive Th2 or the strengthening of Th1 or Th17, causing autoimmune diseases. A normal immune response is expected to maintain a balance between the four CD4+ subsets.[1] The proposed hypothesis of zinc interaction with protein kinase C and lymphocyte tyrosine kinase explains the decreased T cell activation. Zinc deficiency reduces Th1/Th2 ratio, characterized by decreased IFN-γ production. The supplementation can enhance immunity and effectively reduce the chronic inflammatory response.[2] According to the theory of health development or the origins of disease, which states that environmental exposures of individuals during the critical early stages of life (prenatal) influence short-term and long-term health status.

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The developmental plasticity of the immune system depends on the underlying environment, from conception through birth, including exposure to infections, microbiota, diet, and other factors.[3] The innate immune system also determines the developmental pattern of the adaptive system. Limited T cell function is associated with the normal maturation of activation signals required for B-T cell crosstalk, which can interfere with the germinal centers in lymphoid tissue.[4,5]

Zinc is an essential trace element reported to have immunomodulatory properties. However, high concentrations of this trace element have the potential to impair T cell function. Previous research reported inconsistent results due to differences in methodology, including variations in dose. In this context, further research is needed to investigate the detrimental effects of high dose of zinc on T cell activation.[5] Inflammatory reactions affect intracellular homeostasis, and evidence of decreased zinc levels was found in association with allergic inflammation, such as atopic dermatitis, bronchial asthma, and chronic rhinosinusitis. Allergies and infections reduce intracellular zinc levels through the expression of ZnT (zinc transporter) gene.[6] Recent research has shown a connection between zinc deficiency and the development of allergies, as well as the positive effects of supplementation in modulating the immune system and reducing symptoms.[7] The administration of zinc-alpha-2-glycoprotein peptide (ZAG) to a cut wound model suppressed the expression of TGF-β and p-Smad2/3, preventing hypertrophic scars, although zinc dose was not mentioned.(8) An in vitro analysis on PBMC (Peripheral Blood Mononuclear Cell) with zinc deficiency conditions led to significantly lower IFN-γ expression.[9] Zinc deficiency and topical administration reduce IFN-γ expression and TGF-β. Allergic diseases are characterized by a predominance of Th2 subset (the primary cytokine IL-4) over Th1 (the primary cytokine IFN-γ). Previous research showed that zinc supplementation reduced allergy symptoms. This is related to the strengthening of IFN-γ, which can suppress the activities of IL-4 from Th2 cells.[10] The opposite results were reported in vitro research on PBMC cultures incubated with 50 µM zinc sulfate. The results showed lower levels of IFN-γ compared to the control. However, there was a

higher percentage of CD4+Foxp3 cells and IL-10 levels than in the control.[10] This research examines the effect of zinc on TGF-*β* as a unique regulatory cytokine promoting immunoreactivity and inflammatory immunity.

TGF-*β* was considered an immune-regulating cytokine due to its capacity to suppress pro-inflammatory cytotoxic cells and promote the activity of immunosuppressive Treg cells. Recent research showed that TGF-*β* played a role in regulating tolerance and enhancing inflammation mediated by Treg and Th17 cells in a manner dependent on the state of the immune response. In the intestinal lamina propria, a balance is maintained by TGF-*β* during T cell activation, with CD4+ cells co-expressing TGF-*β*, Foxp3, and RORγt in a continuous counterbalance. Low concentrations of TGF-*β* synergize with IL-6 and IL-21 to support Th17 differentiation by promoting the expression of IL-23R. High concentrations of TGF-*β* suppress Th17 and promote Foxp3 to support iTreg (FoxP3).[11] Furthermore, Foxp3 inhibits the differentiation of Th0 cells into Th17, Th1, and Th2 subsets through a mechanism including IL-10–mediated suppression of MHC epitope presentation by dendritic cells (DCs) to $CD4^+$ T-cell receptors, halting further effector Th0 differentiation. Another pathway of differentiation inhibition by IL-2 storage (IL-2 sequestration) is by high CD25 expression on the surface of Treg cells.[12] Autoimmune and allergic diseases are chronic inflammatory conditions, requiring the activation of Treg cells. However, allergic diseases tend to require Th1 (IFN-*γ*) activation with Treg. Zinc supplementation significantly inhibits the production of allergic cytokines (IL-4), reduces eosinophil activity, suppresses mast cell activity, and improves allergic symptoms. [13,14]

Treg cells play a crucial role in maintaining normal immune responses by producing the cytokines TGF-*β* and IL-10. These cells suppress autoimmunity and are also included in tissue maintenance, repair, and regeneration under physiological and pathological conditions.[12] In vitro experimental research on human PBMC cultures incubated with 50 µM zinc sulfate showed a higher percentage of CD4+FoxP3 (Treg) cells compared to controls.[15]

The question remains regarding the specific high dose of zinc required to elicit optimal enhancement of IFN-*γ*, while careful consideration must also be given to the effects on TGF-*β* in influencing Treg subset. TGF-*β* and Treg are required to suppress Th1, Th2, and Th17 when their activities are excessive. In addition, TGF-*β* induces the expression of Foxp3, a transcription factor essential for promoting Treg formation, which suppresses inflammation and prevents autoimmune reactions. Surface expression of Treg CD28, CTLA4, TCR, and PD-1 ligand inhibits co-stimulatory signals from APCs to effector T cells. Effector T cells also differentiate into Th1, Th2, and Th17 depending on the extent of inhibition from Treg and the other cytokines present.[16]

Research on the role of zinc in IFN-*γ* cytokines and Tregs remains contradictory and inconsistent, respectively. In vivo research is needed to determine the most effective dose for enhancing IFN-*γ* and providing a safe effect on the regulatory cytokine TGF-*β*, specifically in allergic cases. The effects of different zinc dose on IFN-γ and TGF-*β* cytokines need to be investigated. Therefore, this research aims to determine the effects of various dose of zinc supplementation on IFN-*γ* and TGF-*β* cytokines in healthy young rats.

Materials And Methods

Ethical clearance
This research was conducted in the laboratory of the Center for Food and Nutrition Studies at Gadjah Mada University, and an Ethical Clearance permit was obtained from the Medical/Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University, Semarang, with clearance number: No. 366/XI/2022/Bioethics Commission.

Experimental Design
This experimental research used a pre-post-test control group design. Data collection was conducted before and after treatment by comparing the groups that received treatment with the control group. Zinc dose was adjusted according to the Recommended Dietary Allowance (RDA) of 11 mg/day for adults,[17]reporting the body weight of an adult (200 g) to young rats (50 g). The adjustment for 50 g rats was obtained, including a maintenance dose of 0.05 mg. Subsequently, the dose was increased to two (0.1 mg), three (0.15 mg), and seven times the maintenance dose i.e. 0.35 mg. The Zinc solution used was zinc sulfate monohydrate from Kimia Farma pharmaceutical factory.

The experimental animals used were 2-week-old 30 male Wistar rats of *Rattus norvegicus* strain. Wistar rats were acclimatized for 7 days. The rats were randomly divided into five groups, namely the control group (group 1), rats administered 0.05 mg of zinc sulfate (group 2), rats administered 0.1 mg of zinc sulfate (group 3), rats administered 0.15 mg of zinc sulfate (group 4), and rats administered 0.35 mg of zinc sulfate (group 5). Each group consisted of 6 healthy male rats, and oral supplementation of zinc sulfate was given for 14 days in 4 different doses of 0.05 mg, 0.1 mg, 0.15 mg, and 0.35 mg, for group 2, 3, 4, and 5, respectively while for the control group (i.e. group 1) did not receive zinc sulfate monohydrate supplementation.

The measurement of IFN-*γ* and TGF-*β* levels were performed before (pre-test) and after treatment (post-test). Measurement of IFN-*γ* and TGF-*β* levels were carried out using an ELISA Kit (MyBioSource MBS2019134, USA). Histopathological examination of the spleen was performed using Hematoxylin and Eosin staining to assess differences in the area and perimeter of the central germinativum among the treatment groups. The procedure used an Olympus CX 21 microscope, with 4x magnification, equipped with an Optilab advance & V2 camera. A simple assessment of the activity of the splenic lymphoid Germinal Center was performed by measuring the circumference (µm) and area (cm^2), which can be seen on the imaginary line in Figure 3.

Statistical analysis
Statistical analysis was conducted using SPSS version 25. One-way Analysis of Variance (ANOVA) was used to evaluate differences in cytokine levels between the control and treatment groups. Also, a paired t-test was used to identify differences in levels before and after treatment, where a p-value <0.05 was considered significant.

Results and Discussion

The development of the immune system early in life is influenced by host, environmental, and microbial factors.The disruption in the regulation of this critical period during early postnatal development leads to diseases that adversely affect health across the lifespan. [18] Zinc deficiency during pregnancy is also associated with developmental abnormalities in the anatomical structures of embryonic tissues and the immune system. [18,19] This trace element provides important benefits from the fetus to adulthood. The metabolism also plays a crucial role in regulating intracellular signaling in innate and adaptive immune cells. Zinc can influence key immune system mechanisms, including antibody formation, inflammatory signaling, and lymphocyte differentiation. This element is implicated in various infectious, allergic, and autoimmune diseases.[20]

Intracellular pathogen-induced IFN-*γ* cytokine analysis can be related to zinc deficiency. Previous research proposed the hypothesis that zinc had a strong effect on the post-transcriptional stage of IFN-*γ* mRNA.[21] Another investigation on healthy, pre-school children aged < 3.5 years showed an increase in IFN-*γ* levels after administering 10 mg of zinc for 4 months compared to the placebo group.[22] The previous cohort analysis reported a relationship between newborn zinc levels and the onset of allergic reactions at 3 years of age.[23] This research was conducted on young rats aged 2 weeks. Zinc supplementation at higher dose strengthens the immune response against infection or Th1 amplification. IFN-*γ* levels increased when the administered high dose is equal to or more than three times the maintenance dose. However, the results were inconsistent at maintenance and twice the required dose. This condition is influenced by the levels before treatment, where IFN-*γ* cytokine levels in the control group are significantly different from the others. Previous cohort analysis proved that lower levels of zinc in newborns were associated with the occurrence of allergies before 6

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months of age. In this context, allergic diseases were influenced by the greater dominance of Th2 (IL-4) compared to Th1 cytokines (IFN-*γ*).[24]

IFN-γ Analysis

The conditions before zinc supplementation intervention varied but were relatively homogeneous. In Table 1, the results of the homogeneity test before treatment showed that there are indeed differences in levels between the control and intervention groups. The levels in the three intervention groups are similar, while the control group is equivalent to group 4. Generally, the conditions before treatment are relatively homogeneous. Table 2 shows that there are significant differences in IFN-*γ* levels before and after zinc supplementation among the five groups (p = 0.000). Zinc supplementation tends to increase IFN-*γ* levels, while the control showed a decrease in the levels. The results of the post hoc analysis showed no significant difference in IFN-*γ* levels between the control group and the 0.05 mg (group 2) or 0.1 mg (group 3) dose, with p> 0.05. IFN-*γ* levels were significantly higher than the control at high dose of 0.15 mg (group 4) and 0.35 mg (group 5) with a p = 0.000. This can be seen from figure 1.

Table 1: Results of the statistical analysis of the Post-Hoc test on the difference in mean IFN-*γ* levels between groups before zinc supplementation

	p-value				
Groups	Group 1 (Control)	Group 2	Group 3	Group 4	Group 5
Group 1	-	0.001	0.003	0.161	0.000
Group 2		-	0.570	0.021	0.289
Group 3			-	0.070	0.110
Group 4				-	0.002
Group 5					-

Table 2: Differences in IFN-γ levels pre and post-zinc supplementation

	Mean (SD)	Mean Difference (95%C.I)	p*
Pre-test IFN-γ group1	31.21 (0.91)	0.36(0.25-0.47)	0.000
Post-test IFN-γ group1	30.85 (0.94)		
Pre-test IFNγ group2	28.76(1.02)	-1.10(-1.25- -0.95)	0.000
Post-test IFN-γ group2	29.86(0.95)		
Pre-test IFN-γ group3	29.12(1.27)	-1.56(-1.79- -1.33)	0.000
Post-test IFN-γ group3	30.68(1.25)		
Pre-test IFN-γ group4	30.31(1.09)	-5.06(-5.18- -4.94)	0.000
Post-test IFN-γ group4	35.37 (1.05)		
Pre-test IFN-γ group5	28.09 (1.10)	-7.19(-7.34 - -7.03)	0.000
Post-test IFN-γ group5	35.27 (1.12)		

* Paired t-test

results in Table 3 show a comparison between groups. The high-dose zinc intervention group had different levels compared to the others. IFN-*γ* levels were significantly higher at 0.15 mg/day and 0.35 mg/day, with p=0.000. The results differed when the research was conducted on unhealthy rat models with infections, allergy, or autoimmune diseases.[25, 26] Animal research showed that zinc deficiency in a rat model of sepsis reduced IFN-*γ* production, which led to a shift towards Th2 and subsequently worsening the immune imbalance. [27] Zinc acts as an intracellular signaling molecule in dendritic monocytes and macrophages. Additionally, this trace element plays a crucial role in cellular immunity and in mitigating oxidative stress. The deficiency is associated with increased pro-inflammatory cytokines, oxidative stress, and immune disorders. [28]

TGF-*β* Analysis

The levels of Transforming Growth Factor-*β* (TGF-*β*) before zinc intervention are relatively the same. Table 4 shows that TGF-*β* levels of the control group before treatment are similar to others. Therefore, the condition of rats before treatment was homogeneous. Table 5 shows that supplementation reduces TGF-*β* levels. In groups 3, 4, and 5, TGF-β levels decreased significantly (p = 0.00). The control group experienced a significant increase in the levels of TGF-*β*. The maintenance dose of zinc did not show a significant decrease in TGF-*β* levels, p = 0.403. Figure 2 reports a significant decrease in TGF-*β* levels at higher dose of zinc sulfate supplementation (0.1 mg, 0.15 mg, and 0.35 mg) compared to the control (p = 0.000). However, there was no difference in TGF-*β* levels between controls and doses ranging from 0.05 mg (p = 0.059).

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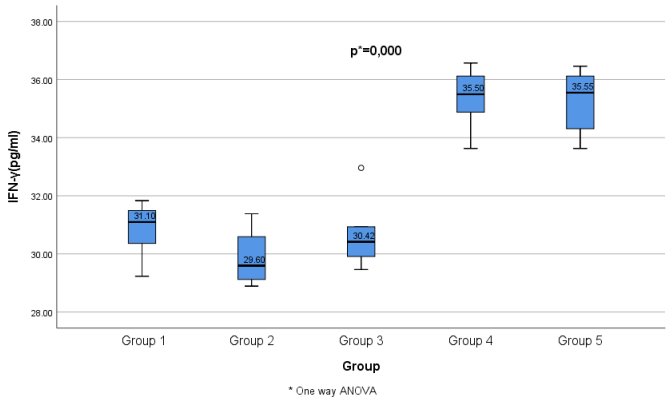

Figure 1: The average IFN-γ levels after zinc sulfate supplementation showing that the increase in zinc dose is followed by increase in IFN-γ levels. Description: Group 1: control; Group 2: zinc dose of 0.05 mg; Group 3: 0.1mg dose; Group 4: 0.15mg dose; Group 5: 0.35mg dose

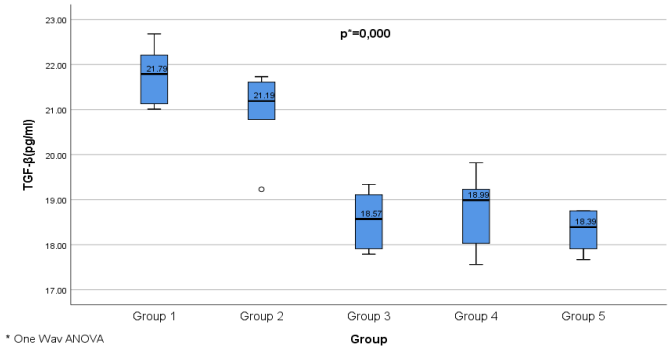

Figure 2: The average TGF-β levels after zinc sulfate supplementationshowing the significant decrease in TGF-β levels at higher doses of zinc sulfate supplementation (0.1 mg, 0.15 mg, and 0.35 mg) compared to controls (p=0.000), but there was no difference in TGF-β levels at a maintenance dose of 0.05 mg (p=0.059). Description: Group 1: control; Group 2: zinc dose of 0.05 mg; Group 3: 0.1mg dose; Group 4: 0.15mg dose; Group 5: 0.35mg dose

IFN-*γ* levels were significantly higher in the high-dose zinc supplementation group (0.15 mg and 0.35 mg) compared to the control, as well as 0.05 mg and 0.1 mg, p = 0.00. There was no significant difference in IFN-*γ* levels between the control at 0.05 mg and 0.1 mg, p > 0.05. After zinc supplementation, TGF-β levels were significantly lower than the control group at doses of 0.1 mg, 0.15 mg, and 0.35 mg, p = 0.000. Table 6 does not report any difference in TGF-*β* levels between groups 3, 4, and 5. However, there were differences in TGF-*β* levels between the control and the groups 3, 4 and 5. The levels of the control group and group 2 (i.e. the maintenance dose of zinc were not significantly different. From Figure 2, groups 3, 4, and 5 have lower levels than the control group and group 2.

The decrease in TGF-*β* levels was lowest or decreased in groups 3 and 5. Therefore, the high dose of group 4 (0.15 mg) was the best for increasing IFN-*γ* levels optimally. The decrease was smaller when compared to groups 3 or 5, even though TGF-*β* was reduced. TGF-*β* regulates the activity of effector cells in adaptive immunity. This regulator is important for generating the presence of Tregs, inhibiting the function of other effector T cells, and antigen-presenting dendritic cells (DCs).[29]

Table 3: Results of the statistical analysis of the Post-Hoc test on the difference in mean IFN-*γ* levels between groups after zinc supplementation

	p-value				
Groups	Group 1 (Control)	Group 2	Group 3	Group 4	Group 5
Group 1	-	0.120	0.786	0.000	0.000
Group 2		-	0.194	0.000	0.000
Group 3			-	0.000	0.000
Group 4				-	0.878
Group 5					-

Table 4: Results of the statistical analysis of the Post-Hoc test on the difference in mean TGF-β levels between groups before zinc supplementation

	p-value				
Groups	Group 1 (Control)	Group 2	Group 3	Group 4	Group 5
Group 1 (Control)	-	0.346	0.004	0.636	0.370
Group 2		-	0.033	0.162	0.073
Group 3			-	0.001	0.000
Group 4				-	0.668
Group 5					-

Table 5: Differences in TGF-β levels pre- and post-zinc supplementation

	Mean (SD) pg/mL	M.D (95% CI)	p*
Pre-test TGF-β group 1	21.57(0.58)	-0.19(-.38- -0.01)	0.042
Post-test TGF-β group 1	21.77(0.64		
Pre-test TGF-β group 2	21.17(0.92)	0.22(-0.39 - 0.83)	0.403
Post-test_TGF-β group2	20.96 (0.92)		
Pre-test TGF-β group 3	20.24 (0.62)	1.69(1.64 -1.74)	0.000
Post-testTGF-β group 3	18.5483 (0.62)		
Pre-test TGF-β_group 4	21.77(0.84)	3.0(2.95 -3.05)	0.000
Post-testTGF-β group 4	18.77(0.83)		
Pre-test TGF-β group 5	21.9500 (0.53)	3.64 (3.54-3.75)	0.000
Post-test TGF-β group 5	18.31(0.47)		

*Paired T-test

Previous research stated the combined effect of zinc with TGF-β1 on PBMC and mixed lymphocyte cultures (MLC) in increasing Foxp3 expression. [30] Another analysis stated that the administration of 200 µM zinc to hepatic stellate cells reduced TGF-β RI, without affecting TGF-β RII. [31] Therefore, there is a need to investigate the effect of zinc on TGF-β cytokine. An increase in the dose of zinc can reduce TGF-β levels. This is because if the zinc dose is equal to or more than twice the maintenance dose then, TGF-β levels decreases. In assessing the influence of the subject's condition before treatment, they were no significant difference between the levels of cytokines of the control group and the other groups i.e. groups 2, 4 and 5,except for group 3. In this context, zinc should be administered cautiously since excessive supplementation can trigger a decrease or an increase in specific cytokines, with undesirable effects. As an anti-inflammatory cytokine, TGF-β decreased in levels when higher dose were administered compared to the maintenance dose. Suggesting that zinc supplementation should be preceded by a measurement of the individual's zinc status.

Table 6: Results of the statistical analysis of the Post-Hoc test on the difference in mean TGF-β levels between groups after zinc supplementation

	p-value				
Groups	Group 1 (Control)	Group 2	Group 3	Group 4	Group 5
Group 1	-	0.059	0.000	0.000	0.000
Group 2		-	0.000	0.000	0.000
Group 3			-	0.595	0.568
Group 4				-	0.275
Group 5					-

Table 7: The area and perimeter of the germinal center of the spleen

Area (cm^2)	N	Mean	p*	Perimeter(µm)	N	Mean	p
Group 1	6	0.16		Group 1	6	14506.46	
Group 2	6	0.13	0.19	Group 2	6	13306.00	0.14
Group 3	6	0.15		Group 3	6	14429.84	
Group 4	6	0.24		Group 4	6	17494.06	
Group 5	6	0.17		Group 5	6	16459.18	

*One-way ANOVA test

Figure 3 provides a basic assessment of B-cell and T-cell activity in lymphoid tissue using Hematoxylin and Eosin staining. This assessment measures the area (cm^2) and circumference (µm) of the central germinativum of the spleen. The results showed no significant difference based on the statistical analysis in Table 7.

Table 7 shows that there is no significant difference in the area or perimeter of the germinal center of Lien among the five groups ($p > 0.05$). However, the germinal center activity of the spleen in the 0.15 mg dose group was the largest in both area and perimeter length. The area of the central germinal region was assessed to approximate the activity. The germinal center served as the site for B-cell maturation after antigen contact, including the presence of Follicular Dendritic Cells (FDCs).[32] The lack of significant differences in the central germinal spleen area was because rats were uninduced or healthy.

In human when PBMCs were purified to obtain T cells and subsequently stimulated with zinc aspartate at concentrations ranging from 40 µM to 140 µM for 72 hours, a dose-dependent decrease in IL-1ra, TGF-β1, and IL-10 was observed, and this was accompanied by an increase in IL-6. Increased apoptosis occurred at zinc aspartate concentrations exceeding 100 µM.[33] The decrease in TGF-β levels associated with high zinc dose showed possible inhibition of Treg cells and increased Th1 activity. The administration of zinc to rats with pathogenic TH17 cells did not activate Th17 response or inflammation. Kitabayashi described the binding of zinc to Signal Transducer and Activator of Transcription 3(STAT3) in reducing Th17 polarization and suppressing autoimmune diseases.[34] A meta-analysis of 14 observational research and three Randomized Controlled Trials (RCTs) concluded that a significant decrease in serum, hair, and erythrocyte zinc levels exist in atopic dermatitis when compared to controls [14]. Even though several research have proven the benefits of zinc supplementation in allergic and autoimmune diseases, the dosage should be administered with caution. This is because the levels of zinc fluctuate in cells and affect T cell differentiation.[5, 35]

Zinc deficiency and supplementation affect the development and function of T cells, B cells, natural killer (NK) cells, monocytes, and macrophages. In this context, compromised immune cells lead to impaired elimination of bacteria or an increase in the initial inflammatory response destructive to host tissues. Zinc deficiency inhibits NK cell activity, T helper 1 cytokine production, and B cell response. The supplementation enhances the immune response during infection by mitigating excessive production of pro-inflammatory cytokines and preventing uncontrolled inflammation.[35] Control of excessive inflammation requires tolerance mechanisms. Dendritic cells inhibit T cell effector responses through mechanisms that include the production of anti-inflammatory factors such as transforming growth factor (TGF-β) and interleukin (IL)-10. These cytokines suppress the function of effector T cells and induce Treg cells to reduce inflammation, maintain tolerance to self-antigens, and control autoimmunity. Th17 is a subset of T-helper cells that primarily secretes IL-17 in the development of autoimmune diseases. Therefore, an adequate balance of effector T and Treg cells is required to maintain tissue homeostasis under pathological conditions.[36]

An in vitro research was conducted on C57BL/6 mice aged 8-12 weeks, which were treated with 100 µM ZnSO4 and *H. capsulatum* (strain 217B) on Dendritic Cells (DCs) derived from bone marrow. The results provided evidence of an increase in the percentage of PD-L2+ MHCIIlo DCs in groups infected with *H. capsulatum* (217B) compared to the controls. Tolerogenic DCs expressed PD-L1, PD-L2, and possessed low expression of MHC II (MHCIIlo), as well as immunosuppressive mediators such as Indoleamine 2,3-Dioxygenase (IDO), TGF-β, and IL-10.[35] The results reported that excessive zinc administration reduced the anti-inflammatory cytokine TGF-β, inhibiting the tolerance function of immune response. The ability to induce immune tolerance is necessary to inhibit Th2, as evidenced by the effect of corticosteroids in increasing FOXP3 (Treg) and reducing GATA-3 (Th2) expression.[37] Meanwhile, this research proved that the administration of high zinc reduced TGF-β levels.

A double-blinded, randomized controlled trial research suggested a faster decrease in the pediatric respiratory assessment measure (PRAM) score in the group receiving zinc bis-glycinate 30 mg/day for a maximum of four days compared to the group without treatment .[38] Further investigation should also be conducted on the average zinc levels of a population associated with various disease complaints. The role of zinc supplementation in populations with low levels of the element reduces the incidence of disease or health disorders. Previous research analyzed the effects of zinc on early childhood growth, but the results were inconsistent with others. A combination of zinc and vitamin B complex, or other multivitamins, did not reduce the incidence of underweight, stunting, and severe malnutritionin infants in Tanzania.[39] Another interesting result proved the benefits of zinc in a rat model of polycystic ovarian syndrome, where a combination of curcumin and Zinc improved reproductive hormone levels to normal.[40]

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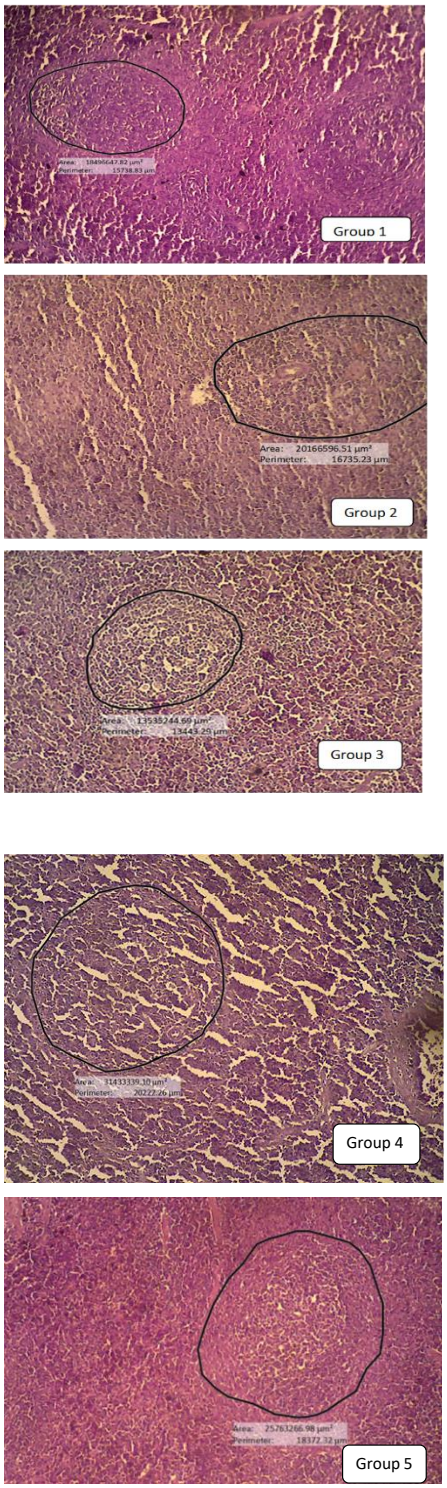

Figure 3: Histology of the lymphoid tissue of the spleen organ stained with Hematoxylin and Eosin. showing a simple assessment of spleen activity by assessing the average area and circumference of the central germinativum between groups, the results did not show significant differences.

The form of ZnO Nanoparticles made using *Terminalia catappa* (Almond) methanol leaf extract was effective as an antioxidant and antibacterial agent.[41] The appropriate zinc dose should be in line with the therapeutic indication. Although daily requirements have been determined for children through adulthood to achieve the desired effect, supplementation should be for a specific purpose, such as growth, infection control, or immune system disorders. In this context, an analysis of zinc supplementation is needed to determine the specific dose for certain diseases.

Conclusion

In conclusion, the dose of zinc sulfate had different effects on IFN-γ and TGF-β levels. High-dose zinc sulfate supplementation increased IFN-γ and decreased TGF-β levels. Therefore, high-dose zinc supplementation of 0.15 mg/day was best for cases requiring Th1 (IFN-γ) enhancement. The next phase of research will use the same methodological design, but in mouse models of allergies or autoimmune disorders. The goal of future research is to examine the effects of various zinc doses on the cytokines IFN-γ and TGF-β in immune system disorders. Concerning the limitation of this study, this research did not measure serum zinc levels before and after supplementation. Also, the initial zinc levels influenced the supplementation of the element in rats before treatment. Moreover, the oral zinc intake was influenced by intestinal absorption capacity and substances inhibiting absorption. Additionally, the levels of IL-4, as a Th2 cytokine, were not measured. Further research should also include a randomized controlled trial with three different doses, with 0.15 as the middle dose and a placebo group. The effects assessed will be cytokine and zinc levels before and after zinc supplementation.

Conflict of Interest

The authors declared no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and any liability for claims relating to the content of this article will be borne by them.

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