Comparative Clinical Pathology (2021) 30:847–856
https://doi.org/10.1007/s00580-021-03276-3

ORIGINAL ARTICLE

Protective effect of selenium in prednisolone testicular toxicity

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Received: 18 April 2021 / Accepted: 3 August 2021 / Published online: 10 August 2021

Abstract

The most common side effects of corticosteroids are adrenal suppression and the subsequent complications that can be attributed to testicular tissue degradation and dysfunction. Selenium or sodium selenite concentration indicates the protective role of this rare element and its related enzymes during spermatogenesis. The concentration of selenium in testis is regulated by a homeostatic mechanism that precedes the capacity of selenium in male gonads from other tissues. Therefore, the aim of this research was to investigate the protective effects of selenium on tissue structure and performance of testis in mice. In this research, 40 NMRI mice were treated in 5 groups (each including 8 mice) for 8 weeks under darkness–lightness conditions (12–12) at 25 C. The study groups were control group, prednisolone control group (1.5 mg/kg), prednisolone control group (2.5 mg/kg), and two treatment groups with selenium (0.2 mg/kg). Data resulting from investigation of sperm parameters and histological studies were analyzed using ANOVA software. The results showed that the group treated with sodium selenite along with prednisolone showed a significant decrease in testicle weight ($P<0.05$). In surveying the average of sperm number in the groups treated with prednisolone and sodium selenite, it was shown that prednisolone at concentrations of 1.5 and 2.5 mg/kg can decrease the number of sperm production, which is a significant difference compared to the control and other groups ($P<0.05$). Finally, selenium is one of the most essential elements in testicular tissue and essentially required for spermatogenesis and male fertility. Also it can act as a protective barrier in preventing the deleterious effects of corticosteroids.

Keywords Prednisolone · Selenium · Testicular tissue · Corticosteroids · Mice

Introduction

In order to prevent allergic reactions, many immunosuppressive drugs have been used. A group of immunosuppressive drugs is corticosteroids such as methyl prednisolone and prednisolone that are practically included in all immunosuppressive treatment regimens (Masuda et al. 2003). Corticosteroids inhibit the production of interleukin-1 and produce potent anti-inflammatory effects. Side effects can occur due to drug use or as a result of excessive immune suppression (Turk et al. 2010). Taking corticosteroid drugs causes the hypothalamic center of the brain to order the pituitary gland, and that gland also orders the adrenal gland to stop producing steroids anymore, as this material is sufficiently absorbed by the body (Turk et al. 2010). Selenium (Se) is an important trace mineral having many essential roles at the cellular and organismal levels in animal and human health. It is evident from observations of past studies (both animal and human) that Se is essentially required for spermatogenesis and male fertility, presumably because of its vital role in the modulation of antioxidant defense mechanisms and other essential biological pathways (Qazi et al. 2019). Selenium is a component of antioxidant enzymes, including glutathione peroxidase which plays a role in neutralizing free radicals and relieving oxidative stress (Agarwal et al. 2004; Mirone et al. 2013; Rezzani 2006). Since prednisolone has negative effects on production of spermatogenesis through decreasing safety levels of the body, increasing cortisol, and subsequently decreasing the production of good prostaglandins, it seems that consumption of selenium can stop these effects (Durak et al. 1998).

Among the reproductive tracts, the testis has the highest selenium concentration, the amount of which is even higher than that of the liver. Decrease in selenium concentration

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will likely make testicular tissue more vulnerable. Mitosis rate and different stages of meiosis in the seminiferous tubules make the germ cells susceptible to localized scavenging effects of free radicals (Fukushima et al. 2005).

With regard to the mentioned contents, the aim of the present study was to investigate the protective role of selenium regarding the effects of prednisolone drug on spermatogenesis in mice.

Materials and methods

Animals

In the present experimental study, 40 NMRI mice aged 5–6 weeks were used. The animals were kept in the animal shelter at the Ferdowsi University School of Veterinary Medicine under standard conditions (12 h light, 12 h dark) and 24 ± 2 °C with free access to food and water. These animals were randomly divided into five groups as the following: control group which did not receive any medication, group (1.5P) which received prednisolone intraperitoneally at a concentration of 1.5 mg/kg, group (2.5P) which received prednisolone intraperitoneally at a concentration of 2.5 mg/kg, the treatment prednisolone group (1.5P + Se) received intraperitoneally 0.2 mg/kg of sodium selenite with 1.5 mg/kg prednisolone, the treatment prednisolone group (2.5P + Se) received intraperitoneally 0.2 mg/kg of sodium selenite with 2.5 mg/kg prednisolone. The daily treatment was administered to the animal at approximately 10–11 a.m. for 8 consecutive weeks. The clearance to conduct this study was provided by the Ferdowsi University of the Mashhad Ethics Committee (2/45443–15/11/2017).

Chemicals

Selenium was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Also prednisolone was supplied by Sigma Chemical Co, USA/Canada.

Morphological and histological procedures

Body weight and weight of testes to body weight ratio. On the first day of the experiment, the weight of mice before gavage and injection was measured and recorded using digital scales. On the last day of the experiment, the mice were weighed again. Then, by anesthetizing the animal under sterile conditions, the right and left testes and the right epididymis were excised by creating a cleavage in the lower part of the abdomen. Then, using Sartorius scales with the accuracy of 1000 g, the testicles were separately weighed. Right testicle was examined until the start of work to get the amount of sperm. The left testicle was placed in a Bouin's fixative solution for histological study. To determine the percentage of live spermatozoids, 1 cm of the end of epididymis was immediately cut by sterile scissors and placed in an isotonic solution (1 ml of saline phosphate buffer) after removal. The tissue was homogenized by the homogenizer and was incubated for 5 min at 37 °C. This time was set to completely remove sperm from the lumen.

Morphometric evaluation of seminiferous tubules

From each section, seminiferous tubule diameter and area (essentially from circular tubular cross sections) were determined using a pre-calibrated measuring eyepiece. About 20 sections of seminiferous tubules that were round or nearly round were chosen randomly and measured for each group. The tubular diameter was measured at × 400 magnification. The diameter of the seminiferous tubule was measured across the minor and major axes, and the mean diameter was obtained (Fig. 1).

Investigation of sperm motility percentage

A drop of sperm suspension obtained in the previous step was placed on the slide, and then it was investigated with the help of an optical microscope from 5 microscopic fields with a magnification of 40, and the mean of this motion was recorded as motility percentage.

Checking sperm count

One drop (10 µl) of sperm suspension obtained in the preceding steps was placed on the Neobar slide, and the total number of spermatozoa was counted in four large chambers of the slide (four chambers related to white blood cell counts). Then the average number of sperms in a chamber was calculated.

The method to measure testosterone and LH

Testosterone hormone and LH were measured using VIDAS kit and VIDAS device.

Data analysis

Statistical analysis was performed with SPSS Version 11.0 statistic software package. Data were expressed as means ± standard deviation (SD). Comparisons between groups were performed with an analysis of paired t test. A value of $P < 0.05$ was considered statistically significant.

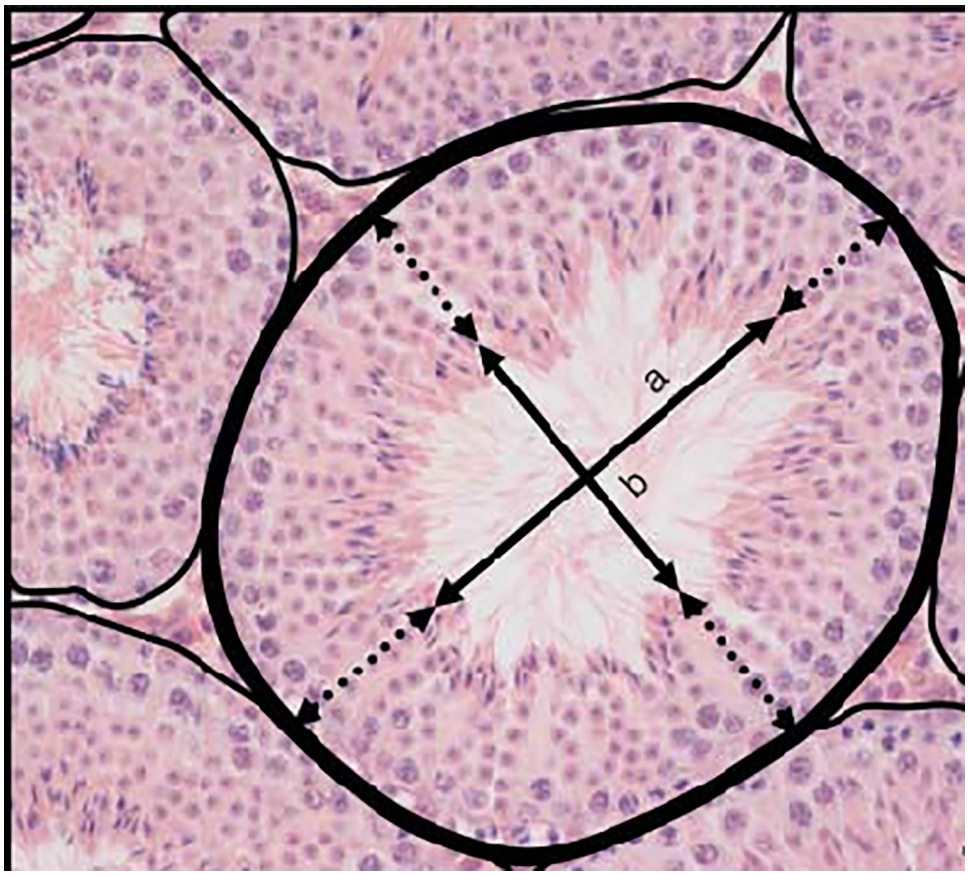

Fig. 1 Measurements of components of the seminiferous tubules and the proportion of seminiferous tubules. 'a' is the major axis, and 'b' is the minor axis

Ethics

The guidelines for the care and use of the animals were strictly followed in accordance with the Ethics and Regulations guiding the use of research animals as approved by the Ferdowsi University of Mashhad, Mashhad, Iran (2/45443–15/11/2017).

Results

Based on the findings regarding body weight and testicles' weight to body, weights are shown in Table 1. According to the findings and changes in testes weight, it is concluded that although the testicular weight of animals treated with prednisolone has decreased, this decrease is not significant compared to the control group and the other groups. By comparing two treatment prednisolone groups, the group treated with sodium selenite along with prednisolone (1.5P + Se) indicates a significant increase in testicular weight ($P < 0.05$). It seems that prednisolone with lower doses can play a better protective role and improve testicular tissue. Also, the body weight of the animals in different groups did not show a significant difference compared to each other. Although the weight in the groups treated with prednisolone has decreased, but this difference was not significant.

In investigating the mean of the sperm count in groups treated with prednisolone and sodium selenite, it was shown that prednisolone at concentrations of 1.5 and 2.5 mg/kg can decrease the number of sperm production, which is a significant difference compared to the control and other groups ($P < 0.05$). Also, in groups treated with sodium selenite along with prednisolone, the rate of sperm production is close to the control group (Fig. 2) (Table 2). Sperm motility percentages in groups treated with sodium selenite showed improvement of motility status of live and movable spermatozoids compared to prednisolone-treated groups, which

Table 1 Mean and standard deviation (mean ± SD) of body weight and testicular weight in different groups

Groups	Weight of Testis (g)	Body weight (g)
Control	0.21 ± 0.28	36.61 ± 2.08
1.5P	0.11 ± 0.01	34.2 ± 2.41
2.5P	0.13 ± 0.03	29.5 ± 1.33
1.5P + Sel	0.18 ± 0.03*	38.16 ± 0.9
2.5P + Sel	0.12 ± 0.03	34.55 ± 2.28

* Indicates a significant difference in the specified group regarding other groups

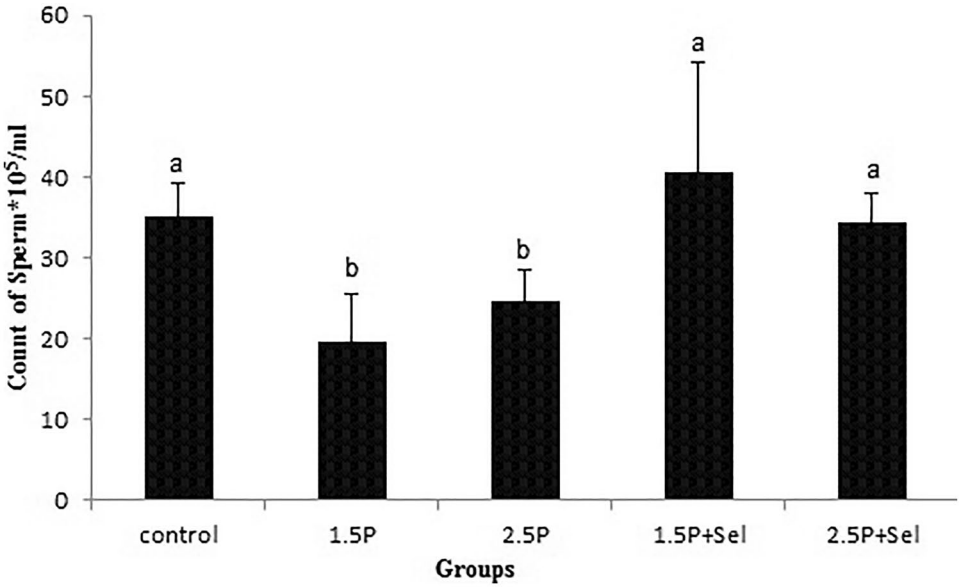

Fig. 2 Mean of counted sperms per each ml 10^{-5}. * That are not significantly different are assigned a common letter, and different letters indicate significant difference in expression ($P<0.05$)

shows a significant decrease in groups treated by prednisolone in comparison to other groups ($P<0.05$) (Table 2).

In an investigation related to serum concentrations of LH testosterone hormone, there was a significant change in serum LH in groups treated with prednisolone ($P<0.05$). Also, in groups treated with sodium selenite along with prednisolone treatment, there was no difference in testosterone (Table 3) (Figs. 3 and 4).

In the histological study of testicular tissue in the group treated with 2.5 mg/kg of prednisolone, there was a significant decrease in the diameter of the seminiferous tubules compared to the other groups and control ($P<0.05$) (Table 4). In the histological study, in the control group, testicular tissue was normal in appearance with plenty of sperms. Epithelium was composed of several layers (Fig. 5). In the treated group (prednisolone 1.5 mg/kg), epithelium was reduced compared to the normal group, and there are a few spermatozoid accumulations in the seminiferous tubules (Fig. 6). In the treated group (prednisolone 2.5 mg/kg), epithelium was greatly reduced and vacuolated, and spermatozoa were not seen in the seminiferous tubules (Fig. 7). In the treated group (prednisolone 2.5 mg/kg + 0.2 mg/kg selenium), although the number of layers in the previous group was reduced and vacuolated but with the injection of selenium, the layers of epithelium have been repaired and increased (Fig. 8).

Table 2 The mean of sperm count and the percentage of sperm motility in different study groups with prednisolone and sodium selenite

Groups	Count (10^5/ml)	Motility (%)
Control	35 ± 4.1	59 ± 3.2
1.5P	19.6 ± 5.9*	26.5 ± 6.7*
2.5P	24.5 ± 4.1*	19.6 ± 4.2*
1.5P + Sel	40.6 ± 13.6	49.5 ± 4.6
2.5P + Sel	34.3 ± 3.6	41.3 ± 10.5

* Indicates a significant difference in the specified group with others

Discussion

According to this study and the fact that prednisolone, in addition to reducing cortisol, can induce free radical production in the cell (Choi et al. 2004), it can be argued that production of free radical in testicular germ cells that are highly sensitive may cause their removal and loss of testicular weight; in addition, it can be said that one of the possible causes of testicular atrophy is an unknown factor that interferes in the flow of spermatogenesis, and as a result with the reduction of the number of sexual cells, the loss of testicular weight happens. In addition, other studies have found that ROS production reduces the quantity and quality of semen (Rayman 2012; Ahsan et al. 2014) and, by increasing cell permeability, leads to sperm death

Table 3 The levels of testosterone and LH in different groups studied with prednisolone and sodium selenite

Groups	LH (mIU/ml)	Testosterone (ng/ml)
Control	0.33 ± 0.01	7.7 ± 0.15
1.5P	0.17 ± 0.05*	7.5 ± 2.8
2.5P	0.18 ± 0.03*	7.6 ± 0.2
1.5P + Sel	0.30 ± 0.05	7.4 ± 0.3
2.5P + Sel	0.30 ± 0.03	7.5 ± 0.3

* Indicates a significant difference in the specified group with other groups ($P<0.05$)

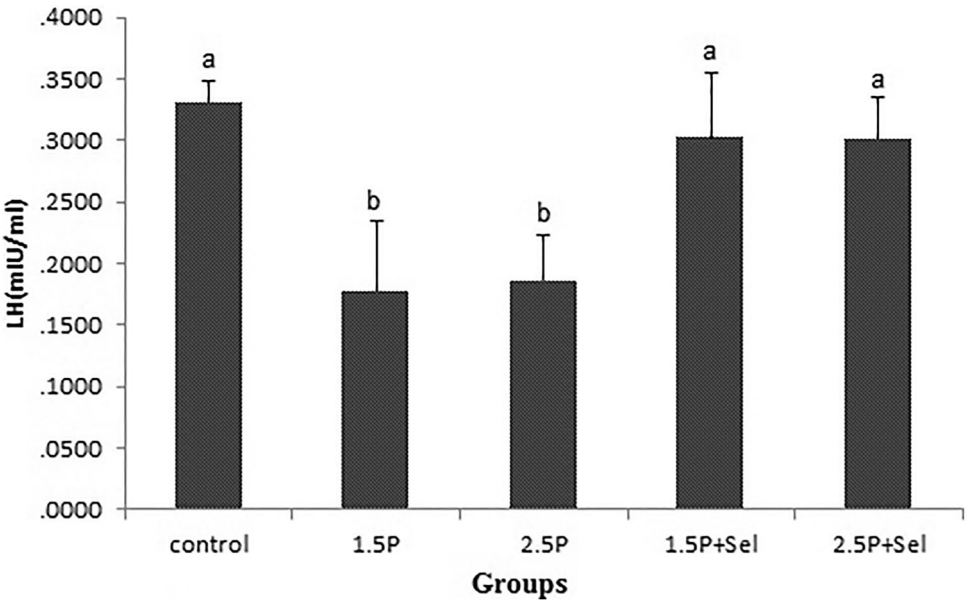

Fig. 3 The rate of LH hormone in different groups studied with prednisolone and sodium selenite. * That are not significantly different are assigned a common letter, and different letters indicate significant difference in expression ($P < 0.05$)

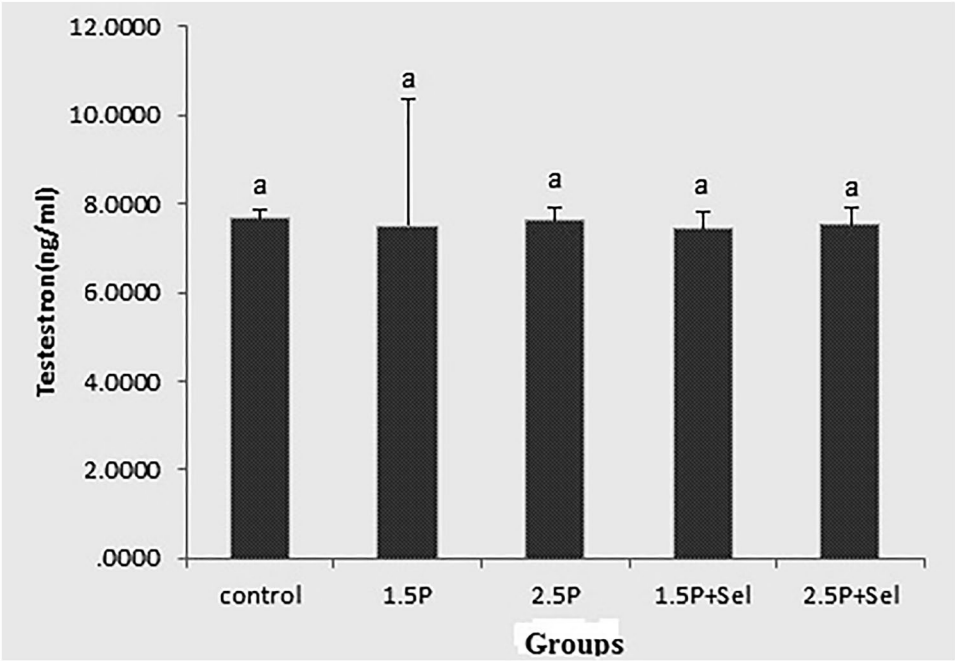

Fig. 4 Testosterone levels in different study groups treated with prednisolone and sodium selenite. * That are not significantly different are assigned a common letter and different letters indicate significant difference in expression ($P < 0.05$)

Table 4 Diameter of seminiferous tubule in different groups treated with prednisolone and sodium selenite

Groups	Diameter of seminiferous tubule (μm)
Control	221.1 ± 7.5
1.5P	180.5 ± 7.8
2.5P	157.4 ± 19.9*
1.5P + sel	183.1 ± 14.1
2.5P + sel	198.4 ± 17.1

* Indicates a significant difference in the specified group with other groups ($P < 0.05$)

(Fukushima et al. 2005; Hammerstedt 1993). Other studies have shown that ROS is produced from two different sources of sperm fluid called damaged spermatozoa and activated white blood cells, the high levels of which by damaging DNA, decreasing the percentage of live spermatozoa and lack of attaching sperm to the egg surface, leads to infertility in men (Hawkes and Turek 2001; Sharma and Agarwal 1996). Studies by Kobayashi et al. (2001) showed that there is a continuous decrease in the number of live and active sperm cells associated with increased amount of ROS.

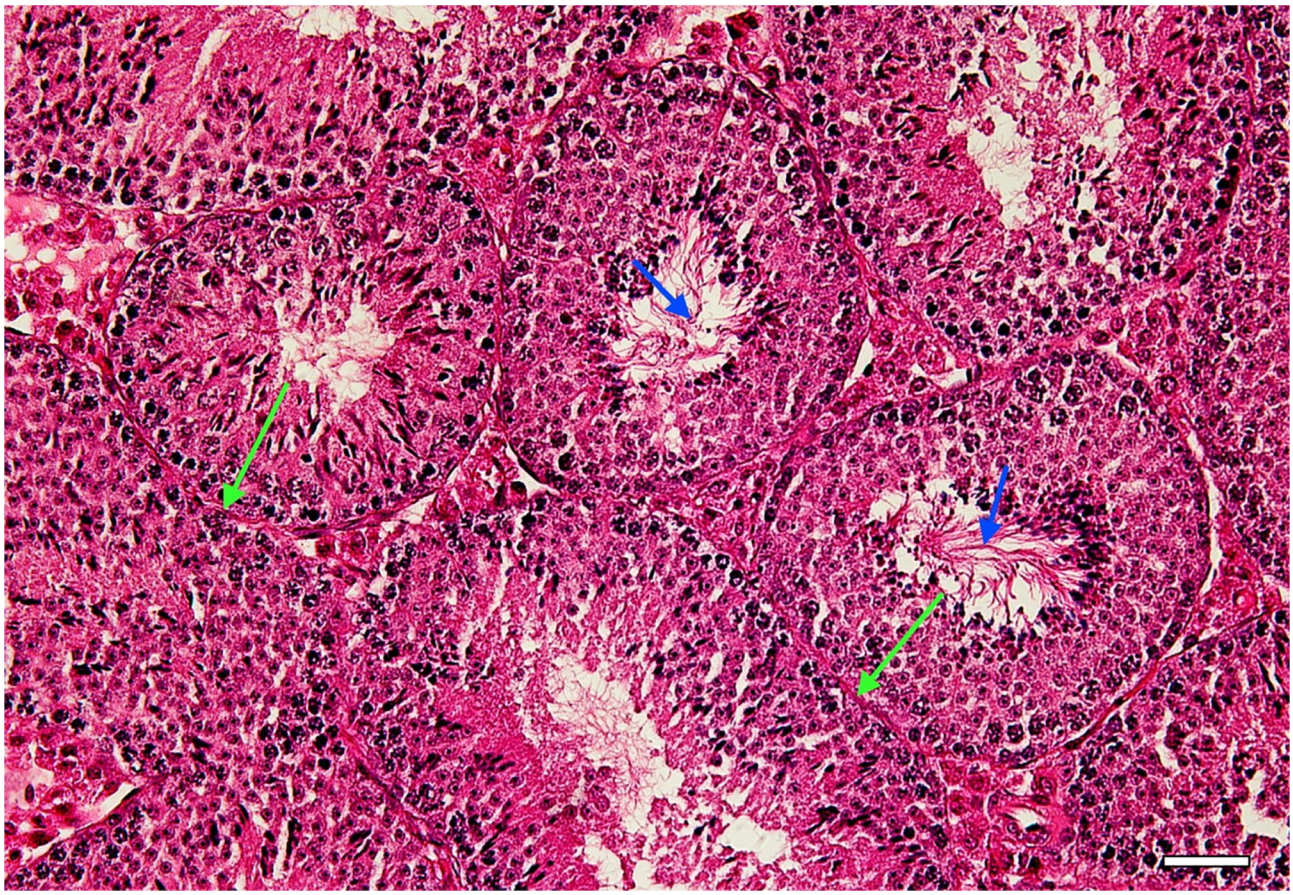

Fig. 5 Histological section of the mice testis in control group. The testicular tissues is normal in appearance with plenty of sperms (blue arrows); also indicates cells of normal spermatogonia stacked in several layers on seminiferous tubules (green arrows). H&E stain, bar = 5 µm

According to Colborn et al. (1993) endocrine disruptive chemicals (EDCs) by producing free radicals are capable of causing oxidative damage to biological molecules such as DNA and protein. Prednisolone is likely to cause free radicals and mutations in testicular tissue, especially spermatogonial cells, primary spermatocytes, spermatids, and spermatozoids, which result in serious injury and destruction of these cells (Choi et al. 2004; Kristen et al. 2009). In this study, on the other hand, as prednisolone and sodium selenite did not alter testosterone, but the interstitial connective tissue is decreased, there is a likelihood of reduction of germ cells. For this reason, it is also possible to damage and destroy interstitial and Sertoli cells in this way. Also, the function of interstitial cells is affected by Sertoli cells (Dennis and Kempton 2010; George et al. 1989). Selenium is one of the components of antioxidant enzymes, including glutathione peroxidase, which plays a significant role in neutralizing free radicals and relieving oxidative stress.

In the present study, the role of selenium as a protector of testicular tissue as well as a factor to improve testicular function against the deleterious effects of prednisolone was quite evident. As an important micronutrient, Se has many essential roles at the cellular and organismal levels in animal and human health and relevancy to various patho-physiological conditions. The effect of this element on spermatogenesis and sperm parameters was also well documented, and in confirming this study Brown and Burk (1973) also showed that selenium is essential for spermatogenesis (Rayman 2012; Flohe 2007).

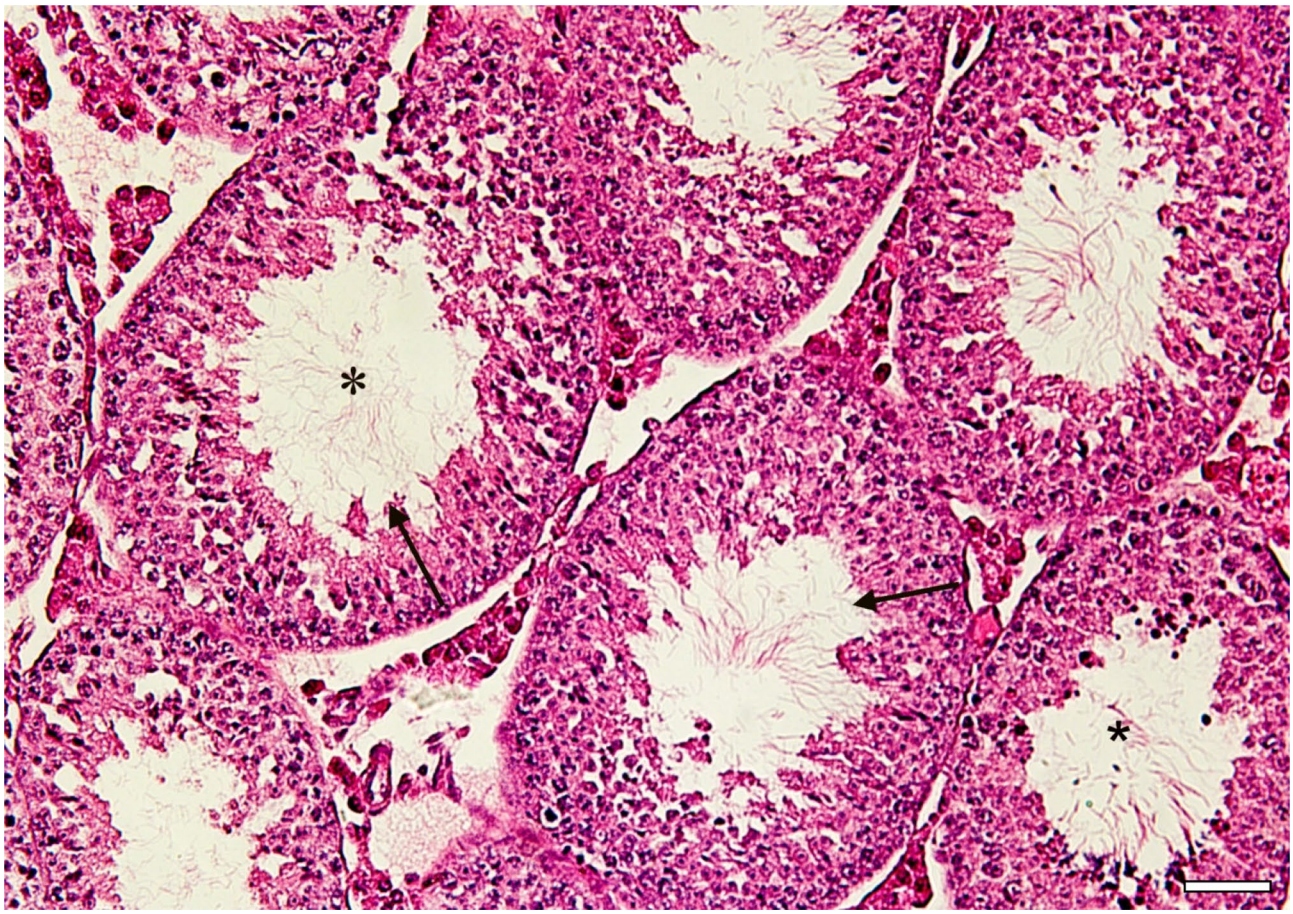

Fig. 6 Histological section of the mice testis in treated group (prednisolone 1.5 mg/kg). Epithelium composed of several layers but reduced compared to the normal group (black arrow). There are very few spermatozoid accumulations in the seminiferous tubules (*). H&E stain × 400. Bar = 5 µm

In the present study, we found that the diameter of testicular seminal tubes decreased in prednisolone-treated groups and, also, in this regard, the amount of connective tissue has decreased. According to Iwasaki et al. (1991), consumption of immunosuppressive drugs in transplanted patients in addition to nephrotoxic and hepatotoxic effects, causes destructive and atrophic changes in seminal tubes, reduces sperm count and motility in sperm and malignant sperm, and as a result leads in male infertility, which confirms our study.

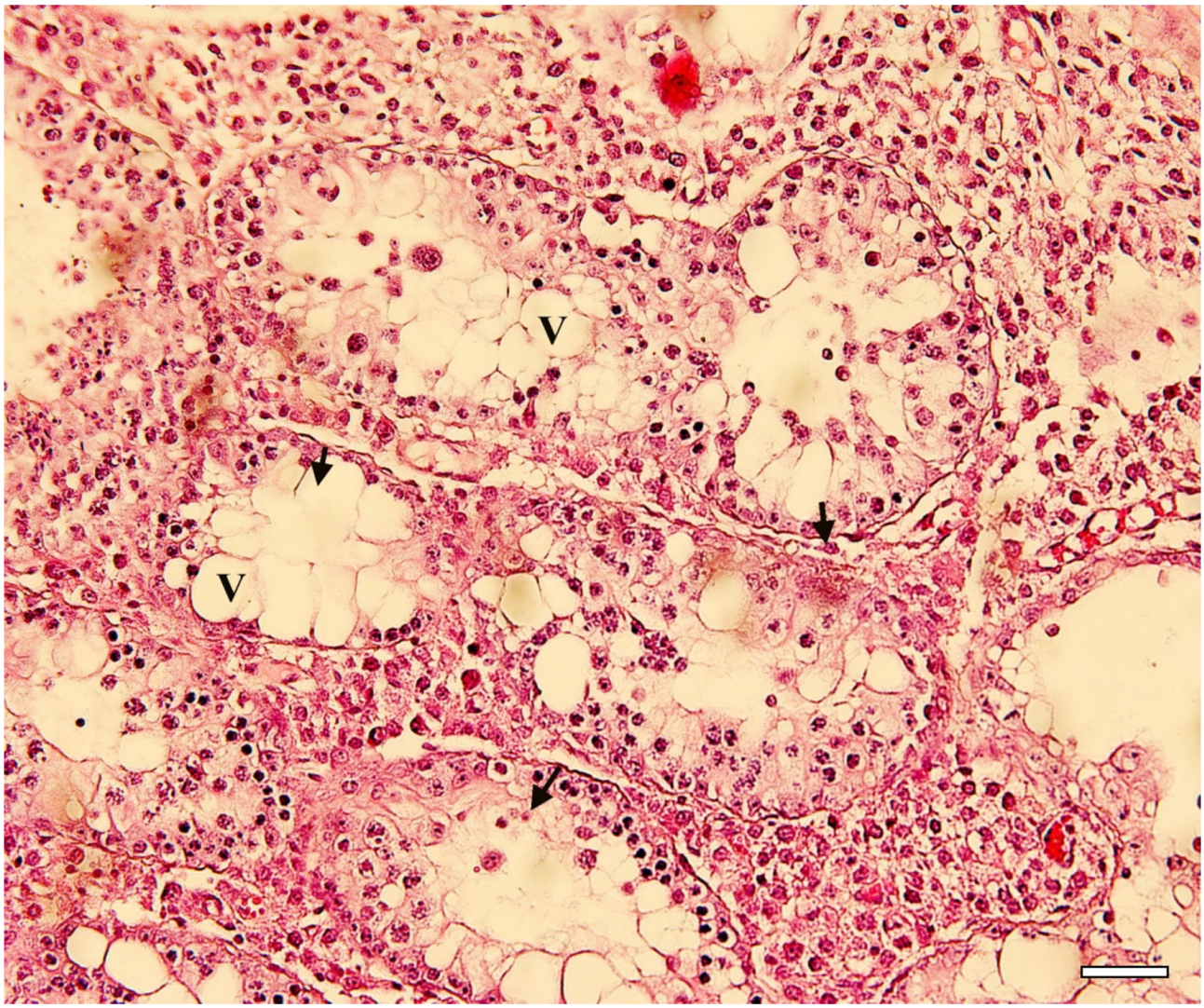

Fig. 7 Histological section of the mice testis in treated group (prednisolone 2.5 mg/kg). The number of layers of epithelium is greatly reduced (black arrow) and vacuolated (V). Spermatozoa are not seen in the seminiferous tubules. H&E stain × 400, bar = 5 μm

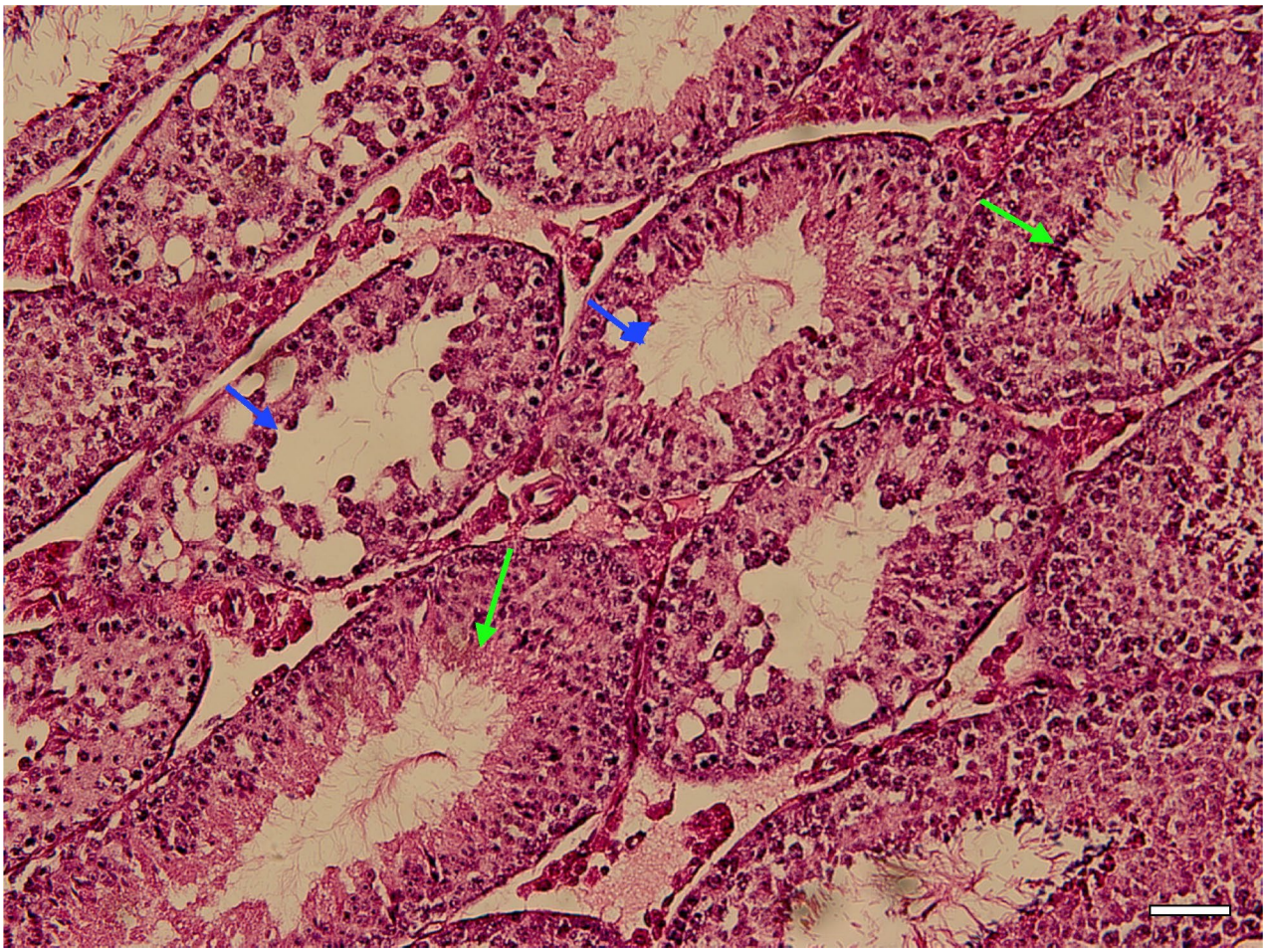

Fig. 8 Histological section of the mice testis in treated group (prednisolone 2.5 mg/kg + 0.2 mg/kg selenium). Although the number of layers in the previous group was reduced and vacuolated (blue arrows) but with the injection of selenium, the layers of epithelium have been repaired and increased (green arrows). H&E stain, bar = 5 μm

Conclusion

In conclusion, the present study has demonstrated that selenium is one of the most essential elements in testicular tissue and essentially required for spermatogenesis and male fertility. Also, it can act as a protective barrier in preventing the deleterious effects of corticosteroids.

Funding The authors acknowledge the University Grants Commission of Ferdowsi University of Mashhad (FUM) for providing financial assistance for the research. This study was supported by the Ferdowsi University of Mashhad (FUM), (Project Number: 45443).

Declarations

Ethical approval The guidelines for the care and use of the animals were strictly followed in accordance with the Ethics and Regulations guiding the use of research animals as approved by the Ferdowsi University of Mashhad, Mashhad, Iran (2/45443–15/11/2017).

Conflict of interest The authors declare no competing interests.

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