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Omega-3 Fatty Acids Ameliorate Sleep Deprivation-Induced Histomorphological Alterations In The Rat Prostate

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Abstract

Objective: The primary objective of this study was to observe the impact of chronic sleep deprivation on the histomorphological features of the rat prostate gland and to evaluate the potential protective effects of omega-3 fatty acid supplementation.

Materials & Methods: Thirty male Sprague-Dawley rats (200-300g) were randomly divided into three groups (n=10 per group): Group A (control, maintained on a normal sleep-wake cycle), Group B (sleep deprivation, subjected to 16 hours of sleep deprivation followed by an 8-hour sleep opportunity daily for 60 days), and Group C (sleep deprivation + omega-3, subjected to the same sleep deprivation regimen as Group B, supplemented with 260 mg/kg/day omega-3 fatty acid via oral gavage). Post-euthanasia and after dissection, prostate tissues were processed for histological observation using H&E and Masson's trichrome staining to assess epithelial apoptosis, collagen deposition, and smooth muscle architecture. Statistical analyses were performed using SPSS software version 21, with $p<0.05$ considered statistically significant.

Results: Group A (control) showed normal histological structure. Group B (sleep deprivation) exhibited a significantly higher frequency of epithelial apoptosis ($p<0.05$), increased interacting collagen deposition ($p<0.001$), and marked irregularities in the smooth muscle layer surrounding the acini ($p=0.002$). Group C (sleep deprivation + omega-3) demonstrated reduced epithelial apoptosis, collagen deposition, and smooth muscle distortion relative to Group B.

Conclusion: The present study observed that sleep deprivation caused significant changes in the histomorphology of the rat prostate gland, including epithelial apoptosis, collagen fibre deposition in the interacting space, and irregular arrangement of the smooth muscle fibres around the acini. Additionally, omega-3 fatty acid supplementation had an ameliorative effect on these histomorphological alterations induced by sleep deprivation.

Keywords: Sleep Deprivation, Prostate, Histology, Omega-3 Fatty Acids, Apoptosis, Collagen, Smooth Muscle.

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Introduction

Sleep deprivation has emerged as the most commonly encountered cause of behavioural shifts and oxidative stress. It imposes radical effects on various psychological and physiological systems. Numerous researches have shown the damaging consequences of inadequate sleep, with the earliest experiments dating back to the late 19th century.[1] Sleep deprivation causes histomorphological changes in various organs and disrupts hormonal balance, including altering male sex hormone levels.[2] Remarkably, sleep disturbances in men have been strongly linked with an augmented risk of prostatic cancer.[3] It is strongly associated with alteration in the male sex hormone levels causing detrimental effects on the prostate gland and leading to cancer (Sigurdardottir et al., 2013). This highlights the potential vulnerability of the prostate gland to sleep disturbances. Chronic sleep deprivation can disturb the diurnal rhythm and decrease the androgen levels leading to decreased prostate gland weight, signifying impaired steroidogenesis

or diminished androgen expression in the target tissues.[4] In contrast, omega-3 fatty acids, a class of essential polyunsaturated fats, are known to have anti-inflammatory and antioxidant benefits. This may help to alleviate the stress response on the body's organs. It has been anticipated to reduce the levels of the stress hormone i.e. cortisol and has a protective role for the prostate.[5] Omega-3 fatty acids, which include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), significantly impact cell growth and membrane integrity. These compounds bind to specific receptors in the prostate, which helps inhibit cancer cell proliferation and lower the risk of prostate cancer.[6] Remarkably, omega-3 supplementation has also been implicated in extenuating inflammatory responses.[7] Given the adverse effects of sleep deprivation on the prostate and the potential protective role of omega-3 fatty acids, investigating the relationship between these factors is of paramount importance.

To detect histomorphological aberrations, including epithelial apoptosis, interacting collagen deposition, and smooth muscle architecture distortion, brought about by chronic sleep deprivation in the rat prostate gland was the main objective of this study. Additionally, the study aims to evaluate the potential protective effects of omega-3 fatty acid supplementation on these histological changes.

Materials And Methods

In this study, thirty adult male Sprague-Dawley rats, of 4 months of age, having 200-300g weight, were obtained from the National Institute of Health (NIH), Islamabad, Pakistan. These rats were housed in the NIH animal facility under controlled conditions i.e. temperature: 20-26°C, humidity: 50-60%, 12h light/dark cycle with *ad libitum* access to standard diet and water. All experimental protocols were approved by the Ethical Committee on Animal Experiments at Army Medical College, Rawalpindi, Pakistan. A randomized controlled trial was conducted. The rats were randomly divided into three groups (n=10 per group):

Group A (Control): Maintained on a normal 12h light/dark cycle and fed a standard rodent diet.

Group B (Sleep Deprivation): Subjected to 16 hours of sleep deprivation followed by an 8-hour sleep opportunity daily for 8 weeks, fed a standard rodent diet.

Group C (Sleep Deprivation + Omega-3 supplementation): Subjected to the same sleep deprivation regimen as Group B, fed a standard diet supplemented with omega-3 fatty acids (260 mg/kg body weight),[8] administered daily via oral gavage.

To ensure that any observed effects are due to the omega-3 fatty acids and not the gavage process itself, Groups A and B were administered an equal volume of distilled water via oral gavage. This practice served as a control for the gavage procedure, ensuring that the effects observed in Group C were specifically due to the omega-3 supplementation rather than the act of gavage itself.

Sleep deprivation was induced using the modified pendulum technique described by Hulzen et al.[9] Rats were placed in individual compartments of a cage fitted with oscillating trays driven by a mechanical device. The trays oscillated at ~2-minute intervals to disrupt sleep for 16h, followed by an 8h undisturbed sleep window. Uninterrupted power supply ensured continuous operation.

After 8 weeks, rats were anaesthetized with ether inhalation and euthanized. Prostate glands were excised, weighed, and fixed in 10% neutral buffered formalin. Tissues were processed, embedded in paraffin, sectioned at 5µm thickness, and stained with hematoxylin and eosin (H&E) and Masson's trichrome.

Epithelial apoptosis in prostatic acini was assessed by evaluating nuclear hyperchromasia and fragmentation in 10 random fields per slide. Grading of apoptosis was performed on a number scale, where: 0= no lesion, 1= a mean of 1-5 apoptotic cells/field and 2= a mean of 5-10 apoptotic cells/field

Interacinar collagen deposition was evaluated using Masson's trichrome staining, comparing the presence or absence of collagen fibres in the interacinar spaces of the experimental groups with the control group. Jones, M. L., Bancroft, J. and Gamble, M. (2008). Connective Tissues and Stains. Theory and Practice of Histological Techniques. 6th ed, Churchill Livingstone Elsevier, Nottingham. 135-160.The smooth muscles around the acini were stained with Masson’s Trichrome to determine whether they were regularly or irregularly arranged.Data was analysed using IBM-SPSS version 21.

Apoptosis was semi-quantitatively scored on a 0-2 scale. Collagen deposition and smooth muscle morphology were qualitatively assessed. Data was expressed as mean ± standard deviation. Statistical comparisons between groups were made using ANOVA followed by appropriate post-hoc tests. ANOVA test was applied for intergroup comparison of quantitative variables followed by Post Hoc Tukey`s Test which was taken as means and standard deviations (mean±SD) with $p<0.05$ considered significant.

Results

Group A (Control): Epithelial apoptosis was absent in all prostatic acini. Interacinar collagen deposition was not observed. The smooth muscle layer exhibited a regular, continuous arrangement surrounding the acini.

Group B (Sleep Deprivation): Epithelial apoptosis was evident, with grades of 0 (30%), 1 (60%), and 2 (10%), a significantly higher frequency compared to Groups A and C ($p=0.003$). Interacinar collagen deposition was present in 70% of animals, a significantly higher incidence than Group A ($p=0.000$) but comparable to Group C ($p=0.074$). Marked irregularities in the smooth

muscle architecture surrounding acini were observed in 70% of animals, a significantly higher proportion than in Groups A (p=0.002) and C (p= 0.074).

Group C (Sleep Deprivation + Omega-3 Supplementation): Epithelial apoptosis was absent in 70% of animals, while 30% displayed Grade 1 apoptosis, a significantly lower frequency compared to Groups A (p=0.001) and B (p=0.003). Interacinar collagen deposition was present in 30% of animals, a non-significant difference compared to Groups A (p=0.074) and B (p=0.074). The smooth muscle layer displayed a regular arrangement in 70% of animals, a significantly higher proportion than Group B (p=0.004) but lower than Group A (p=0.004).

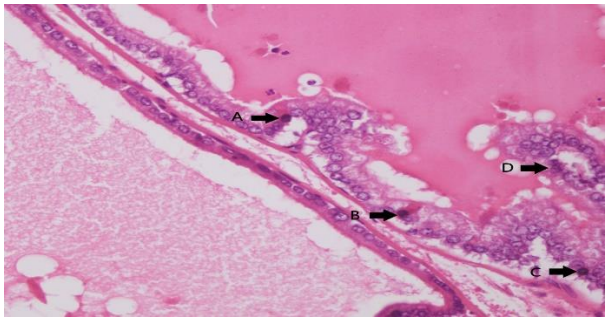

Figure 1: H&E stained histological image of rat prostate of group B, illustrating apoptosis. Hyperchromatic nuclei, marked as A, B and C, indicate early apoptotic events. Label D denotes the Fragmentation of Nuclei that denotes further progression of apoptosis.

Table 1: Percentage of apoptosis, collagen fibre deposition and smooth muscle cells around the acini among control group A and experimental groups B and C

		Group A (n =10)	Group B (n =10)	Group C (n =10)	p-value
Apoptosis	0 lesions	10	3	7	
	0-5 apoptotic cells	0	6	3	
	5-10 apoptotic cells	0	1	0	
	Present	0%	70%	30%	.003
	Absent	100%	30%	70%	
Interacinar Collagen Fiber Deposition	Present	0%	70%	30%	.003
	Absent	100%	30%	70%	
Smooth Muscles Around Acini	Irregular	0%	70%	30%	.003
	Regular	100%	30%	70%	

Table 2: Intergroup comparison showing the difference in *p*- p-values of quantitative and qualitative parameters among control group A and experimental groups B and C

	Group A Vs Group B	Group A Vs Group C	Group B Vs Group C
	p-value	*p*-value	*p*-value
Apoptosis	0.002*	0.156	0.022*
Interacinar Collagen Fiber Deposition	0.000*	0.073	0.074
Smooth Muscles Around Acini	0.002*	0.073	0.074

The present study unveiled a marked increase in epithelial apoptosis within the prostatic acini of sleep-deprived rats, as evidenced by nuclear fragmentation and hyperchromatism. The hyperpigmentation of nuclei in apoptosis is a result of a combination of factors, including chromatin condensation, DNA fragmentation, altered staining affinity, and increased dye penetration. These changes contribute to the enhanced staining intensity of the nucleus, making it a hallmark of apoptotic cells.

This observation aligns with previous reports implicating sleep deprivation in neuronal apoptosis and suggests a potential disruption in androgen homeostasis, consequently triggering apoptotic cascades crucial for prostate survival and function.[10,11] Corroborating these findings, a study on rat brain tissue demonstrated that sleep loss induces neuronal damage and triggers apoptotic pathways.[12] The prostate gland's reliance on a continuous supply of androgens for cellular viability and functional integrity provides a plausible explanation for the observed apoptotic response.

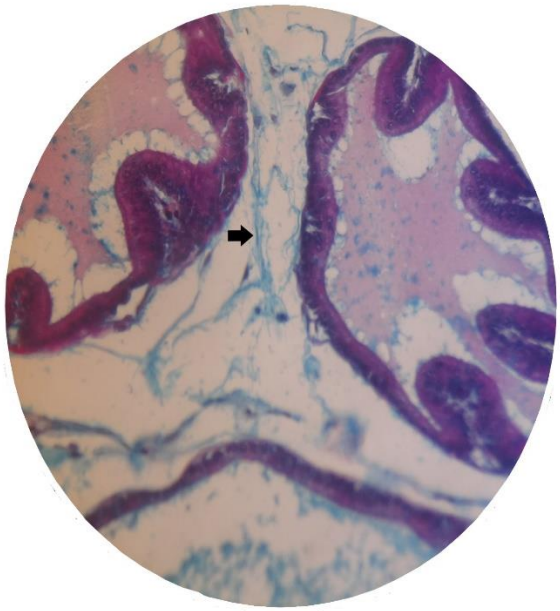

Figure 2: Masson Trichrome stained image depicting collagen fibre deposition in the interacting space (indicated by black arrowhead) of prostate glands in the sleep-deprived group B. The arrow indicates the presence of collagen accumulation, suggesting tissue remodelling associated with sleep deprivation.

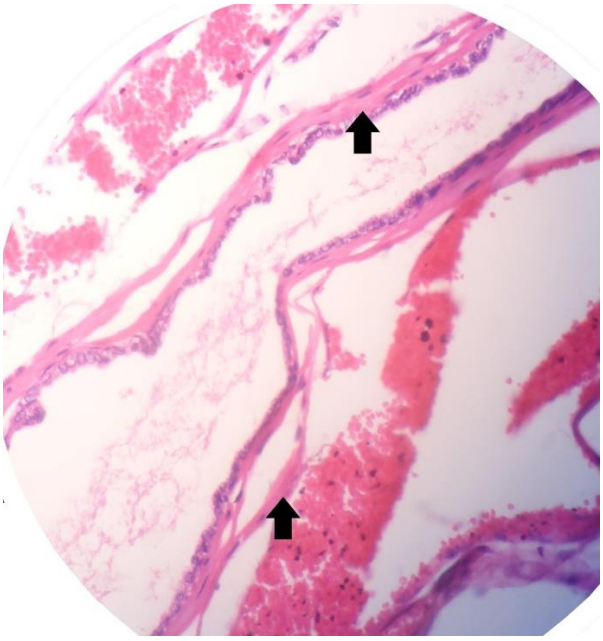

Figure 3: H & E stained photomicrograph displaying distortion in the architecture of smooth muscle in the rat prostate of group B, indicated by the arrowhead. This distortion suggests alterations in tissue integrity and function associated with sleep deprivation.

Discussion

A reduction in androgen levels activates programmed cell death mechanisms in the prostate, which manifests as condensed, hyperchromatic nuclei and gradual cellular shrinkage.[10] Notably, the administration of omega-3 fatty acids effectively attenuated epithelial apoptosis in the present study, a finding consistent with reports of their anti-apoptotic effects in neuronal cell lines.[13,14]

The histological analyses revealed a significant accumulation of collagen fibres within the inter-acinar spaces of the prostate glands in sleep-deprived rats, a phenomenon largely absent in control animals and markedly reduced in the omega-3 supplementation group. Sleep deprivation is associated with systemic inflammation. Chronic inflammation can stimulate fibroblast activity and collagen production, leading to increased collagen deposition in the prostate. These observations corroborate existing evidence linking inflammation to increased prostatic collagen content.[15] Omega-3 fatty acids possess anti-inflammatory properties, counteracting the inflammatory cascade and subsequent collagen fibre deposition in the prostate induced by the stress of sleep deprivation. This finding aligns with a study conducted on rat liver tissue, where omega-3 supplementation exhibited a preemptive role in mitigating fibrosis development.[16] Numerous researches have demonstrated a positive correlation between prostate inflammation and increased collagen content, with inflamed prostates exhibiting substantially higher collagen levels compared to non-inflamed glands.[17] Furthermore, previous research on the rat ventral prostate has implicated collagen fibre deposition as a growth-limiting factor in prostate gland development.[18]

A striking observation in the present study was the distortion and irregularities in the smooth muscle layer surrounding the prostatic acini in 70% of unsupplemented sleep-deprived rats. These alterations manifested as detachment, increased intercellular spacing, and multilayering of the smooth muscle cells, in contrast to the regular, single-layered arrangement observed in control animals.[19] Remarkably, omega-3 supplementation mitigated these architectural disruptions, with only 30% of the supplemented group exhibiting smooth muscle distortion. These findings align with previous reports linking decreased testosterone levels to an increased fibromuscular content and epithelial degeneration in the prostate.[20] The androgen dysregulation induced by sleep deprivation may underlie the observed smooth muscle distortion in the sleep-deprived group, while omega-3 supplementation potentially modulates smooth muscle cell proliferation and maintains tissue homeostasis.[21]

In summary, these compelling findings collectively suggest that chronic sleep deprivation adversely impacts prostate histomorphology through multifaceted mechanisms involving androgen dysregulation, inflammation, consequent epithelial apoptosis, collagen deposition, and smooth muscle architectural distortion. Conversely, omega-3 fatty acid supplementation

exhibits ameliorative effects, potentially by modulating inflammatory, apoptotic, and proliferative pathways. These insights underscore the critical importance of sleep homeostasis and dietary interventions in preserving prostate gland integrity and optimal health.

Conclusions

This study observed that sleep deprivation caused significant changes in the histomorphology of the rat prostate gland. It was also shown that omega-3 fatty acids had ameliorative effects on the histomorphology of the rat prostate gland. The prostate glands of the rats in the sleep-deprived group showed detrimental effects on the prostate gland including apoptosis, collagen fibre deposition in the interacinar space and irregular arrangement of the smooth muscle fibers around the acini. the prostate glands in rats that received omega-3 supplementation, did not show such marked changes as compared to the sleep-deprived group, supporting the protective role of omega-3 fatty acids.

References

1. Coco M, Buscemi A, Guarnera M, La Paglia R, Perciavalle V, Di Corrado D. Sleep deprivation and physiological responses. A case report. Journal of Functional Morphology and Kinesiology. 2019 Apr 3;4(2):17. https://doi.org/10.3390/jfmk4020017
2. Rizk NI, Rizk MS, Mohamed AS, Naguib YM. Attenuation of sleep deprivation dependent deterioration in male fertility parameters by vitamin C. Reproductive Biology and Endocrinology. 2020 Dec;18:1-3. DOI: 10.1186/s12958-020-0563-y
3. Mangar S, Abbadasari M, Carollo A, Esposito G, Ahmed H, Shah T, Dimitriou D. Understanding Sleep Disturbances in Prostate Cancer—A Scientometric Analysis of Sleep Assessment, Aetiology, and Its Impact on Quality of Life. Cancers. 2023 Jul 4;15(13):3485. doi: 10.3390/cancers15133485
4. Chen JF, Lin PW, Tsai YR, Yang YC, Kang HY. Androgens and androgen receptor actions on bone health and disease: from androgen deficiency to androgen therapy. Cells. 2019 Oct 25;8(11):1318. DOI: 10.3390/cells8111318
5. Allam-Ndoul B, Guénard F, Barbier O, Vohl MC. A study of the differential effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on gene expression profiles of stimulated Thp-1 macrophages. Nutrients. 2017 Apr 25;9(5):424 DOI:10.1016/j.nutos.2023.10.004
6. Allam-Ndoul B, Guénard F, Barbier O, Vohl MC. A study of the differential effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on gene expression profiles of stimulated Thp-1 macrophages. Nutrients. 2017 Apr 25;9(5):424. doi: 10.3390/nu9050424
7. Chang JP-C, Pariante CM, Su K-P. Omega-3 fatty acids in the psychological and physiological resilience against COVID-19. Prostaglandins, Leukotrienes and Essential Fatty Acids.. doi: 10.1016/j.plefa.2020.102177
8. Abdou HM, Hassan MA. Protective role of omega-3 polyunsaturated fatty acid against lead acetate-induced toxicity in liver and kidney of female rats. BioMed research international. 2014;2014:435857.
9. Nor NUR, Nawi A, Wan Ahmad WAN, Noordin L. Sleep Deprivation Models in Rodents. Jurnal Sains Kesihatan Malaysia. 2021;19:29-34.
10. Pelliccia A, Capradossi F, Corsi F, Tarquini GD, Bruni E, Reichle A, et al. Androgen Deprivation Freezes Hormone-Sensitive Prostate Cancer Cells in a Reversible, Genetically Unstable Quasi-Apoptotic State, Bursting into Full Apoptosis upon Poly(ADP-ribose) Polymerase Inhibition. 2023;24(3):2040.
11. Citalingam K, Abas F, Lajis NH, Othman I, Naidu R. Anti-Proliferative Effect and Induction of Apoptosis in Androgen-Independent Human Prostate Cancer Cells by 1,5-Bis(2-hydroxyphenyl)-1,4-pentadiene-3-one. 2015;20(2):3406-30.
12. Xie G, Huang X, Li H, Wang P, Huang P. Caffeine-related effects on cognitive performance: Roles of apoptosis in rat hippocampus following sleep deprivation. Biochemical and Biophysical Research Communications. 2021;534:632-8.
13. Wu Y, Zhang J, Feng X, Jiao W. Omega-3 polyunsaturated fatty acids alleviate early brain injury after traumatic brain injury by inhibiting neuroinflammation and necroptosis. Translational neuroscience. 2023;14(1):20220277.
14. Koppelmann T, Pollak Y, Ben-Shahar Y, Gorelik G, Sukhotnik I. The Mechanisms of the Anti-Inflammatory and Anti-Apoptotic Effects of Omega-3 Polyunsaturated Fatty Acids during Methotrexate-Induced Intestinal Damage in Cell Line and in a Rat Model. Nutrients. 2021;13(3). doi: 10.3390/nu13030888
15. Golanski J, Szymanska P, Rozalski M. Effects of Omega-3 Polyunsaturated Fatty Acids and Their Metabolites on Haemostasis—Current Perspectives in Cardiovascular Disease. 2021;22(5). doi: 10.3390/ijms22052394
16. Al-Attar AM, Al-Rethea HA. Chemoprotective effect of omega-3 fatty acids on thioacetamide induced hepatic fibrosis in male rats. Saudi journal of biological sciences. 2017;24(4):956-65. DOI: https://doi.org/10.18585/inabj.v16i3.3077
17. Schneider AJ, Serrell EC, Grimes M, Wang S, Bushman W. Histologic inflammation and collagen content are not positively correlated in human BPH. The Prostate. 2023;83(16):1529-36. https://doi.org/10.1002/pros.24611
18. Liu TT, Thomas S, McLean DT, Roldan-Alzate A, Hernando D, Ricke EA, et al. Prostate enlargement and altered urinary function are part of the aging process. Aging. 2019;11(9):2653-69. doi: 10.18632/aging.101938
19. Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, Morgan KG. Mechanisms of Vascular Smooth Muscle Contraction and the Basis for Pharmacologic Treatment of Smooth Muscle Disorders. Pharmacological reviews. 2016;68(2):476-532. DOI: 10.1124/pr.115.010652

20. Mbaka G, Ogbonnia S, Sulaiman A, Osiagwu D. Histomorphological effects of the oil extract of Sphenocentrum jollyanum seed on benign prostatic hyperplasia induced by exogenous testosterone and estradiol in adult Wistar rats. Avicenna journal of phytomedicine. 2019;9(1):21-33. PMCID: PMC6369320
21. Alvarenga T, Hirotsu C, Mazaro-Costa R, Tufik S, Andersen M. Impairment of male reproductive function after sleep deprivation. Fertility and sterility. 2015;103. DOI: 10.1016/j.fertnstert.2015.02.002

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N.M - Experimentation/Study Conduction
K.A, A.A - Analysis/Interpretation/Discussion
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N.M, A.Q, M.M.K, A.A - Critical Review

All authors approved the final version to be published & agreed to be accountable for all aspects of the work.