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Effects of Zinc Sulfate and Vitamin D3 in Young Male Rats with Zinc Deficiency; Hemogram Criteria

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Abstract | The zinc (Zn) is one of the vital elements that are needed for body growth and important physiological processes as well as it improves immune competence and productivity. The current work was undertaken to assess the ameliorating role of zinc sulfate and Vitamin D3 on hematological alterations in male rats with induced zinc deficiency. For this purpose, forty healthy male rats (immature) were randomly divided into two groups; First group (n = 10; control) acted as healthy male rats and received 0.5 ml of dimethyl sulphoxide (DMSO) intraperitoneally (I.P). The second group (Zinc deficiency group) consisted of 30 male rats; received single adose of 1,10 phenanthroline 30 mg/kg dissolved in 0.5 ml dimethyl sulphoxide (DMSO) I.P. After 12 h-fasting, zinc deficiency was determined by measuring the serum zinc level after 24 hrs from 1,10 phenanthroline injected. After treatment, animals were distributed into four groups, 1st group healthy rats acted as negative control treated with physiological saline solution(1ml), 2nd group carried zinc deficiency as positive control treated with physiological saline solution, 3rd group zinc deficiency rats were treated with zinc sulfate 20mg/kg dissolved by physiological saline solution; 4th group zinc deficiency treated with vitamin D 500 IU/kg; The treatment was performed in all groups orally and continued for 30 days. Our findings indicated that zinc deficiency induced hematological changes decreasing erythrogram values in concomitant with increased thrombocytes and leukogram count except LYM value which decreased. In conclusion, zinc deficiency led to moderate anemia whereas the oral administration of zinc and vitamin D corrected the zinc deficiency-induced inflammatory response, consequently improved blood profile erythroproiesis and leukogram.

Keywords | Zinc deficiency, Erythrogram, Leuckogram, Anemia, Young rats**Received** | September 25, 2024; **Accepted** | December 02, 2024; **Published** | December 09, 2024***Correspondence** | Ahlam A. Al-Rikaby, Department of Physiology, Pharmacology and Biochemistry, University of Basrah, Iraq; **Email:** ahlam.abdulnabi@uobasrah.edu.iq**Citation** | Mohammed MA, Al-Rikaby AA (2024). Effects of zinc sulfate and vitamin D3 in young male rats with zinc deficiency; hemogram criteria. J. Anim. Health Prod. 12(s1): 284-291.**DOI** | <https://dx.doi.org/10.17582/journal.jahp/2024/12.s1.284.291>**ISSN (Online)** | 2308-2801**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Zinc deficiency is a common problem around the world because it is clearly affecting the overall health status and may eventually lead to systemic health problems which related to various dysfunctions and alterations of normal cell metabolism (Kodama *et al.*, 2020). Nowadays, cases of severe ZD are uncommon, whereas moderate

deficiency has been widely prevalent, ZD during critical periods of growth has become an important health concern worldwide particularly in pregnant women and children having an imbalanced diet (Sun *et al.*, 2011; Kido *et al.*, 2019). ZD can be due to different causes, which can be related to inadequate dietary intake (protein malnutrition, nutrition without supplementation) an increase in the body's requirements (growth, pregnancy, lactation), (In-

Sook *et al.*, 2007; Atasoy and Bugdayci, 2018), also increased excretion (diabetes mellitus, prolonged diarrhea, treatment with diuretics) and genetic diseases (mutation in the solute carrier family 29 member 4 (*SLC29A4*) gene which encodes the ZIP4 transporter in the duodenum and jejunum leading to acrodermatitis enteropathica; mutation in the *SLC30A2* gene, which encodes the ZNT2 transporter, leading to low zinc concentration in breast milk), or a decrease in intestinal absorption (Kechrid *et al.*, 2006; Kamei *et al.*, 2018). Nutritional zinc deficiency associated clinical manifestations include growth retardation, cell-mediated immunological dysfunction, hypogonadism in males, neurosensory disorders, taste abnormalities, delayed wound healing, dermatitis, hair loss and a higher risk inflammatory disease (Nakamura *et al.*, 2019).

Zinc (Zn) is an essential nutrient that is required in humans and animals for many physiological functions, zinc performs important antioxidative functions in biological systems, mainly as a structural component of the antioxidant enzymes (Rasha *et al.*, 2012; Noha and Tag-El, 2019). Zinc up-regulates the synthesis of proteins with oxidant-scavenging capacity (e.g., Thionein) and prevents the binding of redox-active *met als* to oxidation target cell molecules, Hence, it serves as a dietary supplement to reduce the stress (Dzen *et al.*, 2023). Zinc is essential for enzymes involved in DNA synthesis, mitosis, cell division and protein synthesis. Also, it is a component of many transcription factors and proteins that control the cell cycle (Yamaguchi, 2015; Amjed *et al.*, 2024). Moreover, zinc has antioxidant, anti-inflammatory and antiapoptotic effects it is also involved in maintaining immunity, controlling reproduction, erythrocyte production and maturation, regulating gene expression and cell proliferation and providing protection against the deleterious (Kechrid and Bouzerna, 2004; Tomas-Sanchez *et al.*, 2018). Zinc has a vital role in the hormone secretion and present in secretory granules of cells of the endocrine and exocrine organs (O'Connor *et al.*, 2020). On the other hand, Zn has many important functions in the spermatozoa physiological, including effects on lipid flexibility and sperm membrane stabilization (Abdella *et al.*, 2011). It also has a regulated role in capacitation and the acrosome reaction of sperm and is essential for conception and embryonic implantation (Kumar *et al.*, 2013; Juneet *et al.*, 2020).

A century ago, the role of vitamin D in the health of bones and treatment of rickets was first described by (McCollum *et al.*, 1922). Today, it has been established that vitamin D receptors are widely expressed by nucleated cells and diverse health effects not only in bones but also in numerous other organs (Pittas *et al.*, 2019). Vitamin D is a steroid-like hormone that works by binding to its receptors, the function of vitamin D is classically known to be a factor in regulating calcium metabolism

and bone health (Manson *et al.*, 2019; Ayat and Muna, 2020). However, different physiological roles of vitamin D have been shown in recent years as anti-inflammatory, antioxidant, anti-thrombogenic, and anticoagulant activity (Zhou *et al.*, 2019; Della *et al.*, 2023). Furthermore, it plays a role in the widely expression of many genes in cells, therefore, it has important role in modulation of immunity, as well as its role in the differentiation and induction of erythropoiesis in bone marrow, the regulation of cellular growth (De Martinis *et al.*, 2021). On the other hand, Vitamin D plays a role in an increasing bone mass through it promotes calcium and phosphorus absorption from the intestine or through its stimulation effect of osteoblasts which secrete the ALP enzyme this caused an increase in the concentration of phosphate with calcium ion this led to mineralization (Kuehnisch *et al.*, 2017; Martins *et al.*, 2024). Since vitamin D is a potent regulator of monocyte activity and following a tissue transplant the vitamin D can reduce the severe tissue damage associated with inflammation responses (Charoenngam *et al.*, 2022; Infantino *et al.*, 2022). Because there are few studies were carried out to demonstrated the effects of zinc deficiency on physiological performance of blood human and animals in Iraq, so that, the current work was undertaken to assess the ameliorating role of zinc sulfate and Vitamin D3 on hematological alterations in male rats with induced zinc deficiency.

MATERIALS AND METHODS

ANIMALS HOUSING AND MANAGEMENT

A total forty of male Albino rats (pre-pubertal period), 30 days of age and initially weighing from (55-65 gm) were used in the current study. Rats were housed in plastic cages of the animal's house under standard environmental condition with a temperature 22 °C and illuminated for 12 h per day followed by 12 hrs of darkness with free access to plain water in plastic bottles with stainless-steel sipper tubes and fed commercial diet throughout experiment period, after 7 days of adaptation and then the Zinc deficiency induction in rats.

EXPERIMENT PROTOCOL

ZINC DEFICIENCY INDUCTION

Zinc deficiency was induced by a single intraperitoneal dose of 30 mg/kg of 1,10 phenanthroline (Chang *et al.*, 1977) is a zinc chelator, dissolved by 0.5 ml dimethyl sulphoxide (DMSO) into 12 h-fasted rats, and then to determine if zinc deficiency occurred was confirmed by measuring the serum zinc level after 24 hrs from 1,10 phenanthroline injected. The level of serum Zinc was determined using the absorption spectrophotometer according method as described by (Zheng *et al.*, 2014), serum Zn values below (1 µg/ml) indicated Zn deficiency, rats were selected for

experimentation. Male rats were assigned randomly and equally into four groups, 10 rats each, as follows; group one (healthy rats); rats served as a negative control, this group was administered (1ml) of physiological saline solution orally for 30 days, respectively, group two; zinc deficiency group considered as positive control, was administered (1ml) of physiological saline solution orally for 30 days, respectively, group three (zinc deficiency); was treated with zinc sulfate 20mg/kg. b.w dissolved in (1ml) of physiological saline solution orally for 30 days respectively (Nooralhuda et al., 2021). Group four (zinc deficiency); was treated with vitamin D3 at a dose of 500 IU/kg, dissolved in (1ml) of physiological saline solution orally for the same duration of 30 days, according to (Mohammed et al., 2019).

SAMPLING AND HEMATOLOGIC ANALYSIS

Upon completion of the study period, blood samples were collected and prepared for hematological examination, the animals were fasted overnight, 2 ml of blood were taken from each rat in the control group and experimental groups under ether anaesthesia by heart puncture and put into heparinized tubes, analyzed the hematologic indices within one hour, such as erythrocytes count (TEC), percentage hematocrit (HCT), hemoglobin (HGB) and platelets (PLTs) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) total Leukocyte count (TLC) and types of Leukocyte (lymphocytes, monocytes and neutrophil) count, were estimated by using standard procedures with the count 60 hematology analyzer Genix and USA.

STATISTICAL ANALYSIS

Table 1: Total Erythrocyte count, Hemoglobin concentration, Hematocrit percentage and Platelets count in healthy control, zinc deficiency (ZD), treated with Zinc and Vitamin D3 groups.

Groups	TEC count X10 ⁶ /μL	HGB g/dl	Ht %	PLT _s count X10 ³ /μL
Healthy control/ Physiological saline	6.28±0.1 a	13.01±0.16 a	40.41±1.48 a	310.81±16.33 d
ZD positive control/ physiological saline	3.40±0.31 d	7.59±0.29 d	33.76±1.02 d	424.51±37.07 a
ZD treated with zinc 20mg/ kg	5.66±0.37 b	11.53±0.52 b	38.42±0.33 b	340.76±2.01 c
ZD Treated with VD3 500 IU/ kg	5.04±0.03 c	10.39±0.38 c	37.78±0.39 c	365.84±4.14 b

Findings are expressed as Mean ± SD. the symbol represent statistical difference at (p < 0.05) values compared to control animals.

Table 2: MCV, MCH and MCHC values in healthy control, zinc deficiency (ZD), treated with Zinc and Vitamin D3 groups.

Groups	MCV (fL)	MCH pg/dl	MCHC %
HealthyControl/ physiological saline	54.53±2.20 d	22.27±1.28 a	32.96±1.12 a
ZD Positive control/ physiological saline	63.33±2.53 a	16.41±1.82 d	27.62±1.83 d
ZD Treated with Zinc 20mg/ kg	55.84±1.95 c	21.36±1.24 b	31.60±1.40 b
ZD Treated with VD3 500 IU/ kg	57.65±2.81 b	20.76±1.04 c	30.28±1.35 c

Findings are expressed as Mean ± SD. the symbol represent statistical difference at (p < 0.05) values compared to control animals.

The findings are presented as mean±SD, to evaluate statistical significance of the experimental findings by using one-way analysis of variance (ANOVA), Data were considered significant when P < 0.05.

RESULTS AND DISCUSSION

Zinc is an essential micronutrient that is necessary for the proper functioning of the body’s systems. It plays a vital role in various physiological processes, on the other hand, deficiency of Zn is associated with a variety of diseases and could cause systemic health problems including growth and development, neurological dysfunction, poor immune function and decreased reproductive performance, as a result, almost all-important physiological processes in cells depend on content of zinc (Kwon et al., 2024).

As regarded results in the Tables 1 and 2 indicate that a significantly (P< 0.05) declined in blood indices involved (TEC, HGB, Hct% and MCHC) values in concomitant with significantly (P<0.05) increased in platelets number, MCV and MCH in the zinc deficiency group as comparison to healthy group this an indicator to moderate anemia, zinc deficiency anemia is related to an increase fragility of erythrocytes membrane, this could be attributed to the increased inflammatory response or as a results is ability of Zinc deficiency to lowers the plasma membrane sulfhydryl concentration which is inversely related to osmotic fragility (Atasoy and Bugdayci, 2018; Kido et al., 2019). These outcomes are confirmed by results obtained by (King and Fraker, 2002; Fukushima et al., 2009) who revealed that zinc deficiency exacerbated the inflammatory response and consequent increasing the fragility of the erythrocyte

Table 3: Total leukocyte counts, neutrophils (SEGs), lymphocytes (LYMs) and monocytes (MONOs) percentage in healthy control, zinc deficiency (ZD), treated with Zinc and Vitamin D3 groups.

Groups	TLC count X10 ⁶ /μL	SEGs %	LYMs%	MONOs %
Healthy control/ physiological saline	5.41±0.10 d	29.68±0.91 d	55.65±1.23 a	1.59±0.05 D
ZD positive control/ physiological saline	7.29±0.18 a	39.49±1.53 a	46.45±1.77d	1.88±0.02 A
ZD treated with Zinc 20mg/ kg	5.63±0.52 c	30.62±1.66 c	54.22±1.64 b	1.63±0.02 C
ZD treated with VD3 500 IU/ kg	5.99±0.30 b	32.43±1.71 b	52.22±1.29c	1.69±0.01 b

Findings are expressed as Mean ± SD. the symbol represent statistical difference at (p < 0.05). Values compared to control animal.

membrane, On the other hand, the hematopoietic system is highly sensitive to the oxidative stress resulting from zinc deficiency, (El-Hendy *et al.*, 2001; Nakamura *et al.*, 2019) who mentioned that the nutritional zinc deficiency altered the balance between zinc and copper in blood caused the low Cu/Zn-SOD activity, ultimately resulted in the accumulation of ROS therefore the zinc deficiency may aggravate inflammatory reaction via enhancement of ROS thereby causing decreased erythropoiesis and shortened erythrocyte lifespan. Furthermore, the significantly decreased in HGB content, PCV% and MCHC in rats supplies with zinc deficient obviously in parallel to decrease in red blood cells count was either indicative of excessive damage to erythrocytes in the spleen and caused splenomegaly or inhibition of erythrocyte formation, these outcomes are described by (Kido *et al.*, 2021). In rats, the consumption of a zinc-deficient diet lowered erythroid ALAD activity, the first cytosolic enzyme in the heme biosynthetic pathway, thus, the lower levels of porphyrin produced by zinc-deficient erythroid cells may be attributable to the zinc dependence of their ALAD activity (Konomi and Yokoi, 2005). these results are also supporting those in our study. So, in another research by (Bushra *et al.*, 2010) who demonstrated that the dietary Zn deficiency may affect erythrocyte differentiation via loss of function of the zinc finger protein GATA-binding protein 1 (GATA1), a transcription factor involved in erythropoiesis. Therefore, it is possible that the expression of GATA1 as well decreases during the conditions of zinc deficiency. According to findings that seen by (Konomi and Yokoi, 2007). Who recorded that zinc deficiency cause to impairs folate intestinal absorption followed by decreasing in above indices values in rats when fed on dietary Zinc deficient, since Zn is essential for integrity of the immune system, its deficiency results in reduced immunocompetence and decreased resistance to infections, according outcomes in the Table 3 illustrated that the Zinc deficiency cause to a significantly (P < 0.05) elevation in total leukocytes (TLC) number along with increased in neutrophils and monocytes numbers in concurrently with decreased in lymphocytes number, a decrease was markedly as compared with the health group. The results indicate that inflammatory response occurring during zinc deficiency is characterized by increasing white blood cells number especially neutrophils and decreased lymphocytes

in the blood (Zhao *et al.*, 2013), these results are consistent with those of previous studies by (Konomi and Yokoi, 2007; Kido *et al.*, 2018) have shown that an increase in total white blood cells, neutrophils, and platelets counts of the zinc-deficient animals this could be probably due to whole-body inflammatory response. On the other hand, inflammatory responses to zinc deficiency may be affected by increased amount of ROS and macrophage activation, which increases generation of pro-inflammatory cytokines and subsequent increase hematopoietic stem cell proliferation and differentiation into granulocytes (Kodama *et al.*, 2020). Furthermore, enhanced mobilization of neutrophils from the bone marrow and prolonged vascular half-life leading to neutrophilia along with decreased peripheral lymphocytic count, these observations described by (Sato *et al.*, 2022). In previous study, revealed that a significant decrease in lymphocytes in Zn deficient goats may be attributed to the ability of Zinc deficiency to decrease the activity of serum thymine (a thymes hormone), which is required for maturation of T helper cells, resulting in cell-mediated immune dysfunction (King *et al.*, 2005). Also (Prasad, 2014) who found that the immune system function is impaired even in cases of moderate Zn deficiency.

As it is evident from the data, indicate that the administered of zinc and vitamin D3 to rats with zinc deficient can inhibit the zinc deficiency-induced inflammatory response, thereby preventing the occurrence of hemolytic anemia and improved erythropoiesis via increased the amount of erythroid precursor this may be attributable to antioxidant and anti-inflammatory properties which have zinc and vitamin D3 (Ulutas *et al.*, 2020; Keywanloo *et al.*, 2021). Previous studies reported that the zinc supplementation stimulates erythropoiesis in fish and rats, the beneficial effect of zinc may be said to be due to its binding effect on 43-kDa zinc binding protein that is present in the digestive tract of tissue and it is used as a signal to stimulate the formation of new red blood cells in the bone marrow (Chen *et al.*, 2017; Yen-Hua *et al.*, 2018), these results may confirm the results of our study. some studies have shown that zinc supplementation can correct abnormal blood indices this might be attributed to the amelioration the activity of several antioxidant enzymes involved in cellular defense. In addition, the improved Hct percentage might be attributed to improved liver functions (Malhotra and Dhawan, 2008;

Sharifian *et al.*, 2012), these results in agree with those in current study. Another study on rat by (Roozbeh *et al.*, 2009; Kido *et al.*, 2021) who observed that anemia-related factors (RBC, HCB, and Hct) were decreased during zinc deficiency and recovered by zinc supplementation, indicating that the potential antioxidant activity of zinc prevented polyunsaturated fatty acid oxidation in the erythrocyte membrane and suppress premature erythrocyte lysis subsequently increasing erythrocytes number. These results concurs with some authors who experimented on broiler chicks, rats and humans and equally reported that restored the erythrogram and leukogram values to their levels equivalent to those in the standard group following treatment of zinc this may be attributed to the increased in the rate of erythrocyte production and subsequent increased in the blood content (Donmez *et al.*, 2001; Baltaci *et al.*, 2003; Al-Nahal *et al.*, 2024). Also, Chen *et al.* (2013) who demonstrated that the recovery of RBC to the normal level after zinc supplementation was combined with serum transferrin to induce erythropoiesis.

At same time, the administration of VD to rats with zinc deficiency ameliorated blood indices this may be due to the vitamin D has antioxidant and DNA-protective properties and it can control the maturation and production of red blood cells as well iron metabolism and hemoglobin synthesis (Soliman *et al.*, 2012; Refaat *et al.*, 2014). These outcomes in consistent with (Hewison *et al.*, 2001; Marwah *et al.*, 2012; Akbas *et al.*, 2016) who found that the administration of vitamin D to patients with inflammation could improve anemia this may be attributed that vitamin D can stimulate erythrocyte precursor cell receptors, which enhance the maturation and proliferation of congenital erythroid cell, furthermore, it may increase the production of erythropoietin, which is a hormone that regulates the creation of red blood cells (RBC) in the bone marrow. Other studies (Icardi *et al.*, 2013; Park *et al.*, 2017; Mitrasinovic-Brulic *et al.*, 2021) have shown that vitamin D is also effective in erythropoiesis because VDR is expressed in the bone marrow by specific cell subsets such as stromal and accessory cells. Also (Reda *et al.*, 2023) who reported that patients with chronic kidney disease undergoing hemodialysis showed a significant increase in Hb concentration and Hct level after treatment with vitamin D. According to previous reports, vitamin D administration cause to increase the production of erythropoietin, this can raise RBC count, hematocrit and hemoglobin values and mean corpuscular hemoglobin concentration this could possibility be vitamin D has anti-thrombogenic, anti-inflammatory and anticoagulant (Medrano *et al.*, 2018; Megahed *et al.*, 2020), these results are supported those results in current work.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, it can be concluded from findings in this work that zinc deficiency led to moderate anemia whereas the oral administration of zinc and vitamin D corrected the zinc deficiency-induced inflammatory response, consequently improved blood profile erythroproiesis and leukogram.

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NOVELTY STATEMENT

This study presents novel insights in to the zinc deficiency, Zinc is an essential trace element of life that is required in humans and animals for many physiological functions, zinc deficiency led to systemic health problems including growth and development and poor immune function, this work evaluates the effects of zinc deficiency on complete hemogram count and therapeutic role of zinc sulfate and vitamin D3 on hematological alterations. The finding indicate that the zinc deficiency exacerbated the inflammatory response and anemia whereas the oral administration of zinc and Vitamin D corrected the zinc deficiency-induced inflammatory response, consequently improved blood profile erythroproiesis and leukogram, this is first evaluation of zinc deficiency on physiological performance of blood in human and animals in Basrah/Iraq, highlighting adverse effects of zinc deficiency on humans and animals' health.

AUTHOR'S CONTRIBUTION

AAA-R: Methodology, writing original draft preparation, writing review and editing, supervision and project administration. MAM: Investigation, statistical analysis and data curation. AAA-R and MAM: Resources. The authors have reading and agreed to the published version of the manuscript.

CONFLICTS OF INTERESTS

The authors have declared no conflict of interest regarding the publication of this research.

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