Shahid Bahonar University of Kerman
Agricultural Biotechnology Journal
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

Effects of chronic cigarette smoke exposure and alcohol exposure on testicular histopathology, morphometry, and gene expression in adult male rats

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Abstract

Objective

Cigarette smoking and alcohol are two important lifestyle factors that affect male reproductive health. However, the effects of individual and combined chronic exposure to cigarette smoke and alcohol on testicular architecture, selected gene expression profiles, and spermatogenesis remain incompletely understood. The aim of this study was to investigate the chronic effects of cigarette smoke and alcohol on histological, morphometrical and molecular status in testicular tissue in adult male rats.

Materials and methods

Forty adult male Sprague-Dawley rats were randomly divided in four groups (n = 10 per group). These groups were control, cigarette smoke-exposed, alcohol-exposed, and combined cigarette smoke- and alcohol-exposed groups. The exposure period was 8 weeks. Testicular histopathological and morphometric changes were evaluated. Moreover, the expression of genes related to oxidative stress (Nrf2 and HO-1), apoptosis (Bax and Bcl-2), steroidogenesis (StAR and CYP11A1), and spermatogenesis (DAZL and SYCP3) was assessed using quantitative real-time PCR (qRT-PCR).

Results

The results showed that exposure to cigarette smoke, alcohol or their combination significantly reduced the expression of Nrf2, HO-1, StAR, CYP11A1, DAZL, Bcl-2 and SYCP3. While, it increased the expression of Bax ($P < 0.05$). These molecular changes were accompanied by significant histopathological and morphometric changes. For example, it caused a decrease in the diameter of the seminiferous tubules, a decrease in the thickness of the germinal epithelium, a decrease in the density of spermatogenic cells and a decrease in the density of Leydig cells. The most pronounced changes were observed in the combined exposure group.

Conclusions

Chronic cigarette smoking and alcohol drinking negatively impact the structure and function of the testes through mechanisms of oxidative stress, apoptosis, inhibition of steroidogenesis, and disordered spermatogenesis. The combined exposure generates amplifying damage which far exceeds what each factor causes in isolation, suggesting that such lifestyle habits may represent major male infertility risk factors together.

Keywords: alcohol exposure, cigarette smoking, gene expression, oxidative stress, rat model

Paper Type: Research Paper.

Citation: Mekkey, A. M., & Al-Oubaidy, S. A. (2026). Effects of chronic cigarette smoke exposure and alcohol exposure on testicular histopathology, morphometry, and gene expression in adult male rats. *Agricultural Biotechnology Journal,* 18(4), 579-592.

Agricultural Biotechnology Journal, 18(4), 579-592. DOI: 10.22103/jab.2026.27465.1953

Received: May 18, 2026. Received in revised form: July 25, 2026.

Accepted: July 26, 2026. Published online: August 30, 2026.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.

Introduction

The male reproductive system is a biological system that is highly modulated by environmental and lifestyle factors. The male reproductive system is highly susceptible to environmental and lifestyle-related factors. The testes play essential roles in spermatogenesis and male sex hormone production; therefore, the structural and functional integrity of testicular tissue is crucial for normal reproductive function. Hence, structural and functional integrity of testicular tissue is crucial for normal reproductive performance (Aitken & Roman, 2008; Agarwal et al., 2014). Due to the increase in male infertility, lifestyle factors such as smoking and alcohol use have attracted more attention in recent years. Tobacco smoke contains nicotine and heavy metals along with many toxic substances that cause oxidative stress (Creasy, 2001; Harlev et al., 2015; Aitken, 2020). Increased production of reactive oxygen species (ROS) can damage testicular tissue, leading to structural alterations in the seminiferous tubules and apoptosis of germ cells. Conversely, alcohol can interfere with the hypothalamus-pituitary-gonadal (HPG) axis and thereby contribute to an impairment of spermatogenic potential through its action on hormonal balance (Emanuele & Emanuele, 2001; Zhao et al., 2021; Tesarik, 2025). Previous study has discussed the effects of smoking or alcohol independently with more comprehensive histopathological examinations, but studies about interactive/additive/synergistic effects by simultaneous exposure to these two factors are still rare (Oremosu & Akang, 2015). Moreover, the expression of eukaryotic genes is temporally and spatially regulated through multidimensional control mechanisms. Only a limited subset of the entire genome is actively expressed in each tissue type, and gene expression is closely tied to developmental stages. Consequently, gene

expression patterns in eukaryotes are tissue-specific (Arabpour et al., 2021). Additionally, the levels of gene products synthesized within a given tissue, as well as those contributed by other tissues, collectively regulate gene expression. Epigenetic modifications serve as a critical mechanism by which environmental factors leave a lasting imprint on the genome. These modifications can be heritable, influencing successive generations without altering the DNA sequence itself (Kazemipour et al., 2025). For instance, maternal nutrition during pregnancy has been shown to induce epigenetic changes that affect offspring metabolism, growth, and disease susceptibility. A fundamental aspect of genetic research involves the investigation of genes and proteins associated with specific traits, examined at both cellular and chromosomal levels. Advancing our understanding of these regulatory processes holds significant potential for improving biological insights and practical applications in health and production (Alavi et al., 2022). The novelty of this paper includes: Comparison of the effects of both smoking and alcohol factors on testicular tissue. Examine histologically combination synergetic toxic effects of two factors by simultaneous exposures. Quantitative evaluation of microstructural alterations including seminiferous tubule architecture, germ cell degeneration, interstitial edema, vascular congestion and fibrosis. Complicated correlation of lifestyle factors with declining male reproductive function histopathologically. On this basis, the present study will explain the pathological and synergistic effects of single or combined effect of smoking and alcohol on testicular tissue, which may provide a new understanding for the pathogenesis of male infertility.

Materials and Methods

Experimental animals: The current study used a total of forty (40) 6-7 weeks adult, male Sprague-Dawley rats (*Rattus norvegicus*). Male Sprague-Dawley rats were purchased from a licensed animal breeding supplier and maintained for 1 week before the experiment. Animals were kept in controlled environmental conditions (22-25°C, 50-60% relative humidity, and 12 hours light/dark cycle). At the same time, standard laboratory diet and water were provided ad libitum during all study period.

Experimental design: After acclimatization, animals were randomly divided into the four experimental groups. Control group: no treatment, served as a baseline reference, Alcohol group: only received normal ethanol administration, Group exposed to smoke: only cigarette smoke exposure, and Co-exposure group: treated with both ethanol and cigarette smoke exposure. Randomization was applied to avoid the selection bias and groups were made homogenous. Ethanol was dosed by oral gavage (3 g/kg body weight/day) freshly prepared in sterile physiological saline (0.9% NaCl). All administrations were performed in the morning, and the required administration volume was calculated based on the body weight of each animal. Weekly measurements of body weights and weekly adjusted administered volume were performed. All exposure to tobacco smoke was performed in a controlled whole-body inhalation chamber developed for homogenous particle distribution of controlled cigarette smoke exposure. Animals were subjected to mainstream cigarette smoke for two sessions per day, at a duration of 30 minutes each. To ensure reproducibility and controlled combustion, smoke concentration was kept as constant as possible throughout each exposure period through the use of regulated airflow

conditions. In addition to the previous experimental schedule, those animals assigned to the combined group received ethanol (3 g/kg/day) concurrently with exposure to cigarette smoke (30 minutes per session, twice daily). This design was applied to assess possible synergistic toxic effects of their combination. The experimental period was marked by daily observations for any behavioral changes, general health status, and signs of toxicity during the entire experiment. Any abnormal findings were systematically documented throughout the experimental period. The experiment was performed in a controlled laboratory environment over eight weeks (56 days).

Sample collection: Animals were anesthetized at the end of the experimental period following an approved ethical protocol. Animals were anesthetized at the end of the 8-week exposure period. Studied animals were euthanized according to the approved animal welfare protocol. Removing the testes was carefully done. Then, they were weighed and processed for molecular and histopathological analyses. The right testis was used for RNA extraction and immediately snap-frozen in liquid nitrogen before storage at -80°C. The left testis was fixed in 10% neutral buffered formalin for histopathological and morphometric evaluation.

Quantitative Real Time PCR (qRT-PCR) for gene expression analysis: After sacrifice, a small piece of the right testis from each animal was immediately removed, washed with sterile phosphate-buffered saline (PBS), snap frozen in liquid nitrogen and stored at −80°C for subsequent molecular analysis. Total RNA was isolated from testicular tissue using TRIzol reagent according to the manufacturer's instructions. RNA purity and concentration were determined spectrophotometrically using a NanoDrop spectrophotometer at 260/280 nm. Only RNA samples with A260/A280 ratios between 1.8 and 2.0 were used. One microgram (1 μg) of total RNA was reverse-transcribed into complementary DNA (cDNA) using a commercial reverse transcription kit according to the manufacturer's protocol. Quantitative real-time PCR was performed using a SYBR Green-based master mix on a real-time PCR system. Each reaction was performed in triplicate. Relative gene expression was calculated using the 2^−ΔΔCt method, with GAPDH as the reference gene and the control group used as the calibrator (Livak & Schmittgen, 2001). The list of studied genes is shown in Table 1. The sequences of the primers used are given in Table 2.

Table 1. Genes included in the study and their functional categories

Functional Category	Gene	Function
Oxidative Stress	Nrf2	Antioxidant defense regulator
Oxidative Stress	HO-1	Cytoprotective antioxidant enzyme
Apoptosis	Bax	Pro-apoptotic marker
Apoptosis	Bcl-2	Anti-apoptotic marker
Steroidogenesis	StAR	Cholesterol transport for testosterone synthesis
Steroidogenesis	CYP11A1	Catalyzes the first step of steroidogenesis by converting cholesterol to pregnenolone
Spermatogenesis	DAZL	Germ cell development
Spermatogenesis	SYCP3	Meiotic germ cell marker
Housekeeping Gene	GAPDH	Internal control

Table 2. Primer sequences used for qRT-PCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Nrf2	AGCAGGACTGGAGAAGTTCA	TTCTTTTTCCAGCGAGGAGA
HO-1	TCTATCGTGCTCGCATGAAC	CCAACAGGAAGCTGAGAGTG
Bax	GTTTCATCCAGGATCGAGCAG	CATCTTCTTCCAGATGGTGA
Bcl-2	GGTGAACTGGGGGAGGATTG	GAGACAGCCAGGAGAAATCA
StAR	AGCCATGGAGAACTTCCAGA	CCTTGATGACCTGGTTGATG
CYP11A1	TGGAGGAGATGATGGAGACA	AGCAGGTAGGTCAGGATGGT
DAZL	CCTACCTGGAGCTGGACTTT	TGCTGGTTGATGAGGGTAGA
SYCP3	AGAGGAAGAGGAGCTGCTGA	TCTGTTGGCTGTGGTCTTCT
GAPDH	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC

Histopathology: Testicular tissues were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 5-µm thickness, and stained with hematoxylin and eosin (H&E). Histological sections were examined under a light microscope by an experienced histopathologist blinded to the experimental groups.

Statistical analysis: Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using appropriate statistical software. Differences between groups were assessed using suitable tests, and a p-value of < 0.05 was considered statistically significant. The normality of data distribution was assessed using the Shapiro-Wilk test. For non-normally distributed data, the Kruskal-Wallis test followed by Dunn's multiple-comparison test was applied.

Ethical approval: All experimental procedures were carried out in accordance with institutional and international guidelines for the care and use of laboratory animals in Babylon University. Every effort was made to minimize animal suffering and reduce the number of animals used.

Results

Table 3 presents the relative expression levels of genes associated with oxidative stress, apoptosis, steroidogenesis, and spermatogenesis in testicular tissue among the experimental groups. Significant alterations in gene expression were observed following chronic exposure to cigarette smoke, alcohol, and their combination ($P < 0.05$). The expression of the antioxidant genes Nrf2 and HO-1 was significantly decreased in all exposed groups compared with the control group. The greatest reduction was shown in the combined smoking and alcohol group suggesting serious failure of endogenous antioxidant defense system and higher sensitivity to oxidative stress. In reference to apoptosis-related genes, expressions of Bax were significantly induced and Bcl-2 expression was also downregulated in all treated groups. The most significant changes were in the combined-exposure group, suggesting potentiated activation of apoptotic pathways and increased testicular germ cell death. Smoking and alcohol exposure led to a significant downregulation of genes involved in steroidogenesis, particularly the StAR gene and CYP11A1. The combined exposure group consistently showed the lowest expression levels, suggesting obvious disruption of Leydig cell function and testosterone biosynthesis. Likewise, spermatogenesis-associated DAZL and SYCP3 were significantly downregulated in all the

exposed 3 groups compare controls. The largest reduction was seen in smoking plus alcohol group with sever disruption of germ cell development, meiotic progression and overall spermatogenic activity.

Table 3. Relative Gene Expression in Testicular Tissue

Gene	Control	Smoking	Alcohol	Smoking + Alcohol	P-value
Nrf2	1.00 ± 0.08	0.72 ± 0.05*	0.65 ± 0.07*	0.42 ± 0.04*	<0.05
HO-1	1.00 ± 0.09	0.76 ± 0.08*	0.68 ± 0.06*	0.39 ± 0.05*	<0.05
Bax	1.00 ± 0.10	1.68 ± 0.12*	1.94 ± 0.15*	2.85 ± 0.20*	<0.05
Bcl-2	1.00 ± 0.07	0.74 ± 0.05*	0.69 ± 0.06*	0.43 ± 0.04*	<0.05
StAR	1.00 ± 0.08	0.71 ± 0.06*	0.65 ± 0.05*	0.38 ± 0.03*	<0.05
CYP11A1	1.00 ± 0.09	0.76 ± 0.07*	0.63 ± 0.05*	0.41 ± 0.04*	<0.05
DAZL	1.00 ± 0.06	0.73 ± 0.04*	0.66 ± 0.05*	0.36 ± 0.03*	<0.05
SYCP3	1.00 ± 0.07	0.69 ± 0.06*	0.61 ± 0.05*	0.31 ± 0.03*	<0.05

*Significantly different from control group ($P < 0.05$).

Figure 1 shows the relative mRNA expression of Nrf2 and HO-1 in testicular tissue. Both genes were significantly downregulated in smoking, alcohol, and combined exposure groups, with the greatest reduction observed in the smoking + alcohol group.

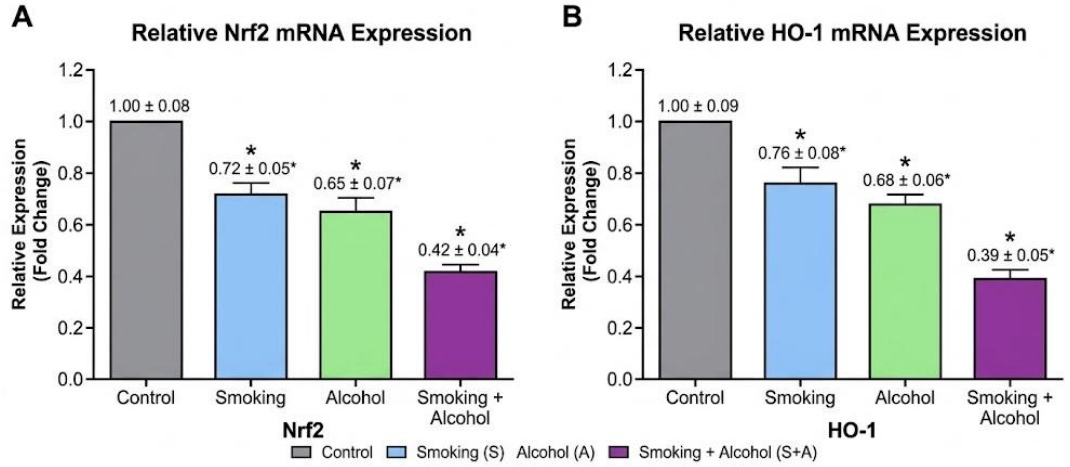

Figure 1. Relative Expression of Oxidative Stress-Related Genes. Relative mRNA expression of Nrf2 and HO-1 in testicular tissue. Both genes were significantly downregulated in smoking, alcohol, and combined exposure groups. The greatest reduction for both antioxidant genes was observed in the combined exposure group. *Significantly different from control group ($P < 0.05$)

Figure 2 represents the relative expression of Bax and Bcl-2 genes in testicular tissue. Bax expression increased significantly, whereas Bcl-2 expression decreased significantly in treated groups, indicating enhanced apoptosis.

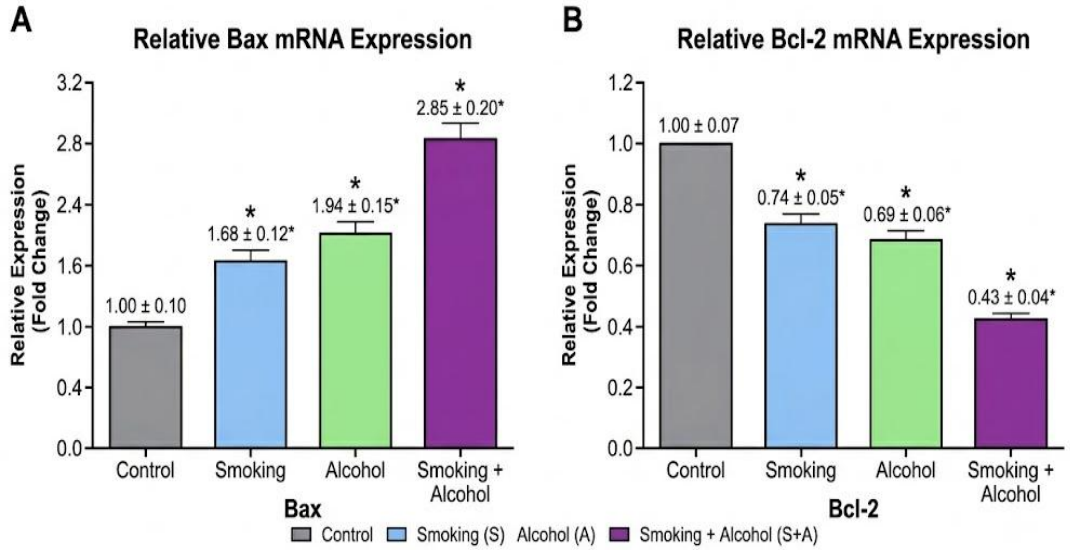

Figure 2. Relative Expression of Apoptosis-Related Genes. Relative expression of Bax and Bcl-2 genes in testicular tissue. Bax expression increased significantly, whereas Bcl-2 expression decreased significantly in treated groups. These results indicate enhanced apoptosis in the exposed groups. *Significantly different from control group (P < 0.05)

Figure 3 shows the relative mRNA expression of StAR, CYP11A1, DAZL, and SYCP3. Significant downregulation was observed in all exposed groups, particularly in the combined smoking and alcohol group.

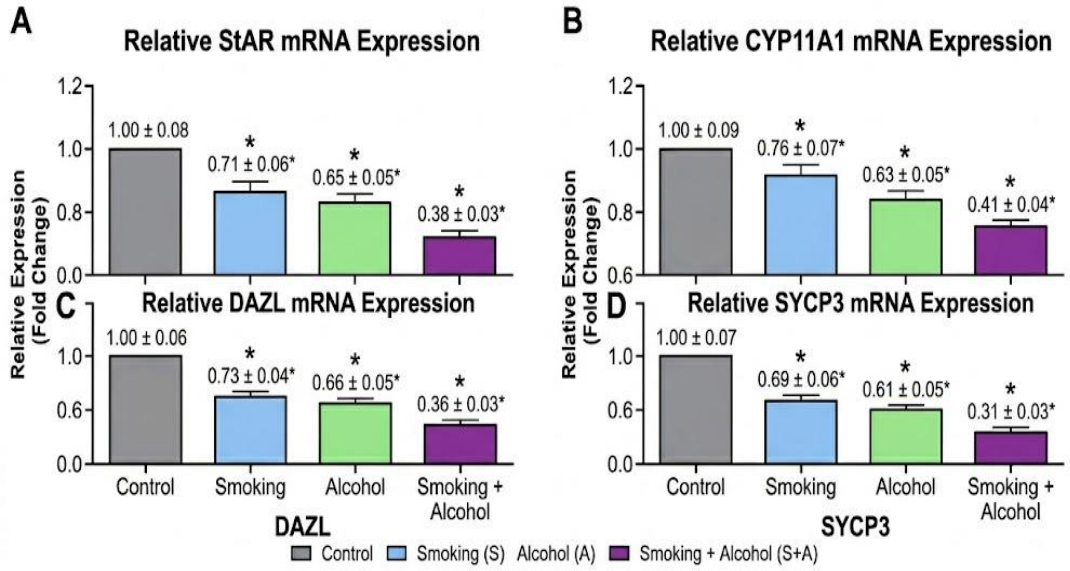

Figure 3. Relative expression of spermatogenesis and steroidogenesis genes. Relative mRNA expression of genes related to steroidogenesis (A, StAR; B, CYP11A1) and spermatogenesis (C, DAZL; D, SYCP3). Significant downregulation was observed for all four genes in all exposed groups. This downregulation was particularly notable in the combined smoking and alcohol group. *Significantly different from control group (P < 0.05)

The histological and morphometric analysis showed that the testicular tissue structure of smoking, alcohol exposure and combined exposure groups was significantly different from the control group. In particular, there were significant differences in the structure of seminiferous tubules, thickness of the germinal epithelium and in the density of the spermatogenic cells. The morphometric analysis revealed that seminiferous tubule diameter and germinal epithelium thickness were significantly reduced in all the exposure groups when compared to the control group ($P < 0.05$). The rate of this decline was greatest among the combined smoking and alcohol group (Table 4; Figure 4).

Table 4. Morphometric and Cellular Changes in Testicular Tissue Among Experimental Groups

Parameters	Control Group	cigarette smoke-exposed group	Alcohol Group	Smoking + Alcohol Group	P-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 spermatozoa count	91.3±4.8	69.4±3.9*	63.5±3.5*	40.7±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 $P < 0.05$.

In addition, the number of spermatogenic cells (primary spermatocytes, spermatids and mature spermatozoa) was also significantly lower in exposed groups ($P < 0.05$). The lowest values were observed in the combined exposure group, indicating that there was significant impairment of spermatogenic function (Table 5; Figure 5). Leydig cell density was also significantly reduced in the exposed groups, particularly in the combined smoking and alcohol group. This result may reflect impaired testicular endocrine function due to oxidative stress and chronic toxic effects (Figure 6). 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 5). Further, the Johnsen score was significantly lowered in all exposure groups, with the lowest score in the smoking+alcohol group and this indicates a significant indicating impaired spermatogenic activity.

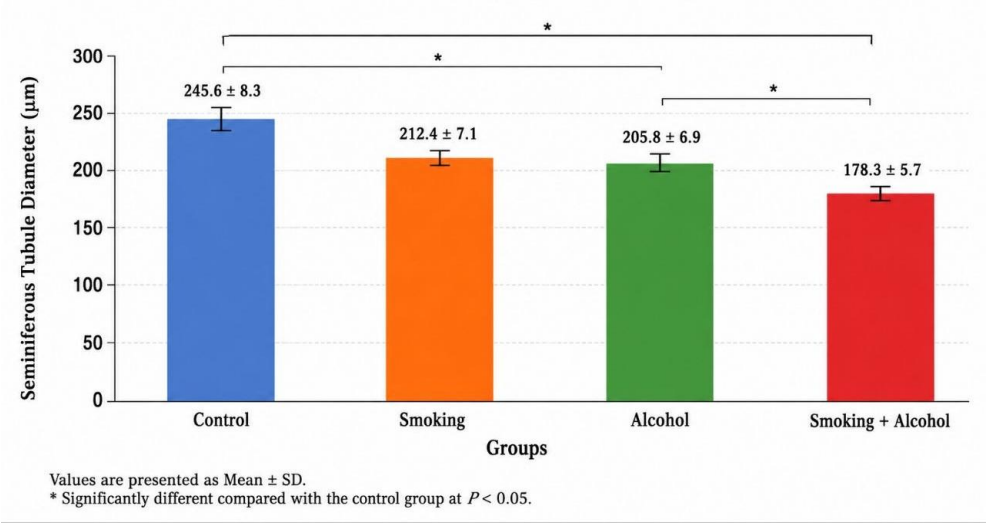

Figure 4. Comparison of Seminiferous Tubule Diameter Among Experimental Groups

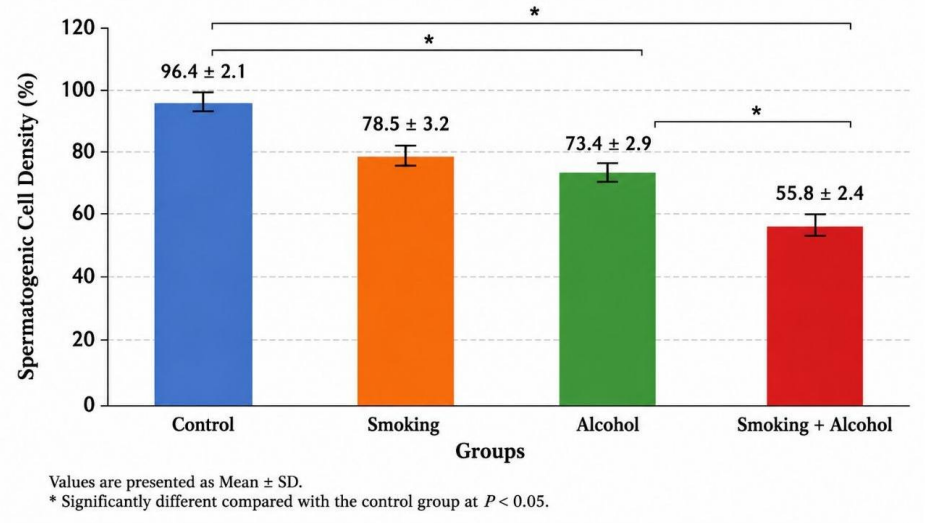

Figure 5. Comparison of spermatogenic cell density among experimental groups

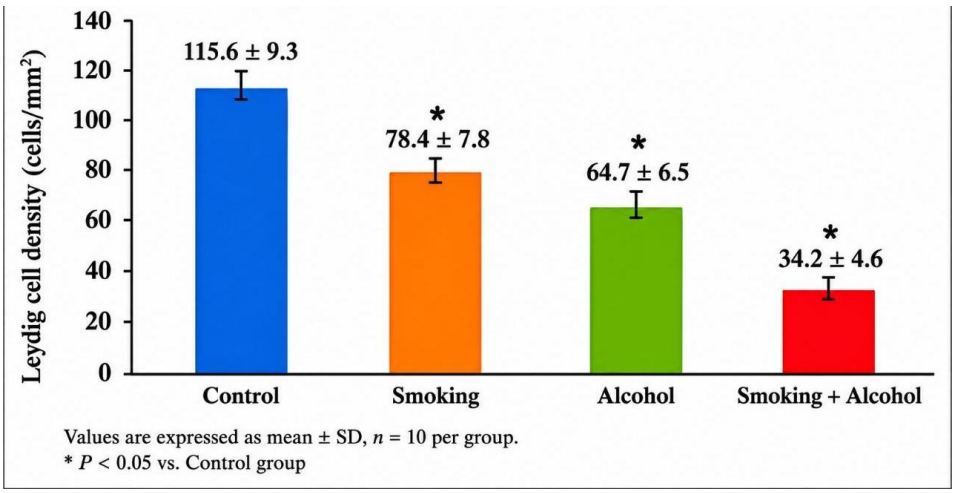

Figure 6. Leydig cell density among experimental groups

Table 5. Histopathological scoring of testicular tissue alterations in experimental groups

Parameter	Control Group	cigarette smoke-exposed 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 = 10 per group). *Significantly different compared with the control group ($P < 0.05$).

A comparison of the Johnsen scoring system for the control, smoking, alcohol and combined exposure groups is shown in Table 5. All treated groups showed a significant reduction in Johnsen score compared to the control group ($P < 0.05$) which means that spermatogenic activity was impaired. Extensive impairment of seminiferous tubule function and significant suppression of spermatogenesis was 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 cigarette smoke-exposed group, respectively, suggesting a gradient of testicular injury across the individual and combined exposure groups. The overall results indicate a progressive reduction in the spermatogenic efficiency with the toxic exposure and combined exposure being the most severe. Histological observations showed that the seminiferous tubule structure was normal in the control group, germ cells were regularly arranged and a large number of mature sperm were observed in the seminiferous tubules (Figure 7). The cigarette smoke-exposed group, on the other hand, exhibited disorganized seminiferous tubules, partial separation of germ cells, mild to moderate vascular congestion and interstitial edema (Figure 8).

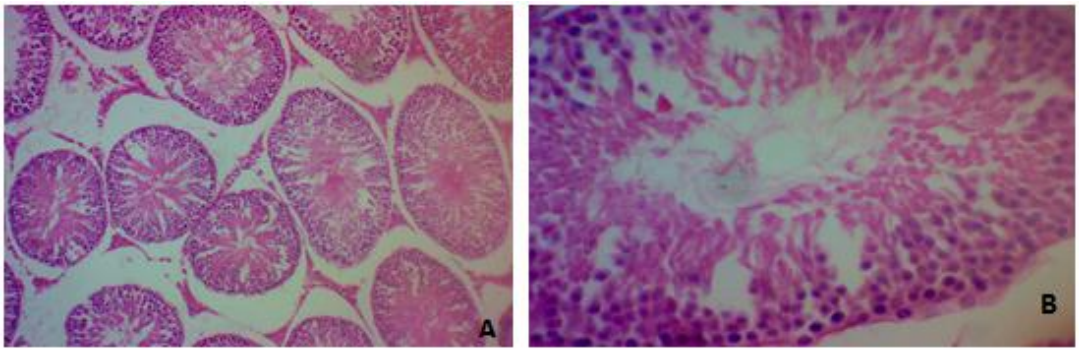

Figure 7. Control Group (Normal Testicular Tissue). A: the seminefours tubules with germ cells were regularly arranged and a large number of mature sperm were observed in the seminiferous tubules (H&E staining, ×40 magnification). B: The spermatogenesis cells well developed (H&E staining, ×100 magnification)

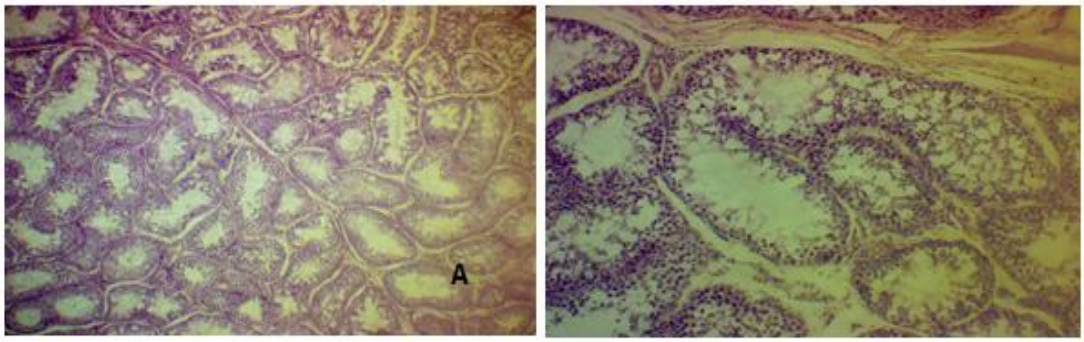

Figure 8. Cigarette smoke-exposed group (Testicular Tissue Alterations): A: 40X and B: 100X exhibited disorganized seminiferous tubules, partial separation of germ cells, mild to moderate vascular congestion and interstitial edema

In the alcohol group, degeneration of germ cells and reduction in the density of the spermatogenic cells, enlargement of interstitial spaces and necrotic changes in some seminiferous tubules were observed (Figure 9). 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 10).

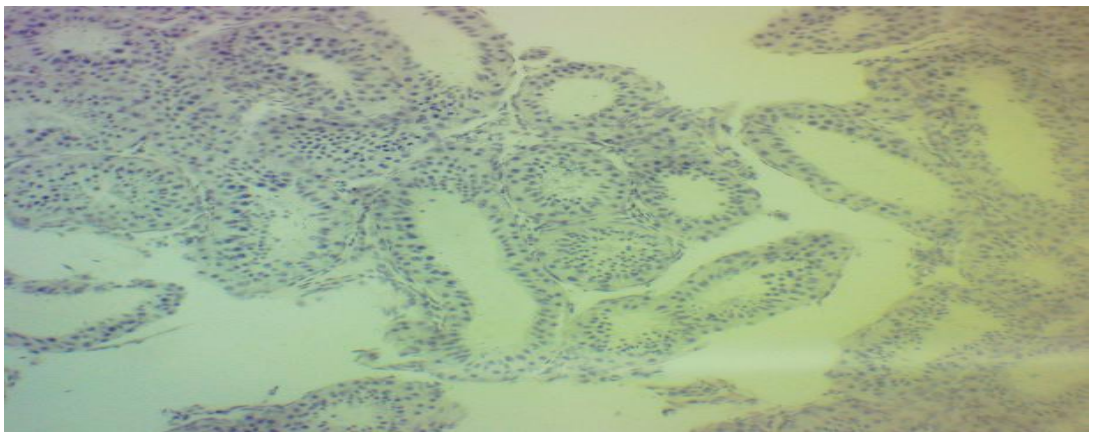

Figure 9. Histological section of testicular tissue in alcohol group showing degeneration of germ cell, enlarged interstitial spaces, and focal necrotic changes in seminiferous tubules (H&E staining, ×100 magnification)

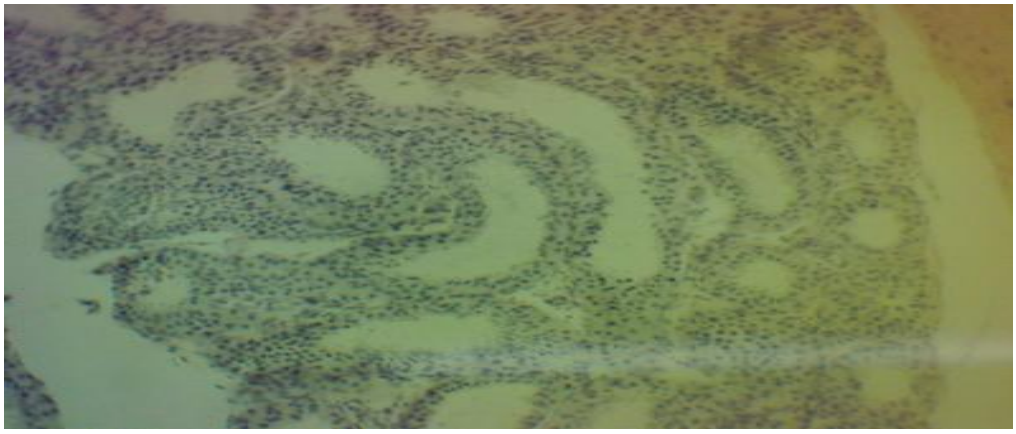

Figure 10. Histological section of testicular tissue in combined smoking and alcohol groups showing severe seminiferous tubular atrophy, extensive germs cell loss, fibrosis, and marked vascular congestion, Including severe impairment of spermatogenesis (H&E staining, ×100 magnification)

Discussion

The results of this study showed that smoking, alcohol exposure and simultaneous exposure to both of them affected significantly the structure of testicular tissue and the spermatogenic function. The results of the current qRT-PCR revealed substantial molecular changes in testicular tissue under continuous cigarette smoke and alcohol exposure. The observed reduction in Nrf2 and HO-1 expression may indicate impaired antioxidant defense and may be associated with increased susceptibility to oxidative stress and damage. Meanwhile, these results corroborate the changes in histopathology such as degeneration of seminiferous tubules and germ cell loss. In addition, the significant upregulation of Bax with down-regulation of Bcl-2 is consistent with activation of apoptotic pathways in testicular tissue. Oxidative stress-induced apoptosis is known as one of the key mechanisms responsible for reproductive toxicity resulting from smoking and alcohol exposure. StAR and CYP11A1 mRNA expressions were both substantially reduced, reflecting impaired steroidogenesis of Leydig cells. The results are similar to a decrease in Leydig cell density and suggesting impaired steroidogenic activity. Likewise, dramatic downregulation of DAZL and SYCP3 illustrates the dysfunction in spermatogenic and meiotic processes. The decreased expression of these genes was accompanied by reduced spermatogenic cell density, lower Johnsen scores, and reduced sperm production-related histological indices. The group receiving combined exposure to both smoking and alcohol showed the strongest molecular changes, which suggested a potentially synergistic toxic effect. In conclusion, these data offer mechanistic insights into how oxidative injury, triggering apoptosis and creating endocrine abnormalities, separately and together contribute to the testicular damage observed in males with infertility. 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 are in line with earlier studies (Jo & Stocco, 2004; Stocco, 2007; Sharma et al., 2016) which concluded that smoking and alcohol are

associated with male reproductive dysfunction. In this study, the diameter of the seminiferous tubule and thickness of the germinal epithelium were both significantly reduced, which may be associated with damage to the cells caused by oxidative stress. Tobacco smoke contains several harmful substances like nicotine, cadmium and carbon monoxide which are known to induce the production of Reactive Oxygen Species (ROS) in the body and cause damage to the lipids and DNA (Li et al., 2019). In addition, acetaldehyde, a metabolite of alcohol, also causes oxidative stress, leading to degeneration and apoptosis of testicular cells (Guo et al., 2025). The reduction of the density of spermatogenic cells and Johnsen score shows that the 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 in line with the finding of impairment in Sertoli and germ cell function in chronic oxidative stress, which affects normal spermatogenesis process (Zhang & Hannink, 2003; Zhu et al., 2025). 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 (Shin et al., 2021). 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 (Xia et al., 2024). In conclusion, this study demonstrated that smoking and alcohol exposure have detrimental effects on testicular tissue and male reproductive function. 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.

Conclusion: The results of this study revealed that smoking and alcohol exposure cause significant histological and morphological changes in testicular tissue. Disturbances of the seminiferous tubule structure, germ cell degeneration and shedding, interstitial edema, vascular congestion, fibrosis and significant reduction in spermatogenesis were observed in the exposed groups as shown in Figures 2-4. In contrast, the control group (Figure 1) showed normal seminiferous tubule structure and germ cell arrangement with a large number of mature spermatozoa observed which is 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, indicating that combined exposure was associated with more severe testicular injury than either exposure alone. 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 cigarette smoke exposure 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. 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 be proactively offering 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 understand the mechanisms of the damage to testicular tissue, including the role of oxidative stress and endocrine dysfunction. The study was conducted in an animal model, and therefore the findings cannot be directly extrapolated to humans. Although the study evaluated selected molecular markers related to oxidative stress, apoptosis, steroidogenesis, and spermatogenesis, direct measurements of oxidative stress biomarkers, circulating testosterone, LH, FSH, and sperm quality parameters were not performed.

Author Contributions

AMM and SAA; Conceptualization and methodology, software and validation, formal analysis and investigation, resources, and data curation, AMM and SAA; writing—original draft preparation, writing—review, visualization, and funding acquisition.

Data availability statement

Data are available from the authors upon reasonable request.

Acknowledgements

The authors thanks all the staff of biochemistry and physiology department, veterinary medicine, Babylon university for their supporting to complete this work.

Ethical considerations

The study was carried out with integrity, with no fabrication, falsification, plagiarism, or any scientific misconduct.

Funding

This study was supported by the Department of Human Anatomy and Histology, Medical College, University of Babylon, Iraq and received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interests

The authors declare no conflict of interest.

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مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸

اثر مواجهه مزمن با دود سیگار و الکل بر هیستوپاتولوژی بیضه، مورفومتری و بیان ژن در موش‌های صحرایی نر بالغ

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تاریخ دریافت: ۱۴۰۵/۰۲/۲۸ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۵/۰۴/۰۲ تاریخ پذیرش: ۱۴۰۵/۰۵/۰۳

چکیده

هدف: سیگار کشیدن و مصرف الکل دو عامل مهم سبک زندگی هستند که بر سلامت تولیدمثل مردان تأثیر می‌گذارند. بااین‌حال، اثرات مواجهه مزمن و منفرد یا ترکیبی با دود سیگار و الکل بر ساختار بیضه، الگوهای منتخب بیان ژن و اسپرم‌زایی هنوز به‌طور کامل شناخته نشده است. هدف این مطالعه، بررسی اثرات مزمن دود سیگار و الکل بر وضعیت بافت‌شناختی، مورفومتریک و مولکولی بافت بیضه در موش‌های صحرایی نر بالغ بود.

مواد و روش‌ها: چهل موش صحرایی نر بالغ از نژاد Sprague-Dawley به‌طور تصادفی به چهار گروه تقسیم شدند (۱۰ موش در هر گروه). این گروه‌ها شامل گروه شاهد، گروه در معرض دود سیگار، گروه در معرض الکل و گروه در معرض ترکیبی دود سیگار و الکل بودند. دوره مواجهه ۸ هفته بود. تغییرات هیستوپاتولوژیک و مورفومتریک بیضه ارزیابی شد. علاوه بر این، بیان ژن‌های مرتبط با استرس اکسیداتیو (Nrf2 و HO-1)، آپوپتوز (Bax و Bcl-2)، استروئیدزایی (StAR و CYP11A1)، و اسپرم‌زایی (DAZL و SYCP3) با استفاده از روش واکنش زنجیره‌ای پلیمراز کمی در زمان واقعی (qRT-PCR) مورد ارزیابی قرار گرفت.

نتایج: نتایج نشان داد که مواجهه با دود سیگار، الکل یا ترکیب آن‌ها، بیان ژن‌های Nrf2، HO-1، StAR، CYP11A1، DAZL، Bcl-2 و SYCP3 را به‌طور معنی‌داری کاهش داد، در حالی‌که بیان Bax افزایش یافت ($P < 0.05$). این تغییرات مولکولی با تغییرات معنی‌دار هیستوپاتولوژیک و مورفومتریک همراه بودند. برای مثال، این مواجهه‌ها موجب کاهش قطر لوله‌های

منی‌ساز، کاهش ضخامت اپی‌تلیوم زایشی، کاهش تراکم سلول‌های اسپرم‌زا و کاهش تراکم سلول‌های لایدیگ شدند. بارزترین تغییرات در گروه مواجهه ترکیبی مشاهده شد.

نتیجه‌گیری: مصرف مزمن سیگار و الکل از طریق مکانیسم‌های مرتبط با استرس اکسیداتیو، آپوپتوز، مهار استروئیدزایی و اختلال در اسپرم‌زایی، تأثیرات منفی بر ساختار و عملکرد بیضه‌ها دارد. مواجهه ترکیبی موجب ایجاد آسیب تشدیدشده‌ای می‌شود که به‌مراتب فراتر از آسیب ایجادشده توسط هر یک از عوامل به‌تنهایی است؛ این امر نشان می‌دهد که چنین عادات سبک زندگی، در صورت همراهی با یکدیگر، می‌توانند از عوامل خطر مهم ناباروری مردان باشند.

کلمات کلیدی: استرس اکسیداتیو، بیان ژن، سیگار کشیدن، مدل موش صحرایی، مواجهه با الکل

نوع مقاله: پژوهشی

استناد: اسماء محمد مکی، شیماء ا. العبیدی (۱۴۰۵). اثر مواجهه مزمن با دود سیگار و الکل بر هیستوپاتولوژی بیضه، مورفومتری و بیان ژن در موش‌های صحرایی نر بالغ. *مجله بیوتکنولوژی کشاورزی*، ۱۸(۴)، ۵۷۵-۵۹۲.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.