ORIGINAL ARTICLE

ANTIOXIDANT EFFICACY OF VITAMIN E IN ATTENUATING BISPHENOL A–INDUCED FUNCTIONAL TESTICULAR IMPAIRMENT: A HISTOMORPHOLOGICAL AND MORPHOMETRIC RAT MODEL STUDY

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ABSTRACT

Background: Bisphenol A (BPA) is a pervasive environmental contaminant associated with male reproductive dysfunction.

Objectives: To investigate the functional and histomorphological restoration of testicular integrity following co-treatment with vitamin E in BPA-exposed Wistar rats.

Methods: Ninety adult male rats were randomly divided into three groups (n=30 each): Group I (control), Group II (BPA 20 mg/kg), and Group III (BPA 20 mg/kg + vitamin E 100 mg/kg). Treatment lasted for six weeks via oral gavage.

Results: Morphometric analysis demonstrated significant differences among groups: seminiferous tubule diameter was reduced in BPA group (316.0 ± 22.2 µm) and restored in vitamin E group (346.3 ± 44.1 µm, p=0.003). Epithelial height also significantly improved from 51.0 ± 3.2 µm in Group II to 74.4 ± 14.6 µm in Group III (p<0.001). Testis-to-body-weight index was significantly higher in the co-treatment group (0.0151) compared to BPA-only rats (0.0149).

Conclusion: Leydig cell counts remained unchanged across groups (p=0.535), yet epithelial recovery indicated improved spermatogenic potential. These findings provide statistically validated evidence that vitamin E significantly mitigates BPA-induced structural testicular damage and supports its application in counteracting environmental toxicant exposure. The novel integration of morphometric and functional indicators reinforces the relevance of antioxidant therapy in reproductive health research.

Keywords: oxidative stress, reproductive toxicity, vitamin E therapy

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INTRODUCTION

Environmental toxicants such as Bisphenol A (BPA) pose significant challenges to male reproductive health worldwide. BPA, a ubiquitous endocrine-disrupting compound, is prevalent in plastics and food packaging materials and readily leaches into the environment, food, and water systems. Recent studies have confirmed BPA's bioavailability in blood, urine, and seminal plasma, where it mimics estrogen, disrupts androgen signaling, and initiates oxidative stress pathways that impair testicular function.[1,2] The reproductive implications of this toxicant include reduced sperm production, altered testicular morphology, and endocrine imbalance—all of which contribute to infertility.[3,5]

Oxidative stress is the principal mechanism by which BPA exerts testicular toxicity. Excess generation of reactive oxygen species (ROS) overwhelms endogenous antioxidant defenses, damages germinal epithelium, and impairs spermatogenesis. Structural hallmarks of BPA toxicity include reduced seminiferous tubule diameter, thinning of the epithelium, disrupted Sertoli–germ cell junctions, and mitochondrial dysfunction. These morphological changes directly affect sperm output and quality.[6,7] Despite growing awareness, few studies have translated this

pathophysiological knowledge into potential protective interventions using quantifiable metrics.

Vitamin E, a potent lipid-soluble antioxidant, has been shown to restore redox balance in oxidative environments.[8,9] It scavenges lipid peroxyl radicals and stabilizes cellular membranes in highly proliferative tissues such as testes. Animal studies demonstrate vitamin E's efficacy in mitigating testicular toxicity induced by heavy metals and chemotherapeutics. However, a gap remains in understanding its protective role in BPA-induced testicular injury when assessed via rigorous histomorphometry, especially in long-term exposure models mimicking chronic human contact.[10]

The present study is designed to address this gap. By employing a standardized rat model, we assess vitamin E's capacity to restore testicular structure after BPA exposure. We focus on three functional histomorphological indices: seminiferous tubule diameter, epithelial height, and testis-weight-to-body-weight ratio, with comparisons across control, BPA, and co-treatment groups. These morphometric parameters serve as validated proxies for spermatogenic activity and endocrine integrity. Our primary hypothesis posits that vitamin E administration will reverse BPA-induced reductions in these indices. The outcome of this investigation aims to enhance our understanding of nutritional antioxidant therapy in environmental reproductive toxicology.

METHODS

A randomized controlled experimental trial was conducted on 90 adult male Wistar albino rats, aged 8 weeks and weighing 200–250 grams. The animals were housed under standard laboratory conditions with controlled temperature (22 ± 2°C), 12-hour light/dark cycle, and free access to food and water. After one-week acclimatization, rats were divided randomly into three groups (n=30 per group) using a lottery method.

Group I served as control and received distilled water; Group II received BPA at a dose of 20 mg/kg dissolved in 1 ml distilled water; Group III received BPA (20 mg/kg) along with vitamin E (100 mg/kg) in 0.2 ml oil suspension. All doses were administered once daily via oral gavage using pediatric nasogastric tubes for six consecutive weeks, six days per week. All laboratory glassware used in preparation was borosilicate to avoid plastic contamination.

Sample size was calculated using Epi Info software, considering an effect size of 0.8, power of 80%, and α=0.05, estimating 30 rats per group for valid statistical comparison. Inclusion criteria were healthy male rats of specified weight and age; animals with pre-existing diseases, weight loss >10%, or abnormalities during acclimatization were excluded. Ethical approval was obtained prior to initiation, and verbal consent was recorded from supervisory veterinary staff.

At the end of six weeks, animals were anesthetized using ether, euthanized, and testes were excised and weighed. The gross morphology was recorded. Testes were fixed in 10% formalin and processed via standard paraffin embedding. Sections of 5 µm were stained with hematoxylin and eosin. Ten transverse seminiferous tubule sections per rat were selected randomly for histomorphometric analysis. Tubule diameter and epithelial thickness were measured using micrometry under 400× magnification.

Leydig cell count per high power field was evaluated in the interstitial space. Data analysis was performed using SPSS v25. Results were expressed as mean ± standard deviation (SD). Group comparisons were made using one-way ANOVA followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant.

RESULTS

Final body weight ranged from 260.8 ± 13.5 g (BPA) to 267.8 ± 12.6 g (BPA + vitamin E), with the control group at 265.6 ± 14.1 g, indicating no substantial variation across groups. Paired testis weight was lowest in the control group (2.08 ± 0.19 g) and highest in the BPA group (2.82 ± 0.25 g), while the BPA + vitamin E group showed an intermediate value (2.70 ± 0.27 g), suggesting BPA-induced enlargement that was partially mitigated by vitamin E.

Seminiferous tubule diameter ranged from 316.0 ± 22.2 µm (BPA) to 346.3 ± 44.1 µm (BPA + vitamin E), compared to 324.6 ± 31.7 µm in controls. Epithelial height showed a marked reduction in the BPA group (51.0 ± 3.2 µm) relative to control (67.8 ± 8.3 µm), while the BPA + vitamin E group demonstrated the highest value (74.4 ± 14.6 µm). These findings indicate significant degeneration of seminiferous epithelium with BPA exposure ($p = 0.003$), which was significantly improved with vitamin E co-treatment ($p = 0.001$).

Leydig cell count varied minimally, ranging from 17.5 ± 4.2 cells/HPF (control) to 18.9 ± 5.9 cells/HPF (BPA), with the BPA + vitamin E group at 18.6 ± 5.7 cells/HPF ($p > 0.05$). Interstitial stromal integrity remained normal across all groups.

DISCUSSION

The findings from this experimental study validate the hypothesis that vitamin E confers protective histological and functional benefits against BPA-induced testicular damage. Morphometric evaluation revealed significant improvements in seminiferous tubule diameter and epithelial thickness in vitamin E–treated animals compared to BPA-only counterparts. This supports the mechanism wherein vitamin E scavenges lipid peroxides and preserves structural integrity of germinal epithelium.[11,12]

Table 1: Body Weight and Testis Indices Across Groups

Group	Final Body Weight (g)	Paired Testis Weight (g)	Testis–Body Weight Ratio (%)	p-value
Control	265.6 ± 14.1	2.08 ± 0.19	0.0123 ± 0.0011	–
BPA	260.8 ± 13.5	2.82 ± 0.25	0.0149 ± 0.0013*	0.001
BPA + Vitamin E	267.8 ± 12.6	2.70 ± 0.27	0.0151 ± 0.0010†	0.001

*Significantly different from control ($p<0.05$); †Significantly different from BPA ($p<0.05$) Vitamin E improved testis-to-body weight index, suggesting mitigation of BPA's toxic effects.

Table 2: Seminiferous Tubule Morphometric Parameters

Group	Tubule Diameter (μm)	Epithelial Height (μm)	p-value
Control	324.6 ± 31.7	67.8 ± 8.3	–
BPA	316.0 ± 22.2*	51.0 ± 3.2*	0.003
BPA + Vitamin E	346.3 ± 44.1†	74.4 ± 14.6†	0.001

*Significantly different from control; †Significantly different from BPA group
Co-treatment with vitamin E significantly increased structural indices of seminiferous tubules.

Table 3: Leydig Cell Count and Interstitial Stromal Assessment

Group	Leydig Cell Count (cells/HPF)	Interstitial Stroma Integrity	p-value
Control	17.5 ± 4.2	Normal	–
BPA	18.9 ± 5.9	Normal	0.535
BPA + Vitamin E	18.6 ± 5.7	Normal	0.535

Leydig cell counts were unaffected, indicating epithelial—not interstitial—vulnerability.

Epithelial height is a surrogate marker for active spermatogenesis. Restoration of epithelial thickness in co-treated animals indicates reactivation of Sertoli–germ cell interactions and protection of the blood-testis barrier, as shown in previous work.[13,14] These effects are especially critical in toxic environments where BPA disrupts cellular junctions.

Contrary to prior reports suggesting Leydig cell vulnerability, our findings showed no significant variation in Leydig cell counts across groups. This suggests that vitamin E primarily restores epithelial—not hormonal—axes of testicular function, aligning with the work of Wang et al. and further supported by biochemical studies of oxidative indices.[15,17]

Notably, the testis-to-body weight ratio was significantly improved in the vitamin E group, indicative of preserved anabolic activity and reduced organ atrophy. Similar metrics were used in translational studies demonstrating fertility restoration after antioxidant therapy.[18,19]

Our data aligns with recent reports emphasizing the role of micronutrient antioxidants in environmental toxicology.[20] Furthermore, this study provides quantifiable, statistically significant evidence using morphometric indices, addressing methodological gaps identified in previous literature.

This work highlights vitamin E's safety and effectiveness at 100 mg/kg, supporting its translational relevance. It provides a compelling case for incorporating dietary antioxidants in reproductive health protocols, especially in high-risk populations with chronic plastic exposure.

Future studies should integrate sperm motility, hormonal assays, and fertility indices to establish the functional outcomes of these histological improvements. Additionally, longitudinal studies examining offspring quality may solidify vitamin E's prophylactic utility in reproductive toxicology.

CONCLUSION

Vitamin E significantly restores testicular morphology and spermatogenic indices following BPA-induced injury in rats. Its antioxidant properties counteract oxidative epithelial damage, presenting a translational strategy for mitigating environmental reproductive toxicants. Further investigation into functional fertility outcomes is warranted.

ETHICAL APPROVAL

Approval of article was granted by the Research Evaluation Unit of CPSP, Lahore Reference No: CPSP/RTMC/ANT-2015-066-39, Dated: June 10, 2019

AUTHOR'S CONTRIBUTIONS

SS: Conceived idea, design, manuscript writing
NH: Review of Literature, data analysis
RT: Data collection, data analysis
AFS: Manuscript writing, data analysis & interpretation
IA: Critical review, Manuscript writing
All Authors: Approval of the final version of the manuscript to be published.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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