International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 22-12-2020; Accepted: 08-01-2021; Published: 15-02-2021
www.foodsciencejournal.com
Volume 6; Issue 1; 2021; Page No. 80-86

Ameliorating effect of zinc on protein supplement induced changes in serum levels of male reproductive hormones of sprague dawley rats

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Abstract

Background: The protein supplements contain the harmful ingredients such as soy protein and silicon dioxide which have the injurious effects on male reproductive system. Addition of Zinc as an antioxidants with the dietary supplements may lessen the harmful effects of Soy protein and silicon dioxide on male reproductive system.

Objectives:

To determine the harmful effect Soy protein and Silicon dioxide and beneficial effect of Zinc on male reproductive parameters of Sprague Dawley rats.

Material and Methods: Total 30 male Sprague Dawley rats were divided into Group 1, Group 2 and Group 3. Group 1 rats were fed on standard laboratory feed, rats of Group 2 were fed on a dietary supplement powder containing Soy protein and Silicon dioxide mixed with standard laboratory feed and rats of Group 3 were fed with a dietary supplement powder containing Soy protein, Silicon dioxide and Zinc mixed with standard laboratory feed.

Serum levels of Testosterone (ng/ml), Follicle Stimulating hormone (ng/ml) and Luteinizing hormone (ng/ml) were measured by ELISA technique.

Results: Group 2 rats showed significant ($P<0.05$) decrease in serum Testosterone level when compared with Group 1 rats. However use of Zinc in Group 3 rats significantly ($P<0.05$) increased serum Testosterone levels. Mean ± SEM of serum Follicle stimulating hormone (FSH) and serum Luteinizing Hormone LH levels in Group 2 rats were significantly decreased ($P<0.05$) as compared to Group 1.Zinc administration caused the significant increase ($P<0.05$) in serum Mean ± SEM of Follicle Stimulating hormone (FSH) levels and Luteinizing Hormone LH in Group 3 rats as compared to Group 1 and Group 2 rats.

Conclusion: Soy protein and Silicon dioxide are found to be harmful for male reproductive system. Zinc has potential to restore normal functions of male reproductive system by increasing serum levels of Testosterone, Follicle stimulating hormone and Luteinizing hormone.

Keywords: protein supplements, soy protein, silicon dioxide, zinc, testosterone, follicle stimulating hormone, luteinizing hormone

Introduction

Dietary supplements are defined as a product taken by mouth that contain a dietary ingredient intended to supplement the diet. About 50% population 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]. Trend of using protein supplements is increasing amongst people who do heavy exercise to gain muscle mass and build a masculine body [3, 4]. Protein is a very important constituent of human diet and plays an important role in metabolism. It is an important constituent of muscles, tissues, hormones, enzymes and serves as an energy source. Protein is obtained from animal and plant sources as well as in the form of synthetic protein powders.[5] 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 [6]. These protein supplements are decreasing the sperm count, male sex hormone levels and effect the male reproductive health [7] Silicon dioxide and soy are constituents of protein supplements which decline the levels of testosterone and other reproductive hormones [8].

Soy is the necessary ingredient of all protein supplements and is a protein itself. Soy protein is used as an energy source, it serves as an emulsifier and helps to facilitate absorption of fats [9] Soy protein helps to increase body weight but it has inverse relationship between soy protein intake and serum levels of male reproductive hormones [2]. Soy proteins are also called as Phytoestrogens that binds to estrogenic receptors which are located in several hypothalamic nuclei including Hypothalamic preoptic area (center of sexual behavior), Pituitary gonadotropes and along lining of Male reproductive tract, Secondary sexual glands, Sertoli cells, Leydig cells and Spermatids [10]. It influences pituitary gonadal axis [11]. Foods rich in Soy decreases the gonadotropins, sperm count and increases the

timing of acrosome reaction and adversely effects the Male Reproductive system [12, 13].

Silicon dioxide is used as preservative in protein supplements and is cytotoxic in nature [14]. 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. Silicon dioxide causes potential harmful effects on sperm parameters including sperm count, sperm motility and normal sperm morphology [15, 16] Silicon dioxide damages mitochondrial cristae in cells leading to decreased ATP generation which causes the oxidative stress leading to the DNA damage.

Zinc is an important dietary nutrient which have a remarkable effect on male reproductive system as it acts as an antioxidant, balances the male reproductive hormones such as testosterone and protects the epithelial linings of the reproductive system. 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. Zinc reduces the amount of free radicals and repairs the DNA [17].

It is a known fact that some constituents in protein supplements and protein rich diet disturbs the male reproductive parameters [18, 19]. Zinc has a protective role for reproductive system [20].

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. Till date there is no research which suggests the regular addition of an antioxidant with protein supplements to avoid the damage to male reproductive parameters. The study aims to unveil the effects of components of protein supplements (Soy protein and Silicon dioxide) on male reproductive parameters and to highlight the role of zinc in relation to these effects.

Gap of Study

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.

Hypothesis

Components of Protein supplements cause damage to the male reproductive parameters and Zinc supplement have a protective role in correcting the damage.

Null Hypothesis

Components of Protein supplements do not cause damage to the male reproductive parameters and Zinc supplement do not have a protective role in correcting the damage.

Materials and Methods

A total number of 30 male Sprague Dawley rats were included in the study for the duration of one year, starting from 1st September, 2019 to 31st August, 2020. The research study was Experimental, randomized control trial (RCT). The research was executed in the department of Physiology and Multidisciplinary Laboratory of Islamic International Medical College, Rawalpindi in association with the Animal House at National Institute of Health (NIH), Islamabad, Federal Laboratory Rawalpindi and Pathology department of Railway Hospital. 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.

Total number of 30 Male Sprague Dawley rats, 8 weeks old in age, weighing 250-300g were included in the study. Male Sprague Dawley rats were distributed into 3 groups as follows: Group 1 called as a control group contained 10 Male Sprague Dawley rats which were fed on a standard laboratory feed. Group 2 called as experimental group contained 10 Male Sprague Dawley rats which were fed on a standard laboratory feed mixed with soy protein and silicon dioxide in powder form. Group 3 also called as experimental group contained 10 Male Sprague Dawley rats which were fed on standard laboratory feed mixed with soy protein, silicon dioxide and zinc in powder form.

Laboratory environment

Male Sprague Dawley rats were acclimatized to the NIH Animal House atmosphere at humidity of 50-70% and at a room temperature of 24 $\pm$ 2 ℃, maintained at 12 hour light and dark cycle. Standard laboratory feed and water was provided ad libitum.

Standard Laboratory feed for Group 1, Group 2 and Group 3

Standard feed for Male Sprague Dawley rats was prepared in pellet form at Animal House of NIH, Islamabad according to the guiding principle given by the Universities federation for Animal welfare [21].

Dietary supplement powder for Group 2 and Group 3

Soy protein (powder) and Silicon dioxide (powder) were used in the preparation of dietary supplement powder for Group 2 and Group 3. Whereas, in addition to Soy protein and Silicon dioxide Group 3 dietary supplement powder also had Zinc tablets. The Dietary Supplement powder was mixed with standard laboratory feed prepared for Male Sprague Dawley rats.

Dietary Supplement powder for Group 2

Dietary supplement powder for Group 2 was prepared by mixing Soy protein (powder) and Silicon dioxide (powder).

Soy protein (Powder)

Soy protein powder (Solarbio Life sciences Cat#S9510 250g) was purchased from Mediplex

International (PVT) Ltd, Islamabad. Amount of Soy protein powder used for preparation of dietary supplement powder was calculated by using the formula according to dose of 2mg/kg body weight [9].

Calculation of amount of Soy protein (Powder)

2mg/kg body weight
1kg=1000gms
2mg/1000gms
1000gms=2mg
Average weight of one Male Sprague Dawley rat=300g
300g=2/1000x300=0.6mg/day
Dose of 1 rat/day=0.6mg/day
Dose of 10 rats/day=0.6x10=6mg/day
Dose of 10 rats for 1 week (7 days) =6x7=42mg/week for 10rats

Silicon dioxide (Powder)

Silicon dioxide powder (SIGMA-ALDAICH Lot# STBGE104Y) was purchased from Mediplex International (PVT) Ltd, Islamabad. Amount of Silicon dioxide powder used for preparation of dietary supplement powder was calculated by using formula according to dose of 0.02mg of the diet.

Calculation of amount of Silicon dioxide (powder)

0.02mg/kg body weight
1kg=1000gms
2mg/1000gms
1000gms=0.02mg
300gms=0.02/1000x300
Dose of 1 rat /day =0.006mg/day
Dose of 10 rats/day =0.006x10=.06mg/day
Dose of 10 rats /week (7 days) =0.06mgx7=0.42mg of silicon dioxide

Dietary Supplement powder for Group 3

Dietary supplement powder for Group 3 was prepared by mixing Soy protein powder (42mg/week), Silicon dioxide powder (0.42mg/week) and Zinc powder. Amount of Zinc for preparation of dietary supplement powder was calculated by using the formula according to dose 10mg/kg per day.

Zinc:

Zinc tablets (GNC Zinc 50mg code 253922) were purchased from Mediplex International (PVT) Ltd, Islamabad. Zinc tablets were grinded to powder form by mortar and pestle. Amount of Zinc powder for preparation of dietary supplement powder was calculated according to dose 10mg per day.

Calculation of amount of Zinc (Powder)

10mg/kg body weight
1kg=1000gms
1000gms=10mg/kg body weight
300gms rat=10/1000x300=3mg/day
Dose of 1 rat/ day=3mg/day
Dose of 10 rats/ day =3x10=30mg/day
Dose of 10 rats /week (7days) =30x7=210mg/week

Storage of Dietary Supplement powder

Dietary supplement powder for Group 2 containing Soy protein powder (42 mg/week) and Silicon dioxide powder (0.42mg/week) was stored in plastic bottle for 1 week which was labelled as #1. Dietary supplement powder for Group 3 containing Soy protein powder (42 mg/week), Silicon dioxide powder (0.42 mg/week) and Zinc powder (210mg/week) was stored in plastic bottle for 1 week which was labelled as #2. Total of 8 plastic bottles labelled as #1 and #2 were stored for both groups for 8 weeks.

Experimental variables

Serum levels of Testosterone (ng/ml), Follicle stimulating hormone (ng/ml) and Luteinizing hormone (ng/ml) were evaluated.

Blood sampling

For estimation of Hormone levels blood collection was done at the beginning of the experiment and at the end of the experiment.

At the beginning of the experiment blood collection from Male Sprague Dawley was retrieved through Tail Vein method.[22].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. After palpation of lower rib cage and sternal margin, a 23 G needle was inserted into the heart and 3mL of blood was drawn by using 3 mL disposable syringe.

The blood was collected in labelled gel tubes and placed in ice box to protect them from light and contamination. The gel tubes were shifted to the laboratory for centrifugation. In the laboratory, the labelled gel tubes having the blood samples were placed in centrifuge machine and were centrifuged at a speed of 3000 rpm for 15 minutes until the serum was separated from blood. The serum was stored at -80°C for analysis of Serum levels of Testosterone (ng/mL), Follicle Stimulating hormone (ng/ml) and Luteinizing hormone (ng/ml) by using ELISA Kits from *(Elabscience Biotechnology Co. Ltd., Japan.)*

Statistical analysis

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

Results

In this study a total of 30 Male Sprague Dawley rats were included. Male Sprague Dawley rats were divided into Group 1(n=10), Group 2 (n=10) and Group 3(n=10). Following interventions were used in these groups for 8 weeks:

Group 1: Male Sprague Dawley rats fed on standard laboratory feed.

Group 2: Male Sprague Dawley rats fed on Soy protein and Silicon dioxide with standard laboratory feed

Group 3: Male Sprague Dawley rats fed on Soy protein, Silicon dioxide and Zinc with standard laboratory feed.

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 of 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 1rats(11.67 ± 1.1ng/ml). While on comparison serum Testosterone levels in Group 3 (11.93 ± 1.09 ng/ml) was significantly increased ($P<0.05$) than 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.

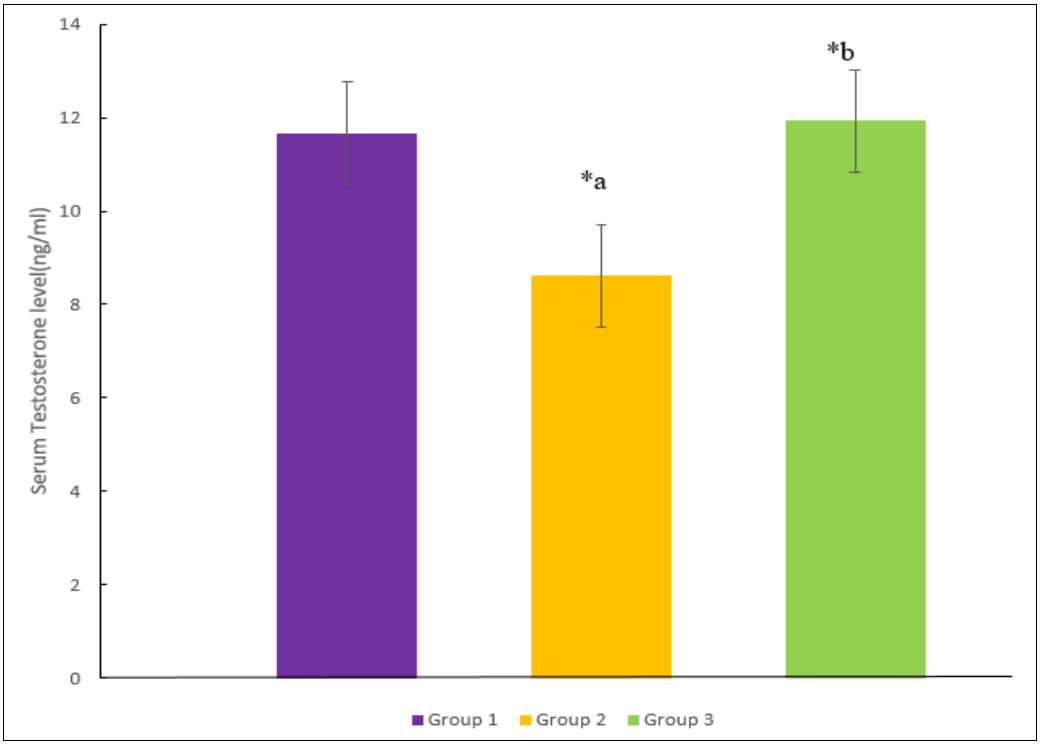

Fig 1: Comparison of Mean ± SEM of Serum

Testosterone levels (ng/ml) in Group 1, 2 and 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
*=P<0.05 is considered statistically significant
*a=Comparison of Group 1 vs Group 2
*b=Comparison of Group 2 vs Group 3

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 Follicle Stimulating Hormone (FSH) levels in Group 1 rats (423.9 ± 10.89ng/ml). Serum Follicle Stimulating hormone (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 Follicle Stimulating Hormone 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.

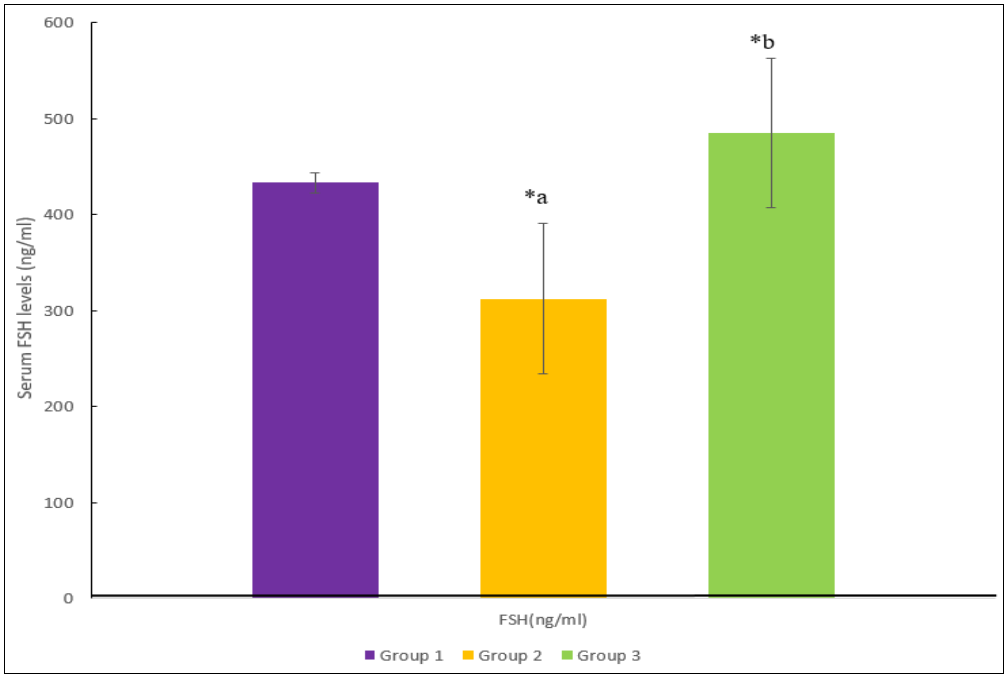

Fig 2: Comparison of Mean ± SEM of Serum Follicle Stimulating Hormone FSH (ng/mL) levels in Group 1,

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

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 were significantly decreased (P<0.05) as compared to Serum Luteinizing hormone levels of Group 1 (11.6 ± 2.48 ng/ml).Whereas, serum Luteinizing Hormone levels in Group 3 rats (13.23± 4.66 ng/ml) was significantly increased (P<0.05) as compared to Group 1 rats (11.6 ± 2.48 ng/ml). Serum Luteinizing Hormone 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 Luteinizing hormone LH levels (ng/mL) of Group 1,Group 2 and Group 3 is shown in Figure 3.

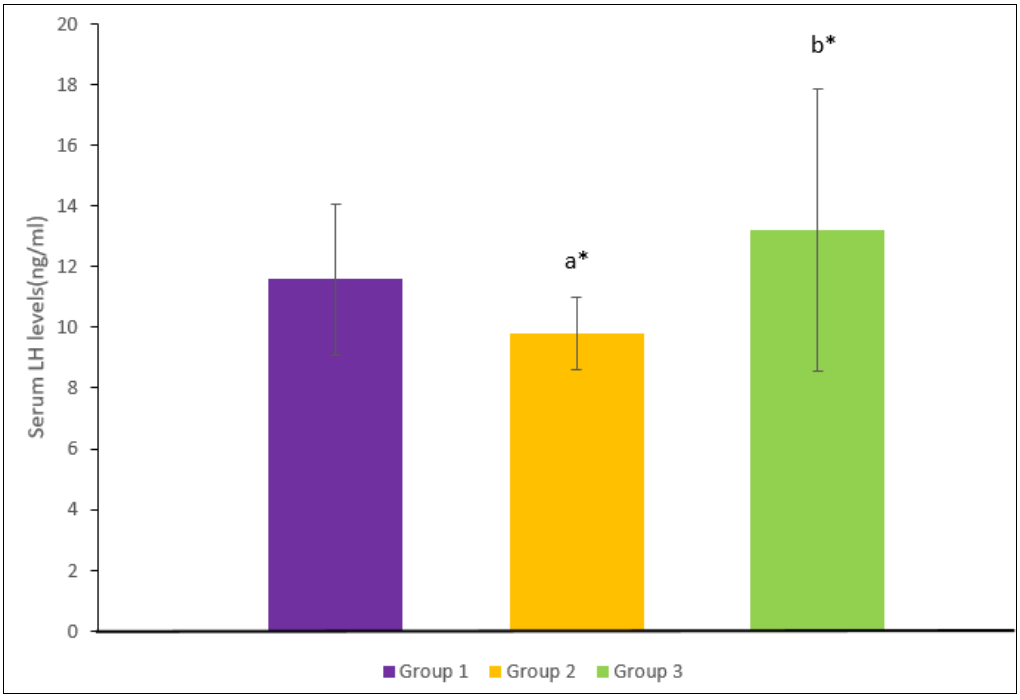

Fig 3: Comparison of Mean ± SEM of serum Luteinizing hormone LH (ng/mL) levels in Group 1, Group 2 and Group 3.

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

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.[6] Studies have proved that some important components of protein supplements like Soy protein and Silicon dioxide are detrimental to male reproductive system. The damage induced by these components to the Male Reproductive system may lead to infertility in males.[23]

In present study, we also measured the Serum Testosterone levels. Disturbance in levels of Testosterone is mainly suspected to trigger the change in process of spermatogenesis. The findings of present study showed that, there is significant difference in levels of Serum Testosterone levels of rats which were fed with soy protein when compared to control group. These results are in accordance with Moaresi, (2011) who proved that soy protein consumption decreases level of Testosterone [24]. While Andriani *et al*, (2019) stated that there is no difference in levels of serum testosterone after administration of Whey proteins.[4] However the usage of Zinc in Group 3 caused significant increase in testosterone levels and these results are in accordance with study done by Fallah., Hasani., and Colagar (2018) explored that use of the Zinc as an antioxidant increases the testosterone levels by downregulating the oxidative stress [25].

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 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 *et al*., (2016), Modaresi., (2011) who stated that there is a marked decrease in Follicle Stimulating hormone levels in the group treated with Soy protein [16, 24]. Findings of present study are different from Luo *et al*, (2019) who reported improvement in male reproductive hormone level after consumption of soy enriched diet [26] Use of Zinc increased the levels of Follicle Stimulating hormone (FSH) and Luteinizing Hormone (LH)

and these results are in accordance with Kim. *et al* (2019) who demonstrated that consumption of antioxidants increases Testosterone, Follicle Stimulating hormone (FSH) and Luteinizing hormone (LH) levels in the infertile men [27]

Conclusion

In conclusion outcomes of the present research showed that Zinc has the protective and antioxidant properties in response to soy protein induced damage to male sex hormones and silicon dioxide-induced 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 improving serum levels of Testosterone, Follicle Stimulating hormone and Luteinizing hormone levels.

Future Recommendations

Although we demonstrated that Soy protein and silicon dioxide induce detrimental effects on serum levels of reproductive hormones. We did not investigate the effects of soy protein on fertilization ability by mating the male rats in the three groups with normal female rats. Furthermore, 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 needs to be clarify via future studies on human

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