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Light physical activity and vitamin D improve sperm quality of male Rattus norvegicus exposed to cigarette smoke

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ABSTRACT

Background: Male infertility is a significant global health issue, with rising prevalence linked to environmental factors such as secondhand smoke, which impairs sperm quality. This study aimed to examine how light physical activity and vitamin D supplementation improved sperm quality in rats exposed to cigarette smoke.

Methods: This study used a randomized experimental design with 38 rats, divided into two groups. The control group was exposed to cigarette smoke and received vitamin D at 18 IU/kg body weight (BW) daily. The treatment group was exposed to cigarette smoke, received the same vitamin D dose, and underwent light physical activity. The 35-day research period involved the analysis of evaluated parameters. Differences between these parameters were assessed using an independent t-test, with statistical significance set at a *P*-value < 0.05.

Results: Compared to the control group (*p*-values< 0.001), the treatment group, receiving light physical activity and vitamin D supplementation, exhibited significantly lower malondialdehyde (MDA) levels (0.96 ± 0.14 nmol/mL), higher vitamin D receptor expression (2.44 ± 0.58 cells/HPF), and increased testosterone levels (4.33 ± 0.76 ng/dL). Sperm quality was also significantly improved in the treatment group, showing higher sperm count ($2.24 \pm 0.81 \times 10^6$/mL), motility ($4.20 \pm 0.23\%$), viability ($3.07 \pm 0.39\%$), and normal morphology ($5.34 \pm 0.17\%$) (*p*-values< 0.001).

Conclusion: Light physical activity and vitamin D supplementation improved sperm quality in cigarette smoke-exposed male rats, correlating with reduced MDA and elevated vitamin D receptor expression and testosterone. Further clinical trials are warranted to explore human applications.

Keywords: cigarette smoke, infertility, light physical activity, sperm quality, vitamin D.
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INTRODUCTION

Infertility is a growing global health concern, particularly in developing countries, affecting an estimated 48 million couples and 186 million individuals worldwide. While historically focused on women, declining sperm quality is increasingly recognized as a significant contributor to male infertility. Infertility, defined as the inability of couples of childbearing age to conceive naturally after one year of regular unprotected intercourse, is a reproductive system disorder impacting both men and women.[1,2]

Globally, smoking contributes to approximately 15% of infertility cases. Among these, male factors account for a significant 40-60%. In men experiencing infertility, nearly 60% have erectile dysfunction, while 40% present with poor ejaculate quality. Semen analysis in infertility cases commonly reveals disorders in sperm concentration, morphology, and motility as key contributing factors.[3,4]

Physical activity encompasses any bodily movement produced by skeletal muscles that expends energy, including activities for leisure, transportation, or work. Light physical activity presents a potential strategy to improve male fertility impaired by obesity and diabetes. Exercise enhances spermatogenesis and semen quality in lifestyle-related infertility by boosting testicular antioxidant defenses, decreasing pro-inflammatory cytokines, and promoting steroidogenesis. Consequently, physically active individuals tend to exhibit a more anabolic hormonal profile and healthier semen production.[4]

Beyond its role in calcium metabolism, vitamin D exhibits significant antioxidant activity. It acts as a membrane antioxidant, inhibiting zinc-induced lipid peroxidation in the central nervous system. Furthermore, vitamin D reduces oxidative stress by enhancing systemic antioxidant defenses, such as glutathione levels and the activity of glutathione peroxidase and superoxide dismutase in astrocytes and the liver. This antioxidant capacity contributes to vitamin D's neuroprotective effects against zinc-induced apoptosis in

the substantia nigra, acting both as a direct free radical scavenger and by inducing the synthesis of neuroprotective proteins against reactive oxygen species.[6,7]

Reactive Oxygen Species (ROS) contribute significantly to infertility by impairing sperm quality. Environmental pollutants, such as cigarette smoke, are a major source of ROS. Studies have demonstrated that cigarette smoke exposure reduces sperm quality; for instance, one study in mice showed a decline in sperm quality after exposure to 10 cigarettes/tail/day for 20, 40, and 60 days.[8]

Endogenous antioxidants, such as catalase (binding Fe), glutathione peroxidase and glutathione S-transferase (binding Se), and superoxide dismutase (binding Cu, Zn, and Mn), counteract oxidative damage from free radicals. However, when free radical production overwhelms cellular antioxidant defenses, exogenous antioxidants become necessary for neutralization. Vitamin D is one such exogenous antioxidant.[6] Vitamin D can mitigate oxidative stress and boost sperm count in mice exposed to cigarette smoke. Additionally, it enhances sperm capacitation and survival, likely by stabilizing chromosome structure and protecting sperm DNA from smoke-induced damage.[9]

This study investigated how light physical activity and vitamin D administration improve sperm quality in cigarette smoke-exposed male Wistar rats by examining their effects on malondialdehyde, vitamin D receptor expression, and testosterone levels.

METHODS

This randomized post-test-only controlled experiment involved 36 male Wistar rats, with the sample size determined using Federer's repeated treatment formula.[10,11] Following a seven-day acclimatization period, the rats were randomly assigned to a control group (n=18) and a treatment group (n=18). Both groups were exposed to cigarette smoke (four cigarettes daily) and received vitamin D supplementation (18 IU/kg BW/day) for 35 days. Additionally, the treatment group underwent light physical activity using a cylindrical roller cage (20 cm height, 200 cm^3 volume, limited to ≤25 rotations per session). On day 36, all rats were euthanized for subsequent analysis.

Rats were exposed to smoke from GGM-brand cigarettes (4.9 mg tar, 2.5 mg nicotine). Vitamin D3 supplementation was administered. This study received ethical approval from the Universitas Udayana Local Research Ethics Committee (Decision No. 2003/UN.2.2.VII14. LT/2024), and all procedures adhered to the NIH Guide for the Care and Use of Laboratory Animals.[12]

Blood samples for testosterone analysis via ELISA were collected from the lateral tail vein. Following euthanasia by cervical dislocation, testes were surgically removed for histological and immunohistochemical examinations. Remaining tissues were incinerated in disposal pits. Testicular malondialdehyde (MDA) levels, an oxidative stress marker, were measured spectrophotometrically using ELISA and expressed as nmol/mL. Vitamin D receptor (VDR) expression, a chromatin interaction transcription factor, was assessed in testes via immunohistochemistry and quantified as cells per high power field (cells/HPF). Serum testosterone levels were measured using ELISA and expressed in ng/dL.

Sperm quality, encompassing count, motility, viability, and morphology, was assessed. Sperm count was determined using an improved Neubauer hemocytometer at 400× magnification. Motility was evaluated by observing 10–15 µL of sperm suspension under 400× magnification to distinguish motile from immotile sperm. Viability was assessed using eosin-nigrosin staining and microscopic examination at 400× magnification, where live sperm remained unstained and dead sperm absorbed the dye. Morphology was evaluated on air-dried smears fixed in methanol, stained with 1% safranin, and examined microscopically.[13–15]

Data, collected from the Analytical Chemistry and Histology Laboratories, Faculty of Medicine, Universitas Udayana, were statistically analyzed using SPSS. Normality was confirmed via the Shapiro-Wilk test, and homogeneity of variance was established with Levene's test ($p > 0.05$ for all variables: MDA levels, VDR expression, testosterone levels, and sperm quality). Group differences were compared using an independent t-test, with significance set at $p < 0.05$.

RESULTS

Micro-anatomical preparations were used to examine MDA levels, VDR expression, testosterone levels, and sperm quality in testicular samples. Experimental rats were acclimatized in the laboratory animal room prior to the study. The average body weight was 208.61 ± 10.33 g in the control group and 199.50 ± 12.58 g in the treatment group, with no significant difference between groups ($p > 0.05$; Table 1).

MDA level data were normally distributed and showed homogeneous variance ($p > 0.05$), justifying the use of an independent *T*-test. The treatment group, which received vitamin D supplementation combined with light physical activity, had significantly lower MDA levels than the control group exposed to cigarette smoke alone (mean 2.83 vs. 3.79 nmol/mL; $p < 0.001$), with a mean difference of 0.96 nmol/mL (Table 2).

VDR expression data also met normality and homogeneity assumptions ($p > 0.05$). The treatment group showed significantly higher VDR expression than the control group (mean difference: 2.44 cells/HPF; $p < 0.001$). Testosterone levels, assessed via ELISA and expressed in ng/dL, were normally distributed with homogeneous variance. The treatment group had significantly higher testosterone levels (mean difference: 4.33 ng/dL; $p < 0.001$).

Sperm quality was evaluated using samples from the cauda epididymis, processed in 0.9% NaCl to obtain a uniform suspension. Parameters assessed included sperm count, motility, viability, and morphology. All datasets met assumptions of normality and homogeneity. The treatment group showed significantly higher values across all measures compared to the control group: sperm count (mean difference: 2.24×10^6 cells/mL), motility (4.20% higher), viability (31.03% higher), and normal morphology (5.34% higher), all with p-values < 0.001.

DISCUSSION

This study examined the combined effects of vitamin D supplementation and light physical activity on oxidative stress, VDR expression, testosterone levels, and sperm quality in cigarette smoke-exposed male Wistar rats.[16] The findings show that this combined intervention significantly reduced the negative impact of cigarette smoke on their reproductive function. The treatment group exhibited significantly lower MDA levels, a marker of lipid peroxidation and oxidative stress, compared to the control group. This indicates that vitamin D and light physical activity effectively reduced oxidative stress induced by cigarette smoke, consistent with studies highlighting elevated MDA in smokers and the antioxidant effects of both interventions.[17]

Significantly higher VDR expression in the treatment group suggests enhanced vitamin D signaling. Since cigarette smoke downregulates VDR while vitamin D and physical activity upregulate it synergistically to support cellular function and reduce inflammation, this enhanced signaling likely contributes to improved testicular health and spermatogenesis in the toxic environment of smoke exposure.[18]

The significant increase in testosterone levels in the treatment group supports previous research indicating that physical activity drives luteinizing hormone and testosterone production, while vitamin D supports testosterone biosynthesis. This improvement likely reflects enhanced testicular steroidogenesis as a consequence of reduced oxidative damage and inflammation.[19,20] Consistent with prior research, this study identified a relationship between physical activity and stress levels, potentially mediated by the hypothalamic-pituitary-adrenal (HPA) axis or changes in circulating glucocorticoids. The HPA axis is crucial for adapting to physical and psychological stress.[5]

The treatment group exhibited marked improvements in sperm parameters—count, motility, viability, and morphology. These positive changes confirm the protective effect of the combined intervention against smoke-induced testicular toxicity, counteracting the known impairments to spermatogenesis caused by cigarette smoke via oxidative stress and hormonal disruption, effects that vitamin D and physical activity have individually shown to reverse.[21–23]

Combining vitamin D supplementation with light physical activity protects against cigarette smoke's reproductive toxicity. This combined approach mitigates damage by reducing oxidative stress, enhancing VDR expression, restoring testosterone levels, and improving overall sperm quality. The superior sperm quality in the combined treatment group likely results from their synergistic enhancement of testicular mitochondrial function and cellular homeostasis. Prior research indicates that physical activity boosts mitochondrial biogenesis and efficiency, thus decreasing ROS production and improving energy for sperm production.[24]

Concurrently, vitamin D protects mitochondrial integrity and modulates antioxidant defenses by upregulating genes involved in redox balance. Essential for male and female reproductive health, vitamin D is crucial for sperm quality and motility in men. Lower levels in men are associated with reduced fertility and poorer outcomes in assisted reproductive treatments. Supplementation with vitamin D and docosahexaenoic acid (DHA) has been shown to significantly reduce seminal oxidative stress in men with poor sperm motility, potentially improving their reproductive chances.[25-26]

This study had limitations. The use of a cylindrical roller cage for assessing

Table 1. Average body weight of Wistar rat

No	Groups	Body weight (g)	p^*
1	Control	208.61±10.33	0.476
2	Treatment	199.50±12.58	
	p^{**}	0.186	

p^* Data normally distributed $p > 0.05$;
p^{**} Varians data were homogeneous $p > 0.05$.

Table 2. Effects of cigarette smoke, physical activity, and vitamin D on Malondialdehyde (MDA) levels, Vitamin D Receptor (VDR) expression, testosterone, and sperm quality

Parameter	Group	Mean ± SD	Mean Difference	*p*-value
MDA Levels (nmol/mL)	Control	1.77 ± 0.64	0.96 ± 0.14	<0.001
	Treatment	0.81 ± 0.22		
VDR Expression (Cells/HPF)	Control	22.19 ± 1.59	2.44 ± 0.58	<0.001
	Treatment	24.63 ± 0.78		
Testosterone Levels (ng/dL)	Control	18.96 ± 0.49	4.33 ± 0.76	<0.001
	Treatment	23.29 ± 1.25		
Sperm Count (cells/mL)	Control	21.75 ± 1.56	2.24 ± 0.81	<0.001
	Treatment	23.99 ± 2.88		
Sperm Motility (%)	Control	47.89 ± 8.17	4.20 ± 0.23	<0.001
	Treatment	52.09 ± 9.26		
Sperm Viability (%)	Control	65.92 ± 6.36	3.07 ± 0.39	<0.001
	Treatment	68.99 ± 7.44		
Sperm Morphology (%)	Control	71.58 ± 5.78	5.34 ± 0.17	<0.001
	Treatment	76.92 ± 5.95		

dL, deciliter; HPF, High Power Field; mL, milliliter; nmol, nanomoles; ng, nanograms; SD, standard deviation

light physical activity may not accurately reflect standardized intensity or duration. Furthermore, the lack of varied vitamin D dosages and long-term follow-up restricts the generalizability of these findings. Future research should employ multiple exercise intensities with standardized protocols, explore different vitamin D doses, and include longer observation periods to evaluate the sustained physiological effects. Incorporating molecular analyses could also clarify the underlying mechanisms connecting vitamin D, physical activity, and reproductive health.

CONCLUSION

This study demonstrates that combining light physical activity with daily vitamin D supplementation (18 IU/kg body weight) enhances sperm quality—count, motility, viability, and morphology—in male rats exposed to cigarette smoke. These improvements correlate with reduced MDA levels, increased vitamin D receptor expression, and elevated testosterone levels.

ETHICAL CONSIDERATION

The Ethics Committee of the Faculty of Medicine, Universitas Udayana reviewed and approved the research protocol with number 2003/UN.2.2.VII14.LT/2024.

CONFLICT OF INTEREST

This study contains no conflicts of interest.

FUNDING

The study received no funding from any institution.

AUTHOR CONTRIBUTIONS

MBA, BKS, IWPSY, and IMS designed the study, collected and processed the data, and wrote the manuscript. IMJ, IBPM, NML, and IGAW collected the data, performed the statistical analysis, and revised the manuscript.

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