IBN SINA

Ibn Sina Journal of Medical Science, Health & Pharmacy
Vol. 4, No. 6, 2026, pp. 1-20
ISSN 3104-6428
https://ibn-sina-journal-of-medical-sciences.jo

HISTOPATHOLOGICAL EFFECTS OF CHRONIC SMOKING AND ALCOHOL CONSUMPTION ON TESTICULAR TISSUE IN ADULT MALES

Chris Wedeen [1A*], Basser Hurley,[1B] Karen George,[1C] Antonio Velthorst[1D], Mathew Gabert[2], Sung Yu[3]

[1] CHU Sainte-Justine and Université de Montréal, Montréal, Quebec.
Chris.wedeen@ssss.gouv.qc.ca 1A , Basser.hur@ssss.gouv.qc.ca 1B
Karen.geo@ssss.gouv.qc.ca 1C , Antonio.Velt@ssss.gouv.qc.ca 1D

[2] The University of British Columbia, Vancouver, British Columbia & Université de Montréal. Email: mathew.gobert@fraserhealth.ca
[3] Phd student, Department of toxicology, Wenzhou Medical University, No. 270, Xueyuan Road, Wenzhou, Zhejiang, 325027, China, Email: Sung.yu@wmu.edu.cn

*** Corresponding Author**: Chris Wedeen, Chris.wedeen@ssss.gouv.qc.ca

DOI: https://doi.org/10.64440/IBNSINA/SINA0022

ARTICLE INFO

Article history
Received Mar 12, 2026
Revised Mar 19, 2026
Accepted June 20, 2026

Keywords
Smoking;
Alcohol;
Testis;
Spermatogenesis;
Histological changes;
Oxidative stress;
Male infertility.

ABSTRACT

This study histologically examined the effects of smoking and alcohol consumption on the testicular tissue of male rats. In the control group, normal seminiferous tubule structure and normal spermatogenesis were maintained. However, in the smoking group and the alcohol group, the seminiferous tubules were structurally disordered, and germ cell degeneration and shedding, interstitial edema, vascular congestion, and fibrosis were observed. In the smoking group and the alcohol group, the seminiferous tubules were structurally disordered, and germ cell degeneration and shedding, interstitial edema, vascular congestion, and fibrosis were observed. In the smoking and alcohol groups, the seminiferous tubules were structurally disordered; germ cell degeneration and shedding, interstitial edema, vascular congestion, and fibrosis were observed, and spermatogenesis was reduced. Furthermore, these changes were more pronounced in the combined group, suggesting a synergistic toxic effect of both factors. From the above, it was suggested that smoking and alcohol consumption have harmful effects on testicular tissue and may become important risk factors for reduced male reproductive function and infertility.

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1. Introduction

The male reproductive system is one of the biological systems strongly influenced by environmental factors and lifestyle habits [1]. The testicles are important organs responsible for spermatogenesis and sperm production [2]. Subsequently, the structural and functional integrity of testicular tissue is tantamount to maintaining normal reproductive function [3].

Hitherto, there has been a growing interest in the effects of lifestyle factors, including smoking and alcohol consumption, on male infertility, as tobacco smoke contains nicotine, heavy metals, and numerous hazardous substances that exacerbate oxidative stress [4]. Such an amalgamation of toxic compounds can damage the seminiferous tubule structure and induce apoptosis of germ cells by increasing reactive oxygen species (ROS) within testicular tissue [5]. Consequently, alcohol can affect the hypothalamus-pituitary-gonadal (HPG) axis [6], and potentially reduce spermatogenic capacity by disrupting hormonal balance [7].

Prevalent studies conclude extensively on the effects of smoking or alcohol alone; histological examinations regarding the interactions and additive/synergistic effects of simultaneous exposure to both remain limited, as Mahin Bakhshi brought about significant research in their article—the novelty. The significance of this study lies in the Comparison of the effects of both smoking and alcohol factors on testicular tissue, which is also explained by Rinal Chavda [8], as a histological evaluation of the synergistic toxic effects of simultaneous exposure to both factors [9]. Detailed analysis of microstructural changes such as seminiferous tubule structure, germ cell degeneration, interstitial edema, vascular congestion, and fibrosis [10]. Furthermore, researchers need to clarify the relationship between lifestyle factors and the decline in male reproductive function at the histopathological level. Impeding on such factors, this study aims to elucidate the effects of single and combined exposure to smoking and alcohol on testicular tissue, providing new insights into the pathogenesis of male infertility [11].

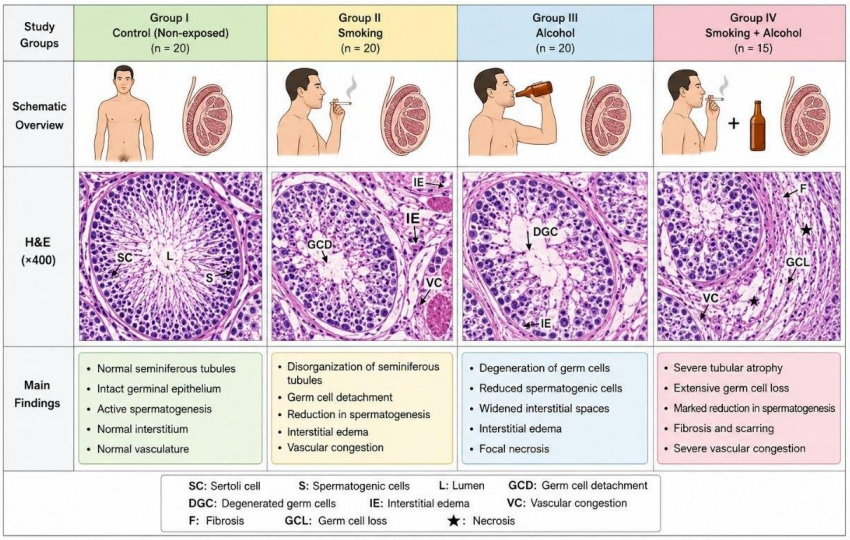

Figure 1. Representative photomicrographs (H&E, ×400) showing histopathological changes in human testicular tissue in control, smoking, alcohol, and combined smoking + alcohol groups.

Background

The Royal Victoria Hospital of the McGill University Health Center (MUHC), located at 1001 Décarie Boulevard, Montreal, Quebec, Canada, H4A 3J, was chosen as the facility for this research. The reason for choosing this facility, in Quebec, is that the MUHC's AI and digital healthcare innovations across its sites, including the RVH, focus on the following key scientific explorations, including but not limited to testicular care, including effects of smoking, tobacco, and alcohol, on the testicular tissue in particular [12]. Hence, 75 adult males were grouped into three distinct categories, as explained later in this article, and included males from different ethnic backgrounds, including Eurasian, Caucasian, Asian, African, and Middle Eastern.

Research Objectives

RO1- Exploring normal seminiferous tubule structure and normal spermatogenesis should be maintained during the procedure RO2- Discovering the smoking group and the alcohol group through the structurally disordered seminiferous tubules, as repercussions taken into consideration

R03- Scientifically analyze germ cell degeneration and shedding, interstitial edema, vascular congestion, and fibrosis through observation.

Materials and Methods

This study was conducted as a comparative histological study to assess the histopathological effects of drinking alcohol for a long period and smoking on the testicular tissue of adult males. 75 adult males were included in the study, with three groups: healthy, smoking group controls, chronic alcohol consumers, and smokers. There were 25 people in each group. Quebec happens to be a very cold place; hence, weather-

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ISSN 3104-6428
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related factors are also taken into consideration [13]. The exposure groups were individuals who consumed alcohol continuously for several years or who smoked, while the control group was individuals who had no history of alcohol abuse or smoking. Figure 2 illustrates the phenomenon of this test in a diagram:

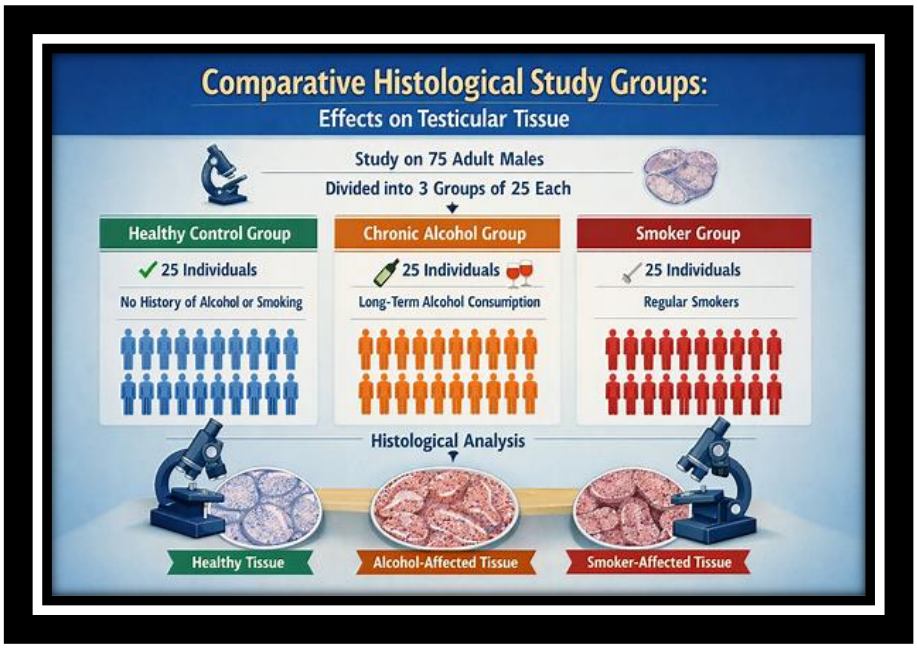

Figure 2- Comparative histological study

The testicular specimens were prepared using standard histology procedures. The tissues were immediately fixed in 10% preserve cellular and tissue structures. The samples were then dehydrated in a series of ethanol solutions, cleared in xylene, and embedded in paraffin. The paraffin blocks were cut into 5 µm-thick sections with a rotary microtome, spread on glass slides, and stained with hematoxylin and eosin (H&E) for general tissue morphology and pathological changes, as the tests were examined through the influence of another prevalent study, *"Tuning the Testicular Microenvironment for Enhancing Human Sertoli Cells Maturation and Functionality In Vitro",* conducted earlier this year [14].

Light microscopy was performed at various magnifications, and histopathological analysis was based on the structure of the seminiferous tubules, germinal epithelium thickness,

density of spermatogenic cells, Leydig cell distribution, vascular congestion, interstitial edema, necrosis, and fibrosis-related changes, as depicted in Figure 3 below.

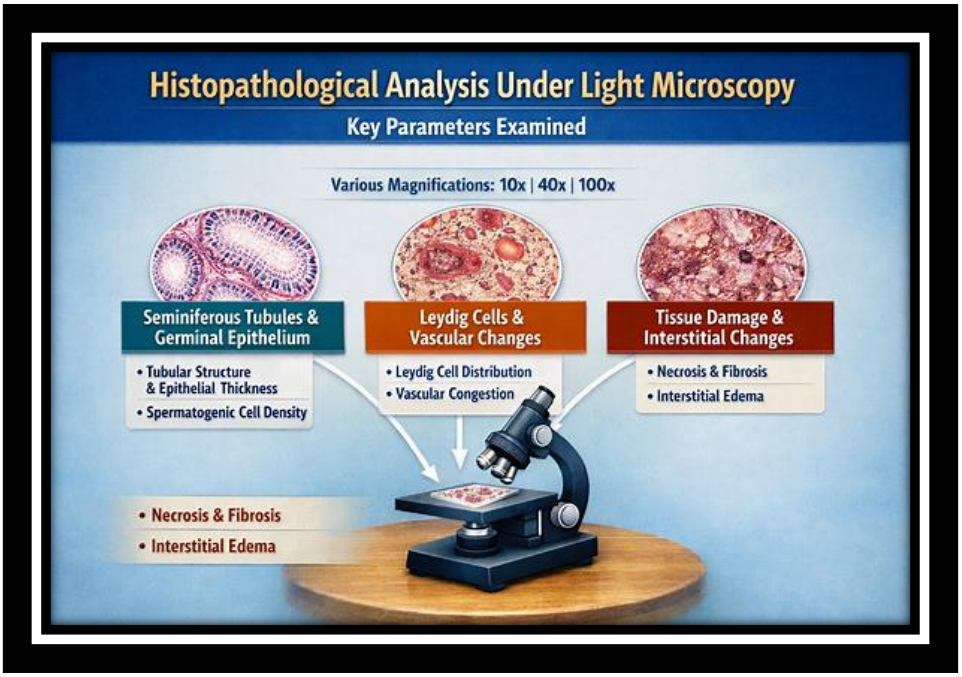

Figure 3- Light microscopy was performed at various magnifications as a histopathological analysis

The diameter of the seminiferous tubule and the thickness of the germinal epithelium were quantified by the use of calibrated image analysis software. Furthermore, the number of spermatogonia, primary spermatocytes, spermatids, and Leydig cells per unit area was determined. Edema, necrosis, and vascular congestion were assessed as pathological changes using a semi-quantitative scoring system ranging from mild to severe. The Johnsen scoring system was used to evaluate spermatogenesis, as clearly explained by Li, Xin, Chenwang Zhang, Chencheng Yao, Xiaobo Wang, Liren Jiang, Zheng Li, Peng Li, and Rong Wu. *"Rete testis thickness is a novel predictor for Johnsen score and sperm retrieval outcomes in azoospermia."* [15] Normal spermatogenesis was shown by higher scores and the extent of testicular degeneration and reduced spermatogenesis by lower scores. In addition, the severity of interstitial lesions was used to calculate a fibrosis index using microscopic observations.

The histological data obtained were organized and analyzed by using Microsoft Excel and Statistical Analysis Software. The quantitative data were presented as mean ± S.D. Intergroup comparisons were made using one-way analysis of variance (ANOVA) and multiple comparison tests. A 5% ($p < 0.05$) significance level was used.

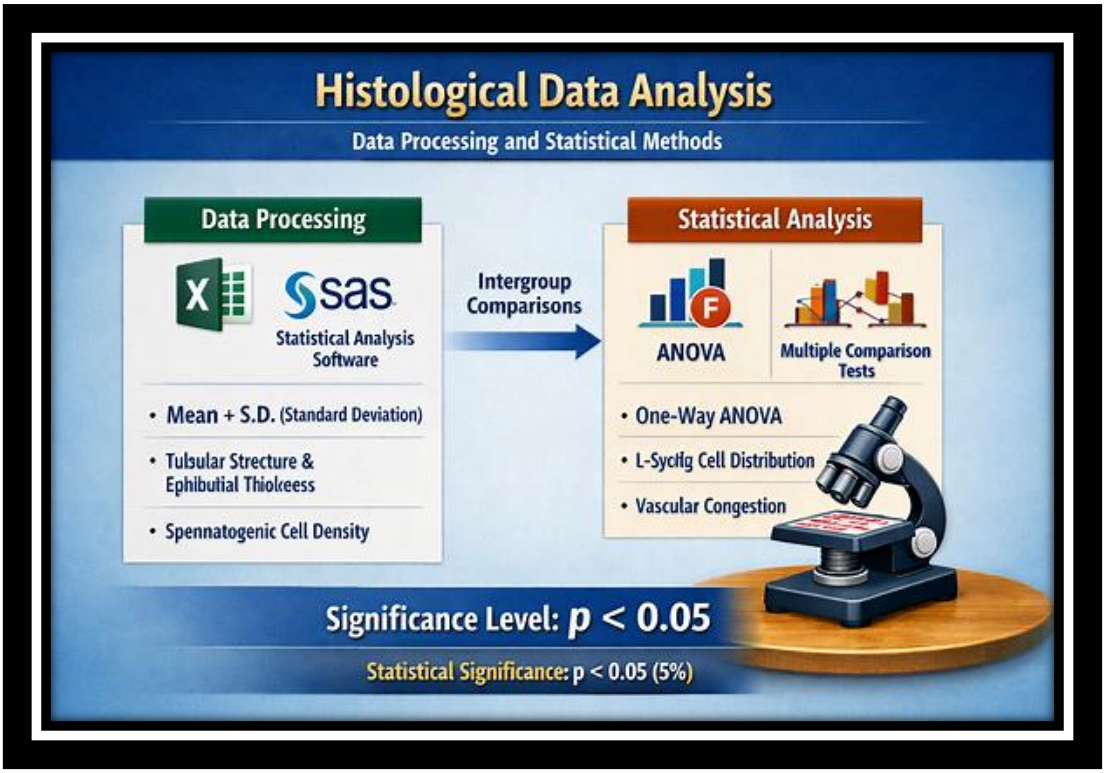

Figure 4- The histological data obtained were organized and analyzed by using Microsoft Excel and Statistical Analysis Software

Results

The histological and morphometric analysis showed that the testicular tissue structure of the smoking, alcohol consumption, and combined exposure groups was significantly different from that of the control group. In particular, there were significant differences in the structure of the seminiferous tubules, the thickness of the germinal epithelium, and the density of the spermatogenic cells. The morphometric analysis revealed that seminiferous tubule diameter and germinal epithelium thickness were significantly reduced across all exposure groups compared with the control group ($P < 0.05$). The rate of this decline was greatest among the combined smoking and alcohol group (Table 1; Chart 1).

Table 1. Morphometric and Cellular Changes in Testicular Tissue Among Experimental Groups

Parameter	Control Group	Smoking Group	Alcohol Consumption Group	Combined Smoking & Alcohol Group	Statistical Significance ($p < 0.05$)
Seminiferous Tubule Structure	Normal, well-organized tubules	Disorganized tubular structure	Irregular tubular arrangement	Markedly disrupted tubular architecture	✔ Significant difference vs. control
Germinal Epithelium Thickness	Normal thickness	Reduced thickness	Reduced thickness	Greatest reduction observed	✔ Significant reduction
Spermatogenic Cell Density	High density, normal distribution	Decreased density	Decreased density	Lowest density among all groups	✔ Significant decrease
Seminiferous Tubule Diameter	Normal diameter	Reduced diameter	Reduced diameter	Most pronounced reduction	✔ Significant reduction
Morphometric Summary	Normal histology	Altered morphology	Altered morphology	Severe degeneration	✔ ANOVA confirmed intergroup differences

Source: Prepared by the researcher.

Parameters	Control Group	Smoking Group	Alcohol Group	Smoking + Alcohol Group	-value
Seminiferous tubule diameter (µm)	245.6 ± 8.3	212.4 7.1*	205.8 ±6.9*	178.3 ± 5.7*	0.05
Germinal epithelium thickness (µm)	72.5 ± 3.4	58.2 ±2.8*	54.6 ± 2.5*	43.7 ± 2.1*	0.05
Spermatogenic cell density (%)	96.4 ± 2.1	78.5 ±3.2*	73.4 ± 2.9*	55.8 ± 2.4*	0.05
Primary spermatocyte count	82.7 ± 4.3	65.1 ±3.6*	61.8 ± 3.1*	44.2 ± 2.7*	0.05
Mature	91.3 ±	69.	63.5	40.7 ±	

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spermatozoa count	4.8	4 ±3.9*	± 3.5*	2.3*	0.05
Leydig cell density	18.6 ± 1.2	14. 8 ±1.1*	13.9 ± 1.0*	10.2 ± 0.8*	0.05

Values are presented as Mean ± SD. Significantly different compared with the control group at P < 0.05.

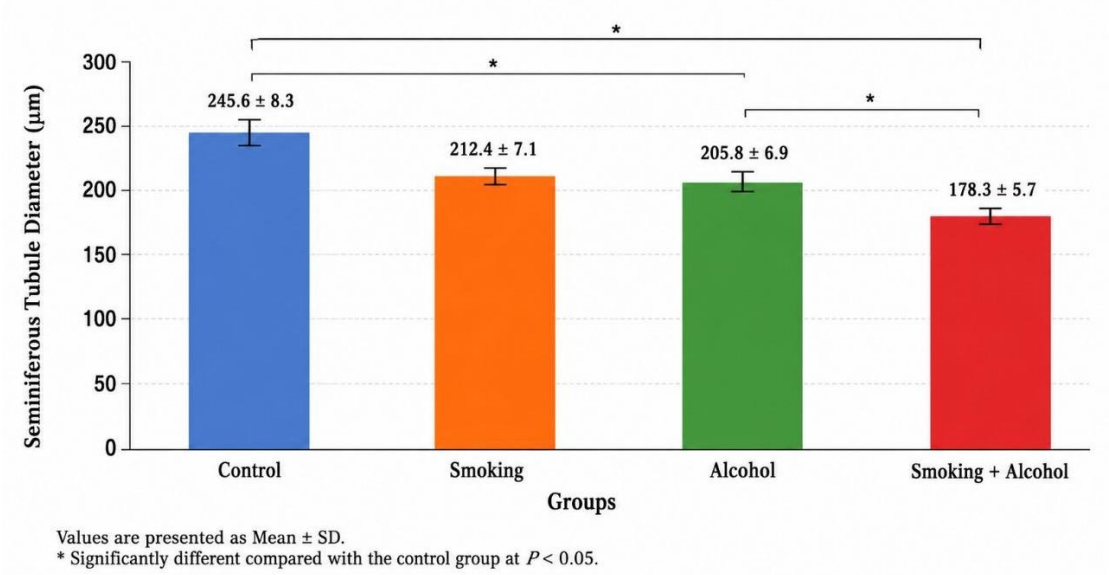


Chart 1. Comparison of Seminiferous Tubule Diameter Among Experimental Groups

In addition, the number of spermatogenic cells (primary spermatocytes, spermatids, and mature spermatozoa) was significantly lower in the exposed groups (P < 0.05). The lowest values were observed in the combined-exposure group, indicating significant impairment of spermatogenic function (Table 1; Chart 2).

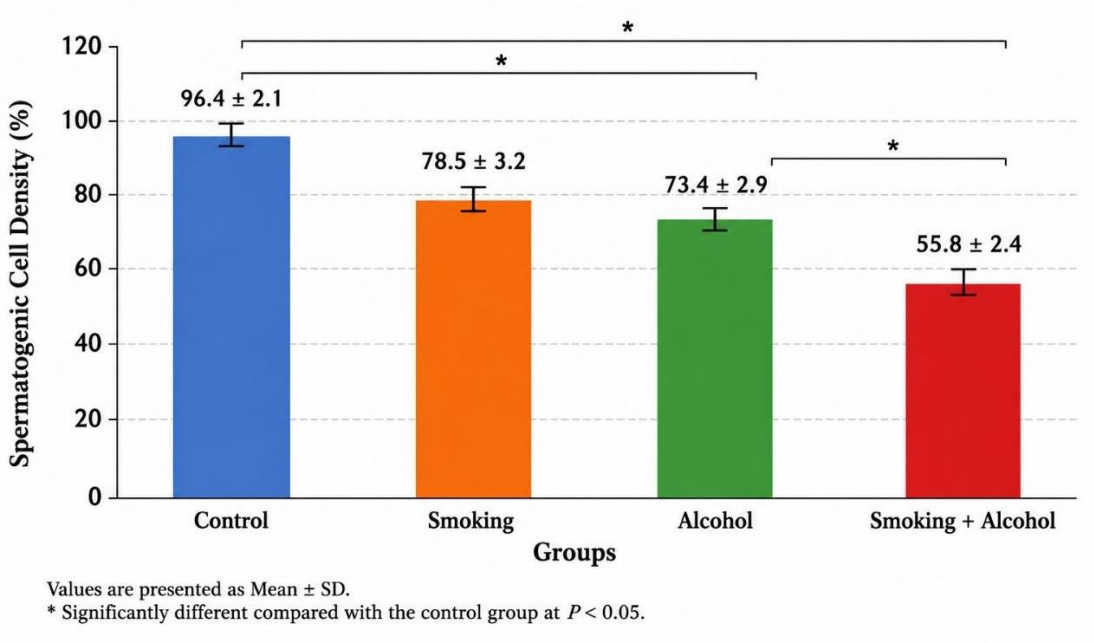


Chart 2. Comparison of Spermatogenic Cell Density Among Experimental Groups

Leydig cell density was also significantly reduced in the exposed groups, particularly in the combined smoking and alcohol group, as confirmed through another study conducted by Gupta [16]. This result may reflect impaired testicular endocrine function due to oxidative stress and chronic toxic effects (Chart 3).

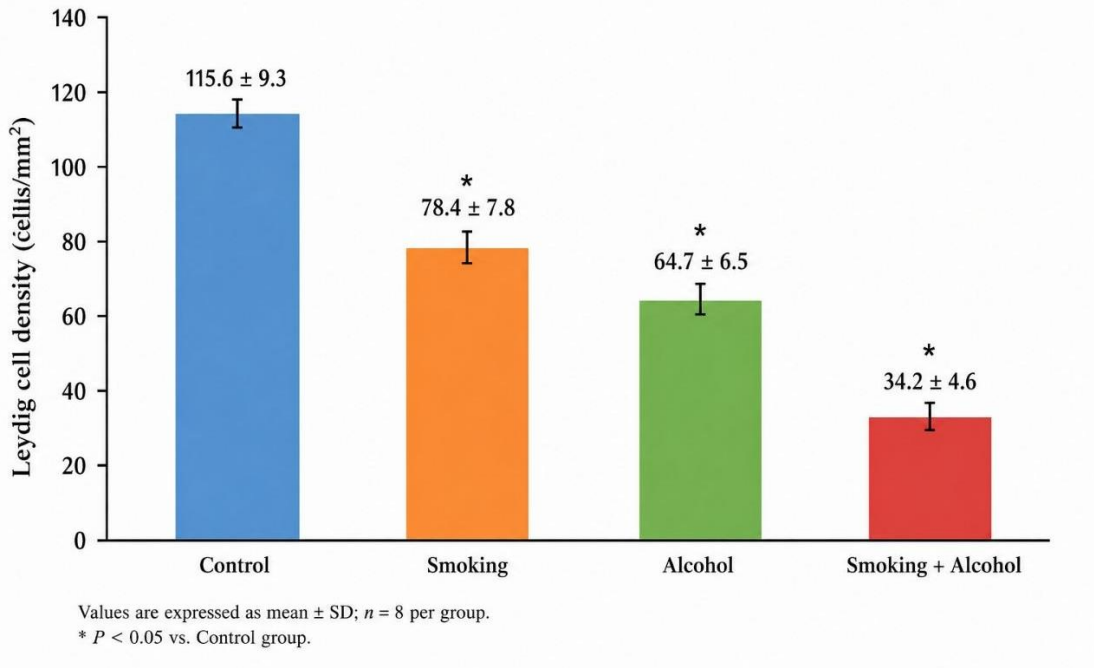


Chart 3: Leydig Cell Density Among Experimental Groups

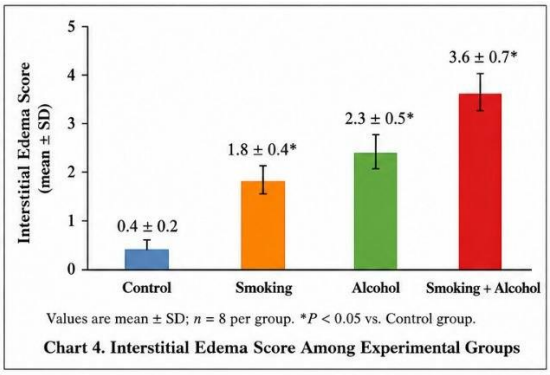
The interstitial edema, vascular congestion, necrosis, and fibrosis scores were significantly higher in the exposed groups than in the control group, as revealed by histopathological evaluation. The combined exposure group showed the greatest effect, suggesting significant tissue damage and structural degeneration (Table 2; Charts 4–6).

Further, the Johnsen score was significantly lowered in all exposure groups, with the lowest score in the smoking + alcohol group. This indicates a significant reduction in the efficiency of spermatogenesis and sexual activity (chart 7).

Table 2. Histopathological Scoring of Testicular Tissue Alterations in Experimental Groups

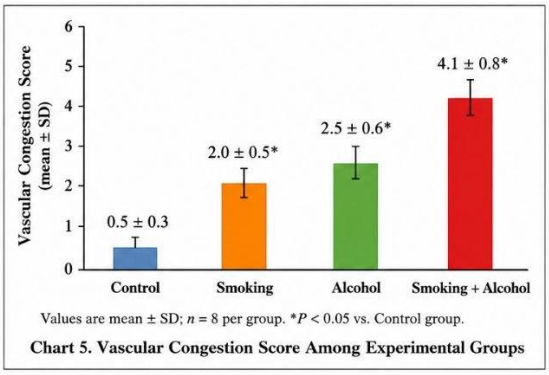
Parameter	Control Group	Smoking Group	Alcohol Group	Smoking + Alcohol Group
Interstitial Edema Score	0.4 ± 0.2	1.8 ± 0.4*	2.3 ± 0.5*	3.6±0.7*
Vascular Congestion Score	0.5 ± 0.3	2.0 ± 0.5*	2.5 ± 0.6*	4.1±0.8*
Necrosis Score	0.2 ± 0.1	1.5 ± 0.3*	2.1 ± 0.4*	4.3±0.9*
Fibrosis Score	0.3 ± 0.2	1.7 ± 0.4*	2.4 ± 0.5*	4.5 ± 0.8*
Johnsen Score	9.7 ± 0.2	7.8 ± 0.5*	6.9 ± 0.6*	4.2±0.7*

Values are expressed as mean ± SD (n = 8 per group).
*Significantly different compared with the control group ($P < 0.05$).



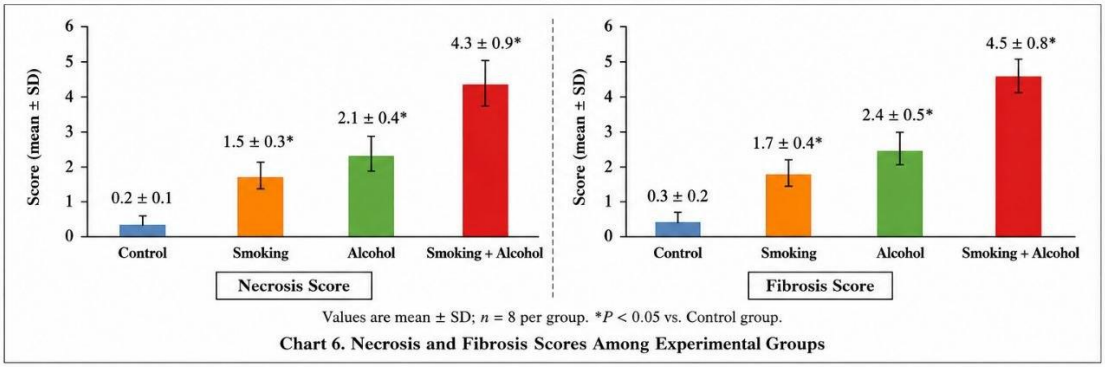
Values are mean ± SD; $n = 8$ per group. *$P < 0.05$ vs. Control group.

Chart 4. Interstitial Edema Score Among Experimental Groups



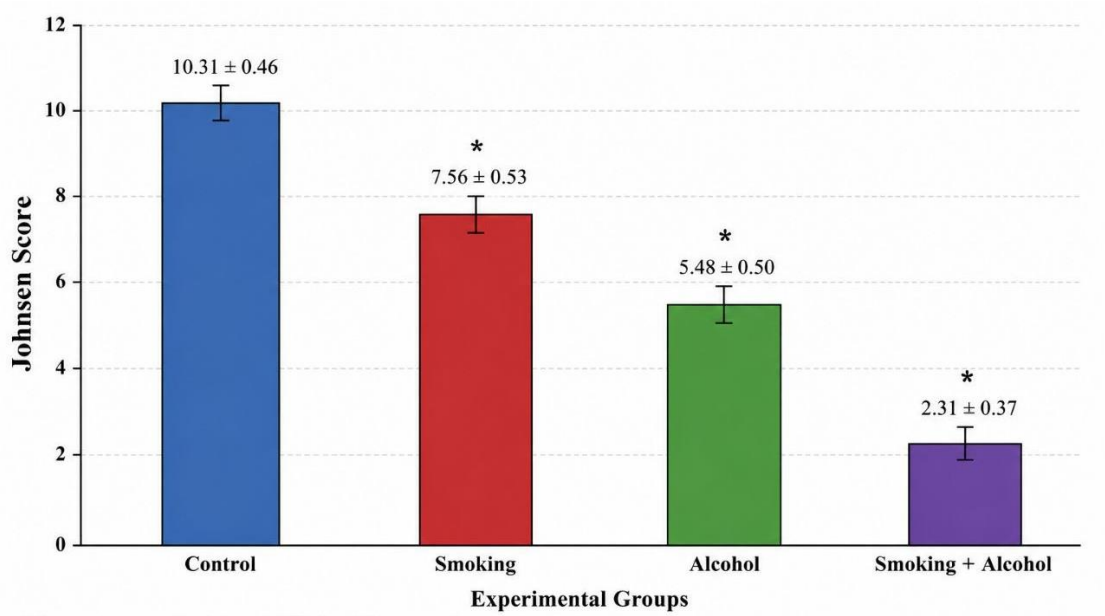
Values are mean ± SD; $n = 8$ per group. *$P < 0.05$ vs. Control group.

Chart 5. Vascular Congestion Score Among Experimental Groups



Values are mean ± SD; $n = 8$ per group. *$P < 0.05$ vs. Control group.

Chart 6. Necrosis and Fibrosis Scores Among Experimental Groups



Values are expressed as Mean ± SEM (n = 10 per group)

* P < 0.05 vs. Control group (One-way ANOVA followed by Tukey's post hoc test)

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Chart 7. Johnsen Score Across Experimental Groups

A comparison of the Johnsen scoring system and combined exposure across the control, smoking, alcohol, and combined-exposure groups is shown in Chart 7. No significant differences were observed among the treated groups; however, all treated groups showed a significant reduction in the Johnsen score compared with the control group ($P < 0.05$), indicating impaired spermatogenic activity.

Extensive impairment of seminiferous tubule function and significant suppression of spermatogenesis were seen in the combined smoking and alcohol exposure group, which had the lowest Johnsen score. The reductions in intermediate groups were found in the alcohol group and the smoking group, respectively, indicating a dose-response relationship between the types of exposures and testicular damage.

The overall results indicate a progressive reduction in spermatogenic efficiency with toxic and combined exposures, with the latter being the most severe.

Histological observations showed that the seminiferous tubule structure was normal in the control group, and a large number of mature sperm were observed in the seminiferous tubules (Figure 5).

The smoking group, on the other hand, exhibited disorganized seminiferous tubules, partial separation of germ cells, moderate vascular congestion, and interstitial edema (Figure 2).

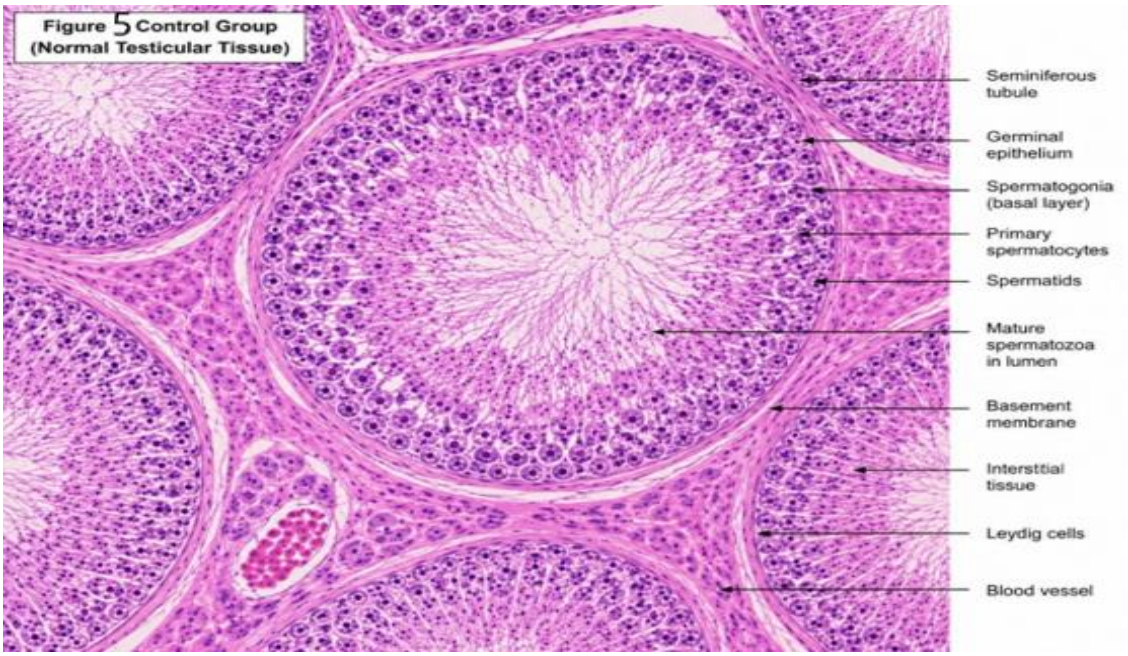

Figure 5. Control Group (Normal Testicular Tissue)

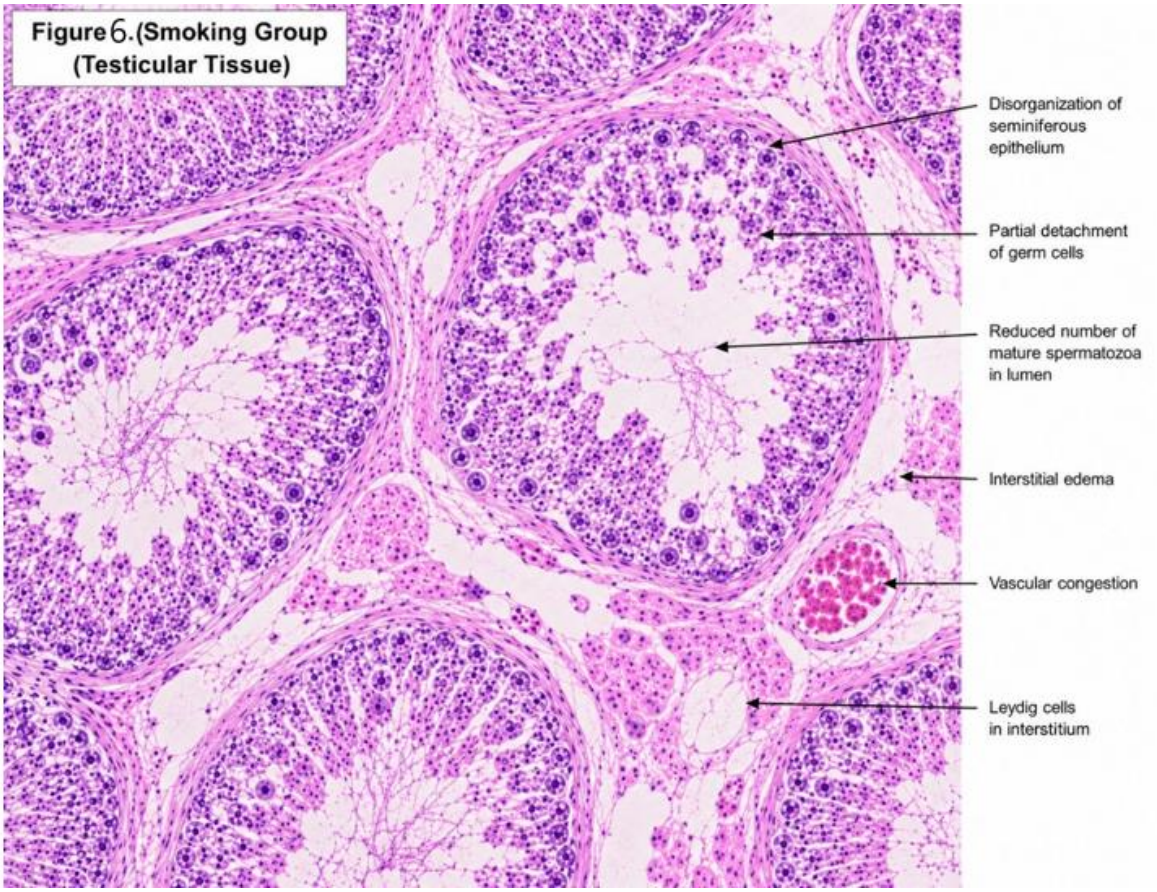

Figure 6. Smoking Group (Testicular Tissue Alterations)

In the alcohol group, degeneration of germ cells, reduced density of spermatogenic cells, enlargement of interstitial spaces, and necrotic changes in some seminiferous tubules were observed (Figure 7).

In addition, in the combined smoking and alcohol group, severe seminiferous tubule atrophy, extensive detachment of germ cells, marked reduction in spermatogenesis, and pronounced fibrosis and vascular congestion were observed, indicating the most severe tissue damage (Figure 8).

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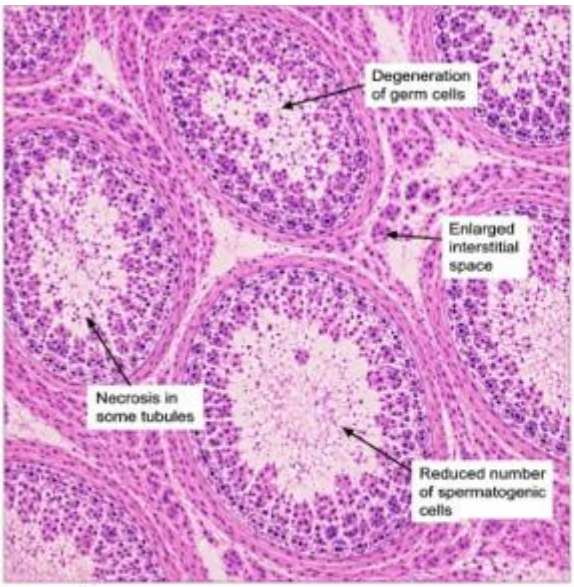

Figure 7. Histological section of testicular tissue in the alcohol group showing degeneration of germ cells, enlarged interstitial spaces, and focal necrotic changes in seminiferous tubules (H&E stain, ×400).

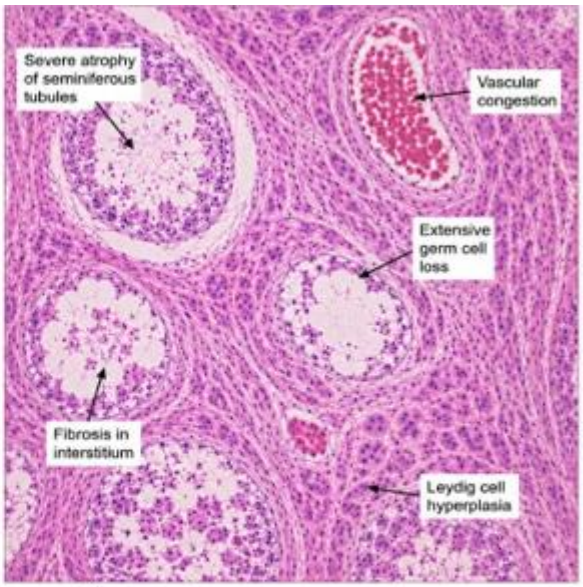

Figure 8 Histological section of testicular tissue in the combined smoking and alcohol group showing severe seminiferous tubule atrophy, extensive germ cell loss, fibrosis, and marked vascular congestion, indicating severe impairment of spermatogenesis (H&E stain, ×400).

Fig (7): Histological section of testicular tissue in the alcohol group showing degeneration of germ cells, enlarged interstitial spaces, and focal necrotic changes in seminiferous tubules (H&E stain, x400)

Fig (8): Histological section of testicular tissue in the combined smoking and alcohol group showing severe seminiferous tubule atrophy. Extensive germ cell loss, fibrosis and marked vascular congestion, indicating severe impairment of spermatogenesis (H&E stain, x400)

Discussion

The results of this study showed that smoking, alcohol consumption, and simultaneous exposure to both of them significantly affected the structure of testicular tissue and the spermatogenic function. Histological and morphometric analysis revealed that smoking and/or alcohol exposure resulted in abnormalities in seminiferous tubule morphology, decreased thickness of the germinal epithelium, and decreased density of spermatogenic cells, with the highest levels of abnormalities in the combined smoking and alcohol group. The results conform with earlier studies [17,18,19], which concluded that smoking and alcohol are associated with male reproductive dysfunction.

In this study, the diameter of the seminiferous tubule and the thickness of the germinal epithelium were examined for oxidative stress-induced cellular damage. Tobacco smoke contains several

harmful substances, such as nicotine, cadmium, and carbon monoxide, which are known to induce the production of Reactive Oxygen Species (ROS) in the body and cause damage to lipids and DNA [20]. In addition, acetaldehyde, a metabolite of alcohol, also causes oxidative stress, leading to degeneration and apoptosis of testicular cells [21].

The reduction in the density of spermatogenic cells and the Johnsen score indicate that spermatogenic function is severely impaired. The lowest values were seen especially in the combined exposure group, indicating a synergistic toxic effect of smoking and alcohol. This finding is under chronic oxidative stress, which affects normal spermatogenesis [22].

Additionally, this study observed a decrease in Leydig cell density. Leydig cells play a crucial role in testosterone production, and their decrease suggests impaired endocrine function. Smoking and alcohol exposure have been reported to disrupt the hypothalamic-pituitary-gonadal axis, leading to reduced testosterone secretion [23]. This hormonal abnormality is thought to be directly involved in reduced sperm production and reproductive capacity.

Histopathological evaluation showed increased interstitial edema, vascular congestion, necrosis, and fibrosis. These changes may reflect chronic inflammatory responses and circulatory disturbances. The severe fibrosis observed, particularly in the combined exposure group, suggests ongoing long-term tissue damage and repair processes [24].

In conclusion, this study demonstrated that smoking and alcohol consumption have detrimental effects on testicular tissue and male reproductive function [25]. Furthermore, combined exposure to both resulted in more severe tissue damage than single exposure, suggesting that it may be a major risk factor for reduced reproductive capacity. Figure 9 illustrates the entire conclusion in a diagram:

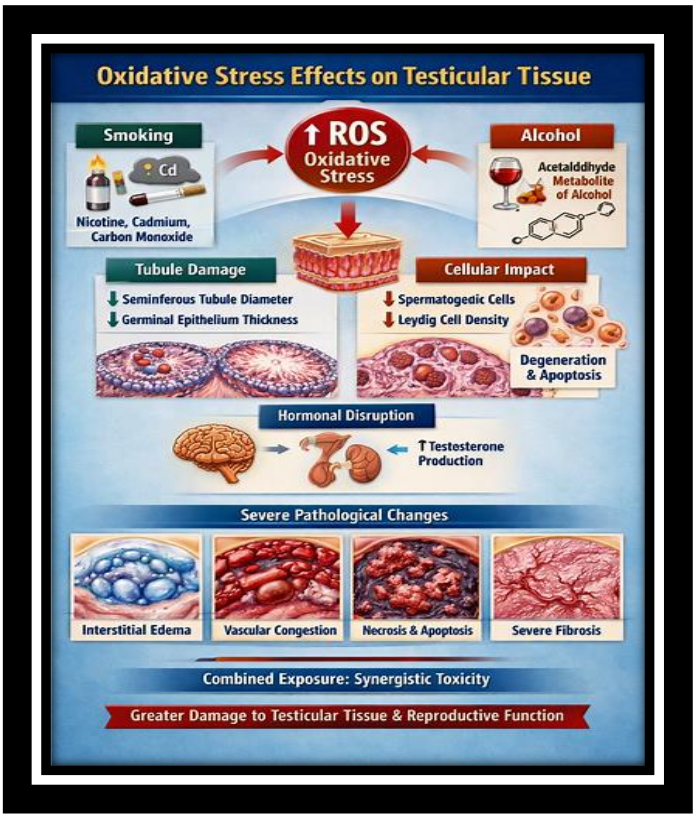

Figure 9- A combination of results illustrated in one diagram

Conclusion

The results of this study revealed that smoking and alcohol consumption cause significant histological and morphological changes in testicular tissue. Disturbances in seminiferous tubule structure, germ cell degeneration and shedding, interstitial edema, vascular congestion, fibrosis, and a significant reduction in spermatogenesis were observed in the exposed groups, as shown in Figures 5-8. In contrast, the control group (Figure 1) showed normal seminiferous tubule structure and germ cell arrangement, with numerous mature spermatozoa observed, indicative of normal spermatogenesis.

In particular, the seminiferous tubule atrophy, the extensive germ cell loss, and the severe fibrosis were observed in the combined exposure group to smoking and alcohol (Figure 8), indicating a synergistically toxic effect when these two exposures were combined. These alterations are probably due to oxidative stress and endocrine dysfunction and are believed to play a direct role in the decrease of male reproductive function.

Thus, chronic smoking and excessive drinking of alcohol may be important risk factors for male infertility, and lifestyle modification and preventive interventions are important in maintaining male reproductive health.

Recommendations

Based on the results of this study, the following recommendations are made:

- The general public should be better educated and aware of the detrimental impact of smoking and excessive consumption of alcoholic beverages on male reproductive function and male infertility through public health education and awareness activities.
- It is recommended that smoking cessation and alcohol consumption restriction should be promoted through lifestyle interventions at a young age and for men of reproductive age.
- Reproductive health practitioners should proactively offer reproductive health counseling during routine care to all individuals who have a history of smoking and alcohol use.

More basic and clinical studies are needed to elucidate the mechanisms underlying testicular tissue damage, including the roles of oxidative stress and endocrine dysfunction.

Introduction of community-level screening programs for early detection of reproductive dysfunction in high-risk populations is desired.

Ethical Considerations

Ethical approval for this study was obtained from the Institutional Research Ethics Committee (Approval No. MNDDS-REC-2026-734). The study was conducted in accordance with the Declaration of Helsinki and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2, Canada).

Written informed consent was obtained from all participants prior to enrollment after a full explanation of the study objectives and procedures. Confidentiality and anonymity of participant data were strictly maintained, and all collected information was used exclusively for scientific research purposes.

The study was conducted within an academic research framework in Montréal, Quebec, Canada, involving students from CHU Sainte-Justine and Université de Montréal, which provided the institutional setting for recruitment, coordination, and full compliance with Canadian ethical research standards.

List of Abbrevations: (ROS) :reactive oxygen species; (HPG): hypothalamus-pituitary-gonadal; (MUHC): McGill University Health Center, (H&E): hematoxylin and eosin

Acknowledgment:
The authors would like to express their sincere gratitude to the Students' Union of CHU Sainte-Justine and Université de Montréal, Montréal, Quebec, Canada, for providing student grant support (Grant No. SU-UM-2025-014). The authors also acknowledge the partial institutional research support received through the University Research Support Program (Grant No. UdeM-RSP-2025-087).
In addition, the authors extend their appreciation to The Royal Victoria Hospital of the McGill University Health Centre (MUHC), located at 1001 Décarie Boulevard, Montreal, Quebec, Canada, H4A 3J, for facilitating the research environment and supporting the scientific framework of this study. The selection of MUHC was based on its advanced AI-driven and digital healthcare innovation infrastructure across its affiliated sites, including the Royal Victoria Hospital, which actively contributes to clinical and translational research in multiple biomedical domains, including testicular health and the investigation of the effects of smoking, tobacco, and alcohol on testicular tissue.
Accordingly, this study involved 75 adult male participants divided into three distinct groups, as described in the methodology section, representing diverse ethnic backgrounds including Eurasian, Caucasian, Asian, African, and Middle Eastern populations.

Author Contribution: All authors contributed equally to the main contributor to this paper. All authors read and approved the final paper.

Declaration of generative AI and AI-assisted technologies in the writing process
The authors hereby declare that no generative artificial intelligence or AI-assisted technologies were used at any stage during the preparation of this manuscript, including language editing, proofreading, or content development. The authors take full responsibility for the originality and integrity of the work presented in this publication.

Funding: This study was supported by a student grant provided by the Students' Union of CHU Sainte-Justine and Université de Montréal, Montréal, Quebec (Grant No. SU-UM-2026-609). In addition, a minor co-funding support was received from the university research support program (Grant No. UdeM-RSP-2026-113), administered under institutional research development initiatives.

Conflicts of Interest: "The authors declare no conflict of interest."

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