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Effect Of Zinc And Ingredients Of Protein Supplement On Sperm Parameters And Male Reproductive Hormones- An RCT Study

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Abstract

Objective: The purpose of this study was to assess the protective effects of zinc against injury to sperm parameters and male reproductive hormones brought on by toxic protein supplement constituents.

Methods: 30 male Sprague Dawley rats were used in the study, of which 10 were in Group 1 (given standard laboratory food), 10 were in Group 2, fed standard laboratory food nutritional supplement powder and 10 were in Group 3, fed standard laboratory food, nutritional supplement powder and zinc. Semen Analysis of Male Sprague Dawley rats of all 3 groups was evaluated after 8 weeks. Sperm parameters and serum levels of Male Reproductive Hormones were measured at the beginning and the end of the study.

Results: There was a significant decrease ($P<0.05$) in Mean ± SEM of sperm count millions /ml, sperm motility (%), and normal sperm morphology (%) in Group 2 rats when compared with Group 1. Zinc supplement in Group 3 caused a significant increase ($P<0.05$) in Mean ± SEM of sperm parameters including sperm count millions /ml, sperm motility (%) and normal sperm morphology (%) as compared to Group 2 rats.

Mean ± SEM of serum level of testosterone Hormone, Follicle Stimulating Hormone(FSH), and Luteinizing Hormone(LH) of Group 2 rats showed a significant ($P<0.05$) decrease when compared with Group 1 rats. However, the use of Zinc in Group 3 rats significantly ($P<0.05$) increased serum Testosterone, FSH, and LH levels when compared to Group 2. No significant difference was noted between serum levels of Testosterone, in Group 1 and Group 3. Serum levels of FSH and LH in Group 3 rats (484.81+ 78.17 ng/ml) were significantly increased ($P<0.05$) as compared to Group 1 rats.

Conclusion: Regular addition of antioxidant Zinc with protein supplements has the potential to restore normal functions of the male reproductive system.

Keywords: Protein supplements, Soy Protein, Silicon dioxide, Zinc, Sperm parameters, Testosterone, Follicle stimulating hormone, Luteinizing hormone.

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1. Introduction

A product containing a dietary element designed to enhance the nutritional content of the diet is referred to as a dietary supplement.[1] About 50% of Americans take these supplements to enhance their health and increase their vitality.[2] Men are becoming more interested in taking oral whey protein supplements to get a healthy body.[3] Protein supplements are one of the commonly used supplements. Whey Protein, Soy (Lecithin or Isoflavone), Whey Peptides, Fat-Reduced Cocoa Powder, Flavorings, Sweeteners, Enzyme Complex, and preservatives are the main components of protein supplements, which are synthetic forms of proteins.[4] These protein supplements have chemicals that are harmful to the health of the male reproductive system.[5] Protein supplements with silicon dioxide and soy as ingredients can harm the male reproductive system.[4,6,7]

As phytoestrogens, soy proteins bind to estrogenic receptors found in the hypothalamic preoptic area (the centre of sexual behaviour), pituitary gonadotrophs, along the lining of the male reproductive tract, secondary sexual glands, Sertoli cells, Leydig cells, and spermatids, among other hypothalamic nuclei.[8] Pituitary gonadal axis is affected. Soy-rich foods hurt male reproductive health.[9]

Silicon dioxide is a cytotoxic substance that is used as a preservative in protein supplements.[10] The production of reactive oxygen species because of oxidative stress is one of the associations connecting silicon dioxide with harm to male reproductive parameters.[11] Sperm characteristics such as sperm count, sperm motility, and typical sperm morphology may be adversely affected by silicon dioxide.

As an antioxidant and protector of the epithelial linings of the reproductive system, zinc is a crucial dietary vitamin that has a notable impact on the male reproductive system.[12] There is a dearth of information regarding the detrimental effects of soy protein, preservatives used in dietary supplements, and the function of zinc in reversing the detrimental effects of protein supplements on male reproductive parameters.

Gap of Study

Male reproductive system issues have been linked to protein supplements, and zinc is known to increase male fertility. No studies have been done to support the frequent supplementation of an antioxidant with protein supplements to protect male reproductive parameters.

2. Materials & Methods

A total number of 30 Male Sprague Dawley rats, 8 weeks old, weighing 250-300g were included in the study. The research study was a randomized control trial (RCT). The research was executed in the Department of Physiology and Multidisciplinary Laboratory of Islamic International Medical College, Rawalpindi. The study was approved by the Ethical Review Committee of Islamic International Medical College and was accomplished under the guidelines, stated by the National Institute for animal experimentations.

Male Sprague Dawley rats were randomly distributed into 3 groups as follows:

Group 1: Group 1 called a control group contained 10 Male Sprague Dawley rats which were fed on a standard laboratory feed.

Group 2: Group 2 called as experimental group contained 10 Male Sprague Dawley rats which were fed on a standard laboratory feed mixed with soy protein 0.6mg/rat/day and silicon dioxide 0.006mg/rat/day in powder form.

Group 3: Group 3 also called as experimental group contained 10 Male Sprague Dawley rats which were fed on standard laboratory feed mixed with soy protein0.6mg/rat/day, silicon dioxide 0.006mg/rat/day and zinc 3mg/rat/day in powder form.

Male Sprague Dawley rats were acclimated to the conditions of the NIH Animal House, which included humidity levels of 50–70%, a room temperature of 24–2 °C, and a 12-hour light/dark cycle. Water and standard laboratory food were always available.

After taking into account the permitted doses of each of the three substances listed in the literature, the formula was used to determine the amount of soy protein, silicon dioxide, and zinc to be utilized in the experiment.[13,14,15]

Semen Analysis:

After dissecting the left testis from the abdomen of Male Sprague Dawley rats, the testis was placed in a Petri dish containing 2 ml of normal saline. The testis was cut into pieces to allow the release of sperm. After 15 minutes, 20ul of suspension containing sperms was taken from the petri dish and added into 3980ul of normal saline to dilute it 200 folds.

After dilution, Sperm count (millions/ml), Sperm motility (%) and Sperm Morphology (%) were measured by Neubauer chamber.

Estimation of Serum Testosterone, FSH and LH levels:

Serum levels of Testosterone, Follicle Stimulating Hormone and Luteinizing Hormone were estimated by using commercially available ELISA Kits from (Elabscience Biotechnology Co.Ltd., Japan.)z

To measure the serum levels of testosterone, FSH, and LH at the start of the trial, blood was drawn from male Sprague Dawley rats using the Tail Vein Method. Male Sprague Dawley rats were confined in a piece of plastic. Rat tails were bathed in water that was 20–30°C to widen the blood vessels. Using gauze and ethanol, the tail was cleansed. At about 2-3 cm from the tip of the tail and an angle of roughly 20°, the 22 G butterfly needle was placed into one of the lateral tail veins. The tail's blood was extracted for about 1.2 ml. The designated gel tubes were filled with the collected blood.[16,17.]

After the trial, blood samples from male Sprague Dawley rats in Groups 2 and 3 were obtained using intra-cardiac puncture. Male Sprague Dawley rats from Groups 2 and 3 were put in a jar with cotton that had been saturated in chloroform. Up until their respiratory movements stopped, the rats were kept in the jar. Once their respiratory movements ceased, male Sprague Dawley rats were killed. Male Sprague Dawley rats that had been sacrificed were laid ventrally on their backs on a dissection board. A 23 G needle was placed into the heart after the lower rib cage and sternal border were palpated, and 3 mL of blood was taken using a disposable 3 mL syringe.

Statistical Analysis

The Statistical Package for Social Sciences version 23 (SPSS 23) was used to statistically analyse the data. The findings were presented as Mean + SEM. A t-test on an

independent sample was used to compare the groups. P values below 0.05 were considered significant.

3. Results

Semen analysis:

Table 1: Comparison of Mean ± SEM of sperm parameters (sperm count, motility and morphology) of Sprague Dawley rats in all three Groups (Group 1, Group 2 and Group 3).

Sperm Parameters	Group 1 Control group n=10	Group 2 Experimental group n=10	Group 3 Experimental group n=10
Total sperm count (millions/ml)	92 ± 1.14	26.34±2.32*a	56.17±4.06 *b
Sperm motility (%)	53.33 ±1.13	19.17±5.44 *a	31.517±2.06 *b
Normal sperm morphology (%)	73.33 ± 3.03	63.06 ± 4.0 *a	70.51±1.86 *b

**=P<0.05 is considered statistically significant.*
**a=Group 1 vs Group 2*
**b=Group 2 vs Group3*

Serum Testosterone Levels (ng/ml):

Mean ± SEM of serum Testosterone levels (ng/ml) of Group 2 Male Sprague Dawley rats (8.61±1.1ng/ml) showed significantly decreased (P<0.05) serum Testosterone levels as compared to Group 1rats (11.67±1.1 ng/ml). There was no change in serum Testosterone levels of Group 3 rats (11.93±1.09 ng/ml) as compared to Group 1 rats (11.67±1.1ng/ml). While on comparison serum Testosterone levels in Group 3 (11.93±1.09 ng/ml) were significantly increased (P<0.05) than in Group 2 rats (8.61±1.1 ng/ml). The comparison of Mean ± SEM of serum Testosterone levels (ng/ml) of Group 1, Group 2 and Group 3 is shown in figure-1.

Serum Follicle Stimulating Hormone FSH Levels (ng/ml):

On comparison of Mean ± SEM serum Follicle Stimulating Hormone (FSH) levels in Group 2 male Sprague Dawley rats (312.1± 78.17 ng/ml) were significantly decreased (P<0.05) as compared to serum FSH levels in Group 1 rats (423.9 ± 10.89ng/ml). Serum FSH levels in Group 3 rats (484.81± 78.17 ng/ml) were significantly increased (P<0.05) as compared to Group 1 rats (423.9 ± 10.89ng/ml). While on comparison of serum FSH levels in Group 3 rats (484.81± 78.17 ng/ml) were significantly increased (P<0.05) as compared to serum FSH levels of Group 2 rats (312.1± 78.17 ng/ml). The comparison of Mean ± SEM of serum levels of follicle-stimulating hormone (ng/ml) of Group 1, Group 2 and Group 3 is shown in Figure 2.

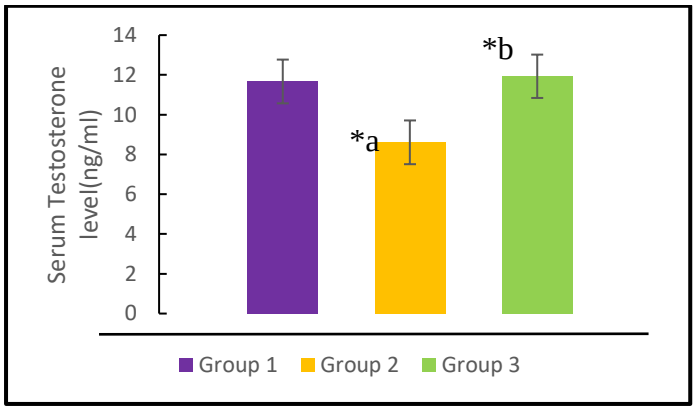

Figure 1: Comparison of Mean ± SEM of Serum Testosterone levels (ng/ml) in Groups 1, 2 and 3.
**=P<0.05 is considered statistically significant*
**a=Comparison of Group 1 vs Group 2*
**b=Comparison of Group 2 vs Group 3*

Group 1: Fed on Standard laboratory feed
Group 2: Fed on Standard laboratory feed mixed with Soy protein and Silicon dioxide
Group 3: Fed on Standard laboratory feed mixed with Soy protein, Silicon dioxide and Zinc

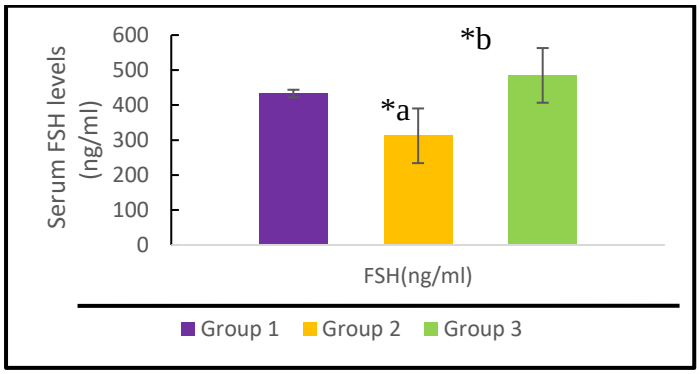

Figure 2: Comparison of Mean ± SEM of Serum Follicle Stimulating Hormone FSH (ng/mL) levels in

**=P<0.05 is considered statistically significant*
**a=Comparison of Group 1 vs Group 2*
**b=Comparison of Group 2 vs Group 3*

Group 1, Group 2 and Group 3.
Group 1: Fed on Standard laboratory feed
Group 2: Fed on Standard laboratory feed mixed with Soy protein and Silicon dioxide.
Group 3: Fed on Standard laboratory feed mixed with Soy protein, Silicon dioxide and Zinc

Serum Luteinizing Hormone LH levels (ng/ml):
Serum Luteinizing Hormone (LH) of Group 2 rats were observed to be (9.83 ± 1.18 ng/ml) which was significantly decreased (P<0.05) as compared to Serum LH levels of Group 1 (11.6 ± 2.48 ng/ml). Whereas, serum LH levels in Group 3 rats (13.23± 4.66 ng/ml) were significantly increased (P<0.05) as compared to Group 1 rats (11.6 ± 2.48 ng/ml). Serum LH in Group 3 (13.23± 4.66 ng/ml) was significantly increased (P<0.05) as compared to Group 2 Male Sprague Dawley rats (9.83 ± 1.18 ng/ml). Comparison of Mean± SEM of serum LH levels (ng/mL) of Group 1, Group 2 and Group 3 is shown in Figure 3.

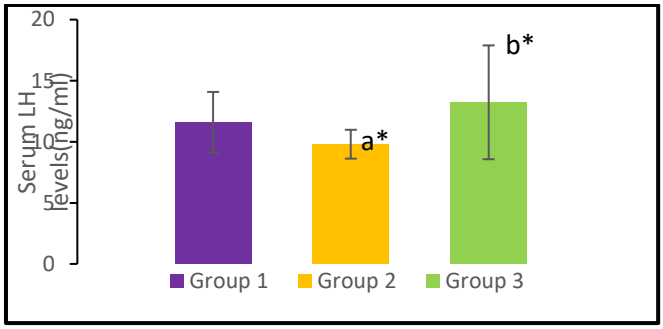

Figure 3: Comparison of Mean ± SEM of serum Luteinizing hormone LH (ng/mL) levels in Group 1, Group 2 and Group 3.

**=P<0.05 is considered statistically significant*
**a=Comparison of Group 1 vs Group 2*
**b=Comparison of Group 2 vs Group 3*

Group 1: Fed on Standard laboratory feed
Group 2: Fed on Standard laboratory feed mixed with Soy protein and Silicon dioxide
Group 3: Fed on Standard laboratory feed mixed with Soy protein, Silicon dioxide and Zinc

4. Discussion

One of the most well-liked nutritional supplements is protein, which has become quite popular among athletes and individuals who engage in vigorous activity to build muscle and improve strength.[4] Studies have shown that some key protein supplement ingredients, such as silicon dioxide and soy protein, are harmful to the male reproductive system and may cause male infertility.[3]
Male Sprague Dawley rats fed with Soy protein and Silicon dioxide powder when compared with the control group, demonstrated decreased sperm count, motility and decrease in normal sperm morphology. Similar findings have been reported in a study conducted by Abo-aloud, Kefer, and Chavarro, which stated that abnormalities in sperm parameters occur after the consumption of soy protein and exposure to free radicals.[18,19,20] The Findings of the present study show that Zinc can reverse the damage caused by Soy protein and Silicon dioxide to sperm parameters. Zinc improves sperm parameters by increasing the sperm count, and sperm motility and increases the number of sperm with normal morphology. These findings are from a study conducted by Adelakun and Fallah who revealed that Zinc is associated with improvement in sperm count, motility and normal morphology.[21,12] Huetos conducted a study and investigated that Zinc and Folic acid supplements improve sperm concentration, motility and morphology. Kefer reported that antioxidants reverse oxidative damage and improve semen quality.[19]
In the present study, we also measured the Serum Testosterone levels. Disturbance in levels of Testosterone is mainly suspected to trigger the change in the process of spermatogenesis. The findings of the present study showed that there is a significant difference in levels of Serum Testosterone levels of rats which were fed with soy protein when compared to the control group. These results are by investigations of Moaresi who proved that soy protein consumption decreases the level of Testosterone.[22] While Andriani stated that there is no difference in levels of serum testosterone after administration of Whey proteins.[2] However the usage of Zinc in Group 3 caused a significant increase in testosterone levels and these results are by study done by Fallah explored that use of the Zinc as an antioxidant increases testosterone levels by downregulating the oxidative stress.[12]
The current study also explored the effect of Soy protein and Silicon dioxide serum levels of follicle-stimulating hormone (FSH) and Luteinizing hormone (LH). The findings of the present study showed that there is a significant decrease in serum levels of follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) in Male Sprague Dawley rats who were fed with Soy protein and Silicon dioxide when compared with Follicle Stimulating hormone levels in Control group Male Sprague Dawley rats. These findings are similar to Nuridiana and Modaresi who stated in their research separately that there is a marked decrease in follicle-stimulating hormone levels in the group treated with Soy protein.[23,22].The findings of the present study are different from Luo et al,. (2019) who reported improvement in male reproductive hormone levels after consumption of a soy-enriched diet.[24] Use of Zinc

increased the levels of Follicle Stimulating hormone (FSH) and Luteinizing Hormone (LH) and these results by Kim demonstrated that consumption of antioxidants increases Testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) levels in infertile men.[25] The limitations of the study were that the sample size was small N=30(10 Male Sprague Dawley rats in Group 1, 10 Male Sprague Dawley rats in Group 2 and 10 Male Sprague Dawley in Group 3). Due to productive cost and the unavailability of rats' the sample size was kept limited.Study duration was short. It was conducted over 8 weeks. But the strengths of the study were that,it is the first study which isolated the potentially harmful ingredients of protein supplements which are very popular these days.It is also the first study which discovered DNA damage produced by harmful ingredients of protein supplements.

We would like to recommend that in fututre that even though we showed that silicon dioxide and soy protein have negative effects on the levels of reproductive hormones in the serum. By mating the male rats in the three groups with regular female rats, we did not examine how soy protein affected the ability to reproduce. To further understand the effects of protein supplements and antioxidants on the male reproductive system, more biochemical and molecular research is required. Future human investigations must clarify the therapeutic benefits of zinc on the male reproductive system.

5. Conclusion

The findings of the current study concluded that regular addition of the antioxidant zinc to protein supplements can protect bodybuilders and gym goers from the harm that harmful protein supplement ingredients like silicon dioxide and soy protein would otherwise cause to the male reproductive system.

CONFLICTS OF INTEREST- None

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Contributions:
A.I - Conception of study
I.A.M - Experimentation/Study Conduction
S.S, S.J - Analysis/Interpretation/Discussion
S.S, N.U.A, S.J, M.H.F, A.I - Manuscript Writing
S.S, I.A.M, N.U.A, S.J, M.H.F, A.I - Critical Review
A.I, I.A.M, S.S, S.J, N.U.A, S.J - Facilitation and Material analysis

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