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Original Article

Does Vitamin E Protect the Male Reproductive System Against the Noise-Induced Stress?

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ABSTRACT

***Objective*:** To investigate how Tocopherols shield testis' histomorphology in adult rats subjected to noise stress.
***Study Design*:** Laboratory based-experimental study.
***Place and Duration of Study*:** National Institute of Health, Islamabad Pakistan, from Sep 2022 to Sep 2023.
Methodology: A total of 90 albino rats, with weights ranging from 250 to 300 grams, were included in the study. Three Groups were created: Group-A, Control, and Group-B, daily noise exposure (100 dB); Group-C was administered with Alpha-Tocopherol 40 IU/kg dissolved in 0.5 ml corn oil and subjected to noise (100 dB) daily. The seminiferous tubules' germinal epithelium's diameter and height were measured using Hematoxylin and Eosin and Periodic Acid Schiff staining.
Results: The study compared gross, microscopic, and biochemical parameters between the Experimental and Control Groups. Group A exhibited an average testicular volume of 1.82±0.05 gms, a mean tubular diameter of 253.72±4.46 µm, and a germinal epithelial height of 56.47±2.31µm. Group-B's testicular volume was 1.13±0.07 gms, accompanied by a decrease in tubular diameter to 182.15±5.05 µm and epithelial height to 36.80±2.79 µm and in comparison to Group-B, Group-C exhibited enhancements in terms of tubular diameter (251.86±2.00 µm, $p<0.001$) and epithelial height (55.63±2.02 µm, $p<0.001$), resulting in a reduced testicular volume (1.181±0.03 gms, $p<0.001$).
***Conclusion*:** Noise-induced histomorphological changes in the testis lowered sperm maturation in the germinal cells and significantly reduced testicular volume. Moreover, α-Tocopherol has a protective effect on histomorphological changes caused by noise stress.

Keywords: Alph-tocopherol, Antioxidant, Infertility, Noise Stress, Testosterone

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INTRODUCTION

Physical stressors include exposure to noise, electric foot shock, cold swimming, and constraint stress. Psychological stressors are mostly social and include sleep deprivation, mother separation, and isolation.[1] A growing body of research has identified environmental pollution and non-chemical stressors, such as exposure to traffic noise or mental stress, as major contributors to chronic non-communicable diseases (e.g. cardiovascular, metabolic, and mental). The most pernicious natural contaminant is noise.[2,3] It can weaken non-auditory systems and its effects on the auditory system.[4]

Reproductive activity is one of the primary processes impacted by the adaptive response to stress. Testicular tissue structure may be impacted by noise-induced stress, according to some research that evaluated testosterone and spermatogenesis levels, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), sperm count, morphology, and viability.[5] Increased apoptosis brought on by pathogenic stress (noise) and suppression of spermatogenesis kinetics have demonstrated how noise stress affects the endocrine or neural systems in humans that preserve the homeostasis of reproductive hormones (i.e., testosterone and LH). These findings suggest a connection between noise stress and male infertility.[6]

Tocopherols, or vitamin E, offer more benefits than just acting as an antioxidant in the diet, even though they are helpful in the prevention and treatment of infertility.[7] The documented lack of antioxidant properties of Tocopherols involves controlling the actions of transcription factors or altering enzymes in signal transduction pathways, particularly protein kinase, to affect various aspects of cellular responses such as survival, inflammation, cellular adhesion, migration, secretion, and immunity. The first line of the chain-breaking antioxidant found in the cell membrane is represented by Vitamin E (Tocopherol).[8] It is a well-known lipid-soluble antioxidant that inhibits

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reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) that can damage cell membranes, including the seminal membrane.[8,9]

Tocopherols as a dietary supplement may help prevent male infertility by enhancing the histomorphology of the testis in rats subjected to noise stress. The study aims to evaluate the histomorphological effects of noise stress on adult rat testes and determine whether Tocopherols can protect the testes from the effects of noise stress in rats.

METHODOLOGY

The laboratory based-experimental study was conducted in collaboration between the National Institute of Health (NIH), Islamabad and the Anatomy Department at Army Medical College, Rawalpindi Pakistan, from September 2022 to September 2023, after approval was obtained from the Ethical Review Committee (IRB 02/09/2022).

Inclusion Criteria: About 90 male Sprague Dawley rats are in good health, weighing 200 grams, and aged between 10 and 12 weeks were included.

Exclusion Criteria: Rats older than 10-12 weeks or weighed between 250-300g, had pre-existing health conditions or difficulties during the acclimatization process, belonged to a non-Sprague Dawley strain, had previously been exposed to noise stress, or had received therapy with Alpha-Tocopherol or antioxidants were excluded.

The rats were housed at NIH for one week throughout their acclimation phase and fed regular laboratory rat food and unlimited water. Soft gel capsules containing Alpha-Tocopherol were purchased from Nature's Bounty, Bohemia. There were 400 IU of Tocopherols in each gel. Thirty rats each were assigned to three Groups. Group A was assigned as the control Group. For 12 weeks, the experimental Group was subjected to a noise stress of 100 dB for a continuous 06 hours per day, from 9:00 am to 3:30 pm. An uninterrupted power source was offered with a nine-volt standby battery and DC adapter. The noise level was measured and maintained using a Radio Shack analogue model 33-4050 decibel meter. It was positioned beyond the cages. For 12 weeks, rats in experimental Group-C were given Alpha-Tocopherol by stomach tube at a 40 IU per kg body weight dose dissolved in 0.5 ml corn oil once daily and subjected to daily noise stress (as in Group-B). The average ± standard error body weight (g) of the animals was noted at the onset and conclusion of the experiment. The testis volume was measured using the Archimedes principle and the formalin displacement method.

After the 12-week experiment, the rats were euthanized. The testicles were removed with care, weighed, and dissected. Following tissue processing, slices five µm thick were cut and stained with PAS and H&E stains. Image J Version 1.49g, a morphometric computer program, was used to examine photomicrographs.

The diameter of the seminiferous tubule was measured between the outer edges of the basement membranes on one side and the other. Two sections were taken from every slide, and three were taken from every specimen. The mean of two testicles from each animal was then computed based on the average of six readings for each testis.

From the foundation membrane (foundation membrane provides stability and protection to a building's structure, the control Group A maintained stable and healthy baseline parameters against which the experimental Groups were compared). The seminiferous epithelium's height was measured to the seminiferous tubule's adluminal boundary. Every slide had two sections taken from it, and every specimen had three slides taken. The data analysis included many sequential processes. Initially, the researchers calculated the average measurements of two testicles from each rat by taking the mean of six observations for each testis. The average of each rat within each Group was computed to get the overall average.

The analysis was conducted using the Statistical Package for the Social Sciences version 22 (SPSS-22). Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Analysis of variance (ANOVA) was used to evaluate the variability within and between Groups. The *p*-value equal to or less than 0.05 was considered statistically significant.

RESULTS

The current study included 90 rats and classified them into three Groups: Groups A, B, and C were assigned as the control Group, the experimental Group exposed to noise stress, and the experimental Group with noise stress and Alpha-Tocopherol intervention, respectively. The study compared the gross, microscopic, and biochemical parameters between the Experimental and Control Groups. Table-I shows that the average testicular volume in Group A was

1.82+0.05 gms. In the control Group A of rats, the mean tubular diameter of the seminiferous tubules measured 253.72±4.46 µm (Table-II). In Group A, the germinal epithelium of seminiferous tubules in rat testis had an average height of 56.47±2.31 µm (Figure).

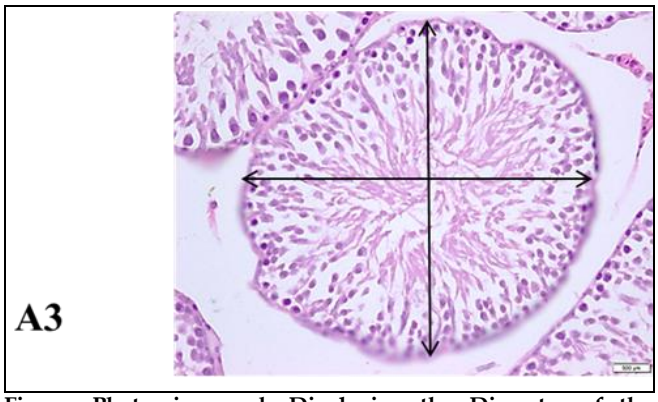

Figure: Photomicrograph Displaying the Diameter of the Seminiferous Tubules in A3 (Control). 40X H and E Stain

In Group B, the average testicular volume was 1.13±0.07, which is significantly less (*p*-value<0.001) compared to Group A (Table I). In Group B, the mean tubular diameter of the seminiferous tubules was 182.15±5.05 µm. Compared to Group A, Group B experienced noise stress, which resulted in reduced diameter of seminiferous tubules (*p*-value<0.001) (Table II). Compared to Group A, Group B's germinal epithelium of seminiferous tubules in rat testis had a mean height of 36.80±2.78 µm, which was less.

Table-I: Testicular Volumes in Experimental Groups B and C, and Control Group A (n=90)

Para-meters	Study Groups (n=30)	Mean±SE	*P*-value		
			Group A/B	Group A/C	Group B/C
Volume of Testis (gms)	A	1.82±0.05	<0.001	0.94	<0.001
	B	1.13±0.07			
	C	1.81±0.03			

1.181±0.03 was the mean testicular volume in experimental Group C. In comparison to Group B, Group C's testicular volume increased, and this difference was statistically significant (*p*-value<0.001). There was a statistically negligible difference (*p*-value>0.05) compared to Group A. The seminiferous tubules in Group C had a mean tubular diameter of 251.86±2.00 µm. When rats were exposed to noise stress, and Tocopherol was administered, the width of their seminiferous tubules increased compared to Group B. Comparing Group C to Group B revealed a statistically significant difference (*p*-value<0.001), while Group A, the control Group, showed no significant change (*p*-value 0.184) (Table-II). In Group C, the germinal epithelium of the seminiferous tubules in rat testis measured 55.63±2.01 µm in mean height. Rats subjected to noise stress with the administration of Tocopherol showed an improvement in germinal epithelium height relative to Group B. Comparing the Groups C and B, statistically significant variances were found between them (*p*-value<0.001).

Table-II: Comparing the Groups' Mean Seminiferous Tubule Diameters in the Rat Testis(n=90)

Parameter	Groups (n=30)	Mean ±SE	*P*-value		
			Group A/B	Group A/C	Group B/C
Diameter of seminiferous tubules(µm)	A	253.72 ±4.46	<0.001	0.184	<0.001
	B	182.15 ±5.05			
	C	251.86 ±2.00			

DISCUSSION

After the study, the study Groups' physical characteristics, such as testicular weight and volume relative to the tissue body weight index of the rat testis, were compared. Compared to Group B, the control Group's mean testicular volume was substantially higher. The fact that rats given Alpha-Tocopherol in addition to noise stress showed a substantial improvement in testicular volume compared to rats subjected to noise stress alone further demonstrated the protective effect of Alpha-Tocopherol. A mere decrease in testis volume can also be associated with generalized testicular damage, even if histological alterations are a more sensitive sign of testicular damage. According to this study, the loss of weight in the testicles and other sex organs might be attributed to either the direct impacts of noise pollution or a drop in testosterone levels. These results are consistent with those of a study by Checa *et al*., which found that decreased sperm count and disrupted spermatogenesis are the causes of the testis' declining weight and volume.[10]

Microscopic criteria such as germinal epithelium height and seminiferous tubule diameter were compared between Groups because proper testicular histology, particularly of seminiferous tubules, is directly linked to the normal spermatogenic function of the testis. The mean seminiferous tubule diameter was considerably lower in experimental Group B than in the control Group. Additionally, the rats receiving Tocopherol and subjected to noise stress had notice-

ably higher mean seminiferous tubule diameters. Sloughing off the epithelial cells explains this morphometric difference, showing gonadal toxicity through cell loss. These results were consistent with a study that found that adult male albino rats exposed to white band noise for six hours a day for thirty days caused chronic noise stress that resulted in testicular histological changes, including a decrease in spermatogenic cells and a moderate reduction in seminiferous tubule size with focal degeneration.[11-13] The morphometric data showed that this Group's seminiferous tubules had higher epithelial heights than Group B's. Tocopherol's antioxidant action on the testes may cause these observations.

The current findings were consistent with research that found that black seed oil protected and enhanced the functioning of the testicular accessory sex glands in rats subjected to hydrogen peroxide-induced oxidative stress.[14] The imbalance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the body's antioxidant defence system is known as oxidative stress.[15] The diameter of seminiferous tubules and the thickness of the germinal epithelium both decrease because of these modifications. In addition, prolonged noise exposure and a drop in testosterone levels may stop the maturation of some stem cells (spermatogonia, spermatocytes, and spermatid). The outcomes also line up with the findings of Sies *et al*. In their research, they discovered that the apoptotic mechanism, which mediates the germ cell degeneration in the testis, is crucial in controlling the quantity of germ cells.[15]

Consequently, these modifications result in a decrease in the diameter of the seminiferous tubules and the thickness of the germinal epithelium.[16,17] Different kinds of gap junctions, connected by similar adaptors or signalling pathways, about the seminiferous epithelial cells. Any change to the gap junction protein, Cx43, can result in the loss of germ cells in both males and females. Gap junction protein is involved in the formation of germ cells. It has been observed that Cx43 expression is downregulated in patients with low testosterone levels, suggesting a reduction in this gap junction protein and the sloughing of germ cells.[18]

Stress also alters the oxidative status, induces testicular apoptosis, and reduces the epithelial area of seminiferous tubules. Prolonged stress results in changes in spermatogenesis and the loss of germ cells. Males under stress with seminiferous tubules displayed several degenerative symptoms, including vacuoles in the basal epithelium and mild to severe exfoliation of degenerative germinal cells in the tubule lumen.[19]

Our results indicate that vitamin E can positively change the levels of reproductive steroids. One important metric for assessing the endocrine function of the testicles is the level of testosterone. In the testis, as in other tissues, androgen activity is mediated via AR transcriptional activation. In Sertoli cells, Tocopherol is preferentially bound to AR. Receptor activation initiates and maintains the spermatogenic process while averting germ cell apoptosis.[20] It has been discovered that oxidative stress reduces the quantity of Sertoli AR cells, which explains the disruptions in maturation and testicular injury that have been seen. Sertoli cells are crucial for determining the testis' structure, organization, and operation of the somatic cell lineages. They frequently support a limited number of germ cells, determining the adult's capacity to produce sperm.

CONCLUSION

This study concluded that 100 dB of noise has made adverse histomorphological alterations, reducing sperm maturation in the proliferating epithelium of seminiferous tubules. Hence, it is established that α Tocopherol does extend a clear defensive role against noise stress-induced histomorphological alterations in rat testis.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MY & HA: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

LA & MA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SI & KN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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