Research Article

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Defensive Role of Vitamin E Against Nitrosamine-Induced Harm to Testicular Tissues in Male BALB/C Albino Mice

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Abstract

Nitrosamines are toxic substances that induce oxidative stress. Oxidative stress results from an imbalance between the formation of free radicals (ROS) and their removal from the body and is a direct or indirect cause of a variety of diseases. The research aimed to assess vitamin E's potential protective role against nitrosamine-induced defect of testicular tissues . A total of forty Albino mice, weighing between 20 To25 grams and aged 25 to 30 weeks, were employed for the study. These mice Which are divided to five groups, each consisting of eight individuals. The initial group served as control, second group received a daily oral dosage of 0.05 mL/ g body weight of nitrosamine over one month. The third group was orally administered 0.1 mL/g of vitamin E. Groups IV and V were provided with vitamin E doses of 0.1 and 0.2 mL/g body weight orally Daily for a month, followed by a nitrosamine dose of 0.05 mL/g. Post the treatment period, blood samples were obtained, and the testis and epididymis tissues were subjected to examination. The study findings revealed notable alterations, encompassing the inhibition of spermatogenesis, reduction in diameters of seminiferous tubules within the testis and epididymis, as well as epithelial cell thickness. Additionally, severe necrosis and germ cell erosion were observed. In contrast, results from the vitamin E-treated animals exhibited favorable changes in the testicular and epididymis tissues when compared to the nitrosamine-exposed group and the control group. Moreover, histological assessments of animals administered a preventive vitamin E dose (0.2 mL/g) alongside nitrosamine indicated enhanced spermatogenesis, evidenced by augmented seminiferous tubule diameters and increased epithelial cell thickness within the testis and epididymis.

Keywards: Nitrosamine, Vitamin E, Spermatogenesis, Testis

الدور الوقائي لفيتامين E ضد الأضرار الناجمة عن النتروزامين لأنسجة الخصية في ذكور الفئران البيضاء BALB / C.

الخلاصة

النتروزامين هو عبارة عن مواد سامة محفزة للإجهاد التأكسدي حيث ينتج الإجهاد التأكسدي عن عدم التوازن بين تكوين الجذور الحرة (ROS) وإزالتها من الجسم حيث يكون سبب مباشر أو غير مباشر لمجموعة متنوعة من الأمراض .يهدف البحث إلى تقييم الدور الوقائي المحتمل لفيتامين E ضد السمية الناجمة عن النتروزامين. استخدمت في الدراسة أربعون فأرا ، تراوحت أوزانها بين 20 و25 غراما، وتراوحت أعمارها بين 25 و30 أسبوعا. تم تقسيم هذه الفئران إلى خمس مجموعات، كل مجموعة تتكون من ثمانية فئران. المجموعة الأولى كمجموعة سيطرة، بينما تلقت المجموعة الثانية جرعة يومية عن طريق الفم قدرها 0.05 مل / غرام من وزن الجسم من النتروزامين على مدار شهر واحد. المجموعة الثالثة أعطيت 0.1 مل/غم من فيتامين هـ عن طريق الفم. أعطيت المجموعتان الرابعة والخامسة جرعات من فيتامين هـ مقدارها 0.1 و0.2 مل/غم من وزن الجسم عن طريق الفم لمدة شهر واحد وبشكل يومي ، تليها جرعة نتروزامين قدرها 0.05 مل/غم. بعد انتهاء فترة التجريع تم التضحية بالفئران وأخذ عينات من انسجة الخصية والبربخ. اوضحت نتائج الدراسة عن تغيرات ملحوظة، بما في ذلك تثبيط تكوين الحيوانات المنوية، وانخفاض في أقطار الأنابيب المنوية داخل الخصية والبربخ، وكذلك سمك الخلايا الظهارية. وبالإضافة إلى ذلك، لوحظ نخر شديد وتنكس الخلايا الجرثومية. من ناحية أخرى أظهرت نتائج الحيوانات المعاملة بفيتامين E حدوث تغيرات إيجابية في أنسجة الخصية والبربخ مقارنة بالمجموعة المعرضة للنتروزامين والمجموعة الضابطة. علاوة على ذلك، أشارت النتائج النسجية للحيوانات التي أعطيت جرعة وقائية من فيتامين (0.2 E مل/غم) مع النتروزامين إلى تعزيز تكوين الحيوانات المنوية، كما يتضح من زيادة أقطار النبيبات المنوية وزيادة سماكة الخلايا الظهارية داخل الخصية والبربخ.

Introduction

Nitrosamines are substances formed and present in the human environment that promote the formation of free radicals that can lead to oxidative stress, undermine the antioxidant protection of cells and cause cellular injury, all of which can lead to cancer initiation [1]. It is a type of disease in which cells are characterized by the ability to divide uncontrollably and spread within the human body, either by invading and growing into neighboring tissues, or they can be transmitted to distant tissues through a malignant tumor that is transmitted through the Also can transmit by transcoelomic and direct seeding [2]. It is difficult to discover the underlying cause of any type of cancer and the reason behind this difficulty is that cancer cells are actually affected by many extracellular and intracellular factors [3]. These factors that lead to an increased risk of cancer include genetic factors, obesity, and ultraviolet radiation, in addition to a lifestyle that includes poor eating habits [4] and various biological factors [5]. As for the chemicals that contain alcohol and tobacco, in addition to the preservatives that are added to fish, meat, and food products, these substances are polycyclic aromatic hydrocarbons and nitrosamines [6].

Nitrosamines are carcinogenic compounds with high potential and functions and are found in many food products [7]. It appears mainly during the storage process, including meat and fish [8]. Nitrosamine-induced injuries are due to nitrites and secondary amines [9]. Nitrite turns into nitrosamines inside the body in cases of acidic stomach [10]. This substance induces oxidative stress and increases the production of free radicals that cause DNA damage and lipid oxidation [11]. Its effect on organs, including the male reproductive system, nitrosamines cause testicular damage and necrosis or degeneration of the seminiferous epithelium [12]. The body's cellular defense system is completely unable to prevent free radical damage, especially in acute cases, so the use of antioxidant agents helps reduce damage and prevent diseases resulting from it [13]. Therefore, vitamin E is a fat-soluble antioxidant present in semen that inhibits free radicals, inhibits the production of lipid peroxides and peroxides, protects sperm from damage and also protects testicular ROS [14]. It plays an essential role in spermatogenesis [15].

Material and Methods

Dosage preparation of nitrosamine and vitamin E

Within the scope of this experiment, nitrosamines were used as an oxidative stress inducing agent, The dose was carefully prepared and then administered to the animals orally ,As for vitamin E, the standard preparation technique recommended by the British Pharmaceutical Society was used to obtain it.

Experimental Design

Forty male albino mice weighing 20–25 g and aged 25–30 weeks were used. It was obtained from the Ibn Sina Center in Baghdad and raised in the Animal House at the College of Veterinary Medicine - University of Fallujah. Mice were distributed randomly in plastic cages with 8 animals per cage. They were provided with the same environmental conditions regarding temperature, light, and food, with a room temperature of 20–25 °C, a light duration of 14 hours, and 10 hours of darkness. The floor of the cages was covered with softwood sawdust, and the cages were cleaned and disinfected regularly. The water bottles and the animal house were also kept clean, and the mice were fed a complete and ready-made diet produced by the General Company for Animal Feed, which consisted of wheat flour, corn, barley, bran, calcium, iodized

salt, milk, and Calvo Tonic. Water was provided to them continuously during the experiment. The Animal was divided into five groups:

- T1: the negative control group, taken daily in a dose of 1 ml of distilled water.
- T2 :was the positive control group, given daily oral nitrosamine at 0.05 ml/g.
- T3 : a daily dose of vitamin E at 0.1 ml / g for a month.
- T4 : a daily dose of vitamin E at 0.1 ml/g for a month, followed by a dose of nitrosamine at 0.05 ml/g daily for a month.
- T5 : a daily dose of vitamin E at a dose of 0.2 ml/gm for a month, followed by a dose of nitrosamine at a dose of 0.05 ml/g of body weight daily for a month

Histopathological Examination

For the purpose of histopathological analysis, the mice were dissected subsequent to being Euthanized using chloroform. the tests and epididymis were meticulously removed and subsequently rinsed with a saline solution, as per the protocol outlined by Bancroft and Gamble [16]. These excised tissues were then subjected to thorough examination and visually documented through photography.

Statistical analysis

The data were analyzed statistically using the analysis of variance table, using the statistical program GenStat- Tenth Edition Version – 10.3.0.0, and the significant differences of the means were tested using the T-test at a probability level ($P \leq 0.01$). The strength and relationship between the variables were measured through a correlation coefficient Pearson for the link. Means were also compared using standard error in addition to Duncan's-multiple test for significant significance among the variables. At a significant level ($P<0.01$) and ($P<0.05$).

Results and Discussion

1. Testes

The histological examination results of the testis of control group revealed a normal tissue composition, consisting of seminiferous tubules and Leydig cells, as depicted in Figure 1.

The histological examination results of the testis in mice treated with nitrosamine at a concentration of 0.05 ml/g through daily oral administration for a duration of 30 days (T2) demonstrated the occurrence of suppressed spermatogenesis. Additionally, a reduction in the thickness of the germinal layer was observed, as illustrated in Figure1(a). The attributed cause of nitrosamine toxicity lies in its metabolism by cytochrome P-450 enzymes, which biologically activate it through alkylation. This activation leads to the formation of radical metabolites, which, derived from nitrosamine, are major catalysts for carcinogenesis. These derivatives tend to interact with various cellular components such as lipids, proteins, and nucleic acids, inducing cellular damage (17). Free radicals produced by P-450-dependent enzymes elevate oxidative stress by generating H202 and the superoxide anion - O2 (18). The increased production of H_20_2 and - O_2 diminishes antioxidant defense mechanisms and generates excess hydroxyl radicals (OH), thereby intensifying lipid peroxidation and causing membrane damage in cells. Consequently, nitrosamine may induce oxidative stress and tissue damage, including oxidative degradation of spermatozoal lipids, disrupting the lipid matrix structure in sperm membranes, reducing sperm motility, and increasing morphological defects within the midpiece(19).

The findings of this study are in agreement with (20), who observed reduced

sperm counts and histological alterations upon administering nitrosamine to mice.

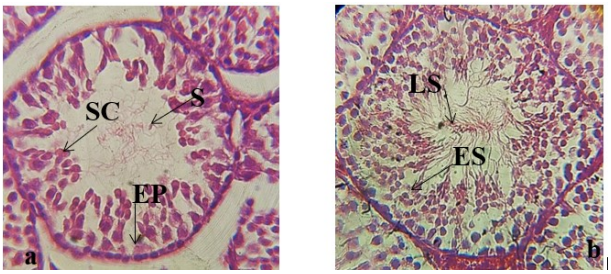

Figure 1 (a) : Histological section of a testis of a mouse from the control group, which shows the normal shape of the testis in which the presence of(S) spermatozoa, the layer of(EP) epithelial cells and(SC) Sertoli cells (H & E X 400), photo No. (b) notes the changes in the testicular tissue in the group treated with nitrosamines, where There is (LS) alack of spermatogenesis, degeneration and a decrease in the thickness of the(EP) epithelial cells (H&E X 400).

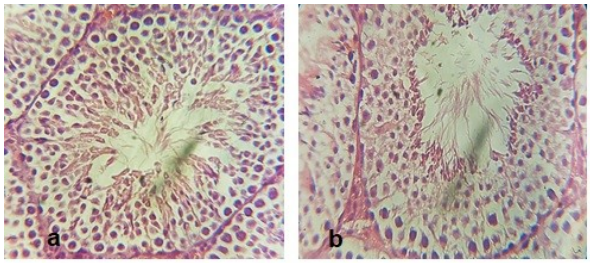

Figure 2 (a): Histological section of testis of a mouse in the group treated with vitamin E (T3), (b): A histological section of the testis of a mouse in the (T4) group that was dosed with vitamin E at a concentration of (0.1 ml / g) followed by nitrosamines, where the normal shape of the testis is observed (H & E X 400).

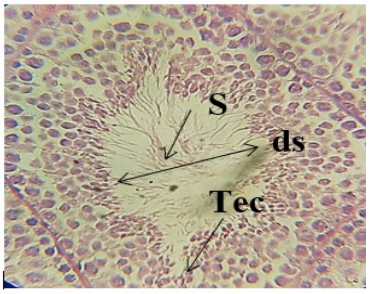

Figure (3): Histological section of a mouse testicle in the (T5) group treated with vitamin E at a concentration of (0.2 ml/g) and followed by nitrosamine, which includes the normal shape of the testis that shows its cavities filled with (S)sperm with an increase in the(ds) diameter of the seminiferous tubules and the(Tec) thickness of the epithelial cells (H & E X 400).

The histological examination results of the testis in the treated mice, which were administered vitamin E at a concentration of 0.1 ml/g daily for a duration of 30 days (T3), revealed the presence of natural tissue with intact seminiferous tubules characterized by proper basal membranes, normal spermatogenesis, and normal Leydig cells (Figure 2 a). This favorable outcome can be attributed to the antioxidative properties of vitamin E, a commonly occurring antioxidant in biological membranes. Vitamin E inhibits oxidative stress and oxidative damage membranes. Moreover, it is a fundamental component of the antioxidant system in sperm cells (21). promotes sperm formation through the inhibition of lipid peroxidation, and safeguards germ cells from oxidative damage due to excessive ROS production (22).

The study findings are consistent with those of Malmir *et al* (23), This indicates that the mice given vitamin E did not show any histological changes in the testicular tissue. When examining the testicular tissue of mice exposed to vitamin E at a concentration of (0.1 ml/g, 0.2 ml/g) daily for 30 days, followed by exposure to the carcinogen nitrosamine at a concentration of 0.05 ml/g daily for 30 days (T4 and T5), respectively. Normal testicular tissue was observed without any histological changes with intact seminiferous tubules and Leydig cells, and the cells did not show any histological changes compared with the nitrosamine-treated group (T2) and the control group (T1), as shown in Figures (4 and 5) respectively.

The reason behind these findings can be attributed to the toxic effects of nitrosamine, which produces reactive oxygen species (ROS) that target cell membranes, DNA, and tissue structures. As a result, germ cells become unable to perform their normal functions (24). Antioxidants inhibit oxidative mechanisms that lead to various degenerative diseases (25). Vitamin E protects cells from oxygen-derived

free radical damage and lipid peroxidation products (26). Vitamin E also reduces reactive oxygen species in the testicle and restores normal testicular function (27) by inhibiting lipid peroxidation in the sperm membrane, thus improving sperm motility and formation (28).

These findings align with the results of (29), who observed that co-administration of sodium nitrite, a precursor of nitrosamine, with ascorbic acid (vitamin C) improved reproductive performance, sperm formation, and histological alterations in testicular tissues. The study results are also consistent with (30), where nitrosamine administration was combined with Pentoxifylline, leading to increased sperm formation and maintenance of normal tubular architecture.

An increase in the height of the seminiferous epithelium

The results indicated statistically significant differences at the P<0.05 level in the average height of the seminiferous epithelial cells. The group (T5) that was administered vitamin E at a concentration of 0.2 ml/g daily for 30 days, followed by nitrosamine administration at a concentration of 0.05 ml/g daily for another 30 days, exhibited a superior performance with an average height of (233 ± 5.38 µm2) compared to group (T4), which was administered a concentration of 0.1 ml/g and subsequently nitrosamine, resulting in an average height of (211 ± 13.38 µm2), compared to the nitrosamine-treated group (T2), which demonstrated a decreased average height of the seminiferous epithelium (104 ± 3.06 µm2) compared to the control group (T1), as presented in the table 1 .

The seminiferous epithelium consists of a complex layered structure lining the seminiferous tubules. The basal region is connected to a fibrous connective tissue, while the deeper layer contains flattened myoid-like cells resembling smooth muscle. The seminiferous epithelium is comprised of supportive Sertoli cells and proliferative germ cells, which can be morphologically distinguished into spermatozoa (31) , The reduction in the height of the seminiferous epithelial cells.

Table 1: The effect of nitrosamines and the protective role of vitamin E on the testicle.

Transactions	Epithelial height (µm)	Seminiferous tubules µm)(
T1	147 ± 7.16 b	250.1 ± 6.44 ab
T2	104 ± 3.06 c	234.5 ± 4.36 a
T3	205 ± 13.4 a	269.4 ± 5.88 b c
T4	211 ±13.38 a	283.5 ± 11.72 c
T5	233 ± 5.38 a	293.6 ± 19.16 c
P- value	<.001*	0.002*
LSD	26.92	31.18

The means with different letters are significantly different from each other.

*(P<0.05)

2- Epididymis

The histological examination results of the vas deferens in the control group revealed its natural tissue composition. The cross-sectional view of the vas deferens appears with a long and highly convoluted tubule, whose function is the transportation of sperm. It is lined with a pseudostratified columnar epithelium, consisting of small basal cells, tall and steady columnar cells, and long steady stereocilia. Additionally, the vas deferens cavity appears normal and filled with sperm, as depicted in fig(4a). The histological examination results of the vas deferens in mice treated with nitrosamine at a concentration of (0.05 ml/g)

through daily ingestion for a duration of (30) days (T2) revealed the absence of sperm in the vas deferens cavities. This was accompanied by a reduction in the diameter of the vas deferens and in the thickness of the superficial layer. Furthermore, erosion in germinal cells and degeneration occurred, as clearly illustrated in fig (4b).

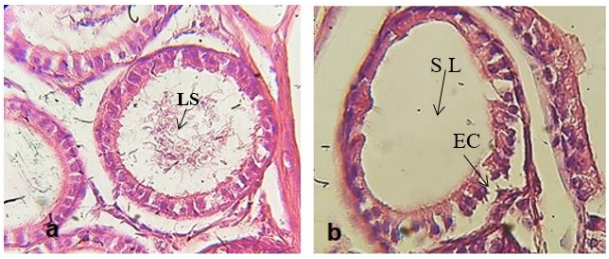

Figure (4)a histological section of a mouse epididymis from a control group, which shows the normal shape of the epididymis, in which the normal tissue of the epididymal canal is noted,(Ls) and the lumen is filled with sperm.(b) As for the nitrosamine group,(EC) degeneration of the epithelial cells and atrophy of the seminiferous tubules was observed and absence of (SL)spermatozoa in the lumen were noted. (H&E X 400).

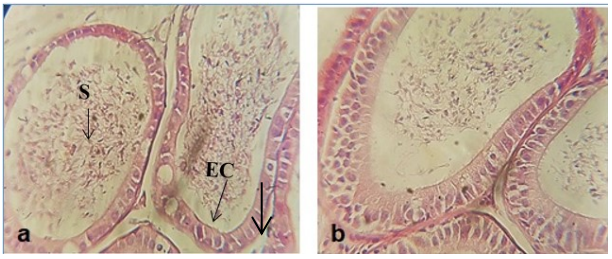

Figure (5) a: Histological section of a mouse epididymis in the group treated with vitamin E (((T3) showing the normal shape of the epididymis with cavities filled with(S) sperms with an increase in the diameter and thickness of(EC) epithelial cells (H & E X400), b: Histological section of a mouse epididymis in the group (T4), which was dosed with vitamin E at a concentration of (0.1 ml / g), followed by nitrosamines, where the normal shape of the epididymis was observed (H & E X 400).

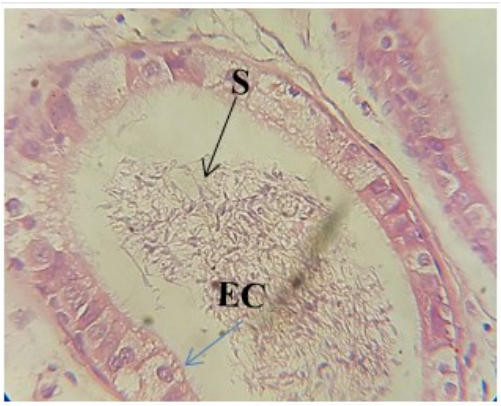

Figure (6) A histological section of a mouse epididymis in group (T5) treated with vitamin E at a concentration of (0.2 ml/g) and followed by nitrosamine, which includes the normal shape of the testis that shows its cavities filled with(S) sperm with an increase in diameter and thickness(ES) of epithelial cells (H & E X 400).

The damage to the tissues of the vas deferens and the impairment of sperm viability can be attributed to oxidative stress and the occurrence of lipid peroxidation, which result in harmful effects including a decrease in membrane fluidity and disruption of mitochondrial and collagen functions. These effects lead to the generation of free radicals and cell death due to toxic substances (32). Additionally, androgens' degradation causes various mechanisms involving the induction of toxicity by causing programmed cell death and degeneration in testicular tissue (33). The study results align with (34), where injecting rats with monosodium glutamate led to histological changes in the vas deferens, affecting its diameter and the thickness of its epithelial cells.

The results of the histological examination of the vas deferens in mice treated with vitamin E at a concentration of (0.1 ml/g) daily for 30 days (T3) showed that the vas deferens returned to its normal shape. This was accompanied by an increase in the diameter of the vas deferens and the thickness of the epithelial cells, as shown in Figure (5a). It was observed from the histological examination of the vas deferens in mice exposed to vitamin E treatment at concentrations (0.1 ml / g, 0.2 ml /

g) respectively daily for (30) days, then the carcinogenic substance nitrosamine was taken at a concentration (0.05 ml / g) daily for (30) days (T4 and T5) respectively, the normal histological state of the vas deferens was detected, which is characterized by an increase in the diameter of the vas deferens, and the thickness of the epithelial layer. cells, and the presence of sperms in the lumen, compared with the T2 group that received nitrosamine at a concentration of (0.05 ml/g), and compared with the control group (T1), as shown in Figures (5(b) ,6) respectively.

The reason for these effects can be attributed to vitamin E's role as an antioxidant, which works in harmony with the antioxidant system present in the vas deferens. This leads to enhanced sperm formation, preservation, and improvement of vas deferens tissue and the regularity of epithelial cells. Vitamin E prevents oxidative stress by inhibiting the production of lipid peroxides and scavenging free radicals (35). The study's findings also align with (36), where vitamin E usage protected vas deferens tissue and increased sperm production

Differences in vas deferens diameter and epithelial cell height were found to be statistically significant at the level of $P < 0.05$. The study results demonstrated superior outcomes in groups (T4) and (T5), which were treated with vitamin E at concentrations of (0.1, 0.2 ml/g) respectively, daily for a duration of (30) days, followed by administration of the carcinogenic substance nitrosamine at a concentration of (0.05 ml/g) for (30) days. These groups exhibited an increase in the average vas deferens diameter, with means of (458.8 ± 25.75 µm and 460 ± 17.58 µm) respectively, compared to the second group (T2), which received nitrosamine at a concentration of (0.05 ml/g) for (30) days, resulting in a decrease in the average diameter ((198 ± 8.41 µm) compared to the control group (T1), as shown in the table 1.

Similarly, significant differences in vas deferens diameter were observed in group (T3), which received vitamin E at a concentration of (0.1 ml/g) daily for a duration of (30) days, with an increase in the average diameter (275.5 ± 10.97 µm) compared to the control group. Furthermore, the results indicated statistically significant differences at $P < 0.05$ in the average height of the vas deferens epithelial cells. Groups (T4) and (T5), which were treated with vitamin E at concentrations of (0.1, 0.2 ml/g) respectively, daily for a duration of (30) days, followed by administration of the carcinogenic substance nitrosamine at a concentration of (0.05 ml/g) for (30) days, showed an increase in the average thickness of the vas deferens epithelial cells, with means of (78 ± 2.9 µm and 64.4 ± 5.19 µm) respectively, compared to group (T2) that received nitrosamine (0.05 ml/g) for (30) days, showing a decrease in cell thickness ((33 ± 1.53 µm) compared to the control group (T1), as presented in the table 2.

Moreover, there were significant differences in the average thickness of the vas deferens epithelial cells in group (T3), which received vitamin E at a concentration of (0.1 ml/g) daily for a duration of (30) days, with an average thickness of (54.4 ± 2.63 µm) compared to the control group.

The decrease in vas deferens diameter and epithelial cell thickness can be attributed to tissue damage in the vas deferens due to interrupted blood supply resulting from the disruption of the vasculature's endothelium in the branches of the internal spermatic artery supplying the testis and its accessories. This leads to degeneration and disintegration of the epithelial cells lining the tubules. Alternatively, it could be due to decreased androgen levels, as the vas deferens relies on androgens, which are vital for the differentiation and proliferation of the epithelial cells lining the tubules, as these

cells have sensitive androgen receptors (37).

The increase in vas deferens diameter and epithelial cell thickness can be attributed to vitamin E's ability to prevent oxidative stress resulting from the imbalance between antioxidants and free radicals. Vitamin E allows free radicals to extract hydrogen from antioxidant molecules rather than unsaturated fatty acids, breaking the chain reaction of free radicals and preventing cell damage (38). Additionally, vitamin E plays a role in testicular development by increasing the diameter of spermatic tubules and the thickness of vas deferens epithelial cells, as well as sperm density(39). The study's results align with (40), where the use of vitamin E improved the tissue changes caused by carcinogenic substances

Table 2: The effect of nitrosamines and the protective role of vitamin E on the epididymis.

Studied Traits	Epithelial height (µm)	Epididymis diameter (µm)
T1	43 ± 1.53 d	267 ±5.97 b
T2	33 ± 1.53 e	198 ±8.41 c
T3	54.4 ± 2.63 c	275.5 ±10.97 b
T4	64.4 ± 5.19 b	458.8 ±25.75 a
T5	78 ± 2.91 a	460 ±17.58 a
P- value	<.001*	<.001*
LSD	8.73	44.1

The means with different letters are significantly different from each other

. *(P<0.05)

Conclusion

The findings indicate significant alterations in testicular and epididymis tissues, with nitrosamine exposure resulting in inhibited spermatogenesis, reduced seminiferous tubule diameters, and epithelial cell thinning. Severe necrosis and germ cell erosion were also observed. However, the administration of vitamin E exhibited a favorable impact on testicular and epididymis tissues compared to both the nitrosamine-exposed group and the control group. Notably, histological evaluations of mice administered a preventive dose of vitamin E (0.2 mL/g) concurrently with nitrosamine demonstrated enhanced spermatogenesis, as indicated by enlarged seminiferous tubule diameters and increased epithelial cell thickness within the testis and epididymis. These findings underscore the potential of vitamin E in mitigating nitrosamine-induced testicular damage, suggesting its promising role as a protective agent against toxic insults in male reproductive tissues.

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Conflict of interest:

The authors declare that there is no conflict of interest

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