Proteative Effects of Zinc Supplement on Chromatin Deficiency and Sperm Parameters in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Objective: Diabetes mellitus (DM) is one of the metabolic diseases that may effect on sperm's health and male reproductive function. Also zinc supplement had many antioxidant agents that may improve adverse effects of diabetes on DNA integrity and sperm chromatin.

Materials and methods: In this study 36 Wistar Rat (200 gr) were randomly divided non-diabetic and Streptozotocin-induced diabetic, then were divided into 4 groups (n = 9): zinc sulfate (25 mg daily) and sailin administration. Then sperm parameters and semen analysis were assessed, thirty-five days later according to World Health Organization (WHO) standards. The sperm DNA fragmentation was measured by sperm chromatin dispersion (SCD) test, protamine deficiency was determined by chromomycin A3 (CMA3) staining.

Results: The sperm count and viability increased in zinc supplement groups, also head and neck deformity were seen less than saline administration groups (p < 0.05). In Zinc administration groups, the sperm motility type C, D reduced; furthermore abnormal morphology, DNA fragmentation and protamine deficiency over than 30% were reduced (p < 0.05).

Conclusion: The results show the protective effects of Zinc supplement on sperm parameters, chromatin efficiency and DNA integrity caused by diabetes. The beneficial effect of Zinc supplement might be associated to the inhibition of oxidative stress accordingly a favorable antioxidant therapeutic candidate for the management of male infertility.

KEY WORDS
diabetes, sperm, infertility, rat, chromatin

INTRODUCTION

Infertility is defined as the inability to have children after at least one year of marriage without the use of any contraceptives methods. About 40-50% of human infertility caused by male factors due to deficiencies in the semen, then semen analysis is the main criteria for male fecundity. Researchers had shown that negative correlation between percentages of spermatozoa health's and levels of reactive oxygen species (ROS) in seminal fluid; The level of seminal ROS is regulated by seminal proteins, vitamins, enzyme and non-enzymatic antioxidants. so these factors have beneficial effects on sperm parameters and developmental capacity. Diabetes mellitus (DM) is a chronic metabolic disorder associated with ROS and other metabolic problems such as disruption of proteins, carbohydrates and lipids metabolism; can alter the structure of sperm chromatin. In diabetic patients hyperglycemia reduces serum levels of LH, FSH and testosterone, that consequently affected sperm quality and their fertility potential. Hyperglycemia increases the levels of ROS trough over generation of superoxide in mitochondria, accordingly the body's antioxidant capacity is decreased. In the normal condition there is a balance between the antioxidant activity and ROS production in male reproductive system by scavenging or removing the ROS. The level of abnormal sperm morphology and DNA fragmentation in diabetic men is higher than normal; subsequently affect male fertility. The endogenous antioxidant of seminal plasma protects the spermatozoa from oxidative damage; however, can't prevent acrosome membranes and sperm tail from lipid peroxidation consequently the sperm cell needs extra antioxidant for protections.

Zinc (Zn) is metalloenzyme cofactors that play an important role in scavenging ROS, DNA transcription, repair of damaged DNA and protein synthesis. In human seminal plasma, the concentration of zinc is higher than other tissues. Many studies in asthenozoospermic patients have indicated the administration of zinc, vitamin E and vitamin C reduces the level of oxidative stress, sperm DNA fragmentation and apoptosis also increases the sperm count and motility. The antioxidant therapy can progress the sperm quality and male fertility. As the antioxidant properties of zinc sulfate could act against reproductive system impairment and the high rate of sperm disorders in diabetic patient, we interested to investigate the zinc supplement protective effects on rat sperm parameters, against oxidative stress and tissue damages in diabetic rat.

MATERIALS AND METHODS

Chemicals

phosphate-buffered saline (PBS), Tris (hydroxymethyl)aminomethane, Dithiothreitol (DTT), Sodium dodecyl sulfate (SDS), Ethylene diamine tetracetic acid (EDTA), borate and Chromomycin A3 (CMA3)
were purchased from Sigma-Aldrich chemical company (St Louis, MO, USA). All chemicals and reagents used were analytical grade.

Animals

In this study, 36 Adult (5 weeks old) male Wistar rats (200 ± 20g) were obtained from the animal house and research center of Gerash University of Medical Sciences, Gerash, Iran. After weighting, the rats were caged in distinct group (n = 9) at animal house with standard air flow and light-dark cycle was adjusted on 12h. Relative humidity and mean temperature was about (40-50 %) and (22-24℃) respectively and the rat was fed with water and food available; the experiment was approved by the institutional Animal Ethics Committee of Gerash University of medical science with referee number[IR.GERUMS 1396-1070].

Study design

The rats were divided into two groups: diabetic induced by Streptozotocin [received a single dose of STZ (150 mg/Kg) intra-perito-

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Protective Effect of Zinc Supplement in Diabetic Rats

The study investigated the protective effect of zinc supplement in diabetic rats, with a focus on spermatogenic function and testicular morphology.

**RESULTS**

**Serum Testosterone Level**

The blood testosterone level in diabetic rats decreased compared with the non-diabetic group, with zinc sulfate administration improving this level (Figure 1). The vital sperms significantly increased within the zinc sulfate administration group, indicating a positive effect on spermatogenic function.

**Morphological study**

The number of Leydig cells was increased in zinc sulfate groups compared to the non-diabetic group, with noticeable improvements in spermatogenic cell series and testicular morphology.

**DISCUSSION**

The present study supports the hypothesis that zinc supplement can mitigate diabetic-induced spermatogenic dysfunctions. Zinc supplementation appears to offer therapeutic potential for improving spermatogenesis in diabetic rats.

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**Figure 4.** Effect of zinc sulfate administration on the types of SCD, in diabetic and non-diabetic Wistar male Rat.

Data were analyzed by one-way ANOVA test followed by Tukey’s post hoc test.

*Significantly different from the control group (*P < 0.05).

# Significantly different from the zinc sulfate administration group (##P < 0.05).

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**DNA fragmentation assay (SCD)**

Sperm DNA integrity was assessed using SCD tests, following the methods of Evenson et al. (2016), with significant differences observed among groups.

**Protamine Deficiency Assessment**

Protamine deficiency was evaluated using CMA staining, with significant improvements noted in the treated groups compared to controls.

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footnotes:

1. Statistical analysis

Data were expressed as Mean ± SD and analyzed by the statistical package SPSS software version 21 for Windows. Analysis was carried out using a One-way ANOVA followed by Tukey’s post hoc test for comparison of data between these groups. Statistical significance was set at p < 0.05.

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fragmentation and prostate deficiency was reduced after zinc sulfate administration in diabetic rats; this may be due to the zinc’s antioxidant role. The generation of reactive oxygen species (ROS) is the most common consequence of diabetes which can induce cell failure and disturb the male reproductive function; furthermore induces an adverse change in chromosomal quality and sperm parameters.\(^\text{30,31}\) Zn is reported to play an important role in glucose homeostasis by enhancing hepatic glycogenesis and improves glucose utilization\(^\text{32}\). Sperm analysis is valuable index for determination of the male infertility reason; many factors including luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone affect male fertility\(^\text{33}\). The overproduction of ROS in seminal plasma in diabetic’s cases, inhibits the aromatase enzyme activity\(^\text{34}\). This research was financially supported by Ministry of Sciences and Medical Sciences. 2015; 5(2): 73-81.

REFERENCES

19) Evenson DP. The Sperm Chromatin Structure Assay (SCSA®) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. Animal reproduction science. 2016; 169: 56-75.