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Research Article

The Effect of Thyme Honey on the Histopathological Changes of the Testis in Relation to the Side Effects of Valproic Acid in Adult Male Wistar Rats

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Introduction. Valproic acid (VPA) is a widely used drug in the treatment of emotional and nervous depression, mental imbalances, epilepsy, and control of bipolar disorders. Despite its therapeutic effects, VPA has toxic effects on male reproduction. The testis is an organ sensitive to oxidative stress. On the other hand, thyme honey contains antioxidant compounds. The aim of this study was to investigate the effect of thyme honey on the histopathological changes of the testis in relation to the side effects of VPA in adult male Wistar rats. *Methods*. In this study, 48 Wistar rats were used, which were randomly divided into equal eight groups and received their desired substances. Including the control group, Th1, Th2, and Th3 groups receiving 1, 2, and 3 g/kg of thyme honey, respectively, VPA (500 mg/kg), VPA + Th1 (500 mg/kg of VPA + 1 g/kg of thyme honey), VPA + Th2 (500 mg/kg of VPA + 2 g/kg of thyme honey), and VPA + Th3 (500 mg/kg of VPA + 3 g/kg of thyme honey). After 60 days, evaluation of malondialdehyde (MDA), total antioxidant capacity (TAC), and histological studies of hematoxylin-eosin and Masson's trichrome staining were performed. *Results*. The use of thyme honey significantly improved some parameters related to spermatogenesis, and in addition, it increased sperm production in the honey groups. In addition, the changes in MDA levels in the groups have shown a decrease in the amount of lipid peroxidation in the VPA + Th2 group. The amount of TAC also increased after increasing the dose of thyme honey. *Conclusion*. Based on the results of this study, it seems that thyme honey has been able to reduce the side effects of VPA to some extent due to its high percentage of antioxidants.

1. Introduction

Valproic acid (VPA), with the chemical formula $C_8H_{16}O_2$ and a half-life of 9–16 hr, is the drug of choice in generalized epilepsy. The mechanism of action of this drug is to compete for the binding sites of phenytoin (another drug used in epilepsy) with tryptophan to plasma proteins (albumin) and increase the entry of this amino acid into the brain tissue metabolism and decrease the abnormal electrical activities of the brain [1]. In addition, it reduces inhibition of the metabolism of other antiepileptic drugs. In fact, increasing gamma-aminobutyric acid in blood circulation is associated with increasing synapses. In high doses, valproate affects sodium channels but has little effect on calcium channels. The relationship between the valproate effect and antibody activity has not yet been determined [2]. The side effects of this drug are tremors and nausea, stomach pain, obesity, swelling in the legs, hair loss [3], the inefficiency of the reproductive system in men, fatal hepatotoxicity due to the production of toxic metabolites, and the induction of allergic immune reactions [4] and teratogenicity (increasing 20 times neural tube defects, cleft lip and palate, cardiovascular abnormalities, reproductive system defects, growth delay, endocrine disorders, organ defects, and autism) [5]. VPA reduces the level of luteinizing hormone, follicle-stimulating hormone, testosterone, reproductive parameters, and tissue changes

and causes tissue changes in the germinal layer of the epithelium of the seminiferous tubules [5, 6]. This drug may interact with several fertility factors, which can lead to sexual dysfunction and fertility in men with epilepsy. It has also been reported that VPA is associated with reduced sperm motility and an increased percentage of abnormal spermatozoa as well as low testicular size in epileptic men [7].

Therefore, due to the side effects of chemical drugs, today the tendency toward the use of herbal drugs has spread. Since ancient times, thyme honey is from the *Lamiaceae* family and famous in Greece for its special aroma and taste [8]. This honey is usually amber, but the colors change according to the dominant flowers used. Greek honey, most of which is made from wild thyme, has a darker amber color and is mostly red agate. Thyme is one of the most well-known complementary herbs from the mint family. This plant has a short and branched shrub with thin and opposite leaves. Thyme also has white (purple and red), umbellate, and solitary flowers. The essential oils in thyme include thymol and carvacrol. It is these essential oils that make the honey so fragrant and famous when the bee eats from this plant. Dark honey, such as thyme honey, contains more antioxidant compounds than other types, and among them, the abundance of phenolic compounds and flavonoids can be mentioned [9, 10]. Thyme honey contains vitamins B, A, and E, strengthens and improves all types of intestinal pain and discomfort, improves shortness of breath and asthma, improves cough and sore throat, improves joint pain, tonic for the brain and nerves, improves epilepsy and convulsions, improves headaches and migraines, reduced painful of menstruation. In addition, this honey is one of the few kinds of honey introduced to treat diabetes patients. In a study in Greece on thyme honey, after analyzing in total 14 compounds were identified as plant markers for thyme honey, 12 of which were phenolic compounds [10]. Thyme honey is a strong antibacterial and antioxidant compound that can play a significant role in reducing fat peroxidation. There are rare studies considering the effects of VPA on spermatogenesis and probably side effects on it. In this case, there are no remarkable studies about the protective role of natural antioxidants against the side effects of drugs. So the aim of this study was to investigate the effect of thyme honey on the histopathological changes of the testis in relation to the side effects of VPA in adult male Wistar rats.

2. Materials and Methods

The present experimental study was approved by the code of ethics IR.LUMS.REC.1399.094 in Lorestan University of Medical Sciences.

The samples used to induce the rats included distilled water, VPA, and thyme honey. VPA was purchased as tablets under the brand name Depakene or Depakote. Each tablet containing 500 mg of sodium valproate is dissolved in distilled water according to the dosage. The dose of VPA is 500 mg/kg daily for 14 days by gavage (po) [11]. According to the manufactured company notice, Ld50 oral of VPA in adult rat is reported 670 mg/kg. First, each tablet was made into a powder and dissolved in 2 mL of physiological serum, and after vortexing, it was transferred to a special gavage syringe. The honey sample was purchased from Meda Company, Iran, and was kept at 5°C.

2.1. Investigating the Antioxidant Properties of Thyme Honey. To evaluate the DPPH radical antioxidant capacity, 100 μL of the honey extract was mixed with 50 μL of folin–ciocalteu reagent and placed at room temperature for 5 min and then mixed with 750 μL of 6% sodium carbonate. After 40 min at room temperature, absorption during the wavelength of 725 nm was read. The read values were calculated after being placed in the standard formula of gallic acid [12].

2.2. Determination of TAC of Thyme Honey. To determine the total antioxidant capacity (TAC) of the thyme honey sample, 100 μg of the honey extract was combined with 100 μg of reagent (including 0.6-micron sulfuric acid, 28 millimicron sodium phosphate, 4 millimicron ammonium molybdate) and placed in a bain-marie for 90 min. After cooling, the absorbance of the samples was read at a wavelength of 695 nm at 15, 30, 60, and 90 min, and TAC was calculated by the formula. The solution of ascorbic acid and quercetin was used as a standard, and the reduction capacity was expressed as the percentage of inhibition equivalent to ascorbic acid and quercetin [13]. DPPH and TAC for thyme honey were calculated as 11.23 mg of gallic acid/g of honey and 1.108 nmol of ascorbic acid/g of honey, respectively. The total flavonoid capacity of thyme honey was calculated as equal to 7.38 mg of quercetin/g of honey.

2.3. Total Flavonoid Capacity on Thyme Honey. The amount of flavonoid was measured by the aluminum chloride colorimetric method. The amount of 500 μL of the honey extract was combined with 500 μL of 2% ALCL3 and after 1 hr it was placed at room temperature. Then the absorbance was read at 420 nm wavelength. The number of total flavonoids in honey was expressed as milligrams equivalent to quercetin. The standard curve was calculated based on the drawn quercetin concentrations and the reading values after being placed in the standard formula [14].

2.4. Animals and Experiment Design. Animals were purchased from the Razi Research Institute (Lorestan, Iran). After acclimatization of all groups to the environment (temperature 25 ± 2°C, relative humidity $50\% \pm 10\%$, light, and dark cycle) and determining the dose of honey and VPA, distilled water was injected into the animals. Forty-eight rats (250 ± 10 g) were randomly divided into eight groups ($N = 6$), group G1: healthy (receive normal saline 0.9%), group G2 (Th1): receiving thyme honey (1 g/kg), group G3 (Th2): receiving thyme honey (2 g/kg), group G4 (Th3): receiving thyme honey (3 g/kg), group G5 (VPA): receiving VPA, (500 mg/kg), group G6 (VPA + Th1): receiving thyme honey (1 g/kg) + VPA (500 mg/kg), G7 group (VPA + Th2) receiving thyme honey (2 g/kg) + VPA (500 mg/kg), group G8 (VPA + Th3): receiving thyme honey (3 g/kg) + VPA (500 mg/kg).

The G1 group received 1 mL of distilled water daily by gavage. Th1, Th2, and Th3 groups received thyme honey in the first 28 days, daily as a solution by gavage. The VPA

group receives valproate in the form of a solution, with a dose of 500 mg/kg in the first 14 days. The VPA + Th1, VPA + Th2, and VPA + Th3 groups also received thyme honey and VPA at the same time, based on the determined doses (VPA was given 1 hr after honey). After 60 days, the rats underwent surgery with a vertical incision in the abdominal area by inducing anesthesia with inhaled chloroform. The right testis was added to Bouin's solution. The microtubes containing the left testicle tissue were placed at −80°C. The left testicle sample was used to perform malondialdehyde (MDA) and TAC tests, and the right testicle sample was used to prepare testicular tissue slides.

2.5. Measurement of Malondialdehyde and Total Antioxidant Capacity. MDA and TAC tests were performed to determine the amount of lipid peroxidation following the oxidative stress reaction and to determine the TAC of the testis after treatment [15]. To determine TAC, about 50–100 mg of tissue sample was weighed and 10 times its weight (500–1,000 μL of lysing buffer) was added. Then it was centrifuged at 10,000 rpm for 10 min and the supernatant was separated and used as a sample. To determine MDA, 300 μL of lysis buffer and 3 μL of BHT100x were added to 10 μg of tissue or 10^7 sperm cells and centrifuged for 3 min at 13,000 rpm. It was centrifuged. Then, the report was calculated based on the kit protocol and the standard formula of MDA and TAC.

2.6. Histomorphometry Testis. In order to count the cells of spermatogenesis (type A spermatogonia, type B spermatogonia, primary spermatocyte, spermatids, and spermatozoa), in all groups, three slides were prepared from each testis and the sections were stained with hematoxylin-eosin (H&E) using light microscopy (Olympus Co., Germany).

The number of active sertoli cells (each of the developing spermatozoa accumulations in the seminiferous tubule pits was considered as an active sertoli cell) in three seminiferous tubules in each tissue sample was counted and the average number of leydig cells in the area of one square millimeter of testicular tissue was reported. For microscopic examination, two types of slides with H&E and Masson's trichrome staining were prepared from testicular tissue samples. The tissue of the testis and epididymis was examined for any edema, hyperemia, and disruption of cellular order in a toxic tube, and the epididymis for fibrosis and loose connective tissue, and for the presence of sperm inside the tube [16].

2.7. Evaluation of Sperm Characteristics and Spermatogenesis in Testicular Tissue. To count sperms that collected from epididymis, a 1 : 20 dilution of sperms was prepared and counted. To measure the percentage of sperm motility, 10 μL of the culture medium containing the desired sperm were evaluated. In order to evaluate the viability of sperm, the staining eosin-nigrosin used for this purpose dissolves 20 μL of the sperm sample on a slide with 20 μL of eosin solution and after 20–30 s, 20 μL of nigrosin-colored solution is added and after preparing the smear, the percentage of live sperms (colorless) and dead sperms (stained) was examined. For this purpose, the number of 100 seminiferous tubules in each testis was evaluated to evaluate the indices of tubular differentiation index (TDI), spermiogenesis index (SI), and regeneration index [17].

2.8. Tubular Differentiation Index %, Spermiogenesis Index, Edema, and Hyperemia. To determine the SI spermiogenesis coefficient (SPI), the average number of seminiferous tubules containing sperm was calculated. To calculate the TDI, the average percentage of seminiferous tubules containing three or more types of spermatogenic cells differentiated from type A spermatogonia cells was calculated. To calculate the recovery factor, the average number of active spermatogonial cells to inactive spermatogonia cells was checked [17].

2.9. Statistical Analysis. Data were analyzed with the SPSS statistical package (24.0 Version). Results are expressed as mean, standard deviation, or 95% confidence interval (95% CI). The Shapiro–Wilk test was used to analyze the normal distribution of the variables ($p > 0.05$). Quantitative data with a normal distribution (spermatogony A, spermatogony B, RI, spermatozoa, leydig, sertoli cell, viability, and dead spermatozoa) were analyzed with parametric tests (one-way analysis of variance). The Tukey test was used for post hoc analyses. The statistical analysis was conducted at 95% CI. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Evaluation of the Effect of Thyme Honey on Histological Changes and Histomorphometry of Testis. In the group that received VPA, edema occurred in the interstitial tissue of most of the samples, and the germinal epithelium in most of the seminiferous tubules had many ruptures. In a number of seminiferous tubules, a considerable distance was observed among most of the cells of the spermatogenesis series. This state of rupture among spermatogenic cells was commonly observed in the samples of this group (Figure 1). TDI of seminiferous tubules is negative. Some tubules are undergoing destruction and cell shedding; however active seminiferous tubules are visible. In the group receiving 1, 2, and 3 g/kg of thyme honey, relatively few spermatozoa were observed in the seminiferous tubules, and the testicular tissue had normal histology. In the groups receiving VPA + 1, 2, and 3 g/kg honey, with increasing the dosage of thyme honey, an improvement was observed in the seminiferous tubules, as in the VPA + Th3 group, the testis tissue and the cellular order of seminiferous tubules are almost normal and close to the control group, on the other hand, the volume of cells is smaller than the other two groups. In the control group, it was shown that there was no edematous state in the interstitial tissue, the germinal epithelium was continuous and the cell arrangement was regular.

The average number of spermatogonia showed a significant decrease ($p < 0.05$) in the group receiving VPA compared to other groups except the group receiving VPA and 1 g of honey. On the other hand, despite the decrease in the number of spermatogonia in the group receiving VPA (Table 1), there is no significant difference between the group receiving VPA and 1 g of honey. The average distribution of active sertoli cells in the wall of the seminiferous

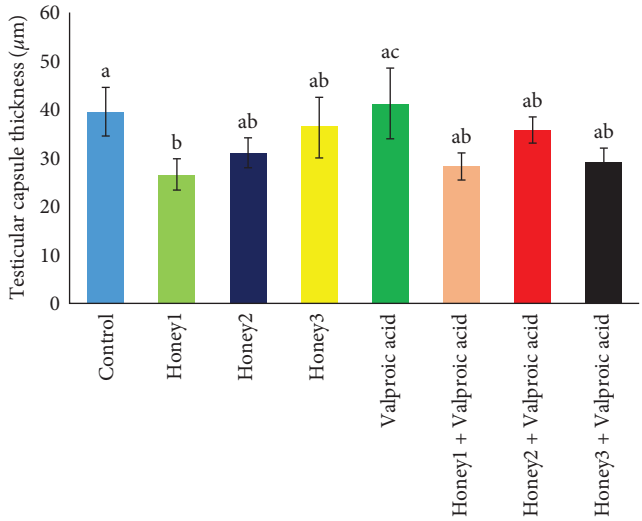


FIGURE 1: Comparison of capsule thickness in the studied groups: measurement of this parameter showed an increases in the VPA group, whereas in groups control and VPA + Th3, the thickness of the testicular capsule is approximately equal. Values with different superscript characters ($^{a,b,\ ab,ac}$) indicate significant difference between the control and treatment groups. Groups with the same superscript letter do not differ significantly ($p < 0.05$).

tubules of the testicular tissue in the groups receiving VPA has decreased significantly compared to other groups, but despite the decreasing trend in the number of sertoli cells in the groups receiving VPA and thyme honey compared to the control group and the groups receiving honey, there is no significant difference between these groups. The average dispersion of leydig cells in the interstitial tissue of the testis in rats receiving VPA compared to other groups was significantly reduced and the number of leydig cells decreased in the groups receiving VPA (Table 1) and thyme honey compared to the control group and the control groups. There is a recipient of honey, and this difference is significant (Table 1).

The TDI is the percentage of seminiferous tubules that contain three or more differentiated layers of germinal cells derived from type A spermatogonia. To detect the RI, the ratio of active (type B) to inactive (type A) spermatogonia was recorded and SI was expressed as the ratio of the number of seminiferous tubules with spermatozoids to empty tubules. Examining the tubular differentiation coefficient in the groups revealed that the tubular differentiation coefficient in the group receiving VPA (82%) showed a significant decrease ($p < 0.05$) compared to other groups and the control group. In addition, the groups receiving VPA and thyme honey had a decreasing trend compared to the groups that only received thyme honey. Changes in the cumulative coefficient of active spermatogonia cells from inactive spermatogonia cells based on morphology, the ratio of RI in the group receiving VPA is lower and there is a significant difference between the control group and the groups receiving 2 g of thyme honey and thyme honey and VPA ($p < 0.05$). However, despite the reduction of the RI coefficient in the group receiving VPA, there is no significant difference between the groups receiving thyme honey and 1, 3 g of honey (Tables 2 and 3). Based on the results of examining the SPI in the experimental groups, the average SPI in the group receiving VPA had a significant difference compared to other groups ($p < 0.05$).

3.2. Histopathology of Testicular Tissue. Group control: the testicular tissue is seen with normal histology, routine staining, and normal capsule thickness. The tissue is free from any kind of edematous and hyperemic. Seminiferous tubules are visible with normal cellular makeup and mostly positive TDI. Group Th1: testicular tissue with normal histology is the same as the control group, routine staining, capsule thickness is normal, and with few visible inflammation, however, TDI of most tubes is positive and spermatogenesis active tubes are visible. Group Th2: testicular tissue with normal histology is the same as the control group, routine staining, and normal capsule thickness. A positive TDI is seen in most tubes. Small amounts of edema are observed in some areas next to the testicular capsule. Group Th3: testicular tissue with normal histology is the same as the control group, routine staining, and normal capsule thickness. A positive TDI is seen in most tubes. Small amounts of edema are observed in some areas next to the testicular capsule. Group VPA: disturbance of the cellular order of the vas deferens along with edema and hyperemia are seen. TDI of seminiferous tubules is negative. Some tubules are in the process of destruction and cell loss; however, active spermatogenesis tubules are visible. Group VPA + Th1: The cell order of the seminiferous tubules is normal. TDI of seminiferous tubules is negative compared to the control group. Tubules have a smaller cell volume. Group VPA + Th2: the testicular tissue is almost normal and close to the control group, and the TDI of most tubes is positive and contains sperm. Hyperemia is not observed in testicular tissue. Group VPA + Th3: tubes have a smaller cell volume. A little hyperemia and edema are visible; however, the cellular order of the seminiferous tubules is normal, the testicular tissue is almost normal and close to the control group, and active spermatogenesis tubules are visible (Figure 1).

3.3. Sperm Characteristics. The evaluation of sperm motility, viability, and dead spermatozoa showed that honey consumption significantly increased the percentage of motility and viability while decreasing % dead spermatozoa ($p < 0.05$) (Table 4).

Based on the results obtained by measuring the thickness of the capsule in the studied groups, it was found that the capsule thickness was the highest in the G5 group that received VPA, while in the groups that received 1 and 2 g of thyme honey was the lowest amount (Figure 1).

3.4. The Results of Macroscopic and Microscopic Studies. During the drug treatment period, the rats did not have any changes in their skin. However, in terms of weight, especially in the groups that received thyme honey alone, an increase in weight was observed in the range of 20–30 g. Based on histological studies (Figure 2), based on H&E and Masson's trichrome staining, it was determined that group 1: testicular tissue with normal histology, routine staining, and normal

Table 1: Evaluation of the effect of thyme honey on histological changes and histomorphometry of testis.

	Spermatogonia A	Spermatogonia B	Spermatozoa	Leydig	Sertoli cell
Control	91.6 ± 6.363^{a}	128 ± 7.527^{a}	270.5 ± 17.914^{a}	29.44 ± 2.297^{a}	95.2 ± 4.022^{a}
Th1	91.5 ± 7.412^{a}	127.7 ± 6.864^{a}	268.2 ± 17.592^{a}	28.77 ± 2.5385^{a}	95.4 ± 5.621^{a}
Th2	90.6 ± 8.435^{a}	126.4 ± 7.876^{a}	249.9 ± 18.823^{b}	28.66 ± 2.5980^{a}	93.5 ± 2.798^{a}
Th3	87.8 ± 7.969^{ab}	121.6 ± 7.676^{a}	245.6 ± 19.597^{b}	25.88 ± 2.147^{b}	92.2 ± 4.917^{a}
VPA	83.1 ± 7.936^{b}	96.09 ± 10.261^{b}	166.7 ± 15.333^{c}	23 ± 2.598^{c}	87.3 ± 5.735^{b}
VPA + Th1	82.87 ± 8.096^{b}	97.75 ± 10.347^{b}	210.3 ± 19.550^{d}	26.37 ± 2.825^{b}	93.44 ± 4.824^{a}
VPA + Th2	88.4 ± 8.474^{ab}	114.66 ± 10.816^{c}	200.7 ± 18.938^{de}	26 ± 2.598^{b}	93.7 ± 4.808^{a}
VPA + Th3	92.1 ± 7.130^{a}	111.6 ± 12.790^{c}	187.6 ± 18.880^{e}	25.22 ± 2.728^{bc}	92.5 ± 4.648^{a}
p-Value	0.033	<0.0001	<0.0001	<0.0001	0.012

Note: In the VPA group, edema in the interstitial tissue and ruptures in the germinal epithelium in most of the seminiferous tubules occurred. In the thyme honey groups, testicular tissue had normal histology. In the VPA + Th3 group, the testis tissue and the cellular order of seminiferous tubules are almost normal. [a,b,c,d,e,ab,ac,bc,de]Values with different superscript characters indicate significant difference between the control and treatment groups. Groups with the same superscript letter do not differ significantly.

Table 2: Evaluation of the effect of thyme honey on histological changes and histomorphometry of testis on TDI %, edema, and hyperemia.

	TDI %	Edema	Hyperemia
Control	98.5	−	−
Th1	95	++	+
Th2	95	+	−
Th3	98	+	−
VPA	82	+++	++
VPA + Th1	88	+	−
VPA + Th2	93	+	−
VPA + Th3	90	++	++
p-Value	<0.0001		

Note: TDI in the group VPA showed a significant decrease compared to other groups and the control group. In addition, the groups receiving VPA and thyme honey had a decreasing trend compared to the groups that only received thyme honey.

Table 3: Comparison of the testicular RI in different groups.

Group	SI	RI
Control	50	1.387 ± 0.112^{a}
Th1	47.61	1.404 ± 0.142^{a}
Th2	45	1.401 ± 0.102^{a}
Th3	44.39	1.395 ± 0.154^{a}
VPA	11.11	1.157 ± 0.158^{b}
VPA + Th1	13.5	1.184 ± 0.135^{b}
VPA + Th2	22	1.310 ± 0.128^{a}
VPA + Th3	23.4	1.209 ± 0.096^{b}
p-Value	<0.0001	<0.0001

Note: [a,b]SI and RI in the groups VPA and VPA + Th1 had a significant difference (reduction) compared to other groups.

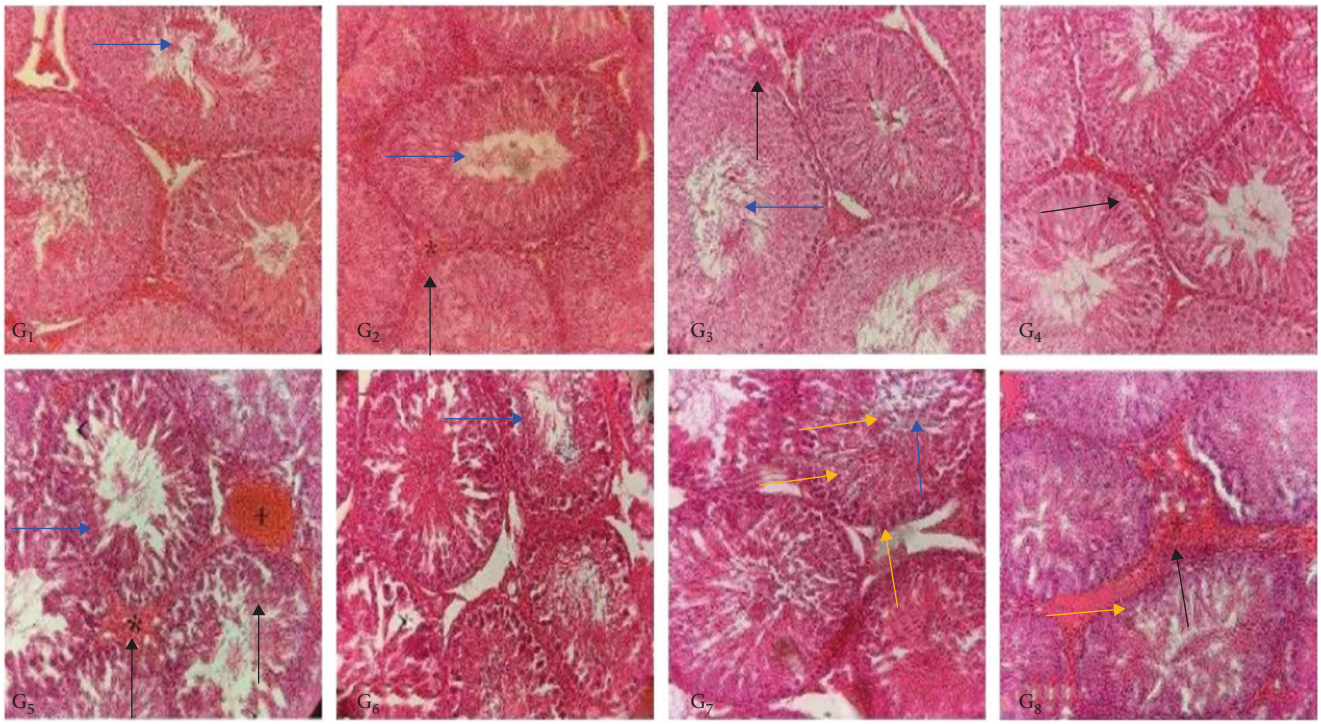
capsule thickness. The tissue is free of any edematous tissue. Seminiferous tubule with normal cell arrangement and mostly positive TDI can be seen. Group Th1: testicular tissue with normal histology such as the control group, routine staining, normal capsule thickness, and visible with little amass, however, TDI of most of the tubes was positive, and the active tubes of spermatogenesis can be seen. Group Th2: testicular tissue with normal histology is the same as the control group, routine staining, and normal capsule thickness can be seen. A positive TDI is seen in most tubes. Small amounts of edema are observed in some areas next to the testicular capsule. Group Th3: testicular tissue with normal histology is the same as the control group, routine staining, and normal capsule thickness. A positive TDI is seen in most tubes. In some areas next to the testicular capsule, small amounts of edema are observed. Group VPA: disturbance of the cellular order of the seminiferous tubule is seen along with inflammation. TDI of seminiferous tubules is negative. Some tubules are in the process of destruction and cell loss; however, active spermatogenesis tubules are visible. Group VPA + Th1: the cell order of the seminiferous tubules is normal. TDI of seminiferous tubules is negative compared to the control group. Tubules have a smaller cell volume. Group VPA + Th2: the testicular tissue is almost normal and close to the control group, the TDI of most tubes is positive and all three cell lines are visible. Group VPA + Th3: tubes have a smaller cell volume. A little inflammation is visible, however, the cellular order of the seminiferous tubules is normal, the testicular tissue is almost normal and close to the control group, and active seminiferous tubules and all three cell lines are visible.

3.5. The Results of the Score Histology. In the histopathological examination of the score, it was found that the A—control group has no edematous tissue and the TDI of most of the tubes is positive. B—In the group Th1, a few inflammation can be seen, however, the TDI of most of the tubes is positive, and active spermatogenesis tubes are visible. C—In the group Th2, positive TDI was seen in most of the tubes along with slight edema in some areas next to the testicular capsule. D—In the group Th3, positive TDI was seen in most of the tubes. It was seen with slight edema in some areas next to the testicular capsule. E—group VPA, TDI of seminiferous tubules was negative. Some tubules are in the process of destruction and cell shedding, and active tubules of spermatogenesis are visible. F—In the group VPA + Th1, the cell order of seminiferous tubules was normal. TDI of seminiferous tubules is negative compared to the control group. The tubes had less cell volume. G—In the

Table 4: Effect of thyme honey and VPA on epididymal spermatozoa.

Parameter (%)	Group								*p*-Value
	Control	Th1	Th2	Th3	VPA	VPA + Th1	VPA + Th2	VPA + Th3	
Viability	70.081 ± 1.57^{a}	78.94 ± 6.1^{a}	75.59 ± 3.78^{a}	80.46 ± 3.97^{a}	55.07 ± 1.13^{b}	69.98 ± 5.04^{a}	73.19 ± 3.10^{a}	70.89 ± 1.21^{a}	<0.0001
Dead spermatozoa	29.92 ± 1.57^{b}	21.06 ± 6.1^{b}	24.41 ± 3.78^{b}	19.54 ± 3.97^{b}	44.93 ± 1.13^{a}	30.02 ± 5.04^{b}	26.81 ± 3.10^{b}	29.11 ± 1.21^{b}	<0.0001
Motility	62.29 ± 4.04^{ab}	73.32 ± 4.84^{a}	75.37 ± 7.22^{a}	72.44 ± 10.75^{a}	45.00 ± 10.26^{b}	59.32 ± 15.58^{ab}	64.68 ± 10.68^{a}	69.42 ± 8.94^{a}	<0.0001

Note: Thyme honey consumption in contrast to the VPA significantly increased the percentage of sperm parameters (motility and viability) and decreased dead spermatozoa. ^{a,b,ab}Values with different superscript characters indicate significant difference between the control and treatment groups. Groups with the same superscript letter do not differ significantly.



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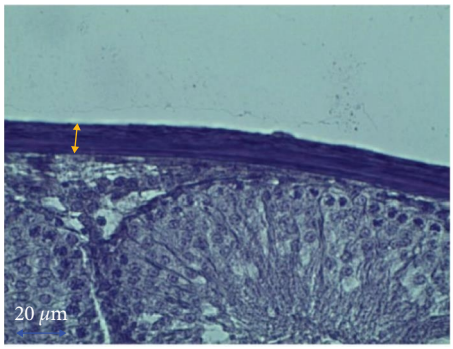

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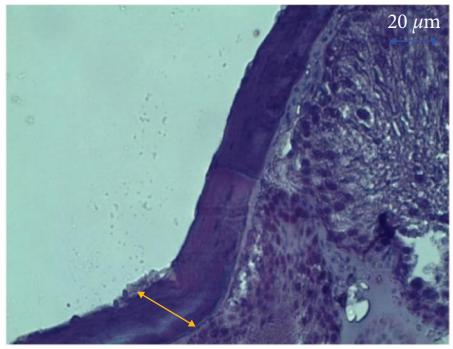

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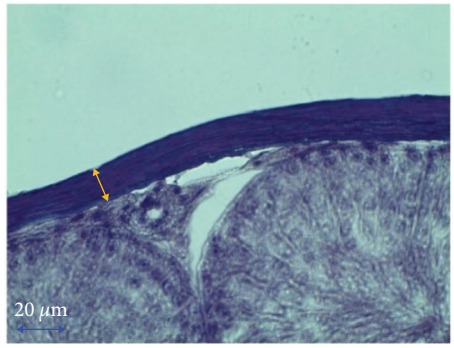

(d)

Figure 2: (a) Histological appearance of the testis in different groups. In some groups, areas of edema * and hemorrhage are observed (indicated by black arrowheads). In addition, in this image, spermatogonia are depicted with a blue arrow and a series of spermatogenic cells with a yellow arrow. Hematoxylin-eosin staining. Magnification ×400. (b, c, and d) Indicating an increase in the thickness of the capsule in the VPA group; (c) whereas in groups control and VPA + Th3 (b and d), the thickness of the testicular capsule is approximately equal. Trichrome Masson staining.

Table 5: Mean and standard deviation, MDA, and total TAC in testicular tissue of the studied groups.

	Testicular capsule thickness (μm)	Malondialdehyde (MDA)	Total antioxidant capacity (TAC)
Control	37.57 ± 4.89^{ab}	1.358 ± 0.575^{c}	0.896 ± 0.022^{a}
Th1	26.67 ± 4.23^{b}	1.997 ± 0.984^{bc}	0.933 ± 0.053^{a}
Th2	31.15 ± 8.09^{ab}	2.508 ± 0.836^{bc}	0.869 ± 0.050^{a}
Th3	36.29 ± 7.95^{ab}	1.613 ± 0.293^{bc}	0.983 ± 0.009^{a}
VPA	41.29 ± 5.68^{a}	5.999 ± 0.756^{a}	0.057 ± 0.078^{e}
VPA + Th1	28.35 ± 7.32^{b}	4.298 ± 0.399^{b}	0.201 ± 0.010^{d}
VPA + Th2	35.83 ± 6.70^{ab}	3.148 ± 0.616^{b}	0.402 ± 0.085^{c}
VPA + Th3	29.17 ± 4.17^{b}	3.020 ± 0.399^{bc}	0.629 ± 0.067^{b}
p-Value	0.001	<0.0001	<0.0001

Note: The groups receiving thyme honey and VPA at the same time prevented the increase of MDA compared to the VPA group. The amount of TAC in the groups receiving thyme honey was not significantly different from the control group. Comparing the control group with the group receiving VPA alone indicates a decrease in TAC. Values with different superscript characters $^{(a,b,c,d,e,\ ab,bc)}$ indicate significant difference between the control and treatment groups. Groups with the same superscript letter do not differ significantly.

group VPA + Th2, the testicular tissue was almost normal and close to the control group and the TDI of most tubes was positive, and all three categories A cells were visible. H—In the group VPA + Th3, the tubes have less cell volume. A little edema was visible and the cellular order of the seminiferous tubules was normal, the testicular tissue was almost normal and close to the control group, and active seminiferous tubules and all three cell lines were visible (* indicates edematous areas and + indicates the areas are hyperemic (Figure 2)).

3.6. Investigating the Levels of TAC and MDA. The amount of MDA in the groups receiving thyme honey (except the Th2 group which $p < 0.05$) was not significantly different compared to the control group ($p < 0.05$). Comparing the control group with the VPA indicates an increase in MDA in the VPA group alone. The results showed that the groups receiving thyme honey and VPA at the same time prevented the increase of MDA compared to the VPA group and could bring the MDA in testicular tissue closer to the results of the control group. The level of lipid peroxidation in the control group was close to 1.5 mmol, while it decreased in groups Th1, Th2, and Th3 which received thyme honey, except in the Th2 group, so that in Th3, MDA was close to reached 1.7 mmol. But in the VPA group, a significant increase in MDA was seen (6 mmol), which indicates an increase in lipid peroxidation due to VPA. In group VPA + Th1, an increase in MDA was observed compared to the control group (4.5 mmol), while in group VPA + Th2 and in group VPA + Th3, it again decreased and reached 2.7 mmol (Table 4).

The amount of TAC in the groups receiving thyme honey was not significantly different from the control group. Comparing the control group with the group receiving VPA alone indicates a decrease in TAC. The simultaneous administration of VPA and thyme honey showed that the compounds used were able to prevent the reduction of TAC, especially in high concentrations, so that in the VPA + Th1, VPA + Th2, and VPA + Th3 groups, this amount increased significantly compared to the VPA group alone. The TAC in the control group reached more than 0.8 mmol, while this amount was not significantly different from the control group in the Th1, Th2, and Th3 groups, and only a slight increase in the TAC level of the Th3 group was observed. In the VPA group, TAC decreased sharply compared to the control group and reached 0.2 mmol, while in the VPA + Th1, VPA + Th2, and VPA + Th3 groups, the TAC level increased, respectively. So that in the VPA + Th3 group, an amount equal to the control group was observed (Table 5).

4. Discussion

VPA as a treatment for epilepsy has a negative effect on male sexual performance. The combination of VPA with its oxidizing properties can disturb the antioxidant balance and as a result damage the testicles, and the results of the present study also indicate oxidative damage to the testicles of the groups receiving this drug. Studies show that thyme honey has antioxidant properties. It is a strong antioxidant that plays a significant role in reducing fat peroxidation [18]. Due to the presence of antioxidant compounds, honey can help maintain the balance of the body's antioxidant system after the increase of ROS, which our results also indicate. Thyme also contains antioxidant compounds that have always been used in traditional medicine. According to the results obtained from the present research, in the groups that received thyme honey, sperm motility increased, which is probably the high antioxidant property of thyme honey [7] that has caused this increase. which was consistent with Hadi [19] study.

Based on the findings of the Zarei et al. [20] study, in the group treated with methotrexate, all sperm quality parameters were significantly affected compared to the control group. Cranberry fruit extract and vitamin E were both able to prevent the effects of methotrexate on DNA damage. In this study, the oxidative stress caused by the administration of methotrexate affected sperm quality, and the protective effects of cranberry fruit extract and vitamin E against the toxicity of methotrexate on sperm quality were also proven, which had similar results compared to the present study. The findings of the present study also showed that in the group that was gavage with VPA, in most of the samples of this group, disruption of the cellular order of the seminiferous tubules along with inflammation is seen. TDI of seminiferous tubules is negative. Some tubules are undergoing destruction and cell shedding; however, active seminiferous

tubules are visible. In the study of Gholami et al. [21] honey improved spermatogenesis and increased the average number of sperm in rats that have undergone ischemia–reperfusion. Dietary antioxidants can improve sperm concentration, and excessive production of ROS may damage sperm cells through several pathways and change sperm morphology, and honey increases sperm count in rats by increasing testosterone. In a study in which all groups experienced apoptosis in the early stages, but after treatment with honey, apoptosis was inhibited [21]. In the present study, the average number of spermatogonia in the group receiving VPA showed a decrease compared to other groups. These findings are consistent with the findings of Tallon et al. [7] study.

Based on the results obtained from measuring the capsule thickness, the capsule thickness was the highest in the group receiving valproic, and the capsule thickness was less in the groups that received honey. Moloody et al. [22] study, by examining the effect of CoQ10 on the testicular tissue in rats treated with busulfan, it was found that the thickness of the testicular capsule increased in the busulfan group and the height of the germinal epithelium decreased significantly compared to the control group, which is consistent with the results of the present study. In the current study, the amount of edema in the group that received only VPA is the highest among the other groups, which is consistent with the results of the study by Fazilatpour et al. [23]. Severe atrophy of seminiferous tubes, the disintegration of germinal cells, and severe edema were observed in the interstitial tissue of the testis. However, administration of bilberry extracts inhibited methotrexate-induced damage in a dose-dependent manner. Accordingly, germinal epithelial differentiation in seminiferous tubules as well as edema decreased in a dose-dependent manner. Comparing the thickness of the testicular capsule (to check fibrosis) did not show any significant difference between the studied groups. But the thickness of the capsule in the methotrexate group was more than other groups. In the present study, the examination of the tubal differentiation coefficient, SPI, and regeneration coefficient revealed that in the group that was only gavage with VPA, it decreased compared to other experimental groups, which was in line with Salahipour et al. [24] study. In this article, after consuming nicotine, a significant decrease in the number and motility of sperm and a significant increase in dead and abnormal sperm were recorded, the obtained results indicated a significant decrease in complete spermatogenetic cells (A and B spermatogonia, primary spermatocytes, spermatids, and mature sperm).

The results of Bakhtiari et al. [25] study which examined the ability of crocin to suppress free radicals and increase sperm quality in animals treated with cyclophosphamide were in line with our research results. In this study, parameters of average number, motility, and viability were quantitatively evaluated. In the control group, the average number, motility, viability, and normal morphology of sperm decreased significantly compared to other groups, and the experimental group was able to increase all these parameters significantly ($p < 0.05$). The results of the present study showed that the administration of VPA caused the disturbance of the balance of the antioxidant system along with the increase of lipid peroxidation and the reduction of antioxidant capacity. Administering honey and thyme decreased the number of free radicals and also decreased testicular tissue damage caused by sodium valproate.

The study of Ourique et al. [26, 27] is in line with our results and showed that VPA disturbs the balance of oxidative stress and as a result, the amount of fat peroxidation and antioxidant capacity also changes accordingly. The combination of honey and thyme in past studies shows that the combination is strongly antibacterial and antioxidant, which can play a significant role in reducing fat peroxidation [28]. Among the properties of honey, we can mention its antibiotic and antioxidant properties, which are due to the function of the glucose oxidase enzyme. In addition, many natural antibacterial compounds have been identified in different types of honey, and flavonoids are one of the main compounds in them. Due to the presence of antioxidant compounds, honey can help maintain the balance of the body's antioxidant system after the increase of ROS, which our results also indicate. Thyme also contains antioxidant compounds that have always been used in traditional medicine to treat infertility problems.

5. Conclusion

One of the most important side effects of taking VPA is the dysfunction of the reproductive system in men. This complication occurs after creating the process of oxidative stress in the testicular tissue. One of the strategies that can reduce these injuries is the use of antioxidants. Based on the results of this study, it seems that thyme honey, due to its high percentage of antioxidants, has been able to improve these testicular complications and reduce the destruction of testicular cells.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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