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ORIGINAL ARTICLE

Protective role of omega-3 fatty acids against energy drink–induced structural changes in the testes of adult albino Wistar rats

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ABSTRACT

Background: Energy drinks (EDs) are widely consumed beverages that enhance alertness and performance but may cause adverse histological and structural testicular changes in males. Omega-3 fatty acids (omega-3 FA) possess antioxidant and anti-inflammatory properties that may protect against such tissue damage.

Objective: To evaluate the protective role of omega-3 FA against energy drink-induced histological and morphological changes in the testes of adult male albino Wistar rats.

Methods: This experimental study was conducted at the University of Health Sciences (UHS), Lahore, and Postgraduate Medical Institute (PGMI), Lahore, Pakistan, for 12 weeks from June 1 to August 31, 2020. Thirty male Wistar rats were divided into three groups (n=10 each). Group A (Control) received distilled water, Group B (ED) received ED (120 mg/kg body weight), and Group C (ED+ Omega-3 FA) received ED (120 mg/kg) plus omega-3 FA (300 mg/kg) orally for 40 days simultaneously. On day 41, rats were sacrificed, and testes were dissected, fixed, and stained with hematoxylin and eosin (H&E) and Masson's trichrome for histological evaluation. Data were analyzed using Fisher's exact test using SPSS version 25, with a p-value ≤ 0.05 considered statistically significant.

Results: Sertoli cell vacuolization was observed in 70% of animals in Group B (ED), 20% in Group C (ED + Omega-3 FA), and was absent in the control group (Group A) ($p = 0.002$). Interstitial fibrosis occurred in 70% of Group B and 30% of Group C, with no cases in Group A ($p = 0.004$). Abnormal sperm head morphology was most frequent in Group B (80%), compared to 30% in Group C and 10% in Group A ($p = 0.008$). These findings indicate that omega-3 FA supplementation substantially reduces ED-induced testicular damage.

Conclusion: Omega-3 FA significantly protected against energy drink-induced histological and morphological changes in the testis.

Key Words: Omega-3 Fatty Acids, Energy Drinks, Testis, Histology, Sertoli Cells, Fibrosis, Interstitial

DOI: https://doi.org/10.53685/jshmdc.v6i2.384

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Received: 19.08.25, 1st Revision: 20.10.25
2st Revision: 06.11.25, Accepted: 16.11.25

How to cite this article: Naqvi QA, Fatima SA, Haider A. Protective role of omega-3 fatty acids against energy drink–induced structural changes in the testes of adult albino Wistar rats. J Shalamar Med Dent Coll. 2025; 6(2): 90-96. doi: https://doi.org/10.53685/jshmdc.v6i2.384

INTRODUCTION

Energy drinks (EDs) have rapidly expanded worldwide, becoming a significant segment of the global beverage market. Their popularity is attributable mainly to aggressive marketing that emphasizes their capacity to enhance physical agility, mental alertness, and overall performance. These attributes have attracted a wide range of consumers, most notably adolescents, university students, athletes, and shift workers who rely on increased energy and focus for demanding tasks or irregular schedules.[1] The formulations of energy drinks typically include high concentrations of caffeine, often surpassing those found in traditional coffee and soft drinks. In addition, they contain other bioactive compounds, such as taurine, glucuronolactone, B vitamins, and herbal

extracts, including ginseng and guarana, which are believed to enhance energy and cognitive function further.[2] The combination of these ingredients has resulted in a growing acceptance and increasing consumption rates worldwide, supported by targeted advertising and easy availability.

However, despite the perceived benefits and widespread use, mounting scientific evidence indicates that excessive consumption of energy drinks can pose serious health risks. Among these concerns, reproductive toxicity has emerged as a critical area demanding attention. The reproductive system is vulnerable to various toxic insults, and beverages containing high levels of stimulants and bioactive additives may adversely affect its function. Studies have demonstrated that energy drink constituents induce oxidative stress, a condition characterized by elevated reactive oxygen species (ROS) that overwhelm the body's antioxidant defenses. This oxidative stress can trigger lipid peroxidation, DNA damage, and cellular apoptosis, which are detrimental to the delicate environment required for spermatogenesis.[3]

In addition to oxidative damage, energy drinks disrupt hormonal homeostasis by interfering with the hypothalamic-pituitary-gonadal axis. This disruption manifests as altered secretion of gonadotropins and steroid hormones, leading to hormonal imbalances that impede normal reproductive function.[4] Testicular histopathology studies have revealed several characteristic alterations related to ED exposure. Sertoli cell vacuolization, considered an early marker of testicular injury, indicates compromised blood-testis barrier integrity and impaired support for developing germ cells. Mitochondrial damage and germ cell apoptosis have also been consistently observed, contributing to defective spermatogenesis and reduced sperm quality. There is also a significant increase in sperm head morphological abnormalities, decreased sperm count, and reduced motility following energy drink administration. These changes at both cellular and systemic levels collectively translate into diminished fertility potential, raising concerns over the long-term reproductive health implications for habitual consumers.[5]

Given the prevalence of energy drink consumption among young males and the potential reproductive risks, it is crucial to explore protective strategies that can mitigate these adverse effects. Omega-3 fatty acids (omega-3 FA) have generated considerable interest due to their well-established antioxidant, anti-inflammatory, and membrane-stabilizing properties.[6] These polyunsaturated fatty acids serve as critical components in maintaining cellular integrity and regulating inflammatory responses, making them particularly relevant in counteracting oxidative stress-induced tissue damage. Experimental data from animal models have demonstrated that omega-3 FA supplementation significantly reduces oxidative stress markers in testicular tissue exposed to harmful agents, preserving the histological architecture and enhancing mitochondrial function.[7] Furthermore, there are improvements seen in sperm count, motility, and morphology following omega-3 FA supplementation in study models of reproductive toxicity, underscoring their functional benefits.[8]

Beyond these biochemical and histological effects, omega-3 FAs help modulate hormonal balance by supporting Leydig cell function and steroidogenesis, thus contributing to the restoration of normal reproductive endocrinology.[9] These findings are supported by clinical evidence highlighting the role of omega-3 FAs in enhancing semen quality and reducing inflammatory markers in infertile men. Collectively, the data provide compelling evidence for the therapeutic potential of omega-3 FAs in preserving male reproductive health against the insults associated with energy drink consumption.[10]

Despite these encouraging findings, the specific protective effects of omega-3 FA against energy drink-induced reproductive toxicity have not been extensively studied. Most existing research focuses on reproductive damage induced by isolated toxins or generalized oxidative stress, with relatively few studies assessing the efficacy of omega-3 FAs in the context of energy drink exposure. This represents a critical knowledge gap that needs to be addressed to develop effective clinical and public health interventions.

Energy drinks have become a routine part of daily life for many people, especially young males, but growing evidence suggests they may quietly be harming reproductive health. Concerns are increasing that these beverages may disrupt normal testicular structure and function, yet the specific cellular changes they induce remain incompletely understood. At the same time, omega-3 FAs are known for their protective, anti-inflammatory effects and may offer a way to reduce this harm, but their role in this context has not been clearly defined. With these concerns in mind, this

study seeks to examine the effect of energy drinks on the male reproductive health in rats and to determine whether omega-3 FA can help protect against these changes.

METHODS

This experimental study was conducted at the University of Health Sciences (UHS) and the Postgraduate Medical Institute (PGMI), Lahore, Pakistan, over 12 weeks, from 1 June to 31 August 2020, after approval from the UHS ethics board. Thirty healthy adult male albino Wistar rats aged 9–12 weeks and weighing 180–220 g were included in this study. Female, diseased, and less than 9-week-old rats were excluded. The sample size was calculated based on a one-way ANOVA for three independent groups. The anticipated group means were 138.7, 147.6, and 121.4 with corresponding standard deviations of 15.9, 15.6, and 14.1, respectively. Using these values, the pooled standard deviation and the variance among group means yielded an effect size of f = 0.715, indicating a significant expected difference across groups. With a significance level of $\alpha = 0.05$ and 80% statistical power, the estimated minimum sample size per group was 9 (total n = 27). To mitigate the risk of data loss, each group comprised 10 albino rats.[11] Animals were obtained from the National Institute of Health (NIH), Islamabad. Rats were acclimatized for one week under standard laboratory conditions (temperature 24 ± 2°C, relative humidity 60 ± 10%, and a 12-hour light/dark cycle). They were housed in stainless steel cages with wire-bar lids, each containing a maximum of five animals, with ad libitum access to standard rat chow and tap water.

Bedding was changed daily to maintain hygiene. Animals were randomly assigned (simple randomization using a random number table) to three groups of 10 rats each: Group A (Control) received distilled water via oral gavage for 40 consecutive days. Group B (ED): received commercially available ED (Red Bull) manufactured by Red Bull Asia FZE, UAE at a dose of 120 mg/kg/day via oral gavage for 40 consecutive days. Group C (ED+ omega-3 FA): received ED at 120 mg/kg/day plus omega-3 FA (Trade name Romega) at 300 mg/kg/day simultaneously via oral gavage for 40 consecutive days.[12]

On day 41, all rats were anaesthetized and sacrificed via decapitation. Testes were dissected and excised, fixed in formalin, and cleared of adherent fat by treating with alcohol and xylene. Testes were embedded in molten paraffin wax, and a microtome was used to trim and section paraffin blocks. These were mounted on wooden blocks for sectioning. Serial cross sections of 5 μm, using a rotary microtome, were obtained from testicular tissues. They were floated in a water bath and picked with glass slides for staining after trimming. The testicular tissues were stained with Hematoxylin and Eosin and Masson's trichrome stain and were examined at 100x and 400x magnification under a light microscope. Sertoli cells were identified by their triangular nuclei at the basal compartment of seminiferous tubules. Vacuolization was recorded as present when one or more clear cytoplasmic vacuoles were visible per cell in at least 10% of tubules examined.[13] Sections stained with Masson's trichrome were evaluated at 100x magnification. Fibrosis was considered present when greenish blue-stained collagen fibers were observed in the intratubular spaces in ≥5% of the examined field area.[14] Epididymal sperm smears were stained with eosin and examined at 400x magnification. Abnormal sperm head morphology included detached heads, bent heads, heads lacking an acrosomal hook, or abnormal angulation between head and midpiece. Two hundred sperms per slide were assessed. Each qualitative variable was scored as present (1) or absent (0) for statistical analysis.

Ethical Approval

The study protocol was approved by the Institutional Research Board/Ethics Committee of the University of Health Sciences, Lahore, Pakistan (IRB #: UHS/ Education/126-20/1049), dated 14th May 2020.

Statistical Analysis

After observations, the data were recorded and entered into Microsoft Word and Microsoft Excel, then summarized in tabular form. The qualitative data, such as sperm head abnormalities, interstitial fibrosis, and sertoli cell vacuolization, were presented as frequencies and percentages. Data were analyzed using SPSS v25. Frequencies of each qualitative variable were compared between groups using Fisher's exact test. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Sertoli cell vacuolization was absent in all rats of the control group (0%). In contrast, it was present in 70% (7/10) of rats in the ED group, indicating substantial injury to the seminiferous epithelium. Omega-3 FA

supplementation reduced this prevalence to 20% (2/10) in the ED+ omega-3 FA group. Statistical analysis confirmed a significant difference among groups (p-value=0.002) (Table 1).

Table 1: Comparison of Sertoli cell vacuolization across experimental groups

Groups	Sertoli cell vacuolization		p-value
	Present n(%)	Absent n(%)	
Group A	0 (0.0%)	10(100%)	
Group B	7(70.0%)	3 (30.0%)	0.002*
Group C	2(20.0%)	8 (80.0%)	

**Fisher's exact test. p-value ≤ 0.05 was considered significant. Group A= Control, Group B= Energy Drink (ED), Group C= ED+ Omega-3 fatty acids*

Table 2: Comparison of interstitial fibrosis across experimental groups

Groups	Interstitial Fibrosis		P value
	Present n (%)	Absent n (%)	
Group A	0 (0.0%)	10 (100%)	
Group B	7 (70.0%)	3 (30.0%)	0.004*
Group C	3 (30.0%)	7 (70.0%)	

**Fisher's exact test. p-value ≤ 0.05 was considered significant. Group A= Control, Group B=Energy Drink (ED), Group C= ED+ Omega-3 fatty acids*

Table 3: Comparison of sperm head morphology across experimental groups.

Groups	Abnormal sperm head morphology		P value
	Present n (%)	Absent n (%)	
Group A	9 (90%)	1 (10%)	
Group B	2 (70%)	8 (20%)	0.008*
Group C	7 (30%)	3 (30%)	

**Fisher's exact test. p-value ≤ 0.05 was considered significant. Group A= Control, Group B= Energy Drink (ED), Group C= ED+ Omega-3 fatty acids*

No interstitial fibrosis was observed in the control group. In the ED group, fibrosis was present in 70% (7/10) of animals, visible as dense blue collagen deposition surrounding seminiferous tubules and perivascular spaces. In the Group C(ED+ omega-3 FA), fibrosis was reduced to 30% (3/10), indicating partial preservation of normal interstitial architecture. The difference among groups was statistically significant (p-value = 0.004) (Table 2).

Abnormal sperm head morphology was detected in only 10% of control rats. In the ED group, abnormalities were present in 80% of rats, including increased incidence of bent heads, acrosome loss, and head detachment. In the Group C (ED+ omega-3 FA), sperm head abnormalities were present in 30% of animals (p-value = 0.008) (Table 3).

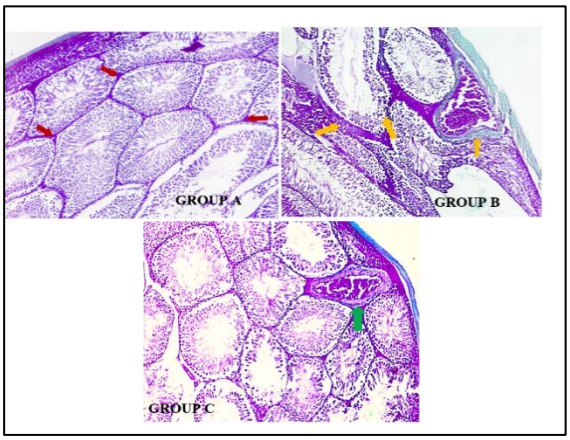

Figure 2: *Photomicrographs of seminiferous tubules of testis from animal in control (Group A) showing normal intertubular space (red Arrows). Group B (given ED) showing fibrosis in interstitial space (orange arrows). Group C (ED+ Omega-3 fatty acids) showing no fibrosis in interstitial space (green arrows) 100x.*

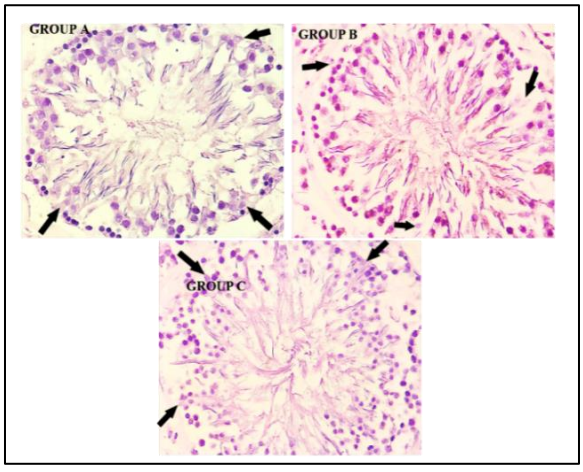

Figure 1: *Photomicrographs of seminiferous tubules of testis from an animal in control (Group A) showing normal Sertoli cells (black arrows). Group B (ED) showing vacuolization in Sertoli cells (black arrows). Group C (ED+ Omega-3 fatty acids) showing no vacuolization in Sertoli cells (black arrows) 400x.*

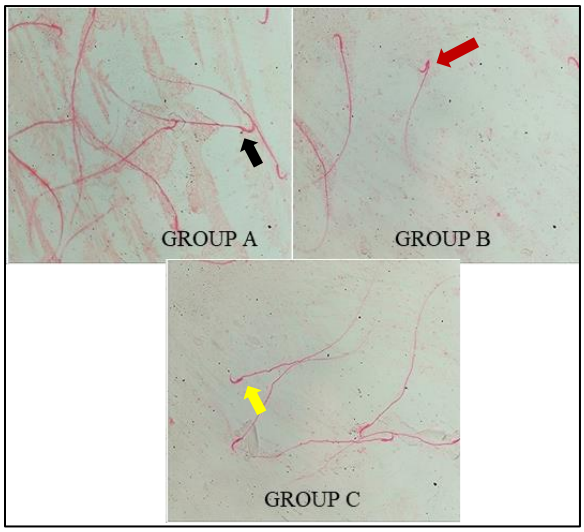

Figure 3: *Photomicrographs of sperms obtained from a smear of the testis of rats. Group A (control) showing sperm heads with normal morphology (black arrow), Group B (ED) showing sperm heads with an abnormal bent head (red arrow), and Group C (ED+ Omega-3 fatty acids) showing sperm heads with normal morphology (yellow arrow) 400x.*

DISCUSSION

In this study, male albino Wistar rats were divided into three groups: Group A (Control), Group B (ED), and Group C (ED + omega-3 FA). Significant histopathological and morphological changes in the testes were observed. Prominent histopathological changes included the pronounced Sertoli cell vacuolization, interstitial fibrosis, and abnormal sperm morphology, each contributing to impaired spermatogenesis and diminished fertility potential. These structural and the cellular abnormalities collectively impair the testicular microenvironment, thereby disrupting the delicate balance necessary for normal sperm development and function. These findings align with another study that provided crucial insights into the molecular underpinnings of ED-induced testicular damage by demonstrating that components of energy drinks markedly disrupt the blood-testis barrier (BTB).[15] This barrier is critically maintained by tight junction proteins that regulate the protective environment for germ cells. Disruption of these proteins by ED constituents increases oxidative stress, which triggers germ cell apoptosis, a process that significantly impairs spermatogenesis. Compromise of the blood-testis barrier is a fundamental insult that increases the vulnerability of spermatogenic cells to toxic agents, facilitating the penetration of harmful substances and inflammatory mediators into the seminiferous epithelium. The cellular pathology extends to the metabolic stress within Sertoli and Leydig cells, mediated notably by caffeine and taurine—the main active ingredients in energy drinks. These compounds disturb calcium homeostasis and mitochondrial function in these somatic cells, thereby inducing elevated reactive oxygen species production.[15] Excess ROS contributes to oxidative stress, an event that provokes apoptotic pathways and disrupts endocrine signaling essential for sperm maturation and testosterone synthesis. Another study reinforced this mechanism by emphasizing the pivotal role of ROS and lipid peroxidation in propagating testicular dysfunction following ED exposure. Lipid peroxidation specifically targets polyunsaturated fatty acids in cell membranes, leading to cellular membrane instability, malfunction, and subsequent cell death.[16]

Hormonal disruption represents another critical dimension of ED-induced testicular toxicity. A different study explicated how caffeine and taurine interfere with hypothalamic-pituitary-gonadal axis signaling, leading to diminished testosterone biosynthesis and the consequent impairment of spermatogenesis. This disturbance results in decreased secretion of gonadotropins and androgens, which are indispensable for maintaining testicular function.[17] Additionally, it has been reported that energy drink exposure enhances the expression of inflammatory mediators such as cyclooxygenase-2 (COX-2) and NF-κB in testicular tissues, thereby intensifying cellular apoptosis and tissue damage. These inflammatory pathways amplify the detrimental effects of oxidative stress, further compromising testicular architecture and function.[18]

Given the key role played by oxidative stress in ED-induced testicular injury, the protective effects of omega-3 FA have been a major focus of recent research. A study demonstrated that omega-3 FA downregulates the NF-κB and TGF-β signaling pathways, thereby reducing testicular inflammation and interstitial fibrosis. By limiting fibrotic changes—such as basement membrane thickening and Leydig cell distortion— omega-3 FA helps preserve the testicular microenvironment. They also suppress pro-inflammatory cytokines and enhance antioxidant enzyme activity, restoring redox balance and mitigating oxidative and inflammatory damage caused by energy drink exposure.[19] Further evidence supports the anti-fibrotic capacity of omega-3 FA, demonstrating their ability to preserve seminiferous

tubule architecture by inhibiting molecular cascades responsible for fibrogenesis. The fibrotic remodeling associated with energy drink toxicity is molecularly linked to activation of the TGF-β pathway, driven by persistent oxidative stress.[20] It has been demonstrated that activation of this pathway triggers excessive extracellular matrix production, resulting in disruption of tissue architecture and function.[21] Evidence indicates that extracellular matrix accumulation not only distorts testicular architecture but also compromises spermatogenic support. Consequently, fibrosis is recognized as a key mechanistic link between oxidative stress and impaired reproductive function.[22] Abnormal sperm morphology, particularly sperm head defects accompanied by reduced motility, is a common outcome of excessive energy drink consumption. These anomalies are primarily associated with oxidative damage and lipid peroxidation targeting the sperm plasma membranes.[23] Since these membranes are enriched with polyunsaturated fatty acids, they are highly susceptible to oxidative insults, which compromise membrane fluidity and integrity essential for motility and fertilization capability. The beneficial role of omega-3 FA in this context is well-documented. omega-3 FA supplementation has been shown to stabilize sperm plasma membranes, significantly improving motility and reducing morphological abnormalities. These benefits are attributed to its modulation of key sperm metabolic pathways, including lactate transport and purine metabolism, which are essential for maintaining sperm energy supply and function.[24] These findings are further supported by evidence showing that omega-3 FA treatment restores sperm membrane structure and function in toxicological models.[25]

CONCLUSION

Energy drink exposure causes clear and measurable damage to testicular structure in rats, including degeneration of seminiferous tubules, disturbed spermatogenesis, and early interstitial fibrosis. These findings reinforce concerns about reproductive risks linked to frequent energy drink use. In contrast, rats receiving omega-3 fatty acids maintained healthier testicular architecture, with better preservation of Sertoli cells and fewer interstitial changes. This highlights a meaningful protective role of omega-3 fatty acids against energy-drink–induced testicular injury.

Limitations and future recommendations

The study duration was relatively short, and hormonal profiling was not performed, which could have yielded deeper insight into structural changes in the testis. Sperm count and motility were not measured. Future studies should integrate molecular analyses, including oxidative stress markers, inflammatory cytokine expression, and assessments of mitochondrial function, to better elucidate the pathways involved. Moreover, well-designed clinical trials are necessary to validate these findings in humans.

Acknowledgments

The authors thank the faculty, laboratory technicians, and staff at PGMI and UHS, Lahore, for assistance.

REFERENCES

1. Erdmann J, Wiciński M, Wódkiewicz E, Nowaczewska M, Słupski M, Otto et al. Effects of energy drink consumption on physical performance and potential danger of inordinate usage. Nutrients. 2021; 13(8): 2506. doi: 10.3390/nu13082506.
2. Górka K, Kruczyńska A, Lenard T, Banach A, Sochaczewska A, Mencfel R, et al. Energy drinks—trend or awareness choice? a mini-review. J Nutr. 2025; 9: 2825-2845. doi: 10.1016/j.tjnut.2025.06.033.
3. Omozee EB. Impact of caffeine and energy drink on some oxidative stress markers and cardiovascular indices in female Sprague-Dawley rats. Sokoto J Med Lab Sci. 2025; 10(3). doi: 10.4314/sokjmls.v10i3.14.
4. Fathy MS, Al Ghonamy NM, Abdelrahman SA, Mostafa SA. Effect of energy drink (Red Bull) on the pituitary-adrenal axis of adult male albino rats: a histological and immune histochemical study. Zagazig Univ Med J. 2025; 31(10): 5073-5089. doi: 10.21608/zumj.2025.409866.4086.
5. Gaballa MMS, Ahmed O, Elsharkawey S, Abdow MF, Shoulah S, Wahba F, et al. Modulation of energy drink-induced hepato-renal and testicular toxicity by pomegranate peel extract: insights from histopathological and immunohistochemical analyses. Pak Vet J. 2025; 45(2): 703-712. doi: 10.29261/pakvetj/2024.293.
6. Manzhulo I, Gromova E, Ivashkevich D, Krasnorutsky A, Tonkoshkur G, Lushnikova I, et al. Antioxidant and anti-inflammatory activity of N-acylethanolamines of omega-3 polyunsaturated fatty acids in vitro. Cell Biochem Biophys. 2025. doi: 10.1007/s12013-025-01942-9.
7. Odetayo AF, Adeyemi WJ, Olayaki LA. Omega-3 fatty acid ameliorates bisphenol F-induced testicular toxicity by modulating Nrf2/NFkB pathway and apoptotic signaling. Front Endocrinol. 2025; 14:1256154. doi: 10.3389/fendo.2023.1256154.
8. Oyakhire FO, Emokpae MA, Esezobor KI, Adejumo BIG, Efenarhua S, Olaniyan JE, et al. Omega-3 fatty acids as a novel antioxidant strategy against cadmium-induced testicular dysfunction: a proteomic approach. Pathol Lab Med. 2025; 9(1): 12-31. doi: 10.11648/j.plm.20250901.12.
9. Hasan MM, El-Shal AS, Mackawy AMH, Ibrahim EM, Abdelghany EM, Saeed AA, et al. Ameliorative effect of combined low dose of pioglitazone and omega-3 on spermatogenesis and steroidogenesis in diabetic rats. J Cell Biochem. 2020; 121(2): 1524-1540. doi: 10.1002/jcb.29388.
10. Rodak K, Kratz EM. PUFAs and their derivatives as emerging players in diagnostics and treatment of male fertility disorders.

Pharmaceuticals.2023; 16(5): 723. doi:10.3390/ph16050723.
11. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009; 41(4): 1149-1160. doi: 10.3758/BRM.41.4.1149.
12. Abd Elwahab NG, Mahmoud FAZ. The role of omega-3 on Red Bull induced skeletal muscle injury in adult albino rat: a histological and immunohistochemical study. Egypt J Histol. 2024; 47(3): 1130-1137. doi:10.21608/ejh.2023.207679.1888.
13. Yang X, He L, Li X, Wang L, Bu T, Yun D, et al. Triptolide exposure triggers testicular vacuolization injury by disrupting the Sertoli cell junction and cytoskeletal organization via the AKT/mTOR signaling pathway. Ecotoxicol Environ Saf. 2024; 279: 116502. doi: 10.1016/j.ecoenv.2024.116502.
14. Labib H, Galal A. Caffeine versus antioxidant combination (Antox) and their role in modifying cadmium-induced testicular injury in adult male albino rats. Andrologia. 2020; 53(7): e13948. doi: 10.1111/and.13948.
15. Al-Shaikh TM, Rajeh NA. Ameliorating effect of blueberry consumption on energy drink-induced testicular damage in rats: histological and immunohistochemical study. J Basic Appl Zool. 2023; 84: 9. doi: 10.1186/s41936-023-00330-0
16. Al-Basher GI, Aljabal H, Almeer RS, Allam AA, Mahmoud AM. Perinatal exposure to energy drink induces oxidative damage in the liver, kidney and brain, and behavioral alterations in mice offspring. Biomed Pharmacother. 2018; 102: 798-811. doi: 10.1016/j.biopha.2018.03.139.
17. Li Y, Peng Q, Shang J, Dong W, Wu S, Guo X, et al. The role of taurine in male reproduction: physiology, pathology and toxicology. FrontEndocrinol. 2023; 14: 1017886. doi: 10.33 89/ fendo.2023.1017886.
18. Ahmed AM. Expression of transcription factor NF-kappa B/p65 and cyclooxygenase-2 (COX-2) in testicular damage induced by Red Bull energy drink in rat. Int J Adv Appl Sci. 2016; 3(10): 49-56. doi: 10.21833/ijaas.2016.10.009.
19. Abdelhaffez AS, Hussein OA, Rateb A, Yousef MS. Omega-3 polyunsaturated fatty acids confer protection against gentamicin-induced testicular injury: novel insights into possible mechanisms. J Physiol Pathophysiol. 2019; 10(2): 17-33. doi: 10.5897/JPAP2019.0126.
20. Qi X, Qin Z, Tang J, Han P, Xing Q, Wang K, et al. Omega-3 polyunsaturated fatty acids ameliorate testicular ischemia-reperfusion injury through the induction of Nrf2 and inhibition of NF-κB in rats. Exp Mol Pathol. 2017; 103(1): 44-50. doi: 10.1016/j.yexmp. 2017.06.005.
21. Xu Y, Hu P, Chen W, Chen J, Liu C, Zhang H. Testicular fibrosis pathology, diagnosis, pathogenesis, and treatment: a perspective on related diseases. Andrology. 2025; 13(6): 1322-1332. doi: 10.1111/andr.13769.
22. Potiris A, Moustakli E, Trismpioti E, Drakaki E, Mavrogianni D, Matsas A, et al. From Inflammation to Infertility: How Oxidative Stress and Infections Disrupt Male Reproductive Health. Metabolites. 2025; 15(4): 267. doi: 10.3390/metabo 15040267.
23. Gualtieri R, Kalthur G, Barbato V, Longobardi S, Di Rella F, Adiga SK, et al. Sperm oxidative stress during in vitro manipulation and its effects on sperm function and embryo development. Antioxidants. 2021; 10(7): 1025. doi: 10.3390/ antiox10071025.
24. Akhigbe RE, Hamed MA, Odetayo AF, Akhigbe TM, Ajayi AFH, Ajibogun FAH. Omega-3 fatty acid rescues ischaemia/perfusion-induced testicular and sperm damage via modulation of lactate transport and xanthine oxidase/uric acid signaling. Biomed Pharmacother. 2021; 142: 111975. doi: 10. 1016/j.biopha.2021.111975.
25. Ahmed MG, Ibrahim ME-D, El Sayed HR, Ahmed SM. Short term chronic toxicity of tributyltin on the testes of adult albino rats and the possible protective role of omega-3. Hum Exp Toxicol. 2021; 40(2): 214-230. doi: 10.1177/096032712094 7451.

AUTHORS' CONTRIBUTION:

QAN: Conceived the study, performed data acquisition and analysis, drafted the manuscript, final approval of the version to be published.

SAF: Contributed to study conception, data collection, data analysis and interpretation, critically reviewed the manuscript, final approval of the version to be published.

AH: Participated in data collection and analysis, manuscript drafting, critical revision of the manuscript, final approval of the version to be published.

All Authors agreed 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

CONFLICT OF INTEREST:

All authors declared no conflict of interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE:

No specific grant was taken for this research from any funding agency in the public, commercial or not-for-profit sectors.

DATA SHARING STATEMENT:

The data are available from the corresponding author upon reasonable request.

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