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Research Article

The Effect of Vitamin E on Testicular Histology and Antioxidant Level in Rats Exposed to Bisphenol A

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ABSTRACT

This study, it was aimed to investigate the effect of vitamin E on testicular tissue and antioxidant levels in rats exposed to Bisphenol A (BPA). Experimental animals were divided into five equal groups. At the end of the experiment, the right testes were kept at -80 degrees to determine the antioxidant level, the left testes were fixed in Bouin's solution. In the microscopic examination, the general appearance of tubules seminiferous contours, epithelial layer, the structure of basement membrane, Sertoli cells, Leydig cells, the structure of capillaries, and tunica albuginea were examined. It was determined that the general histological appearances were similar in the control and sham groups, there was a decrease in tubule diameter in the BPA-treated group, but the seminiferous tubular epithelial layer increased. Epithelial shedding and subbasal vacuoles were detected in some tubules in the vitamin E group. Undifferentiated Embryonic Cell Transcription Factor 1 (UTF-1) positive tubule and cell counts were found to be quite high in the BPA group. The antioxidant parameter SOD value was found to be low in the BPA group and higher in the Vitamin E group. It was noted that the MDA value was higher in the BPA-applied group compared to the other groups. At the end of the study, it was determined that vitamin E applied against BPA exposure had positive effects on testicular histology and antioxidant levels in testicular tissue.

Keywords: *BPA, histology, rat, testicle, vitamin E*

Bisfenol A'ya Maruz Kalan Ratlarda Vitamin E'nin Testis Histolojisi ve Antioksidan Düzeyine Etkisi

ÖZET

Bu çalışmada Bisphenol A (BPA)'ya maruz bırakılan ratlarda Vitamin E'nin testis dokusu ve antioksidan düzeyleri üzerine etkisinin araştırılması amaçlanmıştır. Deney hayvanları beş eşit gruba ayrıldı. Deneyin sonunda sağ testisler antioksidan seviyesini belirlemek için -80 derecede tutulurken, sol testisler Bouin solüsyonunda tespit edildi. Mikroskopik incelemede tübüllerin genel görünümü, epitel tabakası, bazal membran yapısı, Sertoli hücreleri, Leydig hücreleri, kılcal damarların yapısı ve tunika albuginea incelendi. Kontrol ve sham gruplarında genel histolojik görünümlerin benzer olduğu, BPA uygulanan grupta tübül çaplarında azalma olduğu ancak seminifer tübüler epitel tabakasının arttığı belirlendi. E vitamini grubunda bazı tübüllerde epitelyal dökülme ve subbazal vakuoller tespit edildi. BPA grubunda farklılaşmamış Embriyonik Hücre Transkripsiyon Faktörü 1 (UTF-1) pozitif tübül ve hücre sayıları oldukça yüksek bulundu. Antioksidan parametre SOD değeri BPA grubunda düşük, vitamin E grubunda daha yüksek bulundu. BPA uygulanan grupta MDA değerinin diğer gruplara göre daha yüksek olduğu görüldü. Çalışma sonunda BPA maruziyetine karşı uygulanan E vitamininin testis histolojisine ve testis dokusundaki antioksidan düzeylerine olumlu etkileri olduğu belirlendi.

Anahtar kelimeler: *BPA, histoloji, rat, testis, vitamin E*

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Introduction

Bisphenol A(BPA), which is defined as an endocrine-disrupting chemical due to its low steroid-like properties such as polychlorobiphenyls and dioxins, was first used in pharmacy (Robins et al., 2011). Today, it has been widely used in many products such as the plastic industry, construction materials, dental fillings, and toy and beverage cans (Noda et al., 1999).

Studies have shown that BPA affects metabolism and enzyme activity in tissues, and it also disrupts the endocrine system by causing changes in hormone receptor gene activity and several receptors in target tissues (Richter et al., 2007). BPA acts in vivo by altering DNA methylation with enzyme activity and mimicking estrogen and may cause male infertility or spermatogenesis errors and metabolic disorders. Because the testicular tissue is rich in polyunsaturated fatty acids, it is easily affected by oxidative stress. Oxidative damage in the tissue and deterioration of sperm functions can cause infertility (Manfo et al., 2014).

Vitamin E is found in membrane-rich cell structures such as mitochondria and microsomes in tissues. Located between the lipid layers of the membranes, it forms the first line of defense that protects the polyunsaturated fatty acids in the structure of phospholipids from the effects of free radicals (Gupta et al., 2005). It is stated that vitamin E maintains spermatogenesis opposite oxidative stress and rise male fertilization potential (Hasanin et al., 2018). A chemical substance exposed in any period of life can have negative effects on the histological structure of the testis. This study, it was aimed to investigate the effect of vitamin E on testicular histology, spermatogonial stem cells, and antioxidant levels in testicular tissue against BPA toxicity in testicles.

Materials and Methods

Animals and experimental design

Forty adult male Wistar albino rats (240-270 g/90 days old) were used as material in the study. The rats were fed with water and fed *ad libitum* under conventional conditions in 12 hours of light/ 12 hours of darkness during the study. The material rats were divided into five groups with eight animals in each group. 1st Group (Control group): no application was made. Group 2 (Sham group): 0.5 ml corn oil was applied by gavage for three weeks. Group 3 (BPA group): BPA (Sigma-Aldrich) dissolved in 0.5 ml corn oil was applied at a dose of 10 mg/kg/day for three weeks (El-Beshbishy et al., 2012). Group 4 (Vitamin E group): Vitamin E (DL-α-tocopherol acetate, Merck) dissolved in 0.5 ml corn oil was applied at a dose of 300 mg/kg/day for three weeks (Kum et al., 2013). Group 5 (Vitamin E + BPA group): vitamin E (300 mg/kg/day) + BPA (10 mg/kg/day) dissolved in 0.5 ml corn oil was administered to the animals for three weeks. The procedure was arranged by ethical rules (Ethics Committee Approval Decision No: 64583101/2014/023).

Histological and histochemical methods

At the end of the applications, the rats were anesthetized with xylazine ketamine and killed by cervical dislocation, then their testicles were removed. While the right testes were stored at -80 °C to determine their antioxidant levels, the left testes were fixed in Bouin's solution. It is blocked in paraffin. Eight serial sections of 6 µ thickness were taken from the prepared paraffin blocks at 300 µ intervals. Crossman triple staining method and PAS (Periodic acid Schiff reagent) method was applied to determine histological, histochemical, and histometric changes on serial sections. The tubules' seminiferous contours in each section were investigated epithelial vacuolization, subbasal vacuolization, and epithelial eluded. Obtained changes were scored semi-quantitatively and subjective scoring was done according to the images. (-:None, +:Low, ++:Moderate, +++:High).

Determining histometric changes

Eight round or nearly round stages of the 7th and 8th tubules were randomly selected for each section. The diameter and height of the 7th and 8th stage tubules were measured and recorded regarding seminiferous epithelium. Interactive measurements were made with the help of the Olympus cellSens Entry image analysis program and the Olympus BX43F research microscope.

Determination of spermatogonial stem cells

The avidin-biotin staining method was used to examine spermatogonial stem cells (van Bragt et al., 2008). For this purpose, rabbit anti-UTF-1 polyclonal antibody (Rabbit anti-UTRF-1 polyclonal antibody, unconjugated, Bioss bs-12207R) was used.

Biochemical methods

The methods specified in the commercial test kit (Ref. Kot: A2300, Archem Health Ind. Co., Türkiye) were taken into account in the determination of the total protein amount and calculations. Antioxidant and oxidant parameters were determined in testicular tissue. Superoxide dismutase (SOD) analysis was applied according to Sun et al. (1988). Malondialdehyde (MDA) level in tissues was done according to the method of Yoshioka et al. (1979).

Statistical analysis

SPSS© (Statistical Package for Social Sciences) for Windows 22 (SPSS Inc., Chicago, IL, USA) package program was used for statistical analysis of the obtained data. The conformity of the data to the normal distribution was evaluated using the Shapiro-Wilk test. The difference between groups that did not show normal distribution was assessed with the Kruskal-Wallis test and the difference between groups that did show normal distribution was assessed with the one-way ANOVA (Conover, 1980).

Results

Histological findings

In the control group of rats, it was seen that the tubule's seminiferous contortus lumens were prominent, the tubules were smooth, and the spermatogenetic epithelial layer shaped a thick layer. It was observed that Sertoli cells, located between the spermatogonia, were seated on the basement membrane. Single or groups of Leydig cells located close to the blood capillaries in the intertubular space were detected. A single row of myofibroblast cells was seen around the tubules (Figure 1A-B). Capillary vessel structure was normal. Control and sham groups had similar histological appearances. In the BPA-treated group, it was noted that the diameter of the tubules decreased but the seminiferous tubular epithelial layer increased compared to the other groups. While shedding in the epithelial layer of the tubules and vacuoles located close to the basal area were observed in places (Figure 1C-D). Tubules with partially irregular contours were seen (Figure 1E). There was no difference between the groups in the basement membrane structure surrounding the tubules. It was noted that some tubules were separated from the basement membrane (Figure 1F). It was observed that germ cells were poured into the tubule lumen in any tubules (Figure 1G). Epithelial eluded and subbasal vacuoles were also observed in some tubules in the vitamin E group (Figure 1H-I). The general histological appearance was smoother in the group in which vitamin E was administered together with BPA when compared to the group with BPA. However, some tubules were found to have irregular-shaped vacuoles close to the basement membrane (Figure 1K).

Histological and histometric changes

Histological findings are given in Table 1. During the 7th and 8th stages seminiferous tubular epithelial height and seminiferous tubule diameter of the control and experimental groups are dedicated in Table 2.

When tubule diameter values were investigated, it was determined that there was a statistically important difference (P=0.023). This differences between the control group and the BPA group (P=0.012), between the sham group and the BPA group (P=0.041), between the BPA group and the vitamin E group (P=0.003), and between the vitamin E group and the BPA+vitamin E group (P=0.018) were as found to be statistically important. When evaluated in terms of epithelial height, no statisti-

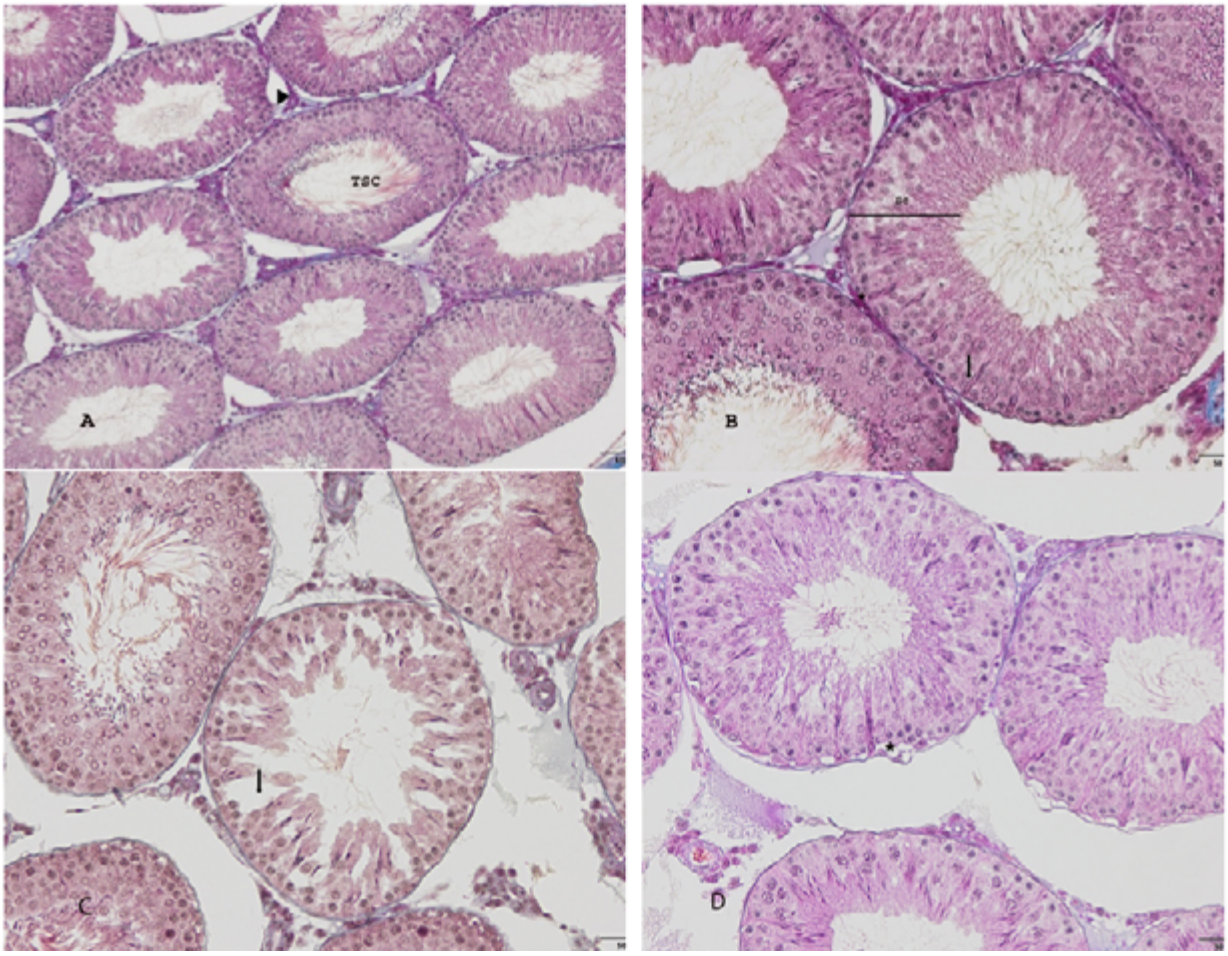

Figure1 A. General histological appearance of testicular tissue of the control group. TSC: Seminiferous tubules. Arrowhead: Leydig cells. Triple stain. B. Control group. The image of seminiferous tubules. Sc: Seminiferous epithelium. Arrow: Sertoli cell. *: Myofibroblast cells. Triple stain. C.D. The image of seminiferous tubules in the BPA group. Arrow: Epithelial shedding *: Vacuoles. Triple stain.

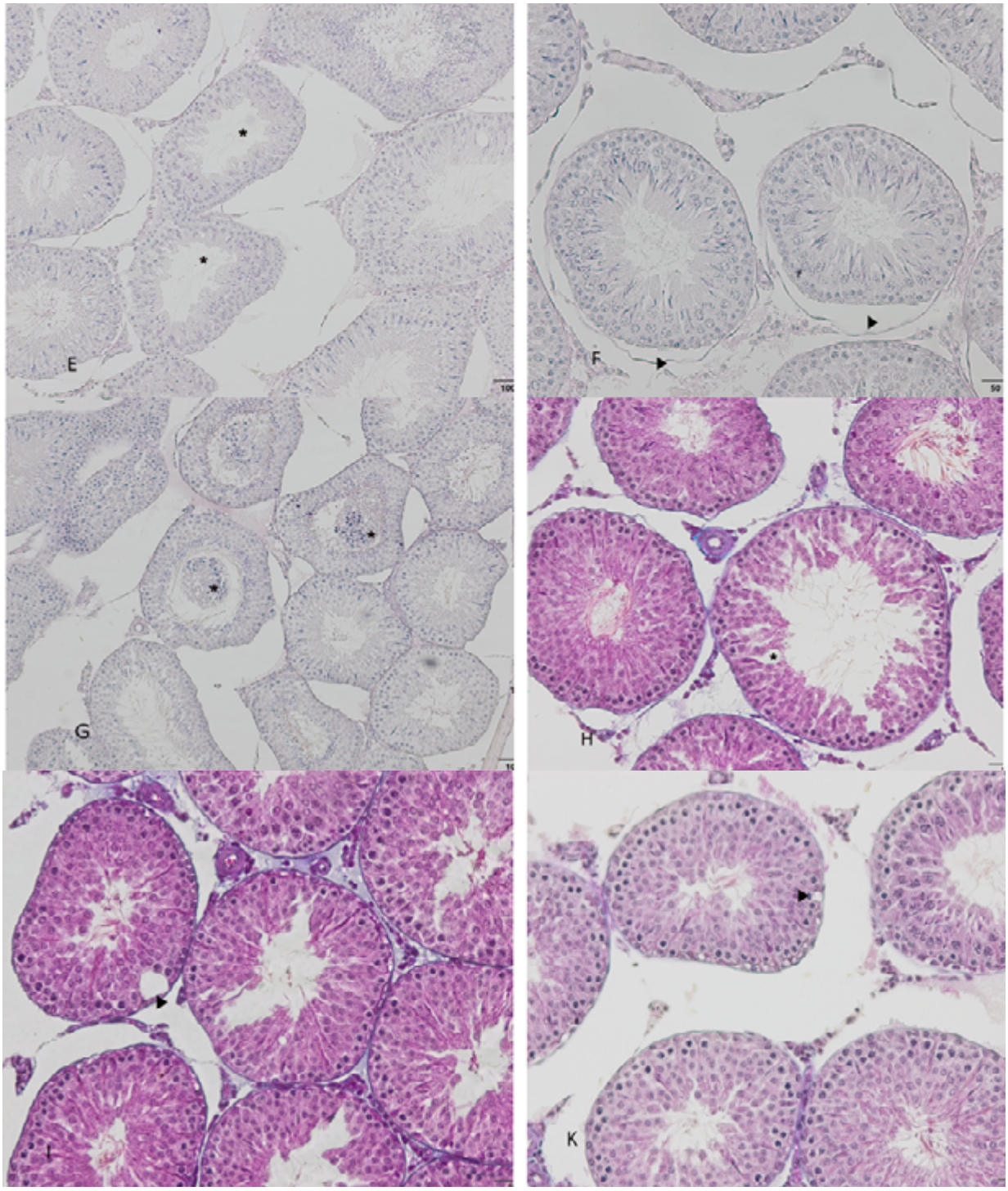

Figure1.
E.F. The image of seminiferous tubules in the BPA group. *: Tubules with irregular contours. Arrowhead: Tubules separated from the basement membrane. PAS
G. The image of seminiferous tubules in the BPA group.*: Germ cells shedding. Ta: Tunica albuginea. PAS
H.I. The image of seminiferous tubules in the vitamin E group. *: Epithelial shedding. Arrowhead: Vacuole. Triple stain.
K. The image of seminiferous tubules in the BPA group + vitamin E group. Arrowhead: Vacuole. Triple stain.

cally important difference was found between the experimental groups.

Immunohistochemical findings

The count of positive tubules and the count of positive cells were determined in three sections of each animal. The data is given in Table 3. The count of UTF-1 positive tubules and cells was found to be quite high in the group given BPA. This difference was also statistically significant. Although the positive tubule count was slightly higher in the sham group than in the control, vitamin-E, and BPA+ Vitamin-E groups, it was not statistically important. In terms of the number of positive cells, it was observed that the other groups were similar to each other except for the group with BPA (Figure 2).

Table 1. Histological changes

Groups	Subbasal Vacuolization	Epithelial Vacuolization	Epithelial Shedding	Germ cells shedding into the tubular lumen
Control	-	-	-	-
Sham	-	-	-	-
BPA	+++	++	+	+
Vit-E	+	+	-	-
BPA+Vitamin E	+	+	-	-

-:None, +:Low, ++:Moderate, +++:High

Histological changes determined as semi-quantitative were assessed with Kruskal-Wallis analysis of variance.

Table 2. Diameter of seminiferous tubules and epithelial height of seminiferous tubules.

Groups	Diameter of seminiferous tubules (µm)	Epithelial height of seminiferous tubules (µm)
Control	67.58±1.86^{ab}	276.18±11.56
Sham	67.51±1.07^{ab}	254.65±13.34
BPA	62.71±1.68^{c}	286.71±7.81
Vitamin E	69.40±1.23^{a}	282.00±10.39
BPA+Vitamin E	64.74±1.31^{bc}	260.60±10.19
P	0.023**	NS*

NS: Not significant

$^{abc:}$ Different letters in the same column indicate a statistically significant difference.

$^{*:}$ Kruskall-Wallis

$^{**:}$ANOVA

Table 3. UTF-1 positive tubule and positive cell numbers

Groups	Number of the positive tubule	Number of the positive cell
Control	7.05±1.60^{b}	6.64±1.99^{b}
Sham	9.70±2.40^{b}	8.10±2.31^{b}
BPA	15.23±0.93^{a}	21.77±4.57^{a}
Vitamin E	6.95±0.48^{b}	7.48±1.42^{b}
BPA+Vitamin E	6.66±0.48^{b}	8.29±0.87^{b}
P	0.009*	0.032*

^{ab}: Different letters in the same column indicate a statistically significant difference.

*:Kruskall-Wallis

Table 4. SOD (antioxidant) and MDA (oxidant) values in control and experimental groups.

Groups	SOD (U/mg protein)	MDA (nmol/mg protein)
Control	5.78±0.90^{b}	150.83±8.70^{bc}
Sham	4.94±0.52^{b}	172.42±9.15^{b}
BPA	1.55±0.39^{c}	206.08±4.42^{a}
Vitamin E	13.81±1.71^{a}	147.78±9.62^{c}
BPA+Vitamin E	5.25±0.97^{b}	163.63±4.61^{bc}
P	0.001*	0.001**

$^{abc:}$ Different letters in the same column indicate a statistically significant difference.

$^{*:}$ Kruskall-Wallis

$^{**:}$ANOVA

Biochemical findings

SOD and MDA values determined in testicular tissue between control and experimental groups are dedicated in Table 4.

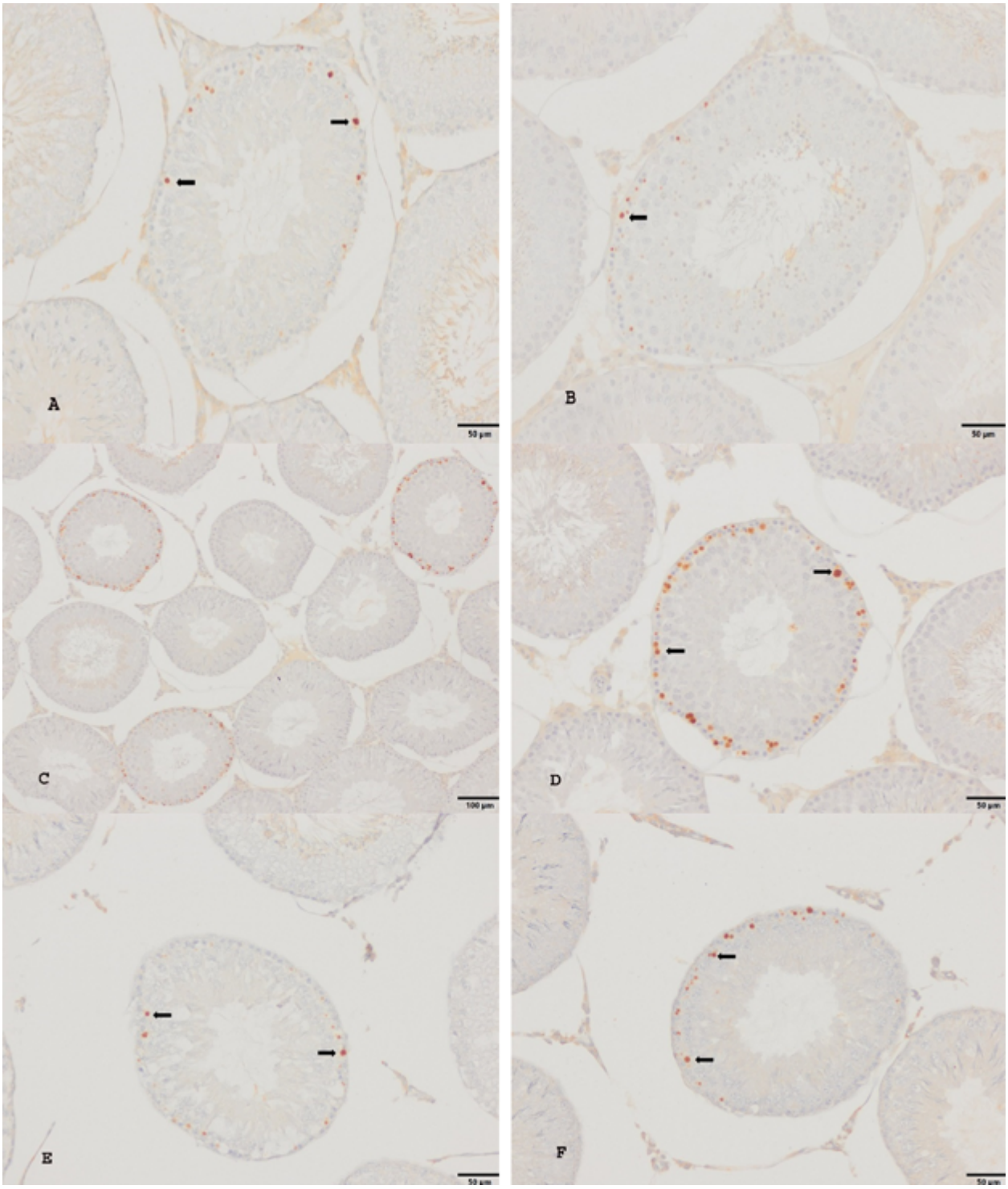

Figure 2. Spermatogonial stem cells (Arrows). A. Control group B. Sham group. C-D. BPA group. E. Vitamin E group. F. BPA+Vitamin E group. Avidin-Biotin Peroxidase.

Discussion

BPA has toxic effects on many organs such as the nervous system, brain, lung, liver, kidney, and reproductive system. In recent years, it has been stated that semen quality, decrease in sperm density, disorders in the male reproductive system, and infertility are increasing (Manfo et al., 2014). It has been reported that the seminiferous tubules lost their normal structures, the interstitium enlarged, spermatids accumulated in the tubule lumen, and degenerated Sertoli cells with pycnotic nuclei were observed in BPA-treated mice (Tolba and Mandour, 2018). It was found that BPA administered to mice impairs spermatogenesis by damaging the connection between the basal lamina of the seminiferous tubules and Sertoli cells (Tian et al., 2017). In the present study,

in the group given 10 mg/kg BPA, although severe findings were not observed, it was observed that BPA caused irregularity in some of the tubules and caused vacuoles in the epithelial layer and areas close to the basal. Epithelial detachments were also observed in some of the tubules. It can be considered that the result is related to the dose given.

The harmful effects of BPA are largely associated with its estrogenic activity (Kurosawa et al., 2002). In mammals, BPA binds and activates the two estrogen receptors ERα and ERb, although it has an affinity 10,000 times weaker than its natural ligand 17β-estradiol. It can also act with the G-protein-coupled receptor (GPR30) acting on a trans-membrane estradiol receptor (Amraoui et al., 2018). Güles et al. (2019), it was found that tubular diameter increased in BPA-treated rats checked to the control group, but this rise was not statistically important and the epithelial height in the experimental group reduced statistically important checked to the others. It has been reported that BPA given at a dose of 250 mg/kg for eight weeks reduces the height of the epithelium (Malmir et al.,v 2021). In this study, while the diameter of the seminiferous tubule decreased in the group given BPA, the height of the tubular epithelium was higher. This result may depend on the dose and time used. The Vitamin E group was similar to the control and sham groups. It has been reported that vitamin E increased the diameter of the seminiferous tubule in rats given sodium arsenide (Momeni et al., 2012). It has been shown that vitamin E increases the tubular diameter and decreased epithelial height in rats in which oxidative damage has been induced by administering para-nonylphenol (Mehranjani et al., 2009). In the presented study, it was noted that vitamin E given with BPA slightly increased the diameter of the tubule, but decreased the height of the epithelium.

Spermatogonial stem cells undergo self-renewal and differentiation to maintain the continuity of spermatogenesis throughout adulthood in men (Jung et al., 2014). UTF-1 detected in spermatogonium A during testicular development in rats can be expressed in all gonocytes in embryonic and newborn testes (van Bragt et al., 2008). It is also expressed in humans during gonadal development and in the spermatogonia of the adult testis (Kristensen et al., 2008). It has been suggested that UTF-1 is a conserved molecule of undifferentiated spermatogonia and may play a role as pluripotent transcription factor in the self-renewal of spermatogonial base cells in mammals and take part in spermatogonial regeneration (van Bragt et al., 2008; Kristensen et al., 2008). Kristensen et al. (2008) stated in their study that UTF-1 is intensely expressed in germ cell tumors, therefore germ cell tumors originate from spermatogonia. Saunders et al. (2001) reported that estrogen has a direct effect on male sex cells, while it has an inhibitory effect on Leydig and Sertoli cells, it has a stimulating effect on germ cells. UTF-1 is a protein associated with the differentiation of spermatogonial stem cells, bound to the N-terminal region of ATF-2 (Mouallif et al., 2014). UTF-1 improves transcription by acting as a co-activator to activate transcription factor-2 (ATF-2) (Fukushima et al., 1998). Vrooman et al. (2015) stated that spermatogonial stem cells were adversely affected and sperm production decreased in rats exposed to BPA. The decrease in sperm production may be due to the suppression of ATF-2 by BPA via UTF-1 (Wu and Zheng, 2013). In the present study, an intense increase in stem cells was detected in the group given BPA. This increase is thought to be due to the estrogenic effect of BPA. Increasing proof suggests that BPA may interact with testicular germ cells and reason infertility as a result of its estrogenic efficiency (Karmakar et al., 2017). Eladak et al. (2018) noticed that while BPA reduced the germ cells expressing pluripotent marker AP-2, it significantly increased the percentage of those expressing the spermatogonial marker MAGE-A4, which is highly expressed in human seminoma tumors.

In recent years, more notice has been drawn to oxidative stress, which has been identified as an important factor in male infertility and moderated by reactive oxygen species and lipid peroxidation (Agarwal et al., 2013). Studies have noticed that BPA reason oxidative stress by disrupting the balance between excessive reactive oxygen species and the antioxidant defense system in the testis (Kabuto et al., 2004). There are many protective ways and mechanisms such as SOD and GSH-Px in the cell against damage caused by free radicals (Shen et al., 2012). It is known that reactive oxygen species are produced by developing germ cells and spermatozoa during spermatogenesis. These generated radicals are cleared by a powerful antioxidant system that helps maintain the balance between the antioxidant/oxidant system. SOD is considered the first line of defense in this balance. It converts reactive oxygen molecules to H^2O^2 and O^2 using hydrogen ions. Malondialdehyde, which is widely used in the evaluation of oxidative stress, is the end product of lipid peroxidation (Bağış, 2013). A remarkable reduction in SOD levels was observed in male mice treated with BPA. Again, in the same study, it was reported that the MDA level in the testis increased (Xie and Li, 2014). It has been reported that BPA given to male rats causes a decrease in antioxidant enzymes such as GSH, GSH-Px, SOD, and catalase (El-Beshbishy et al., 2012). Amraoui et al. (2018) displayed that BPA administered remarkably increased the MDA level, while 0.5 mg/kg Selenium + 100mg/kg vitamin E administered against it decreased the MDA concentration compared to the group with BPA. In this study, the antioxidant parameter SOD value was found to be quite low in the BPA-treated experimental group, while it was found to be high in the vitamin E-administered group. There was no statistical difference between the other groups. It was observed that the MDA value was very high in the group with BPA. It is seen that the findings are compatible with the findings of other researchers.

Conclusion

In this study, it was determined that BPA had negative ef-

fects on the antioxidant levels in the testicular tissue and on the histological structure of the testis. On the other hand, it is seen that vitamin E provides an improvement in the antioxidant level and improves the histological appearance. Attention should be drawn to the use of such chemical substances against the increasing male infertility problems in our age, and new studies should be conducted to solve the problem.

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Conflict of Interest

The authors declare that they have no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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