Investigating the Effects of Shirazi Thyme Extract on Plasma Levels of Testosterone, Dihydrotestosterone, LH and FSH in Adult Male Wistar Rats: An Experimental Study

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Abstract

Thymus vulgaris contains components that are chemically very different. Some of these components have properties as anticonvulsant, antifungal, antioxidant, anti-inflammatory, anti-infection, anti-diabetics and act as a stimulant to central nervous system, stimulating the production of testosterone hydroxylase. The present study aimed at investigating the effect of intraperitoneal injection of hydroalcoholic extracts of Shirazi Thyme on plasma levels of testosterone, dihydrotestosterone, LH and FSH in adult male Wistar rats, conducting histological studies on testis in this regard. In this study, 48 male Wistar rats, weighing about 190-210 gr were randomly divided into 6 groups (n=8). Control group received nothing. Sham group intraperitoneally received 2/0 distilled water daily. For 28 consecutive days, the experimental groups 1, 2, 3 and 4 intraperitoneally received Shirazi Thymus extract with daily doses of 100, 200, 300 and 400 mg/ kg of body weight. At the end of the day 28, blood samples were taken from the heart. The samples were then collected to measure the hormones LH, FSH, testosterone and dihydrotestosterone. The results were assessed using the tests Anova, Tukey and Duncan. After blood sampling, the testes were removed and studied histologically. The mean body weight, testes weight, and plasma concentrations of FSH and dihydrotestosterone in different experimental groups did not reveal any significant differences compared to the control group. Moreover, the histological changes, with respect to the size, the interstitial space, and tubules structure, were not detected in the seminiferous tubules. In terms of size, density, cytoplasm and nucleus features, the histological changes in spermatogonia, primary spermatocytes, Sertoli cells and Leydig were not recognized in the experimental group compared to the control group. However, the mean plasma concentrations of LH and testosterone in the fourth experimental group taking 400 mg/kg/day showed a significant increase in comparison to the control group. The histological changes in the seminiferous tubules were significant in terms of their number, density of tubules, and spermatozoids density in all experimental groups compared to control group which was also much more detected in more cases while increasing the dose. The use of Shirazi thyme leads to an increase in testosterone, influencing LH concentration through its effect on sympathetic nerves and consequently increasing the number of receptors on Leydig cells. Through inducing P450 (cholesterol desmolase enzyme), Cineole existed in Shirazi Thymus vulgaris extract causes increasing the conversion of cholesterol to pregnenolone in the mitochondria which in turn triggers the increase of synthesis of steroids, including testosterone.

Keywords: *Shirazi Thymus vulgaris, testosterone, DHT, LH, FSH, testis, male rats*

Introduction

Medicinal Plants are of the great value and importance in maintaining the communities health in terms of treatment and prevention of diseases. This is why medicinal plants have drawn researchers' attention to find an appropriate alternative for synthetic drugs. Shirazi thymus vulgaris (Zataria multiflora) belongs to mint family (Lamiaceae) growing in different areas of the Mediterrane and Asia (Jamzadeh, 1994). Lamiaceae family has 49 genera in Iran. The extensive cultivation of plants in this family is due to easy reproduction and cultivation, culinary

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use, medicinal, spice and ornamental use (Belyaev, 1999). So far, more than 60 compounds have been identified in thyme oil, the most important of which is a phenolic compound called thymol (Omidbeigi, 2008), with some other compounds including carvacrol, a variety of hydrocarbons monoterpene like terpinene, *p*-cymene, monoterpene alcohols such as linalool and α -terpinen (Leung, 1996). The identified acids in thyme are as rosmarinic, palmitic, stearic, ursolic , caffeic, and caprinic (Duke, 1981; Lemhadri, 2004). Thyme oil contains thymol, cineole, carvacrol, borneol, β-bisabolene, limonene, camphene and minerals including potassium, magnesium, manganese, zinc, copper, iron and tannins such as tannic and vitamins including niacin (nicotinic acid) and β -carotene (Fabio et al., 2003; Soylu et al., 2006).

Formally, from the 16th century so far, Thyme oil is known as germicidal and its anti-microbial effect is due to thymol and carvacrol. This has been greatly used as mouthwash, gargle solutions, toothpastes, soaps, cleaners and disinfectants used for medical products. It is recommended in the treatment of whooping cough, tuberculosis, and bronchitis used at a dose of 3.0 to 6.0 ml (Leung et al., 1996; Zeytinoglu et al., 2003). Thyme reduces aging process and reinforces longevity and health. The dried leaves of this plant affects on body weight, food intake, serum cholesterol concentration in adult women decreasing the chance of the genitourinary system infection after childbirth (postpartum) (Amrik et al. 2004; Tantaoui, 1994). This plant is a very suitable drug for respiratory system and diseases such as the flu, fever, asthma, and dyspnoea (Elgayyar et al., 2001). Considering the medicinal importance of Shirazi thyme, and inadequacy of studies on the effect of the plant on the plasma levels of LH, FSH, testosterone and dihydrotestosterone, the present study has been carried out to evaluate the effects of hydroalcoholic extract of Shirazi thyme on plasma levels of these hormones in male Wistar rats.

Materials and Methods

The method of preparing a hydroalcoholic extract of Shirazi Thyme

Shirazi Thyme was collected from mountains in Arsanjan during spring and dried in the shade and air. After drying and cleaning the plant, it was powdered in order to be used for extraction. 1000 ml of 50% hydroalcoholic was added for each 100 grams of powdered thyme, and was kept for 72 hours at a lab temperature in percolator (made in Iran). After 72 hours, the extract drops were collected from percolator. At the same time, the hydro alcoholic solution was added through a funnel at the top and funnel so that the extract would not have the same color as the plant.

The extract obtained was concentrated by a rotary concentrator (made in Switzerland). To dry the concentrated extract, it was placed in a vacuum desiccator (made in England) for 24 hours (Shariat, 1992). 21 gr dried crystal extract was obtained from each 100 gr of plant.

The obtained dry extract was kept in dark containers in refrigerator. To provide the hydro alcoholic extract at doses 100, 200, 300 and 400 mg/ kg, the sufficient amount of hydro-alcoholic solution 50% was added.

Animals under study and the techniques used for their maintaing

Animals used in this study, were male Wistar rats weighing about 190-210 gr and aged 2/5-3 month, obtained from Razi Institute (Karaj). They were transferred to breeding colonies in Islamic Azad University of Kazerun. The rats were maintained at constant temperature (2 ± 25 ° C) on a 12-hour light/dark cycle, providing them with water and food with no restriction. Animal cages were made of polycarbonate with perforated stainless steel roofs. In order to be adopted to experiment environment, the use of extract doses to animals began one week after their placing in laboratory housing.

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Animals grouping

The total number of rats in the test was 48 divided into 6 groups (n=8) as follows: Control group received nothing. Sham group intraperitoneally received 2/0 distilled water daily. For 28 consecutive days, the experimental groups 1, 2, 3 and 4 intraperitoneally received Shirazi Thymus extract with daily doses of 100, 200, 300 and 400 mg/ kg of body weight. It should be noted that all experimental groups received 0.2 ml extract daily.

Blood samples

At the end of the day 28 after weighting, the rats were anesthetized with ether, and blood samples were taken from the heart, then centrifuged at 3000 rpm for 5 minutes. Serum was separated by Pasteur pipette and stored at -20 ° C. till to measure hormones. Hormones measurement was conducted in the usual laboratory way as radioimmunoassay (RIA) and the amount of hormones LH, FSH, testosterone and dihydrotestosterone were measured.

Removal of the testes

After blood sampling, both testes of mice were removed, weighed, and the tissue sections were prepared and stained employing the haematoxylin and eosin staining of histological studies with light microscope.

Methods of Statistical analysis

The results were examined using SPSS software and statistical tests including ANOVA (Tukey, Sheffe, Duncan) with significant difference at $p \leq 0.05$. The factors in consideration were reported using the diagrams drawn by Excell program.

Results

Examining the percentage of body weight changes in the control and sham groups revealed that there was no statistically significant difference between these two groups. In addition, measuring the percentage of body weight changes in the experimental groups in comparison with the control group showed an increase in the body weight only in the experimental group 4 (dose at 400 mg / kg / day) but the percentage of these changes compared to the control group did not demonstrate any significant difference ($P \leq 0.05$) (Diagram 1).

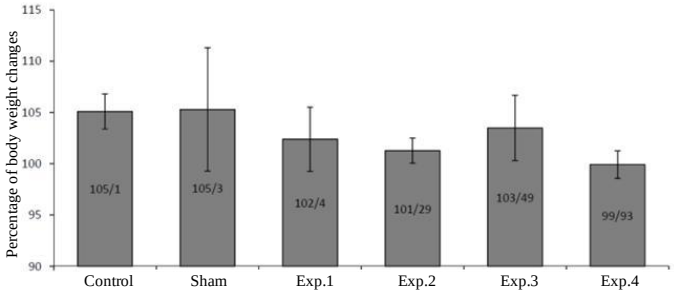

Diagram 1. Comparison of percentage of body weight changes at the end of the experiment in the experimental groups receiving different doses of the extract compared to the control group

Investigating the effect of different doses of Shirazi Thymus vulgaris extract on the weight of left and right testes in the control group and comparing it with the sham group showed no statistically significant difference. Moreover, comparing left and right testis weight in the experimental groups receiving different doses of the extract with that of the control group ($P\leq 0.05$) did not reveal any significant differences (Diagram 2).

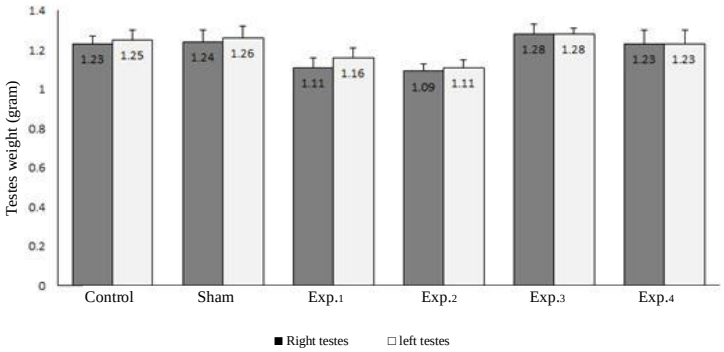

Diagram 2. Comparison of left and right testes weight in the experimental groups receiving different doses of the extract and comparing it with the control group at the end of the experiment

Investigating the effect of different doses of Thymus vulgaris extract on the plasma concentration of FSH in the control group and comparing it with plasma concentration of this hormone in the sham group did not show significant variations. In addition, comparing plasma concentrations of FSH in the experimental groups receiving different doses of the extract with plasma concentration of this hormone in the control group ($P\leq 0.05$) did not demonstrate significant differences (Diagram 3).

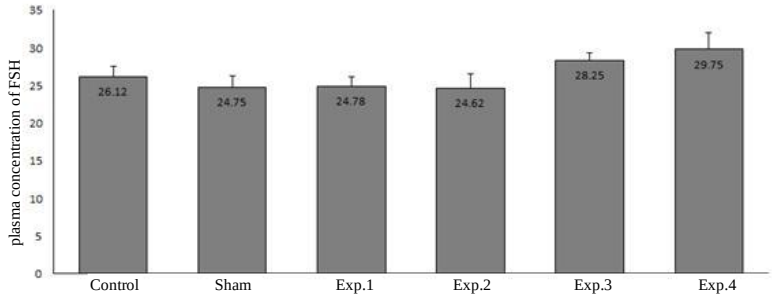

Diagram3. Comparison of the mean value of plasma concentration of FSH in the experimental groups receiving different doses of the extract compared to the control group at the end of the experimental

Studying the effect of different doses of Thymus vulgaris extract on plasma concentration of dihydrotestosterone in the control group and comparing it with the plasma concentration of this hormone in sham group. Also, the comparison of plasma concentration of dihydrotestosterone hormones in the experimental groups receiving different doses of extract with plasma concentration of this hormone in the control group ($P\leq 0.05$) did not reveal any significant differences (Diagram 4).

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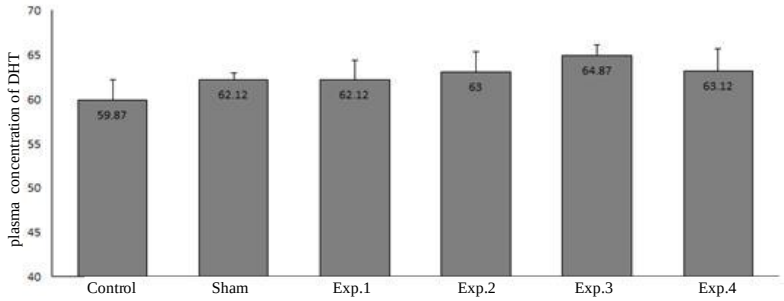

Diagram 4. Comparison of the mean value of plasma concentration of dihydrotestosterone in the experimental groups receiving different doses of the extract compared to the control group at the end of the experiment

Examining the effect of different doses of Thymus vulgaris extract on plasma concentrations of LH in the control group and comparing it with plasma concentration of this hormone in the sham group did not show any significant changes. However, the comparison of the plasma concentrations of LH in the experimental groups receiving different doses of the extract with plasma concentration of this hormone in the control group showed that plasma concentration of this hormone in the experimental groups was increased. As the results showed, this increase in the experimental group 4 (at dose 400 mg / kg / day) was significant (P≤0.05) (Diagram 5).

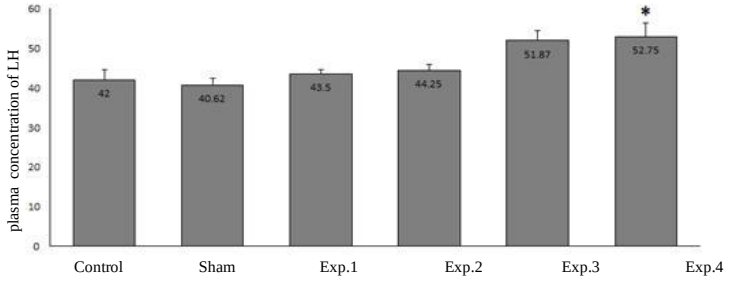

Diagram 5. Comparison of the mean value of plasma concentration of LH in the experimental groups receiving different doses of the extract compared to the control and sham groups at the end of the experiment
* indicates a significant difference (P≤0.05)

Investigating the impact of different doses of Shirazi Thymus vulgaris extract on plasma concentration of testosterone in the control group and comparing it with that of sham group did not show any significant changes. On the other hand, the comparison of plasma concentration of testosterone in the experimental groups receiving different doses of the extract with that of the control group revealed that this hormone was increased in the experimental group. This increase in the experimental group 4 (at dose 400 mg / kg / day) was significant (P≤0.05) (Diagram 6).

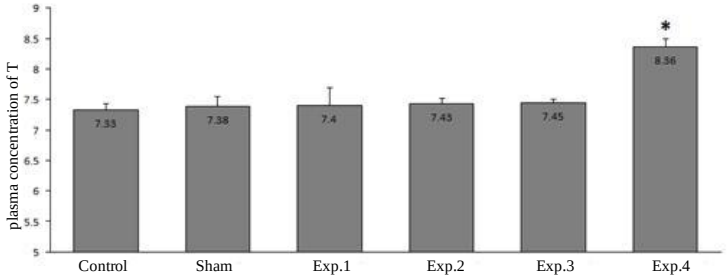

Diagram 6. Comparison of the mean value of plasma concentration of testosterone in the experimental groups receiving different doses of the extract compared to the control group at the end of the experiment
* indicates a significant difference (P≤0.05)

For microscopic studies of testis tissue, the slides of tissue samples taken at a magnification 4x, 10x and 40x, related to the control, sham, and experimental groups 1 to 4 were studied. In order to increase the confidence level, 10 slides were selected from each group and each slide from the control group was randomly compared to one of the slides in the sham group in terms of the spermatogonia cells , primary spermatocytes, sertoli and leydig. In the same way, then, the slides from each of the experimental groups were compared to that of the control group. Investigating the photomicrograph of seminiferous tubules in the sham group and comparing it with the control group, the following results were obtained:

1) In some slides, the number of seminiferous tubules in the sham group showed an increase compared to the control group. In some cases, it occurred in the left testis. This was associated with a decrease in tubular diameter with no change in the size of the interstitial space.
2) Staining of samples in the control group was less than staining of samples in the control group.

It can be stated that the histological changes in the seminiferous tubules in the sham group compared to the control group have not been conducted in terms of the number, the size of interstitial space and the structure of the seminiferous tubules.

Examining the photomicrograph of seminiferous tubules in the experimental groups and comparing it with that of the control group, the following results were obtained:

1) In some slides, the number of seminiferous tubules compared to the control group showed an increase while this event in the experimental groups 1 to 4 and with increasing the amount of extract intake occurred more often. This was associated with a decrease in the tubule diameter with no change in the size of the interstitial space.
2) The staining of samples in the control group was less than the staining of samples in the experimental group.
3) In some slides, the sperm density in lumen tubules compared to the control group demonstrated an increase which in the experimental groups 1 to 4 with increasing the amount of extract intake was observed in more cases.

Generally, it can be mentioned that the histological changes in the seminiferous tubules in the experimental group compared to the control group were performed in terms of numbers and was associated with an increase. However, this phenomenon was not observed in all slides. Moreover, the histological changes in the seminiferous tubules in the experimental group compared to the control group did not take place in terms of the size of interstitial space and the structure of the seminiferous tubules.

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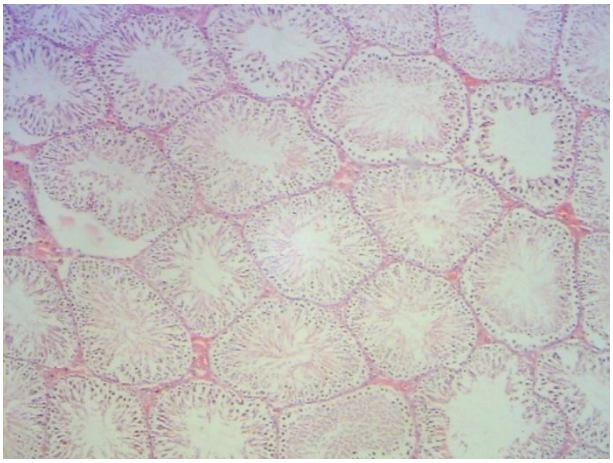

Figure 1. The photomicrograph of seminiferous tubules in the control group (4x)

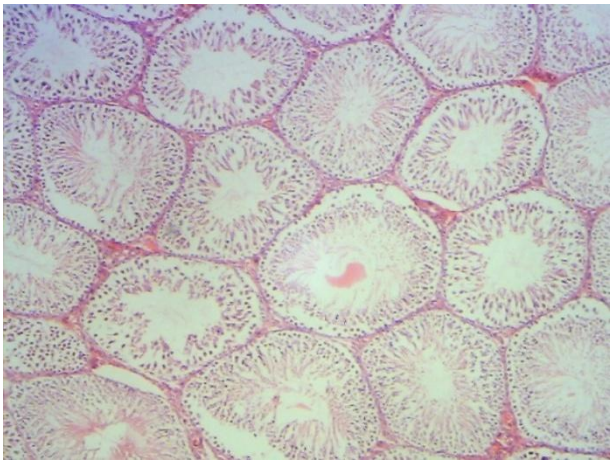

Figure 2. The photomicrograph of seminiferous tubules in the sham group (4x)

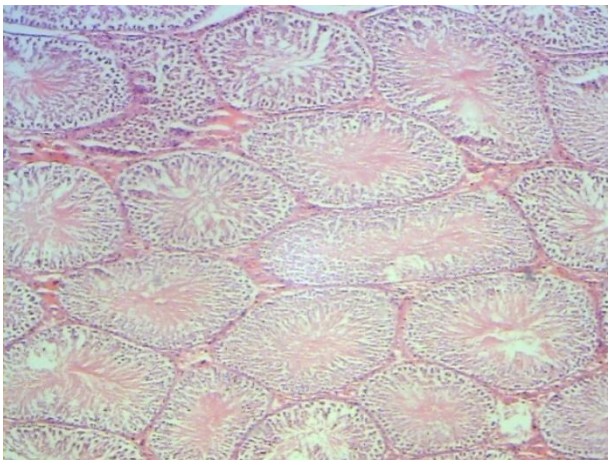

Figure 3. The photomicrograph of seminiferous tubules in the experimental group 1 (4x)

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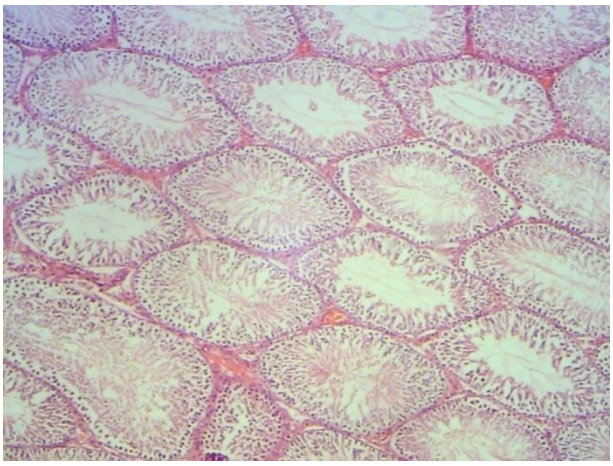

Figure 4. The photomicrograph of seminiferous tubules in the experimental group 2 (4x)

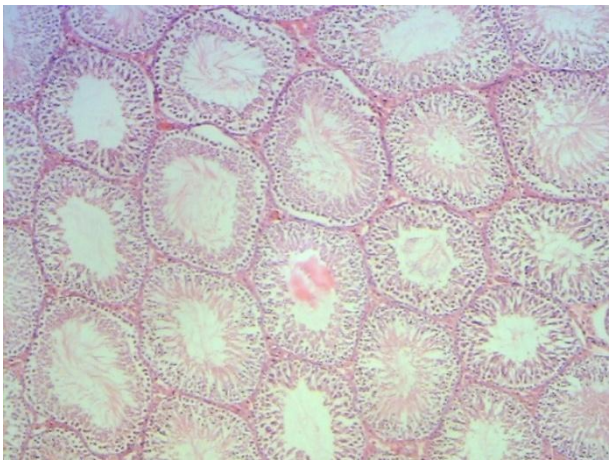

Figure 5. The photomicrograph of seminiferous tubules in the experimental group 3 (4x)

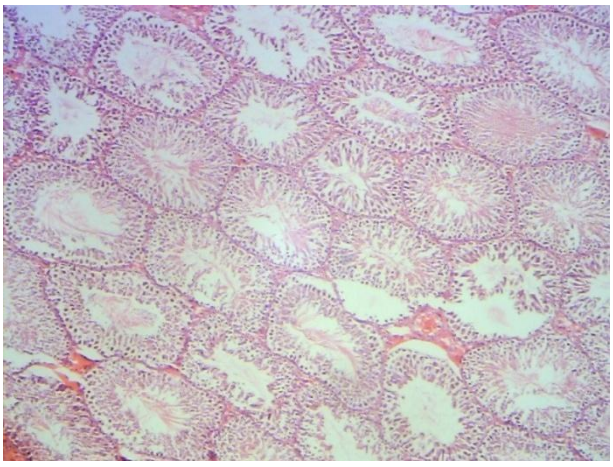

Figure 6. The photomicrograph of seminiferous tubules in the experimental group 4 (4x)

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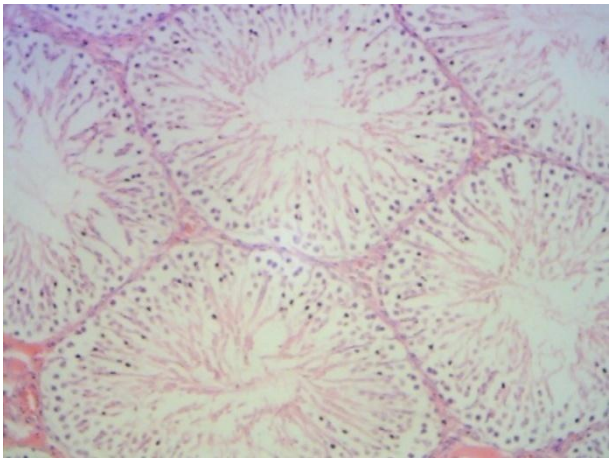

Figure 7. The photomicrograph of seminiferous tubules in the control group (10 x)

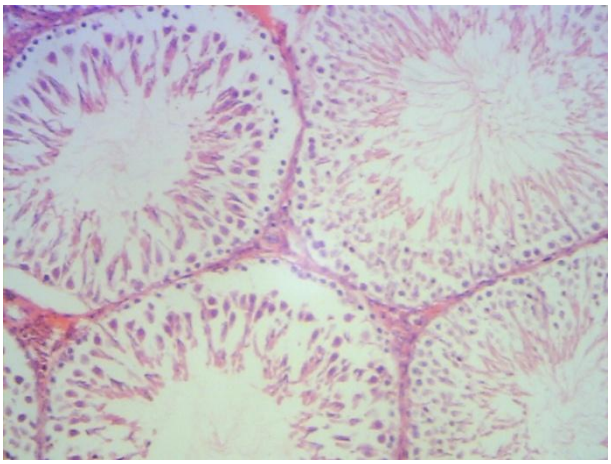

Figure 8. The photomicrograph of seminiferous tubules in the sham group (10 x)

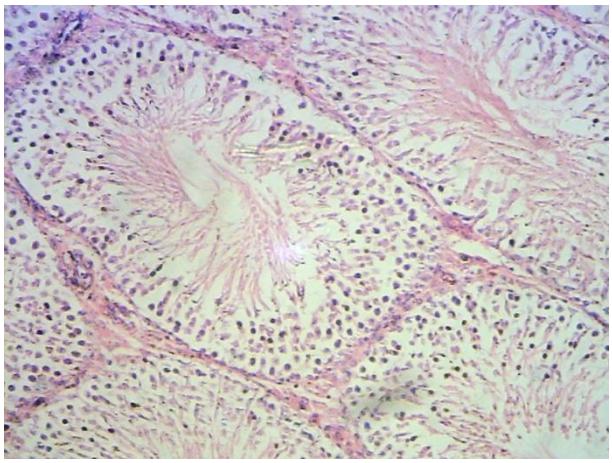

Figure 9. The photomicrograph of seminiferous tubules in the experimental group 1(10 x)

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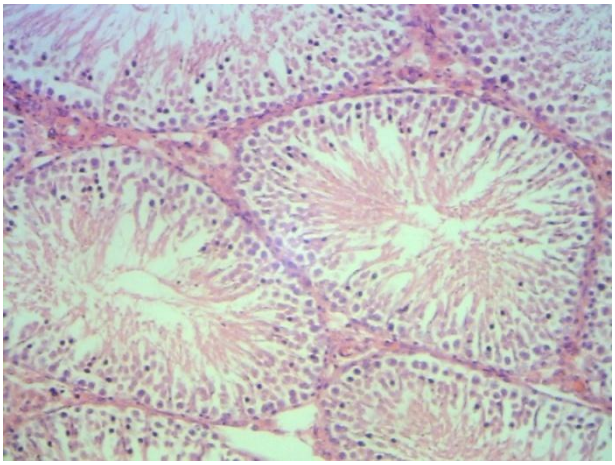

Figure10.The photomicrograph of seminiferous tubules in the experimental group 2 (10 x)

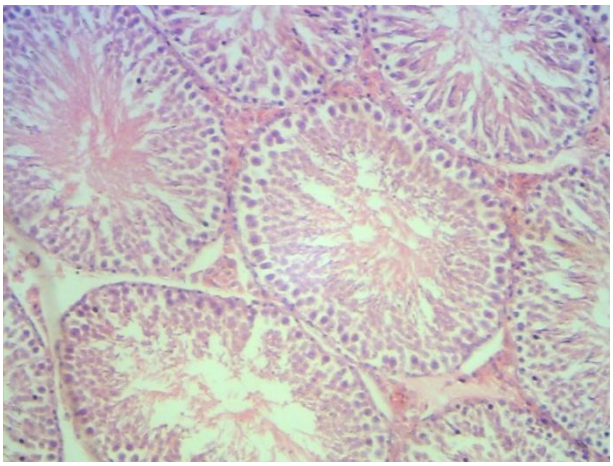

Figure 11. The photomicrograph of seminiferous tubules in the experimental group 3 (10 x)

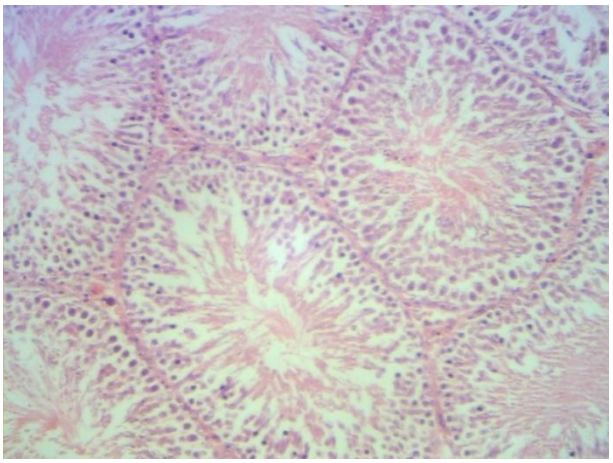

Figure 12. The photomicrograph of seminiferous tubules in the experimental group 4 (10 x)

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Examining the photomicrographs of cross sections of seminiferous tubules with a magnification of 40x in the sham group and comparing it with the control group, the following results were obtained:

1) The histological changes in spermatogonia cells, primary spermatocytes and Sertoli in the sham group compared to the control group were not performed in terms of size, density, the characteristics of cytoplasm and their nucleus.
2) The histological changes in the Leydig cells in the sham group compared to the control group were not carried out in terms of size, density, the characteristics of cytoplasm and their nucleus.
3) The staining of samples in the control group was less than the staining of samples in the sham group.

Investigating the photomicrographs of the cross sections of seminiferous tubules with a magnification of 40x in the experimental groups and comparing them with the control group, the following results were observed:

1) The histological changes in spermatogonia cells, primary spermatocytes and Sertoli in the experimental groups compared to the control group did not occur in terms of size, density, the characteristics of cytoplasm and their nucleus.
2) The histological changes in the Leydig cells in the experimental groups compared to the control group were not done in terms of size, density, the characteristics of cytoplasm and their nucleus.
3) As previously stated, in some slides, sperm density was increased in lumen tubule which was observed more often in the experimental groups 1 to 4 through increasing the amount of extract intake.
4) The staining of samples in the control group was less than the staining of samples in the experimental group.

Generally, it can be said that the histological changes in spermatogonia cells , primary spermatocytes, Sertoli and Leydig cells in the experimental group compared to the control group were not performed in terms of size, density, the characteristics of the cytoplasm and the nucleus. However, the density and the number of spermatozoids in the experimental groups 1 to 4 and with increasing the amount of extract intake showed an increase though this was not observed in all slides.

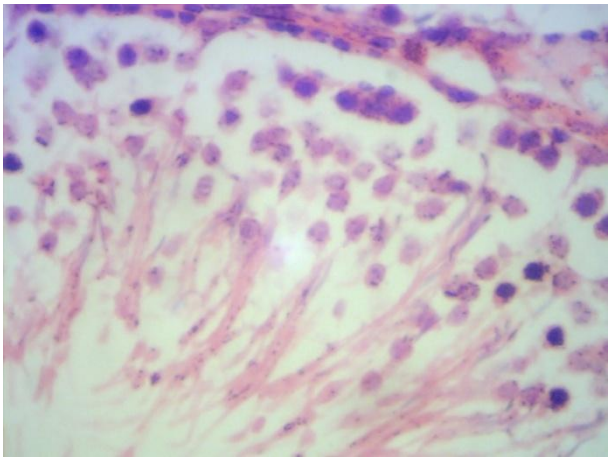

Figure 13. The photomicrograph of spermatogonia cells , primary spermatocytes, spermatit, and sertoli in the control group (40x)

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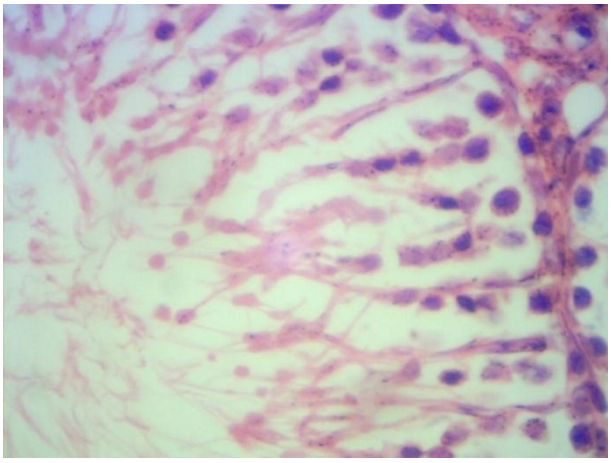

Figure 14. The photomicrograph of spermatogonia cells , primary spermatocytes, spermatit, and sertoli in the sham group (40x)

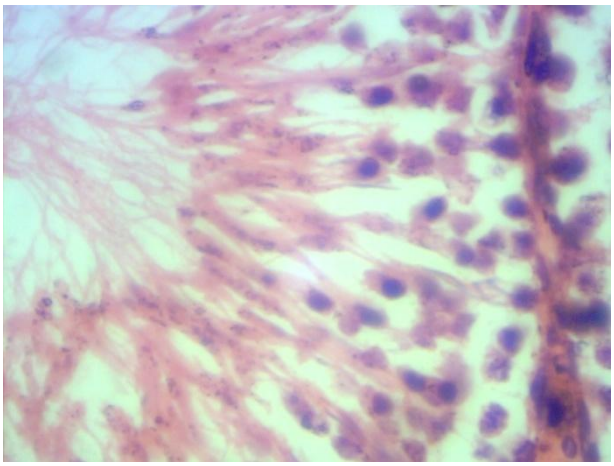

Figure 15. The photomicrograph of spermatogonia cells , primary spermatocytes, spermatit, and sertoli in the experimental group 1 (40x)

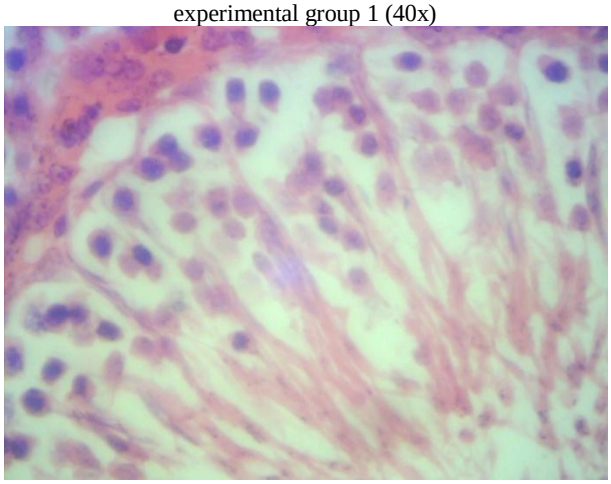

Figure 16. The photomicrograph of spermatogonia cells , primary spermatocytes, spermatit, and sertoli in the experimental group 2 (40x)

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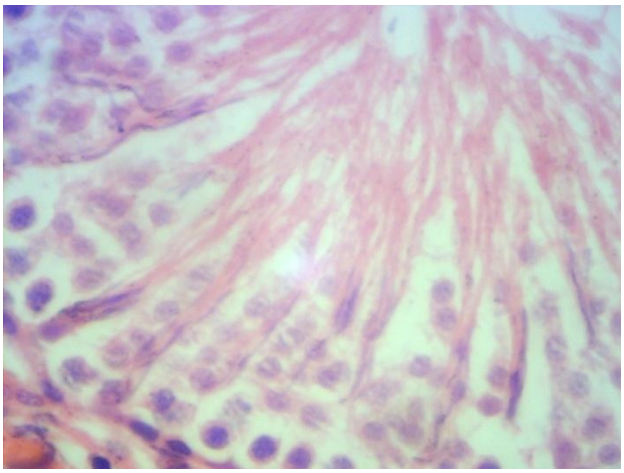

Figure 17. The photomicrograph of spermatogonia cells , primary spermatocytes, spermatit, and sertoli in the experimental group 3 (40x)

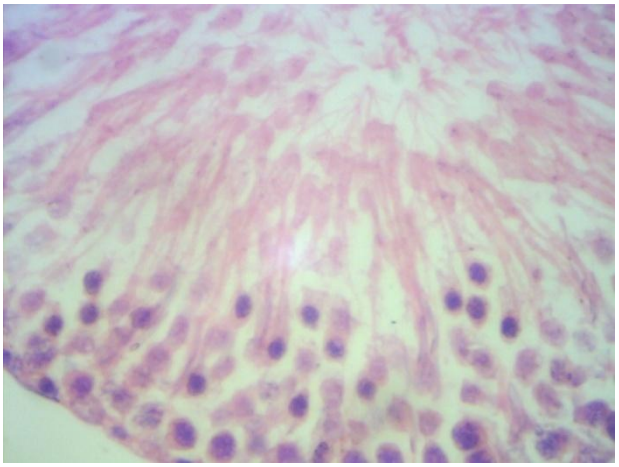

Figure 18. The photomicrograph of spermatogonia cells , primary spermatocytes, spermatit, and sertoli in the experimental group 4 (40x)

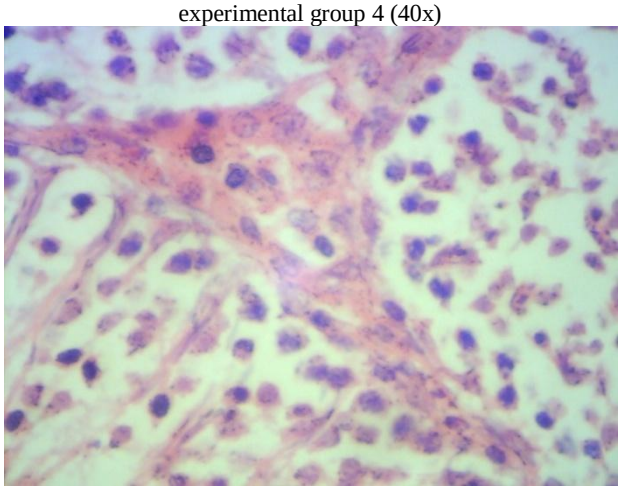

Figure 19. The photomicrograph of leydig cells in the control group (40x)

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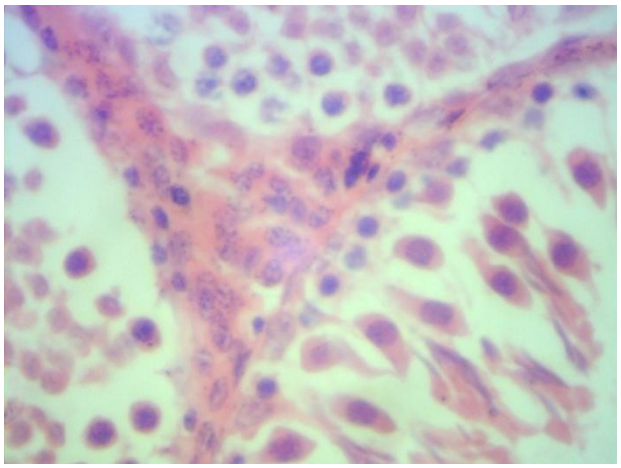

Figure 20. The photomicrograph of leydig cells in the sham group (40x)

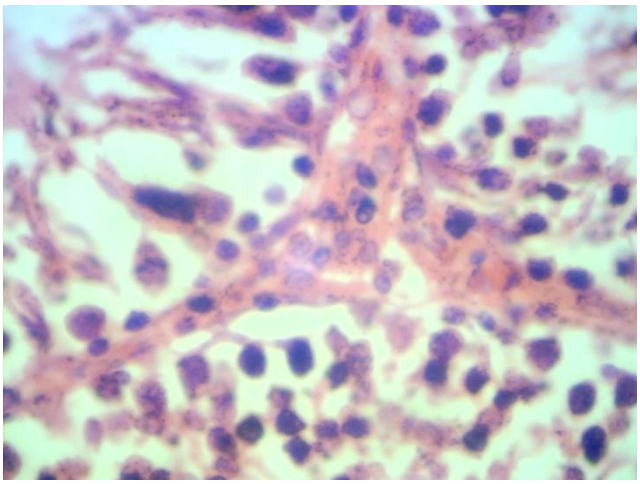

Figure 21. The photomicrograph of leydig cells in the experimental group 1 (40x)

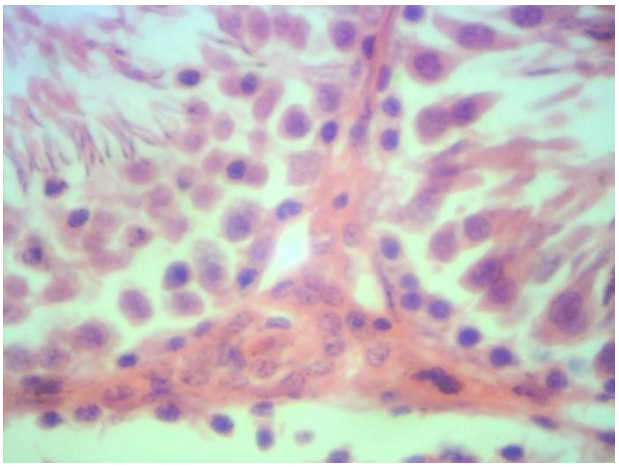

Figure 22. The photomicrograph of leydig cells in the experimental group 2 (40x)

[DOR: 20.1001.1.2717378.2017.1.1.6.2]

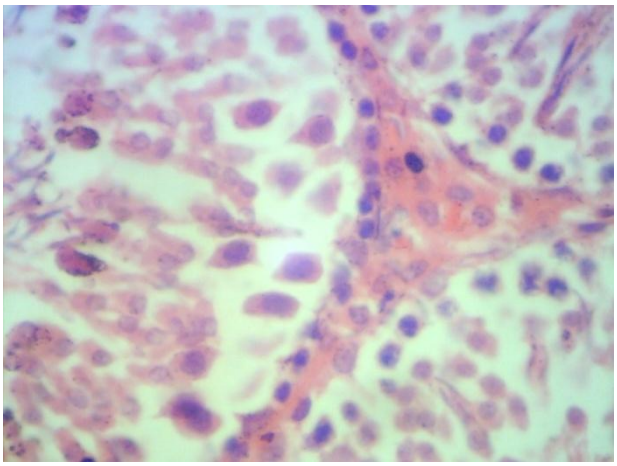

Figure 23. The photomicrograph of leydig cells in the experimental group 3 (40x)

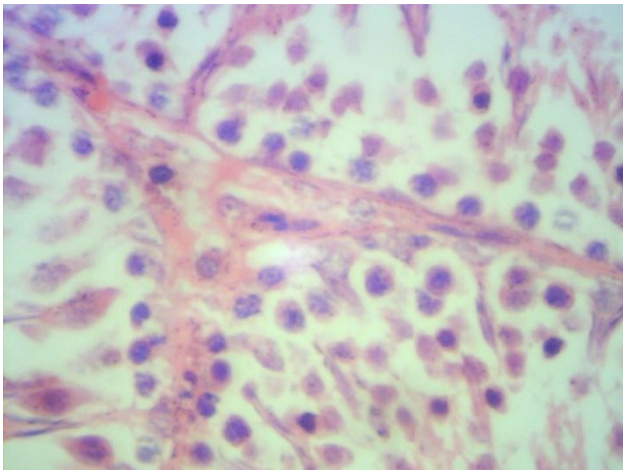

Figure 24. The photomicrograph of leydig cells in the experimental group 4 (40x)

Discussion and Conclusion

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on body weight

Despite the changes in the level of testosterone in different experimental groups, especially in group 4 with a significant increase, and also the effect of testosterone in increasing the volume and weight of muscle tissue through increasing protein synthesis, the absence of significant changes in the mean value of body weight in different experimental groups compared to the control group would be probably due to the short duration of the test and it might lead to changes in the body weight in longer periods of time.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on testes weight

The cineole in the extract by induction of hydroxylase enzyme of testosterone leads to an increase in the androgens (mainly testosterone) which in turn causes an increase in testis weight. However, there was not any significant difference in testes weight, probably due to the short period of experiment (Duke, 2006).

Examining the results related to the effect of the different doses of the Shirazi Thymus vulgaris extract on plasma concentration of LH

Studies show that the secretion of hormone LH is influenced by factors such as LHRH, potassium and serotonin. The direct involvement of serotonin receptors on LH secretion in rats has been approved (Amikishieva et al., 1996). On the other hand, the thyme reduces the number of androgen receptors in FSH cells. Thus probably, the negative feedback of testosterone on these cells is decreased and the secretion of LH is increased (Wang, 1997). Using LHRH antagonists and inhibiting the secretion of LH, Selvage et al. (2003) showed that Corticotropin-releasing factor (CRF) affected Leydig cell function without having an effect on pituitary. There has been a neural pathway between the brain and the testes in which its stimulation by the CRF results in the testosterone secretion (Selvage, 2003).

According to the above mentioned materials, probably, Shirazi thyme extract may influence the sympathetic nervous system, thus increasing the number of receptors on Leydig cells leads to increasing testosterone and affectin the LH concentration.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on plasma concentration of FSH

There is a negative feedback in the regulation of gonadotropin level so that testosterone performs its negative feedback through affecting the hypothalamus and anterior pituitary and accordingly gonadotropin level will bereduced (Amikishieva et al., 1996). On the other hand, thymus vulgaris decreases the number of androgen receptors in the hypothalamus and pituitary gonadotropin cells (Wang et al., 1997). Therefore, this extract probably prevents the inhibitory effect of testosterone on the androgen receptor of hypothalamus and pituitary, which subsequently FSH concentrations will be increased. In this study, the increase in FSH was higher in the experimental groups 3 and 4, though the increase was not significant at ($P \leq 0.05$).

Regarding FSH, the physical mechanism is not merely applied through testicular steroids, but inhibin, activin, and follistatin play a key role through their main effect on the production of GnRH in regulating FSH concentration. So, it is possible that the absence of significant changes in FSH would be due to the modulatory effects of these factors (Wang et al., 1997). On the other hand, Shirazi thyme could probably inhibit adrenergic receptors and prevent the production of inhibin by the sertoli cells, resulting in an increase in FSH. As mentioned before, in the present study the increase in FSH was not significant which could be attributed to the short duration of the experiment.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on plasma concentration of testosterone

The cineole in thymus vulgaris extract by induction of P450 (cholesterol desmolase enzyme) leads to increasing the conversion of cholesterol into pregnenolone in mitochondria leading to an increase in the synthesis of steroids, including testosterone (Ariza-Nieto, et al., 2005). Moreover, the cineole by induction of testosterone hydroxylase enzyme causes the increase of androgens. Some studies have shown that testosterone secretion from Leydig cells is usually regulated by primary control of pituitary LH and its construction in testis will be controlled

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and regulated after the activity of these cells through an independent pathway towards the pituitary gland (a path between the brain and testis). In addition, the survival of LH receptors in the gonads is controlled by the sympathetic nervous system (Meistrich et al., 1997). According to the results in this study, the use of Shirazi thymus vulgaris extract increased LH levels compared to the control group, thus an increase in concentrations of serum testosterone is expected as the result of an increase in LH.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on plasma concentration of dihydrotestosterone

Probably, thymus vulgaris extract did not have any effect on the enzyme 5 alpha reductase which converts testosterone to dihydrotestosterone.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on density and structure of spermatogonial cells

The research shows a direct correlation between the secretion of FSH and the number, proliferation, and the structure of spermatogonial cells (Courot, 1981; Guyton, 2006; kilgour et al., 1998). So probably the ineffectiveness of the extract on the number of spermatogonial cells is due to the ineffectiveness of the extract on plasma concentrations of FSH.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on density and structure of primary spermatocyt cells

Studies show that testosterone is effective in dividing spermatocyt cells (Meistrich et al., 1997). In addition, FSH has direct effects on the production of spermatocytes in leptotene and pachyten stages (Kilgour et al., 1998). It seems that despite the increase in testosterone in the experimental groups (and even a significant increase in the experimental group 4), due to the ineffectiveness of thyme extract on FSH level in these groups; ultimately it did not affect the density and the structure of primary spermatocytes cells.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on density and structure of spermatid cells

FSH causes the production of a number of growth factors and some other factors from Sertoli cells. These factors protect the sperm cells and spermatogenesis action and increase the number of spermatid cells in testis (George, et al. 1989). Due to ineffectiveness of the extract on FSH level and in spite of the testosterone increase in different experimental groups, no change has been expected in the density of spermatid cells in different experimental groups compared to control group.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on density and structure of sertoli cells

The research shows that an increase higher than the physiological level of FSH can increase the frequency of cell division, especially in Sertoli cells (Sharpe et al., 1990; Werbach, 1993). Through binding to its specific receptors on the Sertoli cells and stimulating the production of cAMP, FSH can activate protein kinase and phosphorylation of intracellular proteins, resulting in an increase in Androgen-binding protein *(*ABP*)* and an

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increase in the size of sertoli cells(Larsen et al., 2002; Walker, 2005). Moreover, through the mitogenic effects, FSH causes an increase in the activity of Sertoli cells (sharpe et al., 1990; Swanlund et al., 1995; werbach, 1993).

So in the present study, the reason for the absence of change in the density and the structure of Sertoli cells is a result of no significant change in plasma concentrations of FSH.

Sertoli cells are the most resistant cells during spermatogenesis so that the cells remain in an hypophysectomy of rats (Plant, 2001). Sertoli cells are the only asexual cells that are directly associated with the differentiation of spermatogonial stem cells (Holdcraft, 2004). The proliferation of Sertoli cells is performed in two stages, one in the embryonic stage and the other in the pre-pubertal stage (Walczak-Jedrzejowska et al., 2007). Sertoli cells do not proliferate during reproduction period. Moreover these cells are very resistant against undesirable conditions such as infections, malnutrition, and x-ray and their survival rate is better than the cells in spermatogenic category (Walker, 2003). Thus, due to the resistance of Sertoli cells and their non-proliferation in the reproductive period, no significant changes in the density of Sertoli cells in different experimental groups receiving Shirazi thyme extract compared to control group seems to be logical.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on density and structure of interstitial cells (Leydig)

Studies have shown that the function of interstitial cells is under the influence of Sertoli cells. Moreover, FSH regulates the function of the interstitial cells by Sertoli cells (Boron, 2003; George, 1981). In the present study, the use of thymus vulgaris did not demonstrate a significant effect on FSH and consequently on the density and the structure of Sertoli cells. Accordingly, it is expected that there has been no effect on the density and the structure of interstitial cells.

Examining the results related to the effect of the different doses of Shirazi Thymus vulgaris extract on sperm density level

The cineole in the extract of Thymus vulgaris by the induction of the hydroxylase testosterone enzyme increases androgens and in return it causes an increase in sperm concentration (Duke, 2006). Moreover, the cineole in thyme extract by the induction of cytochrome P450 (cholesterol desmolase enzyme) causes an increase in the conversion of cholesterol into pregnenolone in mitochondria, which in return increases the synthesis of steroids, including testosterone. The increase in the testosterone, therefore, causes an increase in the spherical spermatids of the middle stage of spermatogenesis, the mature spermatids, and the elongated spermatozoa; and thereby it increases the amount of sperm and the transition from spherical to elongated stage (Boron, 2003).

The research has shown that the testosterone causes the survival of spermatogenesis. Therefore, it can be concluded that increasing the density of sperm in the experimental group 4 has been due to an increase in testosterone, affected by Shirazi thyme extract.

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