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Research Article

Effects of Vitamin E on Morphometric Parameters of Testes of Albino Rats Exposed to Tobacco Smoke

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Abstract

The purpose of this study was to investigate the structure of the testes of Albino male rats at the macro and light microscopy level following exposure to cigarette smoke. In addition, the effect of vitamin E on the structure of the testicles of rats exposed to tobacco smoke was investigated. Eighteen rats divided into 3 subgroups were exposed to smoke in a smoking machine for 15 min once daily for 15, 30 and 60 days. Other 18 rats exposed to tobacco smoke received injections of vitamin E. Control group which also included eighteen rats was placed in the smoking machine for the same time but were exposed to room air. The present study showed that exposure to tobacco smoke resulted in a decrease in body weight of animals where an increase of body weight was observed in animals supplemented with vitamin E while exposed to tobacco smoke. However, the latter was not significant. The length and width of the testicles, as well as their weight decreased under the influence of tobacco smoke. Accordingly, their volume also decreased. Histological changes of testicular tissue of the rats that exposed to the tobacco smoke showed thickening of the basal membrane and reduction in diameter of seminiferous tubuli. Some of the seminiferous tubules showed distorted germinal epithelium with its vacuolization and stopping the development of germinal cells. The present results showed that giving vitamin E smoking exposed animals, resulted in a decrease in the morphological and histological changes of testes. So, this data was similar to control. The present work showed that vitamin E can't completely prevent the effects of tobacco smoke but approximates macro and histological parameters to the control.

Keywords: *Anatomy; Rat; Testis; Tobacco Smoke; Vitamin E*

Abbreviations

BM: Basal Membrane; BV: Blood Vessel; Es: Eosinophilic Substance; GE: Germinal Epithelium; L: Lumen of Seminiferous Tubule; Lc: Leydig Cells; RBCs: Red Blood Cells; ST: Seminiferous Tubuli; Sz: Spermatozoa; TBM: Thickness of Basal Membrane; TD: Tubular Diameter; TE: Thickness of Epithelium; Vs: Vacuoles

Introduction

Tobacco consumption is directly responsible for nearly 6 million deaths annually, and a further 600 000 people die each year from exposure to second-hand smoke [1]. Tobacco is killing 1 in 10 adults worldwide and its quantity of consumption is increasing globally especially in developing countries according to WHO statistical data [2]. Many studies published during the past years indicated that tobacco smoking is one of the greatest risk factors of more than 60% noncommunicable diseases [3].

Tobacco smoke is a rich source of oxidants and reactive oxygen species. It has been argued that the increased production of reactive oxygen species associated with smoking may exceed the capacity of the oxidant defense system, resulting in oxidative damage to selected proteins, lipids, and DNA [4,5]. Cigarette smoke is a complex mixture of more than 4000 different compounds that are inhaled with each breath while smoking. These constituents include many that are known to be pharmacologically active, toxic, mutagenic, and carcinogenic. Some of these agents are nicotine, tar, cadmium, carbon monoxide, carbon dioxide, cyanides, various hydrocarbons [6].

Many workers have proved that Vitamin E is quite an effective antioxidant which protects rabbit testis against lipid peroxidation, where testosterone-induced lipid peroxidation could be improved by additional vitamin E treatment [7]. Furthermore vitamin E prevents nonylphenol-induced oxidative stress in testis of rats [8].

Objective of this Study

The objective of this study was to assess the effect of cigarette smoking on structure of testes of the animals and to recognize the possible prophylactic role of the Vitamin E in reducing the effect of cigarette smoking.

Materials and Methods

The method used to evaluate the effects of both cigarette smoke and vitamin E was proposed by Simani., *et al* [9]. In this study, a modified Walton smoking machine was used to generate smoke [10]. Exposure to cigarette smoke was carried out once daily. The rats were placed in a clear chamber connected to the smoking device and subjected to the cigarette smoke. Smoke puffs were drawn from a cigarette by vacuum and then blown inside the chamber. Smoke was released at the rate of 3 cigarette/15 minutes until the end of the study period. The injections of vitamin E were carried out once daily 30 minutes before the start of the exposure to tobacco smoke until the end of the experiment time. The control rats were exposed to normal room air with the smoking machine in a similar fashion.

Animals

The study comprised 54 adult Albino male rats aged 3 months. Body weights of animals at the start of the study ranged from 130 to 150 g. Rats were selected and bred in Laboratory of Lugansk State Medical University, Ukraine. Animals were then assigned to three study groups. Each group consisted of 18 rats and was divided into three subgroups (1^{st}, 2^{nd} and 3^{rd}) depending on the time of exposure (15, 30 and 60 days respectively). Each subgroup consisted of six rats. Group 1 is **control** group. Group 2 (smoking group) includes animals that were exposed to tobacco smoke. Group 3 (vitamin E and Smoking treated group) consisted of animals that were under the influence of tobacco smoke and along with it received vitamin E. The animals were sacrificed by ether anesthesia next day after last exposition, dissected and the testes removed, measured and fixed in 10% formaldehyde.

Housing

Rats were housed in groups of 6 in wire-mesh cages in an environmentally controlled room (22^0C, 30% - 70% relative humidity, 12-h light/dark cycle). Feed and drinking water were provided ad libitum. Rats received standard rat maintenance diet and community tap water ad libitum.

Parameters Evaluated

Survival

Animals were observed twice daily for mortality.

Clinical Observations

Rats were observed once daily. Detailed clinical observations were carried out once weekly during the treatment period.

Measurement of Body and Organ Weights

Rats were weighed twice weekly. Mean body weights and mean body weight changes were calculated throughout the study, and final body weights were recorded on the day of scheduled necropsy.

The volume of the testis was calculated using the formula: ($width^2 \times length \times \pi/6$) [11]. The gonad index was calculated using the formula: (testis weight (g)/body weight (kg) × 100).

Sizes of Testes

Organs were photographed. After that their length and width were measured using ImageJ software.

Hematoxylin-Eosin Staining

The testes were fixed in Bouin's solution, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Sections (4 μm thick) were mounted on glass slides and stained with Karazzi' hematoxylin and eosin.

Quantitative analyses

Quantitative analyses of seminiferous tubules of were performed in all rats. The seminiferous tubular and germinal epithelial parameters were analyzed quantitatively by light microscopy on at least 10 tubules with a round cross-section. The tubular diameter (TD), thickness of epithelium (TE) and thickness of basal membrane (TBM) were measured using micrometer eyepieces. The cell populations were assessed by counting the number of cell layers in each cross-sectioned tubule. The degree of spermatogenic maturation was evaluated.

Statistical Analyses

Statistical analyses were performed by Statistica 6.0 software and done by Student's t test.

Results and Discussion

Survival

The survival of animals in the experiment was 100%.

Clinical Observations

We noticed that, the rats were exposed to cigarette smoke in the smoking chamber, they appeared excited and crowded in the corners of the cage. After inhalation of the cigarette smoke the rats had become more difficult to handle being more aggressive, that manifested by lunge and bite attack, although, this response of the animals seemed to diminish gradually. Clinical signs observed in tobacco smoke treated rats from day 1 and throughout the study included yellow material on various body surfaces (most notably around the nose, mouth, forelimbs).

Body and Organ Weights

Body and organ weights of animals are shown in table 1. A significant reduction in the mean body weight was observed in the rats exposed to tobacco smoke during 30 and 60 days compared to those in control animals. Body weight of 3rd group animals increased in comparison with the group 2. However, these differences were not statistically significant. Absolute testicular weight of rats (group 2) was reduced, although statistical significance was only observed in the subgroup that exposed to tobacco smoke during 60 days (Table 1). Absolute testicular weight of the 3rd group rats was higher than data of the 2nd group, but lower than those of the control group. However, statistically significant differences were not found. There were no statistically significant changes in the gonadal index in all animals.

Groups	Subgroups	Body weight, g	Testis weight, g	Gonadal index, %
Group 1	1 (15 days)	163.17 ± 8.62	0.82 ± 0.04	0.50 ± 0.02
	2 (30 days)	185.33 ± 10.12	0.87 ± 0.03	0.47 ± 0.03
	3 (60 days)	193.67 ± 9.67	0.91 ± 0.03	0.46 ± 0.01
Group 2	1 (15 days)	156.17 ± 5.34	0.79 ± 0.02	0.51 ± 0.03
	2 (30 days)	171.33 ± 6.93*	0.84 ± 0.05	0.49 ± 0.02
	3 (60 days)	172.17 ± 8.28*	0.83 ± 0.04*	0.48 ± 0.03
Group 3	1 (15 days)	158.50 ± 10.61	0.81 ± 0.04	0.51 ± 0.02
	2 (30 days)	179.50 ± 7.13	0.85 ± 0.06	0.47 ± 0.02
	3 (60 days)	177.83 ± 9.72	0.88 ± 0.05	0.49 ± 0.03

Table 1: *The body, testis weights and gonadal index of rats of the studied groups (mean ± SE). Significant differences from the control group are denoted as * p < .05.*

Sizes of Testes

Photos of testes of different groups are presented in figures 1-3. Sizes of testes are shown in table 2. The effect of tobacco smoke leads to a decrease in the size of rat testes in all subgroups. But only in animals exposed to tobacco smoke for 60 days we noticed statistically significant changes. The testis volumes were significantly reduced in all subgroups rats exposed to tobacco smoke. Length and width of testis of the 3rd group rats was higher than data of the 2nd group, but lower than those of the control group. However, statistically significant differences were not found. But the testicle volume statistically significantly exceeded the values of the 2nd group.

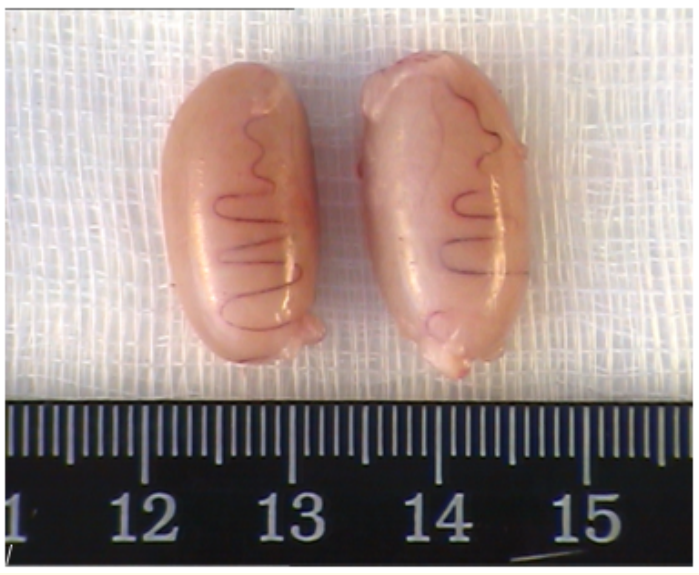

Figure 1: *Testes of control rat (3rd subgroup).*

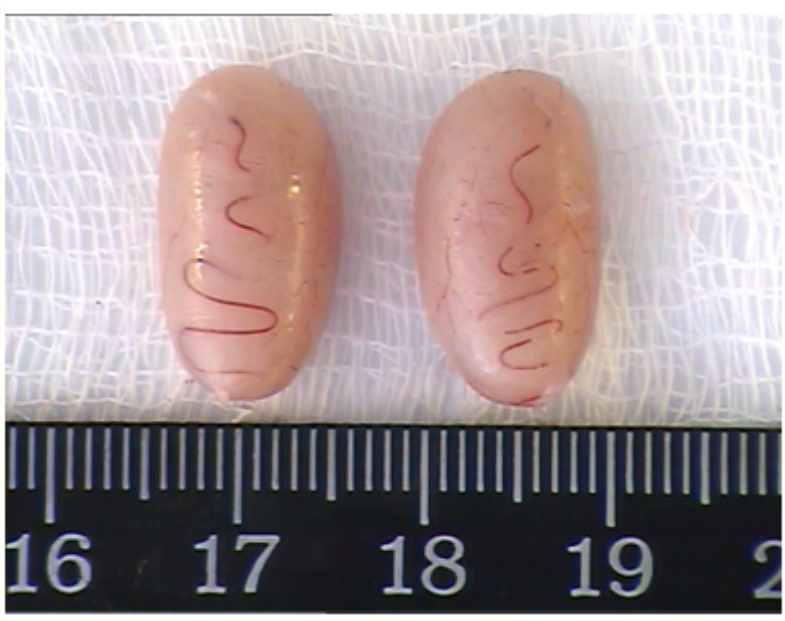

Figure 2: *Testes of rat exposed to tobacco smoke (3rd subgroup).*

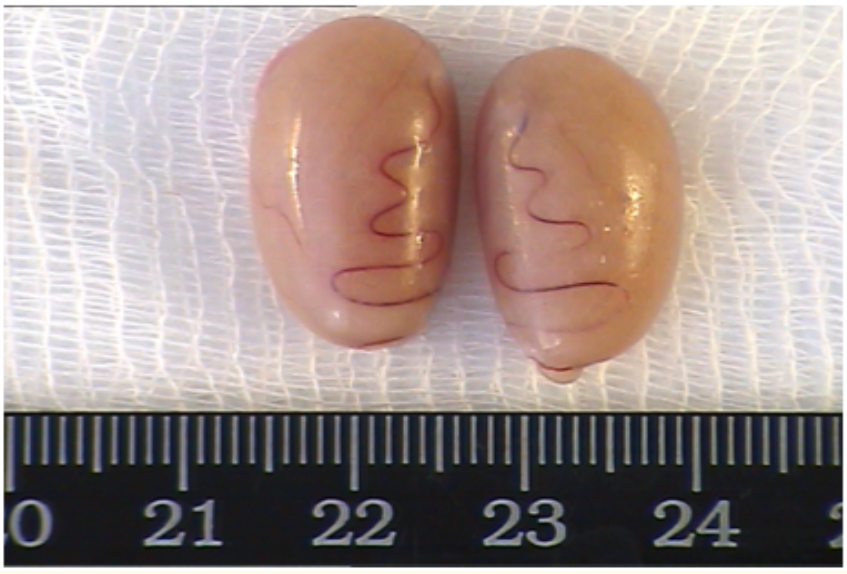

Figure 3: *Testes of rat exposed to tobacco smoke (3rd subgroup).*

Groups	Subgroups	Length, mm	Width, mm	Volume, µl
Group 1	1 (15 days)	15.76 ± 0.92	9.21 ± 0.04	698.31 ± 36.33
	2 (30 days)	16.43 ± 0.75	9.48 ± 0.06	771.42 ± 38.25
	3 (60 days)	17.50 ± 0.82	10.01 ± 0.07	932.86 ± 47.12
Group 2	1 (15 days)	15.17 ± 0.96	8.94 ± 0.05	633.81 ± 42.55*
	2 (30 days)	15.60 ± 0.70	9.14 ± 0.04	680.55 ± 28.61*
	3 (60 days)	16.04 ± 0.87*	9.44 ± 0.06*	747.60 ± 51.39*
Group 3	1 (15 days)	15.35 ± 0.93	9.12 ± 0.07	668.11 ± 36.02^
	2 (30 days)	16.02 ± 1.12	9.26 ± 0.05	717.74 ± 56.08^
	3 (60 days)	16.93 ± 0.91	9.82 ± 0.05	852.19 ± 49.37^

Table 2: *The size and volume of testis of the studied groups (mean ± SE)*
*Significant differences from the control group are denoted as * p < .05.*
Significant differences from the cigarette smoke group are denoted as ^ p < .05.

Histological Structure of Testis

Control group

Histological structure of testis of the control group was similar to normal structural pattern of seminiferous tubular cells and interstitial Leydig cells (Figure 4 and 5).

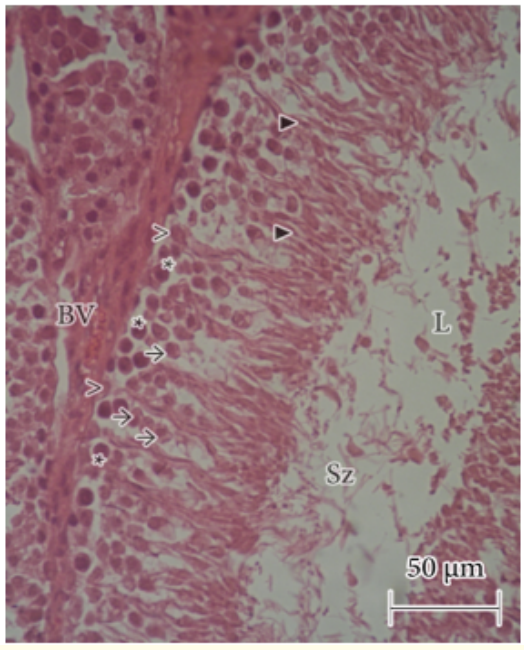

Figure 4: *A photomicrograph of a section in the testis of the control rat showing seminiferous tubule which contains spermatogonia (asterisk), spermatocytes (arrow), spermatids (triangle) and Sertoli cells (arrowhead). Sz: Spermatozoa; L: Lumen of Seminiferous Tubule; BV: Blood Vessel with RBCs (H&E).*

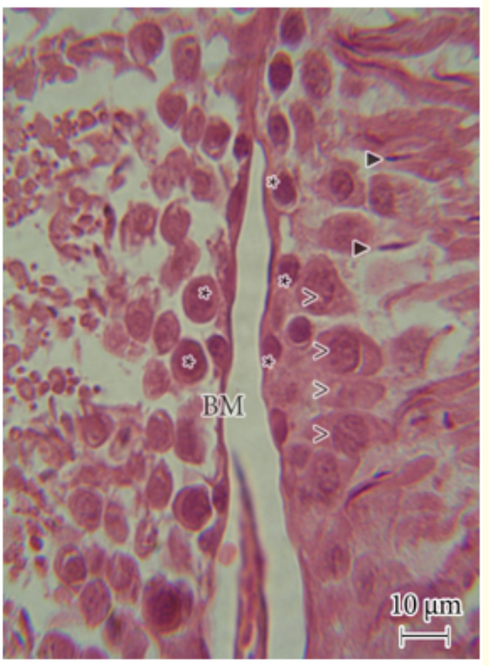

Figure 5: *A photomicrograph of a section in the testis of the control rat showing regions of two seminiferous tubules which contain spermatogonia (asterisk), spermatids (triangle) and Sertoli cells (arrowhead). BM: Basal Membrane (H&E).*

Tobacco smoke group

Compared to that of the control group lumen of seminiferous tubules was delayed and the overall number of germ cells in the tubules was reduced (Figure 6 and 7).

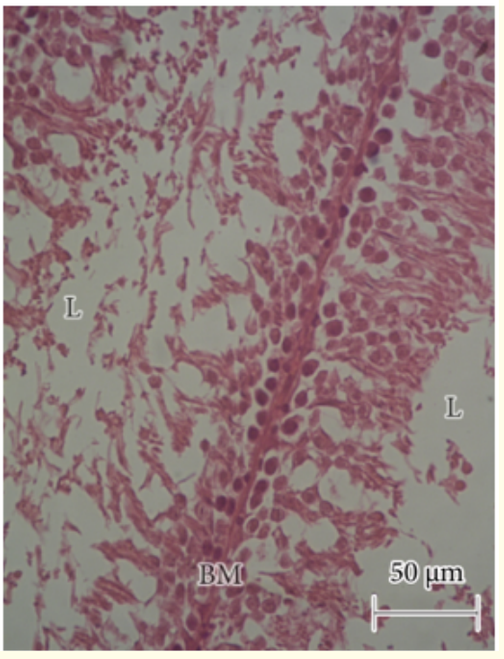

Figure 6: *A photomicrograph of a section in the testis of the rat exposed to tobacco smoke showing regions of two seminiferous tubules. The lumen of one of the tubes is significantly enlarged. Defective cells were fragmented and located in the lumen. The number of spermatozoa is reduced. L: Lumen of Seminiferous Tubule; BM: Basal Membrane (H&E).*

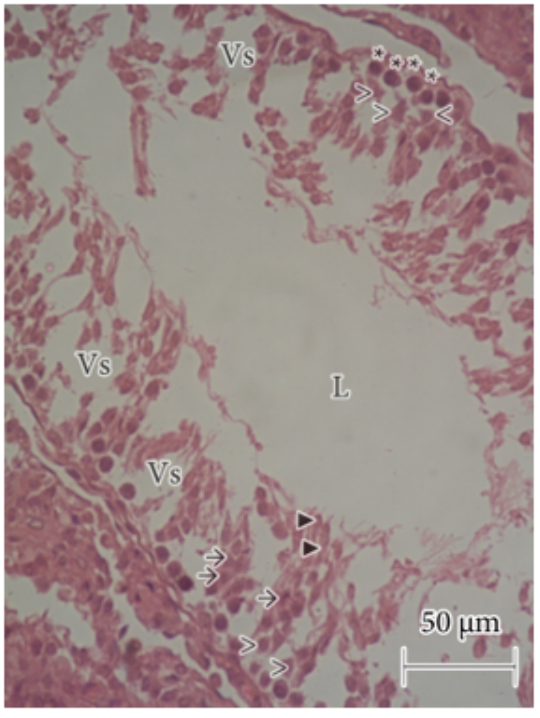

Figure 7: *A photomicrograph of a section in the testis of the rat exposed to tobacco smoke showing seminiferous tubule which contains spermatogonia (asterisk), spermatocytes (arrow), spermatids (triangle) and Sertoli cells (arrowhead). The epithelium contains many vacuoles (Vs). L: Lumen of Seminiferous Tubule (H&E).*

The epithelial cells were undergoing degeneration and necrosis. It was revealed vacuolization of cytoplasm and nuclear changes, such as karyolysis. In some tubules, there was a stop in the development of germ cells. Noticeable change was at the adluminal compartment of epithelium where the cells are at the stage of primary spermatocytes and spermatids. Defective cells were fragmented and located in the lumen of the seminiferous tubules (Figure 6).

The germinal epithelium contains many vacuoles (Figure 7). There is a decrease in the number of Leydig cells in interstitial tissue. These cells also show signs of degeneration. The interstitial tissue was filled with homogenous eosinophilic substance in the region of damaged tubules. The nuclei of Leydig cells were pyknotic. The blood vessels were dilated and filled with a large number of erythrocytes.

Tobacco smoke and vitamin E group

Our results showed that using vitamin E on rats exposed to tobacco smoke was lead to normal histological structure of testis. Cells of the seminiferous tubules, such as the cells of the germinal epithelium and Sertoli cells, were of normal shape and location (Figure 10). The cytoplasmic vacuolization of Sertoli cells was reduced. The number of Leydig cells increased under the influence of vitamin E. The lumen of the tubes decreases in comparison with that in animals exposed to tobacco smoke. We noticed that dilatation of blood vessels decreased. The basal membrane showed a slight thickening (Figure 8-10).

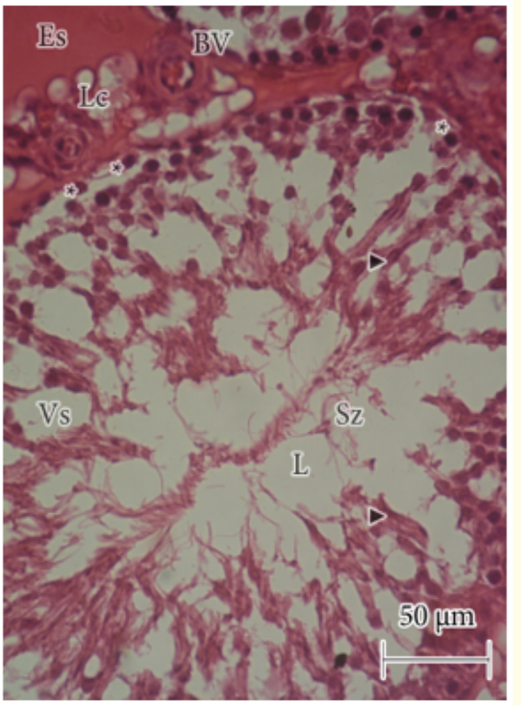

Figure 8: *A photomicrograph of a section in the testis of the rat exposed to tobacco smoke and treated with vitamin E showing seminiferous tubule which contains spermatogonia (asterisk), spermatids (triangle). The epithelium contains many vacuoles (Vs). However, their number and size decrease. The interstitial tissue contains Leydig cells (Lc) and eosinophilic substance (Es). Sz: Spermatozoa; L: Lumen of Seminiferous Tubule (H&E).*

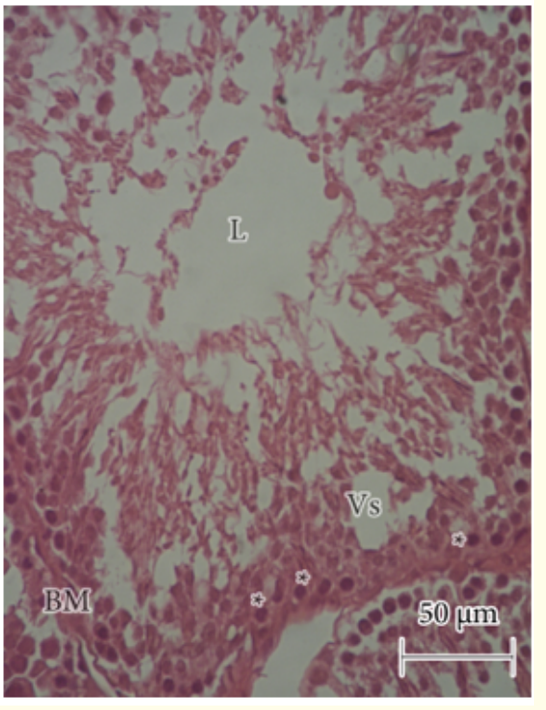

Figure 9: *A photomicrograph of a section in the testis of the rat exposed to tobacco smoke and treated with vitamin E showing seminiferous tubule which contains spermatogonia (asterisk). The epithelium contains few vacuoles (Vs). BM: Basal Membrane; L: Lumen of Seminiferous Tubule (H&E).*

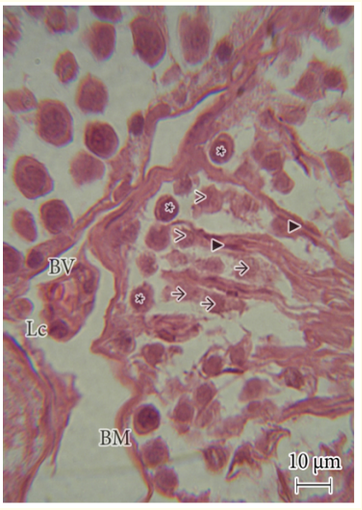

Figure 10: *A photomicrograph of a section in the testis of the rat exposed to tobacco smoke and treated with vitamin E showing seminiferous tubule which contains spermatogonia (asterisk), spermatocytes (arrow), spermatids (triangle) and Sertoli cells (arrowhead). Leydig cell (Lc) is located near blood vessel (BV) in the interstitial tissue. L: Lumen of Seminiferous Tubule (H&E).*

Morphometric Results

In our study tobacco smoke administration showed significant change in the mean diameter of seminiferous tubuli, thickness of germinal epithelium and thickness of basement membrane compared with the control group. We noticed significant changes in all subgroups of rats exposed to tobacco smoke except mean of thickness of germinal epithelium in 1st subgroup, although, this parameter was below control.

In this study shown that administration of vitamin E led to inhibit the fast reduction of diameter of the seminiferous tubuli and the thickness of the germinal epithelium. The thickness of the basal membrane under the influence of vitamin E decreased. We noted more significant changes of these parameters after 30 and 60 days from the start of the experiment.

Groups	Subgroups	Thickness of GE, µm	Diameter of ST, µm	TBM, µm
Group 1	1 (15 days)	78.25 ± 4.22	232.31 ± 12.77	3.81 ± 0.17
	2 (30 days)	80.71 ± 6.13	240.24 ± 16.28	3.66 ± 0.19
	3 (60 days)	79.38 ± 5.83	245.18 ± 15.33	3.74 ± 0.14
Group 2	1 (15 days)	76.10 ± 4.60	217.56 ± 12.72*	4.39 ± 0.18*
	2 (30 days)	73.72 ± 5.19*	218.19 ± 13.56*	4.40 ± 0.21*
	3 (60 days)	69.79 ± 4.82*	205.04 ± 11.08*	4.85 ± 0.27*
Group 3	1 (15 days)	78.52 ± 4.64	223.20 ± 13.05	4.15 ± 0.30
	2 (30 days)	77.20 ± 5.17	227.86 ± 15.19	4.09 ± 0.23^
	3 (60 days)	74.08 ± 6.38^	220.83 ± 14.06^	4.32 ± 0.27^

Table 3: *Results of the analyses of the morphometric parameters in the testis of the studied group (mean ± SE).*
GE: Germinal Epithelium; ST: Seminiferous Tubuli; TBM: Thickness of Basal Membrane.
*Significant differences from the control group (C) are denoted as * $p < .05$.*
Significant differences from the cigarette smoke group (S) are denoted as ^ $p < .05$.

Discussion

Tobacco smoke contains several hundred substances, including nicotine, carbon monoxide, and recognized carcinogens and mutagens, such as radioactive polonium, benzo(a)pyrene, dimethylbenz(a)anthracene, dimethylnitrosamine, naphthalene and methylnaphthalene [6]. It was demonstrated that systemic injections of nicotine reduced the reproductive capacity of male rats [12].

In this study, the pattern of decrease in body weight of the experimental animals, as recorded following the smoke exposure was similar to that recorded by Gentry-Nielsen., *et al.* [13] and the apparent gain in weight post treatment with Vitamin E. Our results agreed with Boitani., *et al.* [14] and Chandra., *et al* [15]. The cause of the demonstrated decrease in weight might be related to a diminished food consumption caused by developed anorexia or decreased utilization of food [13] or may probably be due to the modification of protein metabolism, as greater proportion of the amino acids of the food protein was thought to be used for energy production rather than for growth and this metabolic modification appeared to be related to a considerable extent to the nicotine content of the smoke [16].

Condorelli., *et al.* reported that nicotine suppressed sperm progressive motility in a concentration-dependent manner starting from the relative low concentration (1 ng ml^{-1}) *in vitro* [17]. This study indicated that nicotine may be considered as a toxic component of tobacco smoke that directly impairs male reproductive functions. Other studies revealed that nicotine could induce mouse Leydig cell apoptosis and inhibit androgen biosynthesis in rat Leydig cell [18], suggesting the possibility that nicotine may impaired male reproductive hormone system.

In testes from smoke-exposed rats the normal spermatogenic maturation sequence and mature spermatids were scarce, and many tubules contained spermatocytes with irregular nuclear membranes and abnormal mitotic forms characterized by enlargement and irregular chromatin accumulations of the nuclei. These results suggest that cells in the rat testis have the greatest sensitivity to the cytotoxic effects of cigarette smoke early in the maturation sequence. Similarly, Viczian [19] reported an increase in the number of morphologically abnormal sperm in cigarette smokers compared with nonsmokers. He suggested that the noxious agents generally exert their harmful effects mainly on primary spermatocytes. Because of the rapid rate of cell division, spermatogenetic cells of the germinal epithelium are more sensitive to gonadotoxins than Sertoli or Leydig cells [20].

Güven., *et al.* [21] found that degenerated spermatids sloughed off into the tubule lumen in association with the disintegrated microtubular network in Sertoli cells, raising the possibility that these phenomena are related. Based on ultrastructural studies, microtubules in the Sertoli cell cytoplasm conform with the shape of the developing spermatids [22]. Studies in mice have also shown that an increase in the number of abnormal forms might be expected after exposure to mitogenic agents [23]. More recently Sofikitis., *et al.* [24] concluded that the ratio of the acrosomal region to the surface area of the sperms' head were low in smokers. It was suggested that smoking caused alterations in the sperm cytoskeleton and the formation of morphologically abnormal sperms.

Cigarette smoke results an increase in the level of oxidants and a simultaneous decrease in the level of antioxidants [25]. It was found that cigarette smoke induce changes in the microcirculation of testes and other organs [26].

The present study showed that cigarette smoke caused a decrease in the interstitial tissue with reduced number of Leydig cells, followed by degenerative changes in the germinal epithelium. Our results were further strengthened by the work of Abdul-Ghani., *et al*. [27], who reported that a significant relationship exists between cigarette smoke and impaired testicular histology, which is also manifested as reduced diameter of seminiferous tubules and a decrease in the index of Sertoli cells in rats which is related to the reduction in sperm development process, however this can be generalized to human after careful histological studies.

We noticed that, the germ cells in the earlier stages of spermatogenesis were less affected by cigarette smoke than those in the later stages. This suggests that spermatogonia were more resistant to cigarette smoke toxicity that supported the suggestion of Aydos., *et al* [28].

Yue., *et al.* showed that vitamin E gave a positive role in improving semen quality via protecting testicular cell membrane and mitochondria from antioxidant abilities [29]. Also vitamin E supplementation was shown to increase weight of epididymis and the numeric density of convoluted seminiferous tubules reported by Hong., *et al.* [30], which is in agreement with what we found in our work.

Conclusion

The present study showed that exposure to tobacco smoke resulted in radical effects on the rats' testes and using vitamin E cannot completely prevent these effects of tobacco smoking on the testicular tissue, but it decreased to some extent the degenerative changes.

Conflict of Interest

The authors declare that there is no conflict of interest.

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