Original Article

Effect of Zinc Supplementation on some Biochemical and Hematological Parameters in Alloxan Induced Diabetic Rats

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Abstract

Background & Objective: This study aims to evaluate the effects of zinc sulfate on blood biochemical and hematological parameters in healthy control and alloxan-induced diabetic rats.

Materials & Methods: Experimentally, 40 rats were used in four equal groups, including healthy control, healthy feed with zinc sulfate, diabetic control, and zinc sulfate. The alloxan 120 mg/kg with blood sugar over 140-250 mg / dL was intraperitoneally injected to induce diabetes mellitus. In addition, the value of 0.6 g / L zinc sulfate dissolved in tap water was used for three months to be drunk by the treated group. All animals were killed, and blood and pancreas samples were collected for necessary tests. Serum levels of alpha-amylase, total cholesterol, triglycerides, LDL, HDL, hemoglobin, and hematocrit were measured. Statistical data were analyzed based on the analysis of variance using SPSS software.

Results: Our data showed that in the diabetic control group, serum levels of total cholesterol, triglyceride, LDL increased significantly and hemoglobin, hematocrit, and HDL decreased significantly compared to the healthy control ($P<0.05$). Zinc sulfate consumption in these groups reduces total cholesterol, triglyceride, LDL and increases hemoglobin and hematocrit compared to the control groups. Microscopic examination of the pancreas showed that in the diabetic control group, the percentage of Beta cells was reduced compared to the other groups.

Conclusion: Our findings reveal that dietary zinc sulfate not only exerts no damage to bodies but also zinc sulfate as food supplement seems to have beneficial effects on biochemical and hematological parameters in diabetic and healthy animals.

Keywords: Alloxan, Zinc Supplementation, Biochemical parameters, Diabetic mellitus

Introduction

There are dozens of elements in nature that, in small amounts, perform vital functions in living things. Therefore, the presence of these elements in the diet of living organisms is essential for their growth and survival. In addition, the amount of these elements in the diet should be at a desirable and balanced level so as not to disrupt the life of living organisms. Zinc is one of the 15 essential minerals that is considered an important element in the daily nutrition of humans and animals that plays a major biological role in nature that our body needs (1-3).

Zinc is an antioxidant that is the second most abundant vital metal in the human body and plays a role in vital functions such as growth, immune system activity, reproduction, and response to

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oxidative stress production and storage of insulin in pancreatic beta cells (4-5). Zinc ion also acts as a coenzyme for the activity of many enzymes, including alkaline phosphatase, lactate dehydrogenase, carboxypeptidase, polymerase, and essential enzymes required for the metabolism of sugars and lipids. One of the most important causes of zinc deficiency in the body is the lack of its intake through food. Intestinal absorption disorders can also reduce this important element, i.e., zinc in the body. Zinc deficiency causes immune system disorders, chronic and unhealed wounds, retardation of physical and mental development, diarrhea, and skin lesions (6-8). The amount of zinc ions in the plasma of diabetic patients is lower than healthy individuals, which can be one of the reasons for dietary changes, digestive disorders, reduced digestion and absorption, depletion of body reserves due to accelerated catalytic processes, or the presence of palpable renal failure. In this regard, Jamilian *et al.* (9) analyzed the impacts of magnesium zinc-calcium-vitamin D co-supplementation on parameters of inflammation and oxidative stress and pregnancy outcomes among women with gestational diabetes (GDM). The outcomes of this work have confirmed that magnesium-zinc-calcium-vitamin will reduce biomarkers of inflammation for six weeks. By adopting management of type 2 diabetes mellitus (T2DM), Nazem *et al.* (10) evaluated the impacts of zinc supplementation on the superoxide dismutase gene expression and enzyme activity. The outcomes demonstrated that the zinc supplementation enhanced both gene expression and enzyme activity of SOD and the levels of insulin compared to the control group. In (11), Li and Zhao carried on a systematic review and meta-analysis to ameliorate the metabolic status for gestational diabetes by evaluating the effect of zinc supplementation. In comparison with control action for gestational diabetes, the values of FPG, insulin, HOMA-IR were reduced by zinc supplementation. However, there was no evidence of an impact on LDL-cholesterol and total cholesterol. Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, glycosylation of the erythrocyte membrane, damage to bone marrow stem cells, impaired insulin secretion or function, and impaired metabolism of sugars, fats, and proteins (see Fig. 1)(12-14). Symptoms include hyperuricemia, binge drinking, overeating, weight loss, increased urine glucose, blurred vision, and other symptoms. High blood sugar leads to increased blood lipids and eventually leads to dyslipidemia and increases cardiovascular disease and stroke. Hyperglycemia in people with diabetes reduces antioxidant defense by reducing glutathione and catalase, and superoxide dismutase, thus upsetting the balance between free radicals and the body's antioxidant protection (15-17).

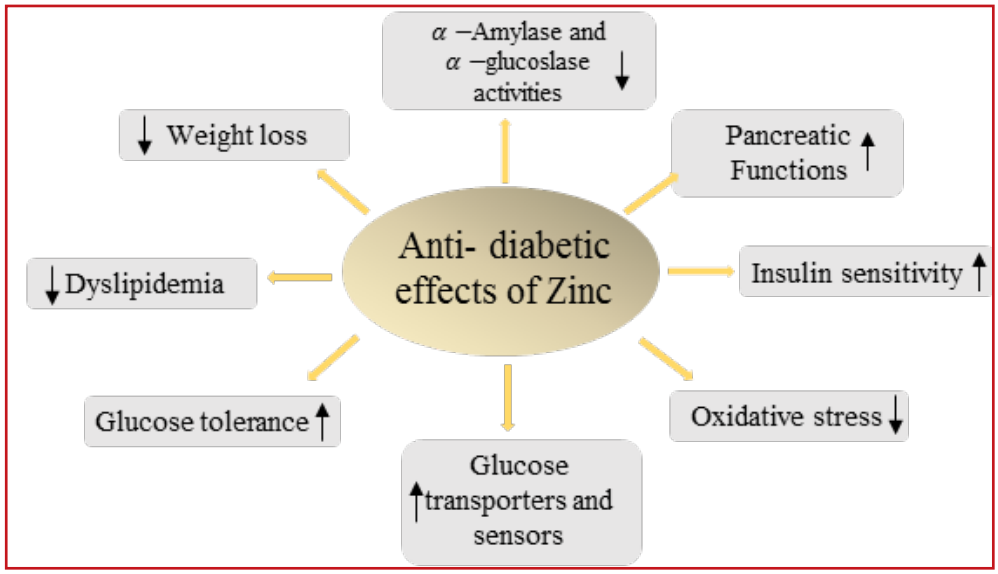

Figure 1. The anti-diabetic effect of zinc

On the other hand, high blood sugar in DM patients and chronic increase in the process of glycosylation of proteins and fat peroxidation leads to the intensification of oxidative stress, and that is why consumption of zinc as a supplement can have an antioxidant effect and reduce the rate of fat peroxidation (18). Recent studies revealed that zinc deficiency increases plasma lipid and cholesterol levels and increases Low-Density Lipoprotein (LDL) sensitivity to lipid peroxidation. In addition, zinc supplementation in DM patients can lead to lower fasting blood sugar and lower serum lipids, as reported in (19- 20).

Due to the increase in oxidants and free radicals in the intercellular space, zinc and intercellular cells are reduced in antioxidant enzymes. Recent studies revealed a complex relationship between zinc and type1 and type-2 diabetes (21-23). The significant effect of diabetes on zinc homeostasis is a decrease in zinc in the blood resulting from an increase in zinc in the urine or a decrease in intestinal absorption of zinc or both. Thus, the progression of diabetes, especially in children, can have adverse and irreversible consequences such as growth retardation, immune system dysfunction, skin disorders, weakening of antioxidant defenses, abnormal mental states and, in general, the occurrence of abnormalities in all biological reactions. Decreasing the amount of zinc in the diet of mice reduces the ability of the pancreas to secrete insulin in response to glucose pressure, which is a sign of DM because Zinc is inherently stored in beta cells. Increased insulin secretion in DM patients decreases the concentration of zinc within beta cells, and this is consistent with a decrease in insulin content of cellular islets in a state of reduced zinc (24-25).

Mammalian RBCs are continuously replenished through erythropoiesis. A deficit in the total amount of RBCs is defined as anemia. Zinc is considered as an essential factor for erythropoiesis in addition to iron, folate, and vitamin B12. Studies have indicated that anemia is significantly associated with zinc deficiency, especially in pregnant women and preschool children. To treat human zinc deficiency diseases, many zinc supplementation studies have been performed (26). Other reports also indicate that different concentrations of potent antioxidants can inhibit red blood cell hemolysis by several mechanisms. Based on these findings and considering the increasing trend of zinc supplementation, the study of the long-term effects of zinc ions on diabetes is one of the most attractive and essential research topics. Thus, this research work is dedicated to evaluating the effects of zinc ions on fat profile and the study of hematological parameters in alloxan-induced diabetic rats.

Materials & Methods

Experimental Animals

In this work, 40 Wistar rats weighing 150 to 180 g were purchased from the Animal Center of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. Before the study, the animals were kept at a constant temperature and humidity level for one week to adapt to the potential stress of relocation and the potential change in physiological conditions of the animal. The conditions used in this study included 12 hours of light and 12 hours of darkness at a temperature of 23±2°C, and the possibility of continuous access to water and food was kept the same. The storage cages were washed three times a week, and thin wood chips were poured on the floor, and the rats were fed with prepared food. At all stages of this study, all ethical considerations and application protocols on laboratory animals were observed (27).

Induction of diabetes in animals

In this study, a single dose of alloxan monohydrate (120 mg/kg b.wt.) was prepared in normal saline solution an injected intraperitoneally to induce diabetes after 15 hours of abstinence. After 72 hours, blood glucose was measured by taking blood from the tail of mice and using a blood glucose meter (glucometer). However, to be surer of being diabetic, blood sampling was repeated one week after the

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injection. The diabetic basis was considered to be blood sugar over 140-250 mg/dL. 20% of mortality was observed 72 hours after alloxan injection (28- 29).

Drugs and chemicals

In this study, zinc sulfate salt was purchased from Sigma Company in a package of 500 g. The amount of 0.6 g of zinc sulfate was dissolved in 2 liters of ordinary water and given to the animals as drinking water. To measure the activity of serum enzymes, diagnostic kits and quantitative Pars test (German biochemistry standard) were adopted (30).

Animal Grouping

In this study, the animals were randomly divided into four groups (n=10), and each group was kept in a separate cage under the same conditions. Healthy control group: The animals in this group did not receive any treatment for three months, and they were given normal water and plate food with full access. The healthy group was fed with zinc sulfate, which was given as drinking water for three months instead of ordinary water containing zinc sulfate at a concentration of 0.6 g / L. The diabetic controls group which became diabetic by intraperitoneal injection of 120 mg/kg alloxan and did not receive any other medication during the test period, were given plain water and plate food with full access. After testing for diabetes and ensuring that they had diabetes, they were given sulfate-containing water at a concentration of 0.6 g /L as drinking water for three months instead of normal water.

Animal caring

To evaluate the effect of zinc sulfate supplement, the animals were kept for three months, and during this period, changes in weight and water and food intake of the animals were recorded daily.

Enzyme activity assay

After a 12-hour starvation period, blood samples were drawn from all animals' left ventricles using anesthetized diethyl ether. Blood samples were taken into heparinized tubes to measure hemoglobin and hematocrit, and serum samples were separated by centrifugation at 3000 rpm for 15 minutes at 25 ° C. Biochemical factors include the level of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) by spectrophotometry and using kits purchased from the company Parsush Azmash (Standard). To obtain the amount of hemoglobin level, samples were mixed with Drabkin solution, and absorbance was measured at 546 nm after 5 minutes. Then, the obtained adsorption was placed in the standard diagram, as illustrated below. To receive the amount of hematocrit, the capillary tube for hematocrit with blood up was filled to three-quarters of its length. By implementing a centrifuge, it was centrifuged at 3000 rpm for 15 minutes. Finally, the total number of red and white blood cells relative to blood plasma was calculated using the plate. The flowchart of the current work is illustrated in Figure 2.

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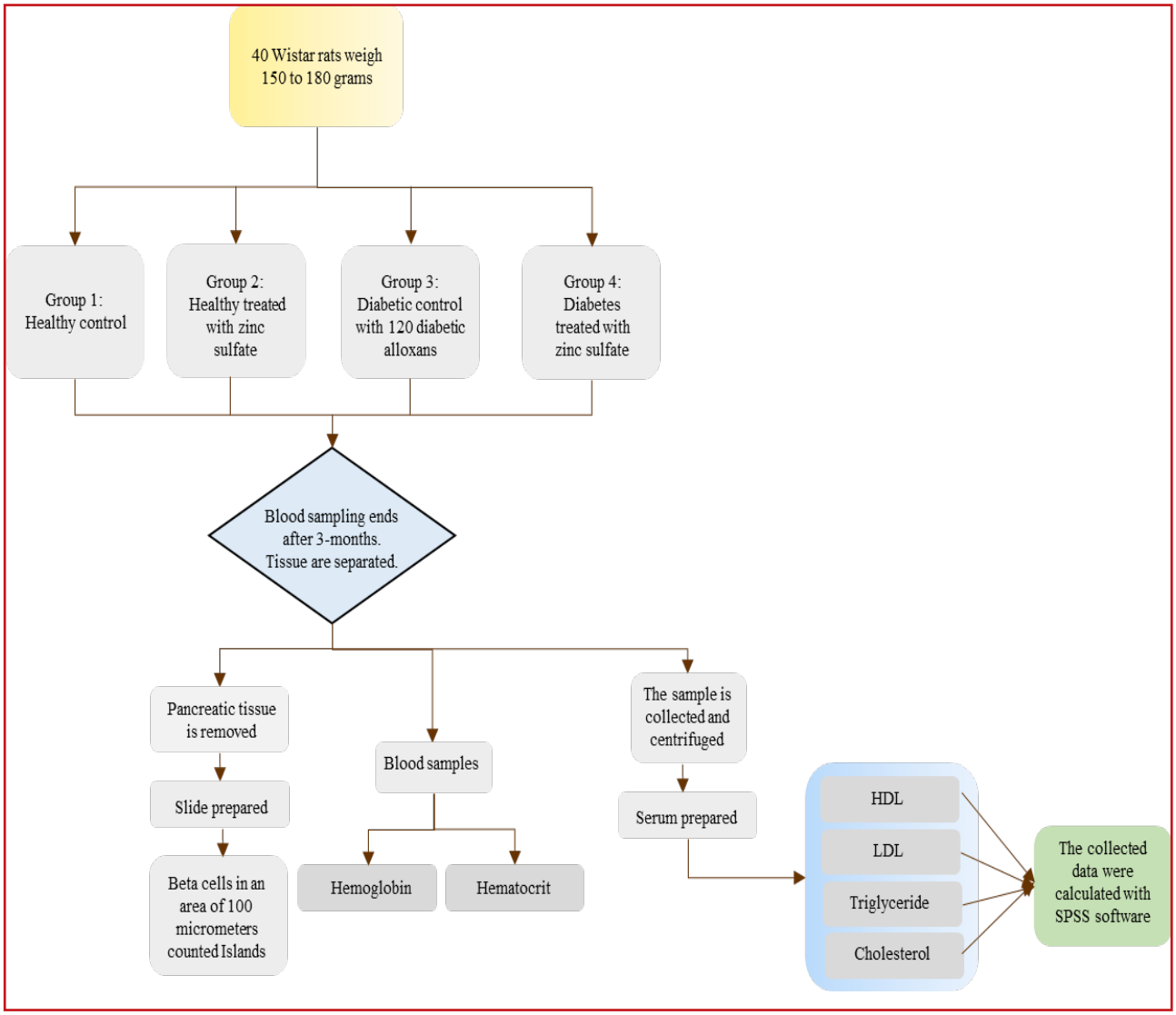

Figure 2. Flowchart of experimental procedure on alloxan rats.

Results

In the present study, to ensure the normal distribution of data, the normality test was performed on the results and in order to compare the data and detect the presence or absence of differences between different groups, an independent t-test, and analysis of variance were performed. In all these comparisons, P-value less than 0.05 was considered a significant difference, and the obtained results are expressed as mean standard ±deviation. All calculations were performed using SPSS software version 22. The diagrams were drawn using Excel software.

Hemoglobin and Hematocrit Levels

The effect of oral zinc on hemoglobin and hematocrit levels was demonstrated in Table 1. In diabetic control mice, the amount of hemoglobin and hematocrit showed a significant decrease compared to healthy control ($P<0.05$). Zinc sulfate treatment in diabetic mice caused a significant increase in hemoglobin and hematocrit compared to diabetic control mice. ($P< 0.05$) showed a healthy group treated with zinc sulfate to increase hemoglobin and hematocrit compared to healthy control, which was not statistically significant ($P > 0.05$).

Table 1. Effect of oral zinc sulfate on hemoglobin and hematocrit levels in control and diabetic rats

Groups	Hb(gm/dl)	Hct (%)
Healthy control	15.01±4.1	35.4±5.4
Healthy + zinc sulfate	16.06±6.9	36.3±4.5
Diabetic control	12±2.27*	32.8±6.2*
Diabetic + zinc sulfate	14±2.28#	34.9±6.3#

One-way analysis of variance, and independent t-test* was significant compared to healthy control, #significant compared to diabetic control. Results as mean± standard deviation

Serum total cholesterol levels

In the diabetic control group, the serum cholesterol level significantly increased compared to the healthy control ($P< 0.05$). In the zinc sulfate-treated diabetic group, the serum cholesterol level decreased compared to the diabetic control ($P > 0.05$). Consumption of zinc sulfate in the healthy group showed a significant reduction in cholesterol than healthy controls ($P<0.05$) (chart1).

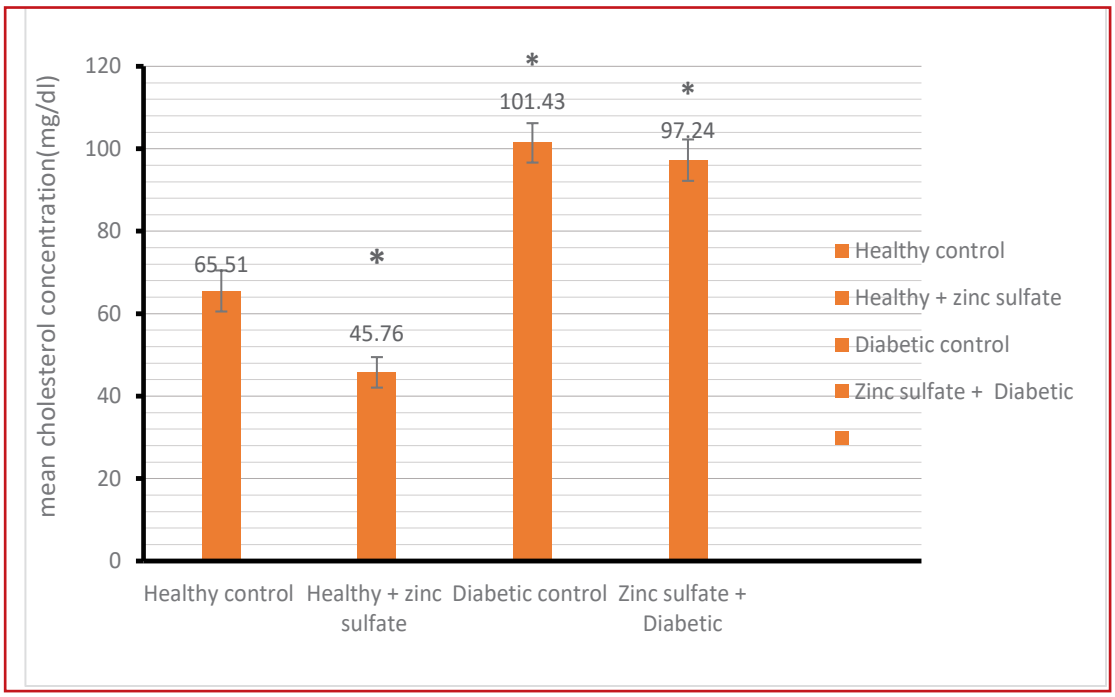

Chart 1. Comparison of mean cholesterol concentration in zinc sulfate treatment groups

One-way analysis of variance and an independent t-test* indicates a significant difference with healthy control

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The serum triglyceride level

In the diabetic control group, serum triglyceride level shows a significant increase compared to the healthy control ($P<0.05$). The diabetic group treated with zinc sulfate the triglyceride level compared to the diabetic control group showed a significant decrease ($P< 0.05$). In the healthy group treated with zinc sulfate, the level of triglyceride showed a considerable decline compared to the healthy control ($P<0.05$) (chart 2).

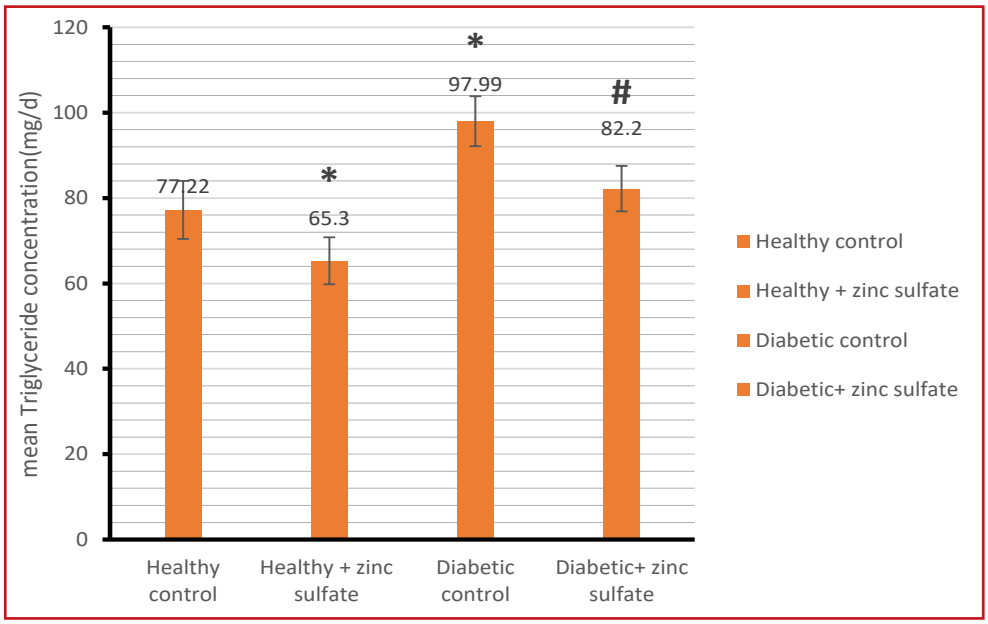

Chart 2. Comparison of mean triglyceride concentration in zinc sulfate treatment groups

One-way ANOVA, and independent t-test* indicate a significant difference with healthy control # indicate a significant difference with the diabetic control group.

The serum LDL level

In the diabetic control group, serum LDL level showed a significant increase compared to the healthy control ($P< 0.05$). Treatment of mice with zinc sulfate showed a 3% decrease in LDL in healthy animals than healthy controls ($P > 0.05$) and a 4% decrease in the diabetic group compared to the diabetic control (-0.05), which was not statistically significant in both groups (chart 3)

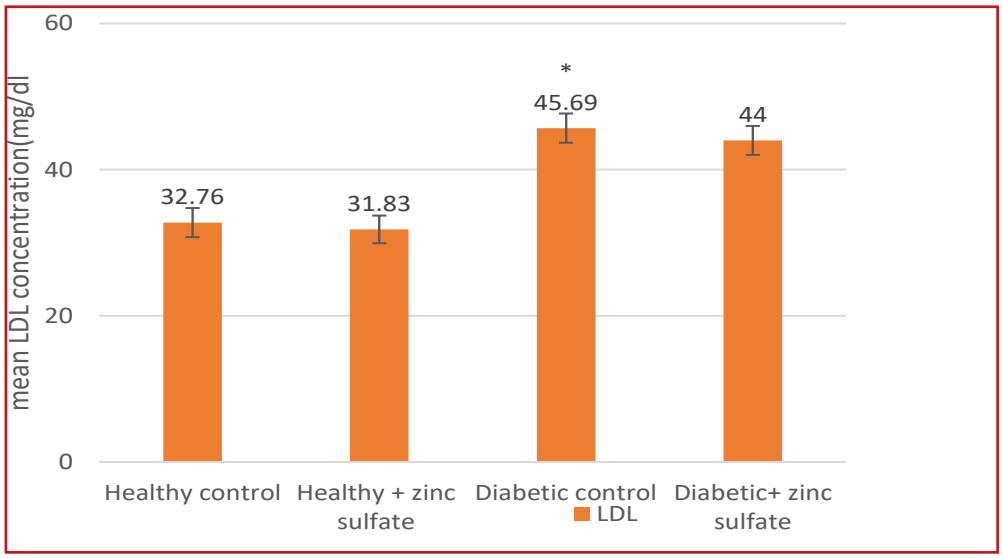

Chart 3. Comparison of mean LDL concentration in zinc sulfate treatment groups

One-way ANOVA, and independent t-test* indicates a significant difference with healthy control

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The serum HDL level

In the diabetic control group, serum HDL level showed a significant decrease compared to the healthy control ($P<0.05$). In the diabetic group treated with zinc sulfate, the serum HDL level showed an insignificant increase compared to the diabetic control group ($P>0.05$). Treatment of healthy animals with zinc sulfate caused a negligible decrease in HDL compared to healthy controls ($P>0.05$) (chart 4).

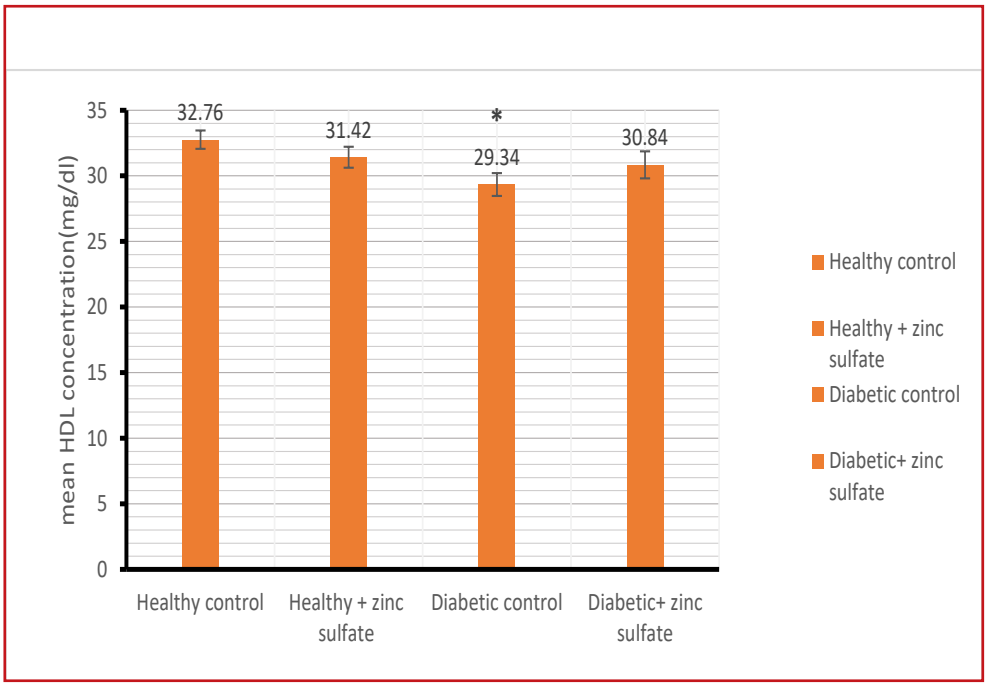

Chart 4. Comparison of mean HDL concentration in zinc sulfate treatment groups One-way ANOVA. An independent t-test* indicates a significant difference with healthy control.

Results of experiments in lipid and lipoprotein changes in zinc sulfatetreatment groups for different groups, including healthy control, healthy + zinc sulfate, diabetic control, and diabetic + zinc sulfate, are furnished in Table 2.

Table 2. Outcomes of lipid and lipoprotein changes in zinc sulfate treatment groups after three months

Groups	HDL (mg/dL)	LDL (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)
Healthy control	32.76± .26	32.76±.11	77.22±12.8	65.51±9.98
Healthy + zinc sulfate	31.42±.08	31.83±.48	65.30±10.5	45.76±6.7
Diabetic control	29.34±1.87	45.69±.99	97.99±8.86	101.43±4.78
Zinc sulfate + Diabetic	30.84±1.53	44±.98	82.20±10.33	97.24±8.02

One-way analysis of variance, and independent t-test. Results as mean± standard deviation

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Histological results of pancreas

Microscopic examination of the pancreas showed that in the healthy control group, Langerhans islets were located in the form of spherical or oval masses in the acinar tissue of the exudative part of the gland. The tissue structure of the islets was natural and consisted of cellular cords that were distinguished from the surrounding by a distinct boundary of the exocrine portion of the gland. Beta cells were located in the central part of the island and accounted for the most significant percentage of cells. Small blood capillaries were visible along with the cell cords. No abnormal changes in the tissue structure of the islets were observed in this group. In the sulfate group, some cells were necrotic and the overall percentage of cells in this group was reduced compared to the healthy control group. In the diabetic control group, the cohesion of islets was disrupted and the percentage of cells was reduced compared to the other groups so that most of the islets were cell-free and a large percentage of the cells became necrotic. The diabetic group receiving sulfate had an increase in the number of healthy cells compared to the diabetic group, but their number decreased compared to the healthy control group and was almost similar to the control group in terms of tissue structure, although cell density decreased. The results of histology of the pancreas and beta cells are shown in Figure 3. respectively.

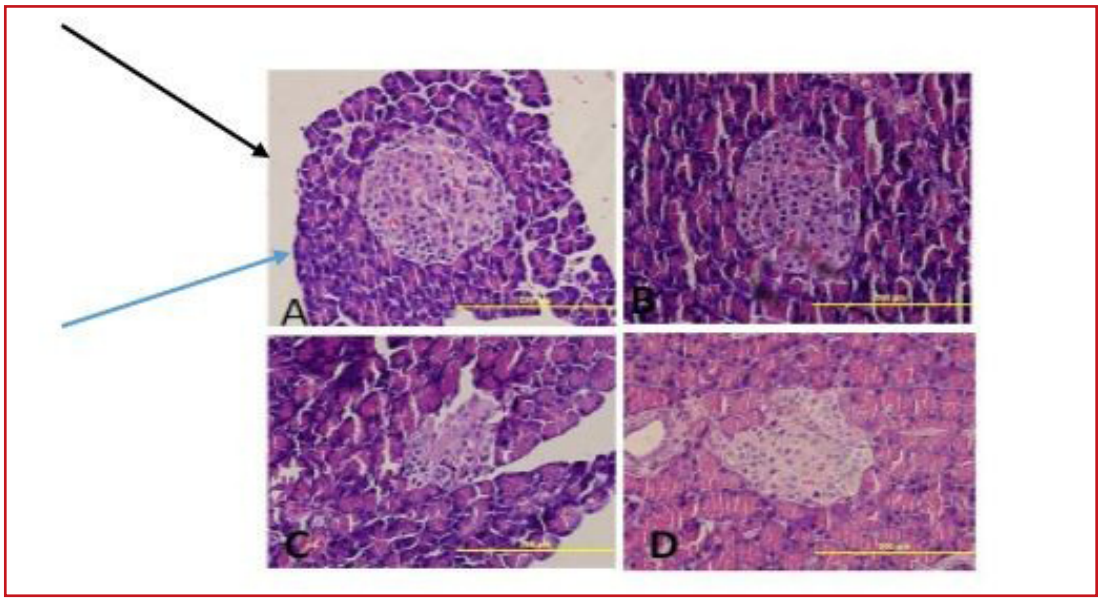

Figure 3. Pancreatic tissue section with H&E staining

A- Healthy control group: islets of Langerhans are normal. B- healthy group fed with zinc sulfate: reducing the number of cells. C- group of diabetic controls: the cohesion of islets was disrupted and the percentage of cells was reduced compared to the other groups. D- The diabetic group receiving sulfate had an increase in the number of healthy cells compared to the diabetic group, but their number decreased compared to the healthy control group. (Flash **Black**: islets of Langerhans, Flash Blue: percentage of cells)

Discussion

This study aimed to experimentally evaluate the effects of zinc sulfate on blood biochemical and hematological parameters in healthy control and alloxan-induced diabetic rats. For this purpose, alloxan monohydrate soluble in physiological serum at a dose of 120 mg/kg body weight of rats was used as an intraperitoneal injection. This substance increases plasma glucose by destroying beta cells of Langerhans islets Alloxan through enzyme inhibition. Protein kinase C

lowers diacylglycerol, which in turn activates protein kinase A, reducing cell tolerance to intracellular calcium and creating the conditions for inducing pancreatic beta-cell apoptosis (31-32). Serum cholesterol, triglyceride, and LDL levels increase with elevated blood glucose levels in alloxan induced diabetic rats. When Insulin decreases, glucagon increases instead and result in lipolysis increasement. On the other hand, the acetyl-coenzyme A cannot enter the Krebs cycle, and cholesterol is formed from a combination of three molecules. Also, elevated levels of serum triglyceride, cholesterol, and LDL in diabetic rats have been previously reported by alloxan, and these results were partially obtained in the present study (33). Serum cholesterol, triglyceride, and LDL levels decreased in the two groups treated with zinc sulfate in healthy and diabetic animals. The reason for the decrease is because zinc is one of the essential elements for insulin metabolism, which improves insulin binding to the receptor and sensitivity, increases serum concentration and insulin utilization, and has a protective role on pancreatic beta cells (34- 35). Studies in some laboratory animals also confirm the effect of zinc on the reduction of plasma lipids. Reiterer in the year 2005 treated a group of diabetic rats with different concentrations of zinc for four months and showed that increase in plasma zinc levels was associated with a decrease in cholesterol and triglyceride concentrations (36). Razmpoosh in the year 2019 reported a decrease in zinc concentration and an increase in plasma lipid concentration in diabetic patients compared to non-diabetic individuals (37). However, they stated that there was no significant difference between triglyceride levels in diabetic animals with healthy control, although zinc consumption increases serum triglyceride concentrations (38). Serum HDL cholesterol levels decreased with elevated blood sugar levels in alloxan-induced diabetic rats. There was no significant change in healthy diabetic animals consuming zinc sulfate, which is consistent with (39). Hemoglobin is a divalent iron porphyrin protein involved in transporting oxygen from the lungs to all tissues. Hematocrit is a percentage of the total volume of blood made from red blood cells and is an essential hematological parameter for diagnosing the disease (40). Hemoglobin and hematocrit levels decrease in diabetic rats, which is consistent with (41). The decrease in hemoglobin and hematocrit in diabetic rats can be explained by the fact that in these animals, a reduction in insulin and impaired glucose metabolism reduced the production of erythropoietin from other sources reducing hemoglobin and hematocrit, another reason for anemia. From diabetes, glycosylation of the plasma membrane of red blood cells occurs if hyperglycemia occurs. Zinc supplementation increases hemoglobin and hematocrit in healthy and diabetic animals. Zinc supplementation also affects hemoglobin synthesis by acting on the synthesis of catalyzing enzymes which is consistent with the findings of (41). Only exposure to high doses has toxic effects. According to numerous available reports such as Mokhtari report, acute and high concentrations of zinc supplements can lead to Seriously damage, but based on our results chronic use of these supplements not only exerts no damage to bodies but also zinc sulfate as food supplement seems to have beneficial effects on biochemical and Hematological parameters in diabetic and healthy animals (30). However, the use of this drug requires more research, and to continue research, the use of various doses and prolongation of the study time is recommended.

Conclusion

Based on the findings of this study, zinc is one of the essential metals for the body. Based on studies, people with diabetes suffer from zinc deficiency. Zinc deficiency can activate the natural metabolic processes of tissues, especially tissues that are more involved in the body's metabolism, such as the liver and pancreas, or cells exposed to oxidative stress, such as red blood cells. It disrupts them and destroys some of the cells in that tissue. Zinc sulfate, with its antioxidant properties, reduces enzymes and fat profiles.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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