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ORIGINAL ARTICLE

The Effect of Marginal Zinc Deficiency on Immune System in Diabetic Fatty Rats

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ABSTRACT
Zinc deficiency is one of the leading risk factors for developing disease and yet we do not have a clear understanding of the mechanisms behind the increased susceptibility to infection. A total of fourty male Wistar rats (120-160 g) were given zinc in the form of $ZnSO4.6H_2O$ at a dose level of 227 mg/L daily in their drinking water and diabetes was induced by giving a single subcutaneous injection of streptozotocin (40 mg/kg body weight). The Feed intake and body weight were recorded weekly. On day 56, blood was collected by retro-orbital puncture for analyzing hematological constituents, glucose, cholesterol, total protein, albumin, and globulin and antioxidant enzyme activities. At 57rd day, rats were antigenically challenged with sheep red blood cell to assay humoral immune response and cell-mediated immune response. The results showed that body weight and feed intake were not affected by dietary groups ($P>0.05$). The zinc supplementation had no effect on various hematological constituents analyzed. The serum cholesterol concentration was reduced by Zn supplementation group than the control group. In liver, TBARS and protein carbonyl concentration are indicative of oxidative stress significantly ($p<0.05$) lowered in Zn supplementation diet than the controls. The antioxidant enzyme activities (including GPx and CAT) were higher and MDA lowered for the animals fed Zn supplemented diet than the animals fed controls ($P<0.05$). The humoral and CMI response were higher ($p<0.05$) with Zn supplemented group than control group. The results showed that the inclusion of Zn supplementation in diabetic-induced rats diet caused to improved the antioxidant status.
Keywords: *Diabetes; Rat; Immune System; Zn*

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INTRODUCTION

Zinc is a mineral that plays an important role in insulin action and carbohydrate metabolism [9]. Zinc deficiency is associated with many diseases, including malabsorption syndrome, chronic liver disease, chronic renal disease, sickle cell disease, diabetes and malignancy [37]. Any decrease in zinc levels can affect the ability of the islet of langerhans cell to produce and secrete insulin, and compound the problem, particularly in type 2 diabetes [9]. Zinc deficiency is more common in developing countries, and although severe deficiency is rare in developed countries, marginal deficiency is thought to be relatively common [5, 25].

Zinc deficiency is indicated which may be secondary to immune-induced cytokine synthesis [14, 15]. The immune function is deftly synchronized by zinc, since both increased and decreased zinc levels result in a disturbed immune function [10, 43]. Zinc deficiency when associated with an immunodeficiency, results in an amplified propensity to a range of pathogens [45]. It has also been demonstrated in rat sympathetic neurons [11].

The effect of zinc supplementation on streptozotocin-induced diabetic rats was examined, which exhibited ghrelin (a 28 amino acid peptide) production and secretion, and lipid metabolism on the gastrointestinal tract [6]. Zinc supplementation also alleviated the hyperglycemia of genetically obese mice, which may be related to its effect on the enhancement of insulin activity [12]. Zinc supplementation

is effective for preventing or ameliorating diabetes in several rodent models of type 1 and type 2 diabetes. Many of the symptoms of zinc deficiency and diabetes are the same, and are related to increased inflammation. Zinc supplementation may improve immunity and glycemic control, and reduce inflammation [41]. The purpose of this study was to determine the effect of zinc supplementation on diabetes and immune parameters of wistar diabetic fatty rats.

MATERIALS AND METHODS

A total of fourty male rats in the weight range of 120-160 g were purchased from market. The animals were housed in polypropylene cages bedded with rice husk in the animal house under hygienic conditions with controlled temperature (22-23°C) and photoperiod (12 h/d) for an experimental duration of 8 weeks. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals, and protocols were followed as approved by NRC (1984). The rats were adapted to a semi-purified control diet containing 25ug Zn/g diet and deionized water for 1 wk (Table 1). The animals were randomly divided into main four groups of 10 animals each:

Group I: Normal Control – Animals in this group was given standard pellet feed and water *ad libitum* throughout the period of experimentation.

Group II: Diabetic – Diabetes was induced in the rats using a single subcutaneously injection of at the dose of 4o mg/kg of streptozotocin (STZ) (Sigma Chemical, St. Louis, MO) in cold 0.1 mol/L citrate buffer, pH 4, to induce diabetes.

Group III: Zinc treated- Rats in this group were given zinc in the form of zinc sulphate at a dose of 227 mg /L mixed in the drinking water of animal [34].

Group IV: Diabetes and Zinc treated- Rats belonging to this group were given Zinc as was given to group III rats and diabetes was induced in a similar way as given to group 2 animals.

The animals in all groups were fed standard laboratory feed and water ad-libitum 8-wk periods (Table 1). Diabetic status of rats was checked by urine reagent strips. Animals with urine glucose higher than 500 mg/dl at 3rd day of injection were considered diabetic. Weekly body weights and daily feed intake were recorded. Blood was collected on 56nd day by retro-orbital puncture to analyze the hematological and biochemical constituents and antioxidant enzymes. On 57rd day of experiment, humoral immune response was examined by antigenically challenging the rats with sheep red blood cell (RBC). On 57th day of experiment, the CMI response was assayed by footpad reaction method.

Hematological and biochemical constituents

For hematology, blood was collected in heparinized vacutainers from all rats on 56nd day. Hemogloubin (Hb) content, and white blood cell (WBC) counts, hematocrit, lymphocyte, monocyte, and granulocyte percentages were determined by Auto analyzer (RA1000 Technicon, USA). Serum was collected and stored at −20°C in Eppendorf tubes for estimation of biochemical constituents, i.e. total protein [42], albumin [24], glucose [13], cholesterol [50].

Oxidative stress index and antioxidant enzyme activity

The blood collected in clean heparinized vacutainers was centrifuged at 2000 rpm for 15 min at 4°C to separate buffy coat and erythrocyte pellet. The erythrocytes were washed thrice with phosphate buffer saline (PH 7.4). The packed RBC obtained was mixed with an equal volume of phosphate buffer saline and then diluted as per requirement with distilled water. The oxidative enzymes viz., RBC catalase (CAT), lipid peroxidation (LPx), glutathione peroxidase, (GPx) and glutathione reductase in hemolysate were estimated as per the procedures of [4], [40], [36] and [9], respectively. The Hb and protein concentration in hemolysate were estimated colorimetrically as per the procedure described by Cannan [8] and Lowry et al. [29], respectively. After 8 weeks of experiment, all rats were sacrificed and livers were collected, which were perfused with normal saline (0.9%) immediately to reduce red blood cell contamination. The samples were then fixed in liquid nitrogen and stored at −20°C for antioxidant analysis. The markers thiobarbituric acid reacting substances (TBARS), reduced glutathione (GSH), and protein carbonyls were estimated as per the procedures of Balasubramanian *et al*. [3], Moron et al. [30] and Levine et al. [28], respectively.

Immune response

The rats were anti-genetically challenged twice with sheep RBC (0.5 × 109 cells/100 g, I/P) at 7 days interval to study the immune response. The blood was collected from retro-orbital plexus of all anti-genetically challenged rats after 1 week of primary and secondary challenge to separate the serum. 25 ml of serum was serially diluted with 25 ml of phosphate-buffered saline (PBS). Sheep RBC (0.025 × 109 cells) were added to each of these dilutions and incubated at 37°C for 1 h. The rank of minimum dilution that exhibited hemagglutination was considered as the antibody titer. The CMI was assayed by footpad reaction method [27]. The increase in the paw volume measured after 48 h of inducing sheep RBC (0.025

× 109 cells), in the subplantar region of the right hind paw. The mean percent increase in paw volume was considered as a delayed type hypersensitivity reaction and considered as an index of CMI. The volume of the left hind paw was injected similarly with PBS that served as control.

Statistical analysis

The experimental results obtained were statistically analyzed using one-way ANOVA according to the method of Snedecor and Cochran [48]. The mean values were compared by Duncan's multiple range tests [18].

RESULTS AND DISCUSSION

In the present study, body weight gains and feed intake were comparable among the groups. Rossi et al. [44] stated that Zn deficiency or low levels of Zn supplementation causes depressed appetite, resulting in decreased feed intake and reduced weight gain. However, in this experiment dietary zinc supplementation did not affect the weekly weight and feed intake of the rats. El Hendy et al. [19] reported that reduced Zn supplementation leads to significant ($P<0.05$) reduction in RBC count, Hb concentration and packed cell volume. Similarly, Akbari et al. [1] reported that addition of 60 mg Zn/kg ZnO to basal diet (BD) significantly ($p<0.05$) increased WBC and lymphocyte count in broiler chicks. However, in the present study, zinc supplementation had no effect on various hematological constituents analyzed (Table 2). Serum total protein and glucose (Table 2) in the present study was comparable among four dietary treatments. Bolkent *et al*. [6] proved the protective effect of Zn supplementation on lipid metabolism indices (total lipids, cholesterol, high density lipoprotein cholesterol) in laboratory rats with streptozotocin-induced Type 1 diabetes. In the present study serum cholesterol concentration was reduced by Zn supplementation compared to control group (Table 2). Lipid peroxidation is a free radical mediated chain reaction, considered as best marker of oxidative stress since its concentration increases during oxidative stress [47]. Though lipid peroxidation is a self-perpetuating reaction, its propagation can be arrested by chain breaking antioxidant. Zn plays an important role in the antioxidant system in two ways: The first is the protection of proteins and enzymes against free radical attack or oxidation [32], the second is through the prevention of free radical formation by other metals, such as iron and copper (39). In liver, TBARS and protein carbonyl concentration are indicative of oxidative stress and these markers significantly ($p<0.05$) lowered by Zn supplementation group than the control group (Table-5). GPx and CAT are involved in the antioxidant defense system and protects from potential oxidative damage [21, 38]. In this experiment, supplementation of Zn significantly ($p<0.05$) increased the activities of CAT and GPx and lowered the lipid peroxidation (malondialdehyde concentration) than the controls (Table 2). Similarly, GSH concentration in liver was significantly ($p<0.05$) lower at Zn supplementation group (Table 5). Several researchers observed better immune response with increasing the level of Zn supplementation [20, 22]. Zn is essential for maintenance of natural killer cells activity and phagocytosis of macrophages and neutrophils. In the present study, the humoral and CMI response were higher ($p<0.05$) with Zn supplemented group than control group, indicating the beneficial effect of supplementing Zn source (Table 2).

Table 1- Composition of the diet

Ingredient	Amounts (g/kg diet)
Protein (spray-dried egg white)	210
Cerelose	595
Corn oil	80
Fiber	40
Mineral premix1	60
Vitamin premix2	15

1. Mineral premix supplied the following (g/kg diet]: $CaCO_3$, 20.33; K_2HPO4, 19.26; NaCl, 10.08; $MgSO_4$/ 5.82; $CaHPO_4$, 3.6;
FeSO4.7H_2O, 0.6; KI, 0.012; $ZnCO_3$/ 0.048; CuSO4-5H2O, 0.0396; $CrK(SO_4)_2$-12H_2O, 0.024; $Mn(SO_4)_2$-H_2O, 0.138.
2. Vitamin premix supplied the following (mg/kg diet): Ca-pantothenate, 37.5; thiamin-HCl, 22.5; pyridoxine-HCl, 22.5; nicotinic
acid, 22.5; menadione, 18.75; riboflavin, 7.5; p-aminobenzoic acid, 7.5; folie acid, 0.45; biotin, 3.9; all-rac-ct-tocopheryl acetate (Rovimix E-50), 178.5; retinyl palmitate (Rovimix A-650], 40.95; cholecalciferol (Rovimix AD3 A650/D325), 3.45; Vitamin B-12 (Merck 12 + mannitol), 22.5; choline chloride (70% solution), 1072.5; inositol, 375; ascorbic acid as a preservative for the vitamin premix, 75. Rovimix was purchased from Hoffman-LaRoche (Nutley, NJ); reagents were purchased from Merck (Rahway, NJ).

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Table 2- Some blood parameters in diabetic-induced rats fed zinc supplemented diets

Parameters	NC	DR	ZTR	DZTR	SEM	P-value
Glucose (mg/dl)	135	140	143	133	2.055	0.445
Cholesterol (mg/dl)	74a	57b	59b	62b	2.055	0.008
Total protein (g/dl)	6.25	6.33	6.32	6.22	0.051	0.469
Albumin (g/dl)	4.41a	4.24b	4.02b	4.06b	0.047	0.003
Globulin (g/dl)	2.83a	1.8b	2.92a	2.73a	0.056	0.002
WBC(× 103/ cu mm)	11.65	10.18	10.56	9.19	0.614	0.506
Lymphocytes (%)	73.41	78.6	78.5	77.3	0.593	0.501
Monocytes (%)	5.87	4.1	4.01	4.62	0.54	0.654
Granulocytes (%)	17.49	16.68	16.8	16.21	0.55	0.312
TBARS (nmol MDA/mg protein)	0.0185a	0.0178ab	0.0131b	0.0132b	0.0021	0.048
Humoral immune response HA(log2 titers)	4.5	4	4.1	4.5	0.167	0.526

NC=Normal Control; DR=Diabetic Rats; ZTR=Zinc Treated-Rats; DZTR=Diabetic and Zinc Treated-Rats; SEM=Standard Error of Mean

CONCLUSION

The results of the experiment showed that the inclusion of Zn supplementation in diabetic-induced rats diet caused to significance improved the immune response and antioxidant status.

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