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Original Article

Protective Effect of Vitamin E against Polyvinyl Chloride Induced Damages and Oxidative Stress in Rat Testicular Tissue

Abbas Sadeghi[1]*, Farah Farokhi[1], Gholamreza Najafi[2], Ali Shalizar Jalali[2]

1. Dept. of Biology, Faculty of Science, Urmia University, Urmia, Iran
2. Dept. of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

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***Corresponding Author**:
Department of Biology, Faculty of Science, Urmia University, Urmia, Iran
Tel: +989145562723
Email:
sadeghiabbas88@yahoo.com

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Abstract

Introduction: Numerous studies suggest that polyvinyl chloride (PVC) has adverse effects on male sexual function and testicular tissue. Vitamin E (Vit E) is a dietary compound with antioxidant scavenging function for toxic free radicals.

Methods: In this experimental study, 36 male Wistar rats were randomly divided into six groups (n=6) including control, Vit E (150 mg/kg/bw/day), PVC (200 mg/kg/bw/day), PVC (1000 mg/kg/bw/day), PVC (200 mg/kg/bw/day) + Vit E (150 mg/kg/bw/day), PVC (1000 mg/kg/bw/day) + Vit E (150 mg/kg/bw/day). The administration route was oral and experiment lasted 40 days. Each rat was weighed at the beginning and at the end of the experiment. Left testis was transferred to 10% formalin. The right testis was transferred to -70 ◦C for determining oxidative stress markers. Data were analyzed in SPSS software using Tukey test.

Results: Oral administration of PVC significantly decreased body and testes weights in the male rats. Furthermore, PVC significantly reduced height of germinal epithelium, diameter of seminiferous tubules, number of Leydig cells as well as catalase and total antioxidant capacity levels ($p<0/05$). However, Vit E minimized PVC-induced testicular toxicities.

Conclusion: Exposure to PVC can cause testicular damage, while Vit E, as an antioxidant, may reduce destructive effects of PVC in rat testis.

Introduction

Studies show that the number of sperms is much lower in men today compared to those who lived 50 years ago. Male infertility is a current issue, especially for those living in industrial societies. The use of polyvinyl chloride (PVC) is daily increasing. It is among the most important polymeric materials in terms of application (1). One of the problems in male reproductive organs may be the result of exposure to PVC which is the raw material for manufacturing plastics, including disposable dishes, plastic tubes, cable and cord covers, flooring, photographic films, electronics, cars, and toys (2, 3). Because of the ever-increasing production of the noted materials, the production of PVC is increased, further exposing workers to this material. Currently, over 81000 workers are exposed to PVC (2). Chemically, PVC does not bind to polymers and is separated from them during production and use (4). Therefore, it can enter the human body through air, water, food, or medical instruments (5). One of the major applications of PVC is the production of medical devices and laboratory instruments (6). Blood bags, injection and hemodialysis instruments, and tracheal tubes contain large amounts of PVC. A high level of materials used in PVC industries is found in the blood and tissues of patients who undergo multiple blood transfusions (7). Nevertheless, the main route of PVC contamination for humans is oral (8). PVC induces oxidative stress, reduces the activity of antioxidant enzymes, and thus causes apoptosis in testicular tissue (9). PVC which is extensively used in plastics, food packaging, laboratory instruments, and hospitals, is a dangerous pollutant of biological environments (10, 11). PVC chemical compound has two major characteristics. First, 56% of PVC's molecular weight is formed by chlorine. Second, in 0%-50% of PVC, bis(2-ethylhexyl) phthalate (DEHP) is used as a plasticizer. Almost all phthalates are employed in the production of PVC and the toxic effects of some of them are confirmed (12, 13). Considering the extensive use of PVC and its toxic effects on many body organs, including the reproductive organ, it is essential to examine these effects. Other organs which are affected by PVC include liver, kidneys, and the central nervous system (CNS). PVC can even lead to visual impairment and CNS disorders (14). Studies conducted in 1997 and 1998 on those working in plastic factories exposed to PVC showed a 6-fold increase in the risk of seminoma (a type of testicular cancer) (15).

Vitamin E (tocopherol) is a strong antioxidant against free radicals (16) with protective effects against DNA oxidative damage. It inhibits lipid peroxidation in cell membranes by limiting the activity of free radicals and thus protects cell membranes from the damage induced by them (17, 18). In the male reproductive

organ, the antioxidant role of vitamin E in inhibiting the destructive effects of free radicals has been reported in testes (19) and sperm (20, 21). Furthermore, vitamin E can sustain the antioxidant defense system in testicular cells and sperm (22). Studies have shown that vitamin E significantly protects testes against oxidative damage (23, 24). Because of the toxic effects of PVC by inducing oxidative stress in the male reproductive system and the role of vitamin E as a strong antioxidant, the consumption of vitamin E seems to reduce the disorders caused by PVC.

Thus, the present study examined the protective effect of vitamin E against the damage caused by exposure to PVC in rat testicular tissue.

Materials and Methods:

Study groups:

The present study examined 36 Wistar rats with the mean weight of 140-160 g. All rats were housed in standard conditions (12:12 h light/dark cycle, 25±5 °C, and 50±10 relative humidity) with ad libitum access to food and water. Rats were randomly assigned to 6 groups (n=6), including control, vitamin E (150 mg/kg bw per day), PVC (200 mg/kg bw per day), PVC (200 mg/kg bw per day) + vitamin E (150 mg/kg bw per day), PVC (1000 mg/kg bw per day), PVC (1000 mg/kg bw per day) + vitamin E (150 mg/kg bw per day). The administration route was oral, and the experimental period was 40 days. PVC (Merck, Germany) and vitamin E were dissolved in normal saline solution and olive oil, respectively (25).

Body and testicular weight:

Rat's body weight was measured on the first day of experiment using Sartorius digital scales (Germany, 0.0001g precision) and recorded. At the end of treatment period, rats were weighed again, anesthetized using a combination of ketamine and xylazine, and sacrificed. Then, in sterile conditions, right and left testes were removed following an incision to the lower abdomen.

Testicular tissue evaluation:

For tissue evaluation, left testes were placed in 10% fixative buffered formalin solution and then underwent testicular processing and paraffin embedding. Paraffin-embedded tissues were sliced to 5 µm in thickness using a rotary microtome (model MICROM, Germany, Serial: 21074) and transferred to slides. Finally, the prepared slides were stained using the hematoxylin-eosin method (H&E). To study testicular tissues, 100 cross-sections of the seminiferous tubules of the testes of each rat were selected. Then, the seminiferous tubule diameter, lumen diameter, and germinal epithelium thickness were evaluated using a scaled ocular lens.

To study the tubular differentiation index (TDI), seminiferous tubules which had 3 or more layers of spermatogenic cells differentiated from type A spermatogonium were evaluated as tubules with a positive differentiation coefficient (26). Spermatogenic tubules in which spermatogenesis had occurred (i.e. included sperms) were considered as tubules with a positive spermatogenesis index (SPI) (27).

Moreover, the number of Leydig cells per testicular surface unit was enumerated using a scaled ocular lens.

Biochemical tests:

After homogenizing the right testes, the level of malondialdehyde (MDA), catalase (CAT), and total antioxidant capacity (TAOC) were evaluated.

Measuring testicular MDA level: To this end, 0.2 g of the testicular tissue was transferred to 0°C 0.05M phosphate buffer with pH=7.4 (10% w/v) and ground by mortar and pestle. Then, the resulting solution was centrifuged at 1000 rpm. Afterwards, 150 µg of the supernatant of the centrifuged specimen was removed, 300 µg of 10% trichloroacetic acid was added, and it was centrifuged at 1000 rpm, 4 °C, for 10 min. Then, 300 µL of the supernatant was transferred to the test tube and incubated with 300 µL of 0.67% thiobarbituric acid at 100 °C for 25 min. After 5 minutes of cooling the solution, the color pink resulting from the reaction between MDA and thiobarbituric acid appeared and evaluated with a spectrophotometer at 535nm wavelength. The concentration of MDA was calculated using the MDA absorption coefficient and expressed in terms of nmol/g tissue (28).

CAT activity evaluation: Catalase activity was determined based on its ability to decompose H_2O_2 using the Aebi method. H_2O_2 decomposition can be examined by absorption reduction at 240 nm. The difference in absorption per unit of time equals catalase activity. First, 10% w/v of 0.2 g of testicular tissue was poured into ice-cold phosphate buffer (pH=6.8) and ground by mortar and pestle. The prepared homogenized tissue solution was centrifuged at 5000 rpm for 5 min. Next, 100 µL of the centrifuged supernatant was added to 2.8 µL of phosphate buffer. Then, 100 µL of H_2O_2 solution was added, and absorption was measured at the 240nm wavelength at 0 and 30 s. Phosphate buffer was used to reset the device. In the end, values were expressed as U/g tissue (29).

TAOC measurement: The TAOC of testicular tissue was measured using the ferric antioxidant power test (FRAP). On the day of evaluation, tissues were homogenized in cold KCL solution. To prepare homogeneous tissue solution, 0.2 g of testicular tissue was taken, 10% (w/v) of it was added to KCL solution and ground by mortar and pestle. Then, the prepared homogeneous solution was centrifuged at 1000 rpm for 5 min. Afterwards, 100 µL of the supernatant was removed and transferred to test tube, 3 µL of FRAP indicator was added, and it was incubated in a 37°C heated bath for 7-10 min. The absorption of the blue complex was read using a spectrophotometer at 593 nm. Values were expressed as mmol/g tissue (30).

Statistical analysis:

Data were analyzed in SPSS 21. Groups were compared using one-way ANOVA and Tukey's post-hoc test at the significance level of 0.05.

Results

Relative testicular weight and body weight: Data belonging to weight difference among rats from the beginning to the end of the experiment as well as the ratio of testicular weight to body weight are presented in Table 1. In this study, all groups receiving PVC showed a significant body weight reduction compared to the control group ($p<0.05$). However, changes in body weight were not significant in the vitamin E group

compared to the control group ($p<0.05$). Moreover, in groups receiving PVC + vitamin E, body weight showed a significant increase compared to groups receiving PVC alone ($p<0.05$). The relative reduction in testicular weight was significant only in groups receiving PVC at the doses of 200 and 1000 mg/kg compared to the control group ($p<0.05$).

Histopathological findings: Results of histological evaluations are presented in Table 2. The diameter of seminiferous tubules showed a significant decrease in groups receiving 200 and 1000 mg/kg bw per day of PVC compared to the control group ($p<0.05$). The diameter of tubules did not show any significant difference between vitamin E and control groups ($p<0.05$).

The height of germinal epithelium of seminiferous tubules had a significant reduction in groups receiving 200 mg/kg bw per day of PVC compared to the control group ($p<0.05$). Nevertheless, no significant difference was observed between groups receiving 200 and 1000 mg/kg bw per day of PVC + vitamin E and the control group ($p<0.05$). Mean height of the germinal epithelium showed no significant difference between vitamin E and control groups ($p<0.05$). In addition, a clear irregularity was observed in the tissue arrangement of the germinal epithelium of seminiferous tubules in the group receiving 1000 mg/kg bw per day of PVC (Table 2, Figure 1).

Lumen diameter in seminiferous tubules showed a significant increase in groups under treatment with PVC compared to the control group ($p<0.05$). However, no such difference was seen in groups under treatment with PVC + vitamin E compared to the control group ($p>0.05$). Moreover, the lumen diameter of seminiferous tubules showed no significant difference between vitamin E and control groups ($p>0.05$). Also, lumen diameter was similar across different groups under treatment with PVC ($p>0.05$). A significant increase in lumen diameter was found in groups receiving PVC alone compared to those receiving PVC + vitamin E ($p<0.05$) (Table 2, Figure 1).

TDI had a significant reduction in groups receiving PVC alone and those receiving 1000 mg/kg bw per day of PVC + vitamin E compared to the control and vitamin E groups ($p<0.05$). However, no such difference was observed in the group receiving 200 mg/kg bw per day of PVC + vitamin E compared to the control group ($p>0.05$). Moreover, no significant difference was seen between the group receiving vitamin E and the control group ($p>0.05$) (Table 2, Figure 1).

SPI demonstrated a significant reduction in groups receiving PVC alone compared to the control and vitamin E groups ($p<0.05$). Nevertheless, no such difference was observed in groups under treatment with PVC + vitamin E compared to the control group ($p>0.05$). Also, the vitamin E group showed no significant difference compared to the control group ($p<0.05$). SPI was significant in higher doses of PVC compared to lower doses ($p<0.05$) (Table 2, Figure 1).

The number of Leydig cells demonstrated a significant reduction in groups receiving PVC compared to control and vitamin E groups ($p<0.05$). However, no such difference was observed in groups under treatment with PVC + vitamin E ($p>0.05$). Also, the number of Leydig cells was similar between vitamin E and control groups ($p>0.05$) (Table 2, Figure 1).

Biochemical test results: MDA concentration was similar between vitamin E and control groups ($p>0.05$). However, it showed a significant increase in groups receiving PVC compared to control and vitamin E groups ($p<0.05$) (Table 3).

CAT activity demonstrated a significant reduction in groups under treatment with PVC compared to the control group ($p<0.05$). Nevertheless, this increase was not significant in vitamin E group compared to the control group ($p>0.05$). No significant difference was seen between groups receiving PVC + vitamin E and the control group ($p>0.05$) (Table 3).

In terms of TAOC, a significant reduction was found in groups under treatment with PVC compared to the control group ($p<0.05$). However, no difference was observed in groups receiving PVC + vitamin E ($p>0.05$). Furthermore, TAOC was significantly higher in rats treated with vitamin E compared to the control group ($p<0.05$) (Table 3).

Table 1. Comparing mean body weight difference and relative testicular weight among different groups

Groups	Variables	
	Body weight difference (g)	Relative testicular weight
Control group	8.01±107.57	0.07±0.78
Vitamin E group	5.59±99.28	0.07±0.82
PVC 200 group	4.74±64.83^{ab}	0.05±0.62^{ab}
PVC 1000 group	3.40±55.81^{ab}	0.04±0.58^{ab}
PVC 200 + Vitamin E group	1.88+77.39^{ab}	0.04±0.71^{b}
PVC 1000 + Vitamin E group	2.49±75.02 ^{ab}	0.04±0.68^{b}

a and b indicate a significant difference (P<0.05) compared to the control and vitamin E groups, respectively, in each column.
Data are expressed as mean±SD.
PVC200: 200 mg/kg bw per day of PVC
PVC1000: 1000 mg/kg bw per day of PVC

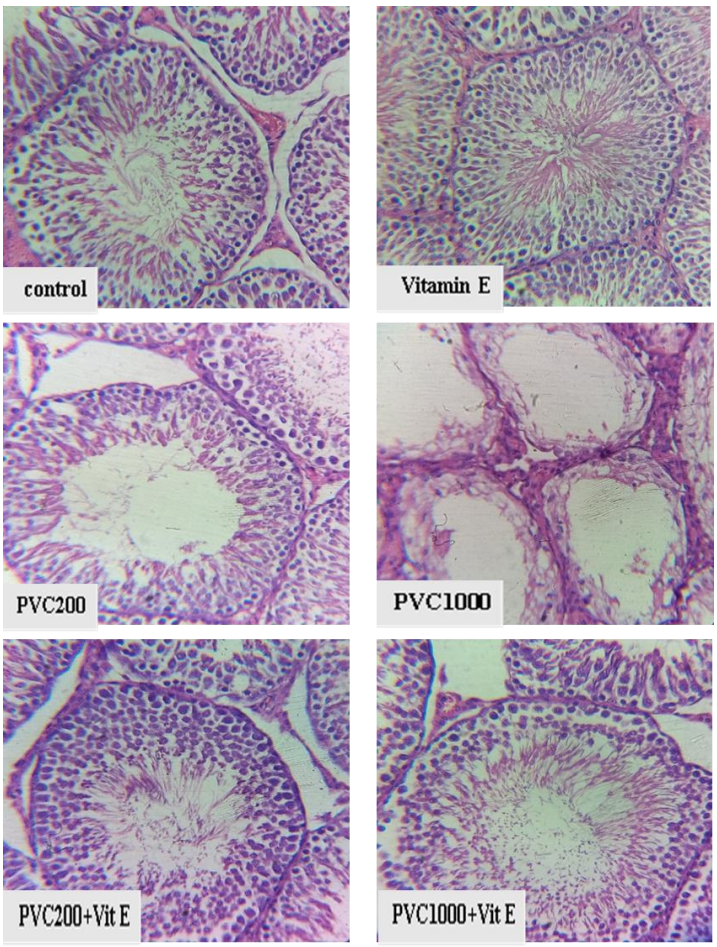

Figure 1. Cross-sections of testicular tissue in different groups, H&E stain, 400x magnification
Seminiferous tubules have normal germinal epithelium in control and vitamin E groups. A reduction in Leydig cells and a reduction in germinal cells are observed in PVC 200 group. The destruction of the germinal epithelium of seminiferous tubules and a marked decrease in Leydig cells can be observed in PVC 1000 group. In groups under treatment with PVC + vitamin E, the structure of seminiferous tubules has become somewhat more natural.

Table 2. Histological parameters in studied groups

Experimental group	Diameter of seminiferous tubules(mμ)	Height of germinal epithelium(mμ)	Diameter of lumen(mμ)	TDI(%)	SPI(%)	Number of Leydig cells
Control group	7.13±324.39	2.65±77.77	2.79±38.46	4.47±85.00	5.16±86.66	0.90±12.24
Vit. E	11.28±321.47	5.63±77.66	3.63±34.36	7.52±91.66	7.52±88.33	0.83±13.00
PVC 200	15.64±276.92ab	1.31±69.10ab	2.29±50.23 [ab]	5.16±73.33 [ab]	8.94±70.00 ab	0.39±8.48 ab
PVC 1000	4.82±208.10 ab	5.26±37.29 ab	2.44±52.12 [ab]	6.50±13.33 [ab]	12.81±15.83 ab	1.68±9.94 ab
PVC 200 + Vit. E	3.97±320.85	3.81±75.67	5.69±34.13	4.08±81.66	6.32±80.00	0.46±11.74
PVC 1000 + Vit. E	15.87±311.26	7.43±73.55	4.63±43.60[b]	7.58±72.50[ab]	16.02±78.33	0.93±11.53

a and b indicate a significant difference ($p<0.05$) compared to the control and vitamin E groups, respectively.
Data are expressed as mean±SD.
PVC200: 200 mg/kg bw per day of PVC
PVC1000: 1000 mg/kg bw per day of PVC

Table 3. Effects of PVC and vitamin E on biochemical indices of testicular tissue in studied groups

Groups	Variables		
	MDA concentration (nmol/g tissue)	Catalase activity (U/g tissue)	TAOC (mmol/g tissue)
Control group	0.37±20.95	0.65±11.30[b]	6.11±267.68[b]
Vit. E	4.11±21.62	0.58±18.77[a]	11.80±333.43[a]
PVC 200	0.39±29.59[ab]	0.51±6.38[ab]	7.60±185.95[ab]
PVC 1000	2.12±38.00[ab]	0.55±5.96[ab]	6.94±155.84[ab]
PVC 200 + Vit. E	1.34±23.92[a]	0.31±15.71	4.97±328.64[a]
PVC 1000 + Vit. E	0.76±26.52[ab]	2.47±12.53[b]	11.15±291.02[b]

a and b indicate a significant difference ($p<05$) compared to the control and vitamin E groups, respectively. Data are expressed as mean±SD.
PVC200: 200 mg/kg bw per day of PVC
PVC1000: 1000 mg/kg bw per day of PVC

Discussion

The present study showed the destructive effects of PVC on body weight, testicular weight, and some histological parameters in adult rats. Vitamin E managed to decrease the toxic effects of PVC to some extent.

The effects of PVC on the testicular tissue can be explained via the induction of oxidative stress and reduction of the activity of antioxidant enzymes (31). Oxidative stress can be highly damaging to tissues like testes which have a high level of metabolism and cell proliferation. Therefore, the antioxidant capacity of this tissue is very important (32). As the oxidant/antioxidant balance in testicular tissue is destroyed, key processes including apoptosis, spermatogenesis, and steroidogenesis are disrupted. By damaging DNA genome and increasing the expression of apoptosis proteins, oxidative stress can lead to gamete death, spermatogenesis disorder, and Leydig cell apoptosis (9). Exposure to PVC fragments testicular DNA and causes apoptosis in rat testes (33). Thus, the effects observed in this study can partly be attributed to the oxidative stress caused by PVC. Results showed that the administration of PVC has destructive effects on certain histological parameters. The diameter of seminiferous tubules is among parameters sensitive to toxic materials (24). By administering high and low doses of PVC, a significant reduction was observed in the diameter and height of germinal epithelium in seminiferous tubules of testes. A direct relationship usually exists between the diameter of seminiferous tubules and testicular spermatogenesis (35).

In the present study, SPI and TDI were used as indicators for evaluating spermatogenesis. Results indicated that the administration of PVC leads to disorders in spermatogenesis and removes germinal cells. With its high mitosis activity, the spermatogenic cycle is targeted by agents with cellular toxicity (36). Also, the present study showed that PVC reduces the number of Leydig cells in experimental groups. Research shows that cell membranes are destroyed by an increase in oxidative stress (31). Thus, PVC has probably destroyed the membranes of Leydig cells by its oxidant effect, leading to degeneration in these cells. According to studies, testosterone has a significant role in maintaining testes (37). Since testosterone is produced by Leydig cells in testes, its level is decreased in testicular tissue as a result of Leydig cell destruction (38). Therefore, a disruption in testosterone level causes a disorder in the process of spermatogenesis (37). Since the presence of sex hormones is necessary for increasing tissue mass, testosterone reduction will definitely reduce testicular weight. Exposure to PVC can cause oxidative stress and reduce antioxidant activity, thereby reducing the weight of animals (39). The observed weight reduction in rats may be related to the reduction in testosterone. This hormone plays a role in increasing weight and muscle growth (40). In fact, testosterone and its derivatives are used by athletes for bodybuilding (41). Results of the present study are consistent with those of some other studies on adult rats (42). Treating adult rats with PVC reduced the volume of seminiferous tubules; increased the diameter of the lumen; reduced the diameter, height, and thickness of seminiferous tubules' basement membrane; and decreased the number of Leydig cells. The reduction in testosterone in testes as a result of Leydig cells' oxidative destruction may affect the process of spermatogenesis and reduce the production of sperm in seminiferous tubules.

In the present study, vitamin E in PVC + vitamin E groups improved the diameter of seminiferous tubules, thickness of basement membrane, height of germinal epithelium of seminiferous tubules, SPI, TDI, and the number of Leydig cells. These findings are in line with previous reports showing that vitamin E as a strong antioxidant can prevent the effects of oxidative stress caused by chemical materials such as cadmium (43), tobacco smoke (44), and para-nonylphenol (45) in testicular tissue. Also, vitamin E inhibits the oxidative stress induced by para-nonylphenol (46) and inhibits the peroxidation of fats in testicular mitochondria and microsomes (47).

The superoxide dismutase (SOD) and CAT enzymatic system form the first line of defense in cells against the toxicity caused by free radicals. SOD turns superoxide radicals into H_2O_2. Then, CAT with a high electron affinity reacts with H_2O_2, neutralizes its toxicity, and turns it into H_2O and O_2 (15, 48). The present study showed that the administration of PVC reduces the activity of catalase and the TAOC of testicular tissue. Therefore, with the reduction in catalase activity, the high concentration of H_2O_2 in testicular tissue induces tissue damage and causes oxidative stress. It seems that vitamin E can prevent the reduction in catalase and TAOC of testicular tissue.

As an oxidative stress index, MDA is the final product of lipid decomposition. Elevation in the level of MDA indicates an increase in lipid peroxidation and cellular membrane damage (49). In the present study, the administration of PVC significantly increased the level of MDA in testicular tissue, indicating the increase in lipid peroxidation and oxidative stress in this tissue. Previous studies have also reported a significant increase in MDA level as a result of PVC administration (31).

Other studies suggest that the administration of vitamin E increases the level of antioxidants in testicular tissue (50). Moreover, a study in 2010 confirmed the increase in antioxidant level in testicular tissue following the administration of vitamin E (19).

As a strong, lipophilic antioxidant, vitamin E is necessary for maintaining the process of spermatogenesis in mammals (51). The effect of vitamin E in preventing damage to the germinal epithelium of seminiferous tubules may be related to its role in stabilizing cell membranes, because this vitamin maintains the bond between Sertoli cells and gametes (52). Moreover, by increasing antioxidant enzymes and decreasing the production of reactive oxygen species (41), vitamin E protects testes from lipid peroxidation, regulates testosterone level, and improves testicular tissue structure and spermatogenesis by affecting the cells in this tissue.

Conclusion

The present study showed that PVC can induce toxicity in the testicular tissue of adult rats, and vitamin E as a strong antioxidant can counter the undesirable

effects of PVC on the testicular tissue. Considering the results of this and other studies, it can be concluded that the undesirable effects of PVC on the testicular tissue of rats are mainly the result of the induction of oxidative stress by this pollutant in testicular tissue. Thus, vitamin E can be introduced as a strong antioxidant to inhibit testicular toxicity caused by PVC.

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