ORIGINAL ARTICLE

AMELIORATING EFFECT OF ZINC ON PROTEIN SUPPLEMENT INDUCED DNA AND SPERM DAMAGE IN MALE SPRAGUE DAWLEY RATS

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Background: Oxidative stress induced DNA damage and impairment in testicular tissue histology are indicative of male infertility. The aim of this study was to evaluate the protective role of Zinc on Protein supplement induced damage to the DNA and testicular tissue histology. **Methods:** The study comprised a total 30 male Sprague Dawley rats divided into Group 1 (n=10) fed on standard laboratory food, Group 2 (n=10) fed on standard laboratory food and dietary supplement powder and Group 3 (n=10) fed on standard laboratory food, dietary supplement powder and Zinc. Serum levels of 8-hydroxy-2′-deoxyguanosine (ng/ml) were estimated by ELISA. Testicular Tissue histology was performed for the detailed examination of Spermatogenic cells, size of seminiferous tubules and lumen of seminiferous tubules. **Results:** Mean±SEM of serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in Group-2 rats was significantly increased (p<0.05) as compared to Group 1 rats. While Mean±SEM of serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels of Group 3 rats was significantly decreased (p<0.05) as compared to Group 2 rats. There was no significant difference in Mean±SEM of serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels between Group-1 and Group-3. Evident pathological changes were seen in the testicular tissue of the Group-2 rats, characterized by atrophic seminiferous tubules with reduced number of spermatogenetic cells and widening of lumen. Rats of Group 3 received Zinc supplement and showed normal seminiferous tubules with increased number of mature spermatozoa in their lumen. **Conclusion:** Soy protein and silicon dioxide which are the ingredients of protein supplements induce DNA damage and destroys testicular tissue. Zinc has potential to restore the DNA damage and testicular tissue structure.

Keywords: Protein supplements, Soy Protein, Silicon dioxide, Zinc, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and testicular tissue histology

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INTRODUCTION

Dietary supplements are the products containing a dietary ingredient intended to improve the nutritional value of food. About 50% population in United States is consuming these supplements to improve health conditions and to boost energy.[1] There is increasing interest of men in oral intake of whey protein supplements to attain a fit body.[2] Protein supplements are synthetic form of proteins which are composed of Whey Protein, Soy (Lecithin or Isoflavone), Whey Peptides, Fat-Reduced Cocoa Powder, Flavorings, Sweeteners, Enzyme Complex and preservatives.[2] These protein supplements contain the ingredients which are negatively affecting the male reproductive health.[3] Silicon dioxide and soy are constituents of protein supplements which damage the male reproductive system.[4–6]

Soy proteins are also called as Phytoestrogens that binds to estrogenic receptors which are located in several hypothalamic nuclei including Hypothalamic preoptic area (centre of sexual behaviour), Pituitary gonadotropes and along lining of Male reproductive tract, Secondary sexual glands, Sertoli cells, Leydig cells and Spermatids.[7] It influences pituitary gonadal axis. Foods rich in Soy adversely effects the Male Reproductive system.[8]

Silicon dioxide is used as preservative in protein supplements and is cytotoxic in nature.[9] One of the association linking Silicon dioxide with damage to male reproductive parameters is the generation of reactive oxygen species as a result of oxidative stress.[10] Silicon dioxide damages mitochondrial cristae in cells leading to decreased ATP generation which causes the oxidative stress leading to the DNA damage.[11]

Zinc is an important dietary nutrient which has a remarkable effect on male reproductive system as it acts as an antioxidant and protects the epithelial linings of the reproductive system.[12]

Zinc is the component of an important enzyme superoxide dismutase which transforms the superoxide to oxygen and hydrogen peroxide so it have the capability to fight against the oxidative stress.[13,14] Zinc reduces the amount of free radicals and repairs the DNA.[15]

There is scarcity of data about the harmful effects of preservatives used in dietary supplements

and about role of zinc in correcting the male reproductive parameters and DNA damage caused by harmful constituents in protein supplements.

The protein supplements have been reported to cause the problems in male reproductive system and Zinc is well known for improving male fertility. There is no research, which suggests the regular addition of an antioxidant with protein supplements to avoid the damage to male reproductive parameters.

MATERIAL AND METHODS

Total number of 30 male Sprague Dawley rats, 8 weeks old, weighing 250–300 g were included in the study. Male Sprague Dawley rats were distributed into 3 groups as following:

Group 1 (control group) contained 10 male Sprague Dawley rats which were fed on a standard laboratory feed. Group 2 (experimental group) contained 10 male Sprague Dawley rats which were fed on a standard laboratory feed mixed with soy protein (42 mg/week for 10 rats) and silicon dioxide (0.42 mg/week for 10 rats) in powder form. Group 3 (experimental group) contained 10 male Sprague Dawley rats which were fed on standard laboratory feed mixed with soy protein (42 mg/week for 10 rats), silicon dioxide (0.42 mg/week for 10 rats) and zinc (210 mg/week for 10 rats) in powder form.

Male Sprague Dawley rats were acclimatized to the NIH animal house atmosphere at humidity of 50–70% and at a room temperature of 24±2 °C, maintained at 12 hour light and dark cycle. Standard laboratory feed and water was provided *ad libitum*.

Amount of soy protein, silicon dioxide and zinc used in the experiment was calculated by using the formula after incorporating the allowed doses of all three ingredients mentioned in the literature.[15–17]

As, a biomarker of oxidative stress induced DNA damage serum levels of 8-hydroxy-2′-deoxyguanosine (ng/ml) was measured. The value was assayed by using commercially available ELISA Kits from (Elabscience Biotechnology Co. Ltd., Japan.)

At the beginning of experiment blood collection from male Sprague Dawley was retrieved through Tail Vein method for detecting the DNA damage. Male Sprague Dawley rats were placed in a plastic restraining holder. Tails of the male Sprague Dawley rats were washed with water (20–30 °C) to dilate the blood vessels. The tail was wiped with ethanol and cleaned with the gauze. The 22G butterfly needle was inserted into one of the lateral tail veins at a position approximately 2–3 cm away from the tip of the tail at an angle of approximately 20°.About 1.2 ml of blood was drawn from the tail. Collected blood was added into the labelled gel tubes.

While at the end of the experiment the blood collection from Group 2 and 3 male Sprague Dawley rats was retrieved through intra-cardiac puncture. Male Sprague Dawley rats of Group 2 and Group 3 were placed in the jar containing cotton, soaked in chloroform. The rats were kept in the jar, until their breathing movements ceased. Male Sprague Dawley rats were sacrificed once their breathing movements were stopped. Sacrificed male Sprague Dawley rats were placed ventrally on their back on dissection board. After palpation of lower rib cage and sternal margin, a 23 G needle was inserted into the heart and 3 mL of blood was drawn by using 3 mL disposable syringe.

Right testis of Group 1, Group 2 and Group 3 of anesthetized male Sprague Dawley rats, were obtained after dissection. Peritoneal cavity was opened through a midline incision along abdominal wall and right testis was dissected out and freed from adherent tissues. Right testis was fixed in formalin saline solution for 24 hours. Ascending grades of ethanol were used to dehydrate the tissue. Tissue was cleared in xylene and embedded in paraffin for 2 hours. Then next day testicular tissue was sectioned by using microtome (thickness of 5 μm). Testicular tissue sections were deparaffinised by xylene, hydrated through an ethanol series of 100%, 90%, 80%, 70%, and 50%. Staining of slides was done using haematoxylin-eosin (HE) stain for the determination of Testicular histology (spermatogenetic cells, size of seminiferous tubules and lumen of seminiferous tubules).

Statistical analysis of the data was done using Statistical package for Social Sciences version 23 (SPSS 23). Results were documented as Mean±SEM. A comparison between the groups was done by using independent sample *t*-test, and $p<0.05$ was regarded as significant.

RESULTS

Comparison of Mean±SEM of serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels of Group 1, Group 2 and Group 3 male Sprague Dawley rats is shown in Figure-1. Serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) in Group 2 rats (5.23±2.62 ng/ml) was significantly increased ($p<0.05$) as compared to serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels of Group 1 rats (2.19±1.84 ng/ml). There was no significant difference in serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels between Group 1 and Group 3. While serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels of Group 3 rats (3.42±2.56) was significantly decreased ($p<0.05$) as compared to serum 8-hydroxy-2’-deoxyguanosine (8-OHdG) of Group 2 rats (5.23±2.62 ng/ml).

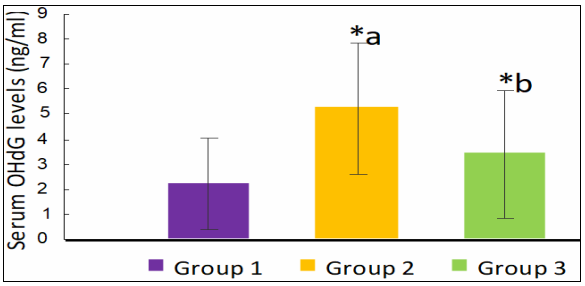

Figure-1: Comparison of Mean±SEM of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) (ng/mL) levels in Group 1, Group 2 and Group 3.

Under light microscope (40× and 100× Magnification), seminiferous tubules of testicular tissue were observed in all three groups. Group 1 (Control group) Sprague Dawley rats showed normal shaped seminiferous tubules, 7 to 9 layers of spermatogenic cells were present and lumen of seminiferous tubules was filled with large number of spermatozoa. There was no atrophy of seminiferous tubules and no widening of lumen of seminiferous tubule. While testicular tissue histology of Group 2 male Sprague Dawley rats who were fed with Soy protein and Silicon dioxide mixed with standard laboratory feed showed atrophic seminiferous tubules with 2 to 3 layers of spermatogenetic cells and widening of seminiferous tube lumen. However, administration of Zinc along with Soy protein and Silicon dioxide in Group 3 male Sprague Dawley rats showed normal seminiferous tubules with increased number of mature spermatozoa in the lumen and presence of 6 to 8 layers of spermatogenetic cells.

The histological changes in testicular segment of all three groups (Group 1, Group 2 and Group 3) are shown in Figure 2, 3, and 4.

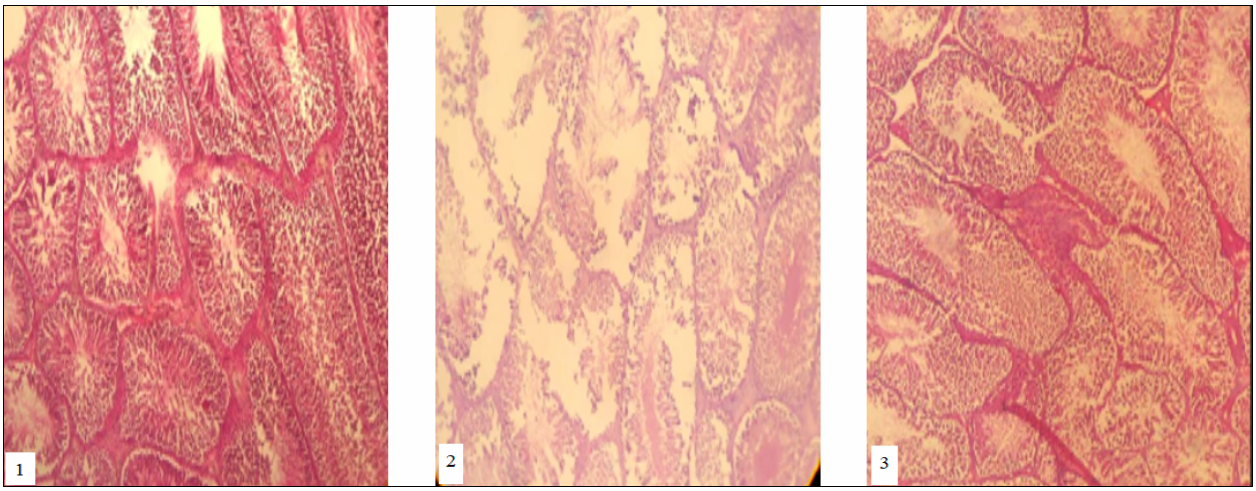

Figure-2: Histological changes in the testicular tissue of Group 1, Group 2, and Group 3. (H&E, ×40)

1. Testicular segment of Group 1 showing normal seminiferous tubules. 2. Testicular section of Group 2 showing atrophic seminiferous tubules. 3. Testicular section of Group 3 showing normal seminiferous tubular.

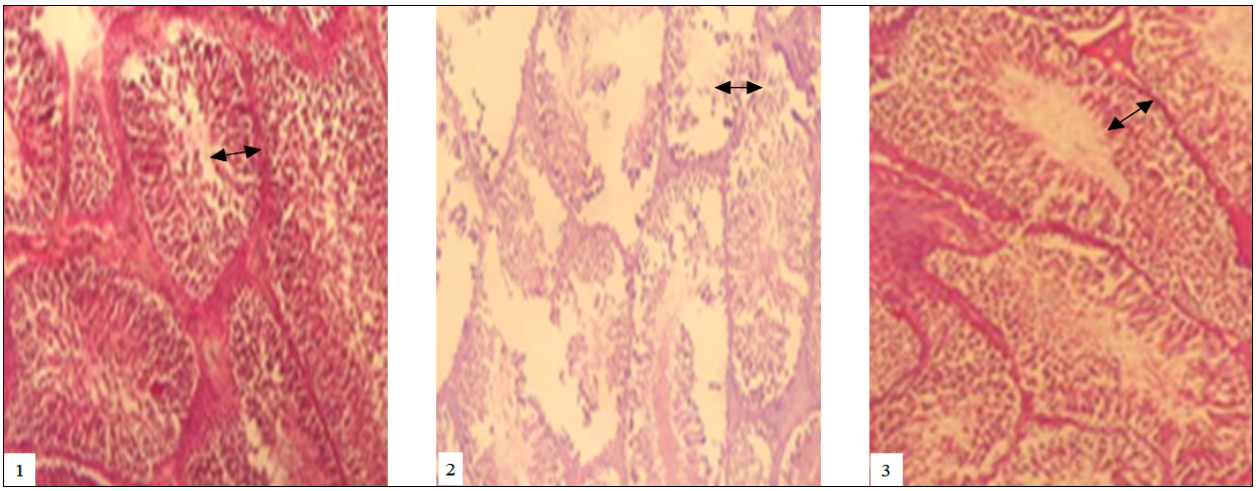

Figure-3: Histological changes in the testicular tissue of Group 1, Group 2, and Group 3. (H&E ×40)

1. Testicular section of Group 1 showing 7 to 9 layers of spermatogenic cells. Testicular section of Group 2 showing 2 to 3 layers of spermatogenic cells. Testicular section of Group 3 showing 5 to 8 layers of spermatogenic cells.

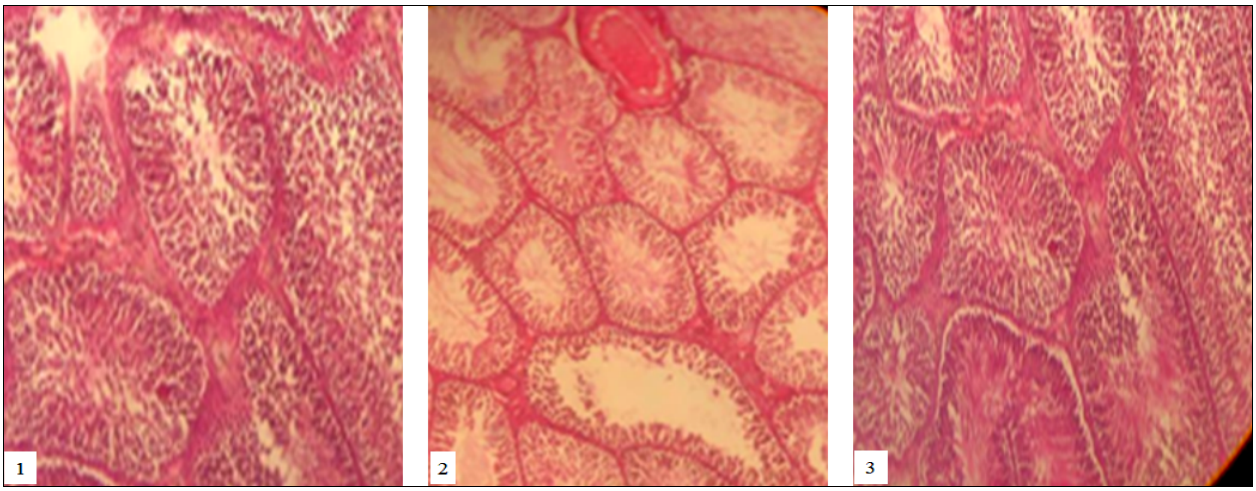

Figure-4: Histological changes in the testicular tissue of Group 1, Group 2, and Group 3. (H&E ×40)

1. Testicular segment of Group 1 showing spermatozoa in the lumen of seminiferous tubules. 2. Testicular section of Group 2 showing decrease number of spermatozoa and widening of lumen of seminiferous tubules. 3. Testicular section of Group 3 showing increase in number of spermatozoa in lumen of seminiferous tubules.

DISCUSSION

Protein is one of the most popular dietary supplements which have gained much popularity amongst athletes and people who do heavy exercise to increase muscle mass and strength. Studies have proved that some important components of protein supplements like Soy protein and Silicon dioxide are detrimental to male reproductive system.[6,16] The damage induced by these components to the Male Reproductive system may lead to infertility in males. This study explored the harmful effects of Soy protein and Silicon dioxide on male reproductive system and the role of Zinc in restoring fertility.

Current study explored the important biomarker of oxidative stress 8-hydroxy-2'-deoxyguanosine 8-(0HdG). Level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was raised in male Sprague Dawley rats who were fed with soy protein and silicon dioxide indicating DNA damage. These results are similar to Guo *et al*[11], and Pearce *et al*[17], who revealed that oxidative stress increases the level of 8-hydroxy-2′-deoxyguanosine (8-OHdG). Levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were remarkably decreased in male Sprague Dawley rats who were fed with Zinc, indicating reversal of DNA damage, these findings are similar to Lee Sr[13], Jarosz *et al*[18], and Kefer *et al*[19].

Observation of testicular tissue histology after HE staining was included in the present study, which demonstrated that Soy protein and Silicon dioxide consumption resulted in atrophy of seminiferous tubules, decrease in number of spermatogenetic cell layers in male Sprague Dawley rats. Similarly, Nurdiana *et al*, showed that administration of soybean extract in male rats resulted in significant damage to the testicular histology.[20] These results are different from Khalida *et al*[21], who concluded that there is no damage to testicular histology of rabbits who consumed body building proteins containing Soy protein. Our study showed that consumption of Zinc improves the histopathological changes in testicular tissue of male Sprague Dawley rats fed with Soy protein and silicon dioxide, Zinc restores the oxidative damage to testicular tissue caused by the production of reactive oxygen species. These results are similar to Fallah *et al*, research outcomes that supported the fact that Zinc have the antioxidant capacity and have the positive influence on the structure and function of male reproductive system.[12]

CONCLUSION

Outcomes of the present research showed that Zinc has the curative and antioxidant properties in response to soy protein and silicon dioxide-induced testicular tissue damage and oxidative stress. Soy protein and silicon dioxide induce damage in the male reproductive parameters. Zinc has the potential to restore normal functions of reproductive system by reducing levels of 8-hydroxy-2′-deoxyguanosine 8-(OHdg) and by improving Histological features of testes.

RECOMMENDATIONS

Although we demonstrated that Soy protein and silicon dioxide induce detrimental effects on testes and increases serum levels and 8-hydroxy-2′-deoxyguanosine 8-(OHdG). The effects of soy protein on fertilization ability should be checked by mating the male rats in the three groups with normal female rats. Sample size needs to be increased. Additional biochemical and molecular studies are needed to clarify the effects of Protein supplements and antioxidants on the male reproductive system. Furthermore, these therapeutic effects of Zinc on male reproductive system and DNA needs to be clarify via future studies on humans.

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SA: Final review and approval
HF: Final review and approval
HM: Data collection and analysis
SM: Data collection and analysis
FA: Data collection and analysis
SJ: Data collection and analysis

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