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Protective effect of *Pistacia lentiscus* extract on ethanol-induced testicular tissue damage in male Wistar rats: Histopathological alterations and fertility index

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ABSTRACT

Background & Aim: Ethanol exposure is known to inflict significant toxic effects on testicular tissue, leading to reduced testosterone levels, impaired spermatogenesis, and ultimately reduced fertility. Given the well-documented antioxidant and anti-inflammatory properties of *Pistacia lentiscus* (mastic gum), the aim of the present study was to investigate the protective effects of its hydroalcoholic extract against ethanol-induced testicular damage in male Wistar rats.

Experimental: Twenty-four adult male rats (weighing 200–250 g) were randomly assigned into four equal groups. The control group received no treatment, while the diseased group was exposed to ethanol (administered intraperitoneally at a dose of 10 mg/kg body weight). Two treatment groups received ethanol in combination with 100 and 200 mg/kg of the hydroalcoholic extract of *P. lentiscus*, respectively. Following a 3‑week treatment period, the animals were fasted, weighed, and blood samples were collected from the right ventricle under diethyl ether anesthesia. Serum testosterone levels were quantified using biochemical assays, and testicular tissue samples were processed for histopathological evaluation via hematoxylin–eosin staining.

Results: Data clearly demonstrated that ethanol exposure significantly reduced serum testosterone levels and adversely affected sperm parameters including the number of seminiferous tubules, spermatogonia, spermatids, and spermatozoa compared with the control group ($P<0.001$). Although the co-administration of 100 mg/kg extract showed notable improvements, the 200 mg/kg dose restored most parameters almost to control values ($P<0.001$). Histopathological evaluations further confirmed a marked recovery in testicular architecture and cellular integrity with the higher dose of the extract.

Recommended applications/industries: This research suggests that *P. lentiscus* extract could be developed as a natural therapeutic agent for reproductive health support in individuals exposed to alcohol-related oxidative stress. The findings may be applicable in the phytopharmaceutical, nutraceutical, and male fertility supplement industries.

1. Introduction

Male infertility is a significant global health issue influenced by multiple factors, including lifestyle, environmental exposures, diet, and contact with toxic substances (Krzastek and Farhi, 2020). Chronic alcohol consumption, particularly ethanol, is regarded as one of the most detrimental factors affecting male reproductive health (Finelli *et al.*, 2021). Ethanol induces reproductive dysfunction through cellular damage, reduction in testosterone levels, disruption of the hypothalamic-pituitary-gonadal (HPG) axis, and increased oxidative stress, ultimately compromising sperm quality and quantity and leading to infertility (Ma *et al.*, 2024; Roychoudhury *et al.*, 2021).

Studies have identified oxidative stress as a key mechanism underlying ethanol-induced testicular damage, as ethanol enhances free radical generation, promotes lipid peroxidation, and triggers structural alterations in germ cells (Maneesh *et al.*, 2005; Yousefi *et al.*, 2025) Consequently, researchers have explored antioxidant compounds as potential therapeutic agents to mitigate ethanol-induced reproductive toxicity (Dosumu *et al.*, 2012; Yousefi *et al.*, 2025).

Among these compounds, *Pistacia lentiscus* extract has emerged as a promising candidate due to its potent antioxidant, anti-inflammatory, and tissue-repair properties, positioning it as a potential protective agent against ethanol-induced testicular damage (Botsaris *et al.*, 2015; Shipa *et al.*, 2022).

Ethanol disrupts the HPG axis, which plays a central role in male reproductive regulation, thereby causing severe hormonal imbalances and testicular dysfunction (Emanuele and Emanuele, 2001). Specifically, ethanol suppresses gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus (Lee *et al.*, 2010). Reduced GnRH levels lead to decreased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion from the pituitary gland (Howard, 2021), ultimately impairing Leydig cell function and reducing testosterone production (Zirkin *et al.*, 2018). This decline in testosterone significantly disrupts spermatogenesis, resulting in impaired male reproductive capacity.

Oxidative stress induction is another primary mechanism of ethanol-related reproductive toxicity, as ethanol alters the balance between free radicals and the body's antioxidant defense system, leading to substantial testicular tissue damage (Asadi *et al.*, 2017). Ethanol metabolism generates oxidative reactions that increase free radical production and promote lipid peroxidation within cellular membranes. Elevated malondialdehyde (MDA) levels, a key marker of lipid peroxidation and cellular damageare indicative of this process (Asadi *et al.*, 2017). These molecular disturbances severely affect sperm function and germ cell integrity.

Furthermore, ethanol decreases the activity of critical antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Asadi *et al.*, 2017; de Freitas *et al.*, 2014). This enzymatic decline weakens the body's ability to counteract oxidative stress, exacerbating cellular injury. Ultimately, oxidative damage triggers apoptotic pathways, whereby ethanol induces programmed germ cell death, significantly reducing sperm count and impairing fertility.(de Freitas *et al.*, 2014; Yousefi *et al.*, 2025).

Numerous studies highlight the beneficial effects of antioxidant supplementation in mitigating ethanol-induced testicular damage (Abu-Khudir *et al.*, 2023; Rosenblum *et al.*, 1989). These compounds neutralize free radicals, restore the oxidative-antioxidative balance, and protect germ cells from oxidative insults. Antioxidants also aid in preserving testicular architecture and enhancing reproductive function (Asadi *et al.*, 2017; Rosenblum *et al.*, 1989).

Several key antioxidants have been identified for their protective effects against ethanol-induced testicular damage. Vitamin E, a major lipophilic antioxidant, plays a crucial role in preventing lipid peroxidation, protecting cellular membranes, and enhancing sperm motility and quality (Malmir *et al.*, 2021; Yousefi *et al.*, 2025). Resveratrol, a natural polyphenol, has been shown to decrease MDA levels, improve overall testicular function, and inhibit ethanol-induced germ cell apoptosis (Gao *et al.*, 2021). Additionally, plant extracts such as *P. lentiscus* contribute to testicular health by regulating antioxidant enzyme activity, mitigating oxidative stress, and modulating inflammatory responses, leading to substantial improvements in testicular function (Chouikh *et al.*, 2025).

As a plant-derived antioxidant-rich compound, *P. lentiscus* extract plays a pivotal role in protecting testicular tissue from ethanol-induced damage. This extract contains bioactive molecules such as

polyphenols, flavonoids, and terpenoids, which exhibit strong antioxidant and anti-inflammatory properties.(Chouikh *et al.*, 2025; Ghzaiel *et al.*, 2021). Experimental studies have demonstrated that these compounds effectively counteract ethanol-induced oxidative stress, enhance antioxidant enzyme activity, and suppress apoptotic pathways, thereby preserving germ cell structure and function (Asadi *et al.*, 2017; Ghzaiel *et al.*, 2021).

In addition to its histopathological benefits, *P. lentiscus* extract positively influences reproductive indices. Research indicates that its administration enhances testosterone levels and improves HPG axis functionality (Hadaya *et al.*, 2020; Suzuki *et al.*, 2017). Furthermore, it significantly improves sperm quantity and quality, increasing the number of spermatogenic tubules, enhancing sperm motility, and reducing the prevalence of morphologically abnormal sperm (Komsky-Elbaz *et al.*, 2019; Sawidis *et al.*, 2010).

Overall, *P. lentiscus* extract represents a promising natural therapeutic agent with potent antioxidant and anti-inflammatory properties, making it a viable intervention for mitigating ethanol-induced reproductive toxicity. This study investigates the protective effects of *P. lentiscus* extract on ethanol-induced histopathological alterations and fertility indices in testicular tissue.

2. Materials and Methods

2.1. Study design and ethical approval

This experimental study was conducted in a controlled laboratory setting using an interventional design with a control group. All research procedures adhered to ethical guidelines for the use of animals in scientific studies. Prior to the commencement of experiments, ethical approval was obtained from the Ethics Committee of Islamic Azad University, Shahrekord Branch (Approval Code: IR.IAU.SHK.REC.1403.161). The experimental design and statistical methods were meticulously planned to ensure the highest level of accuracy and reliability in data collection and analysis, thereby enhancing the scientific validity and reproducibility of the findings.

2.2. Animals and housing conditions

This study utilized 24 adult male Wistar rats, weighing between 200 and 250 g, procured from accredited sources. Before the start of the experiment, the animals were acclimatized for one week under standard laboratory conditions. Environmental parameters were carefully controlled: temperature was maintained at 22±2°C, a 12 h light/dark cycle was implemented, and relative humidity was set at 45–50%. Rats had unrestricted access to tap water and standard laboratory chow. The cages were designed to minimize environmental stress and closely simulate natural conditions, ensuring the welfare of the animals throughout the study.

2.3. Experimental group allocation

The animals were randomly assigned into four equal groups (six rats per group):

1. Control Group: Received no intervention.
2. Ethanol Group: Received ethanol at a dose of 10 mg/kg body weight.
3. Low Dose Group: Received 100 mg/kg *P. lentiscus* extract along with ethanol.
4. High Dose Group: Received 200 mg/kg *P. lentiscus* extract along with ethanol.

Intraperitoneal injection of the extract was performed four times per week.

2.4. Preparation of P. lentiscus extract

The gum and leaves of *P. lentiscus* were obtained from validated sources. After washing with distilled water, the plant material was air-dried in the shade and finely ground into powder. Standardization of the extract was conducted by analyzing key phytochemical components such as phenols and flavonoids using validated methods.

Ethanol 80% was selected as the solvent due to its high efficiency in extracting bioactive compounds and its compatibility with previous studies. A powder-to-solvent ratio of 1:10 was used to ensure optimal extraction. The powdered plant material was immersed in ethanol 80% in sealed glass containers coated with paraffin. The extraction process was carried out over 72 h in a dark incubator at 45°C, with gentle agitation every 12 h. After filtration using filter paper, the extract was concentrated using a rotary evaporator at temperature below 50°C. The final extract was stored in sterile vials at 4°C.

2.5. Blood collection and serum preparation

After three weeks of treatment, the animals were fasted and anesthetized using diethyl ether. Blood was carefully drawn from the right ventricular chamber using a 24-gauge syringe to minimize stress. The collected blood samples were centrifuged (4,000 rpm for 10 min), and the serum was separated. The extracted serum was stored in microtubes at 4°C for subsequent testosterone analysis using a Pars Azmoon diagnostic kit.

2.6. Histopathological processing of testicular samples

Immediately after dissection, testicular tissues were fixed in 10% formalin for 48 h. Samples underwent gradual dehydration using increasing concentrations of ethanol (from 70% to absolute), with each stage lasting one hour.

The specimens were then embedded in molten paraffin to form paraffin blocks. Thin sections (5 µm) were prepared using a microtome and stained with hematoxylin (5 min) and eosin (5 min). Following washing, dehydration, and clearing with xylene, the stained sections were mounted for microscopic examination. This process facilitated the assessment of cellular damage and histopathological alterations.

2.7. Assessment of fertility and biochemical markers

Testicular cell structure, spermatogenic cell count, and microscopic lesions were quantitatively analyzed using digital microscopy and immunohistochemistry. Serum testosterone levels were measured using an ELISA kit. Oxidative stress and inflammatory markers were evaluated using standard biochemical assays as needed.

2.8. Statistical analysis

Data were analyzed using SPSS version 27, employing one-way ANOVA. Tukey's post hoc test was conducted for pairwise comparisons ($P<0.05$). Results were reported as mean ± standard deviation.

3. Results and discussion

The study investigated the protective effects of a hydroalcoholic extract of *P. lentiscus* against ethanol-induced testicular damage in Wistar rats by evaluating both histopathological changes and reproductive parameters.

3.1. Histopathological findings and morphological assessments

Microscopic examination revealed that the control group exhibited densely packed and well-organized seminiferous tubules with normal cellular architecture (Figure 1-A), indicative of intact and healthy testicular tissue. In contrast, the ethanol-exposed group displayed pronounced structural disruption characterized by severe cellular depletion, disorganization, and reduced density of seminiferous tubules. These alterations were accompanied by marked degeneration of spermatogenic cell lines, emphasizing the toxic effects of ethanol (Figure 1-B).

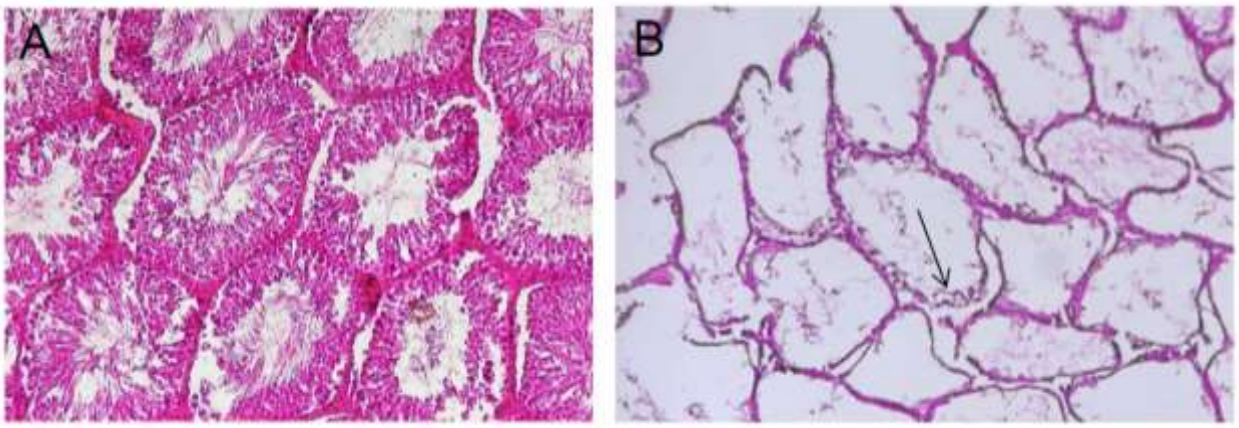

Figure 1. Histopathological evaluation of testicular tissue in the Control and Ethanol groups.
A: Control group – High-density seminiferous tubules with organized cellular architecture. **B:** Ethanol group – Structural disruptions characterized by reduced cellular density and morphological distortions, Degeneration of spermatogenic cell lines is evident (black arrow).

Treatments with *P. lentiscus* extract showed dose-dependent improvements in treatments. In the low-dose group, partial restoration was noted, as evidenced by moderate recovery of tubule density and spermatogenic cells; however, some irregularities persisted such as uneven distribution of the tubules, reduced sperm presence within the lumens, and thinning of the germinal epithelium were also observed (Figure 2-A). In contrast, high-dose treatment resulted in near-complete normalization, with seminiferous tubules regaining a high density and the cellular layers (including mature spermatozoa) closely resembling those of the control group (Figure 2-B).

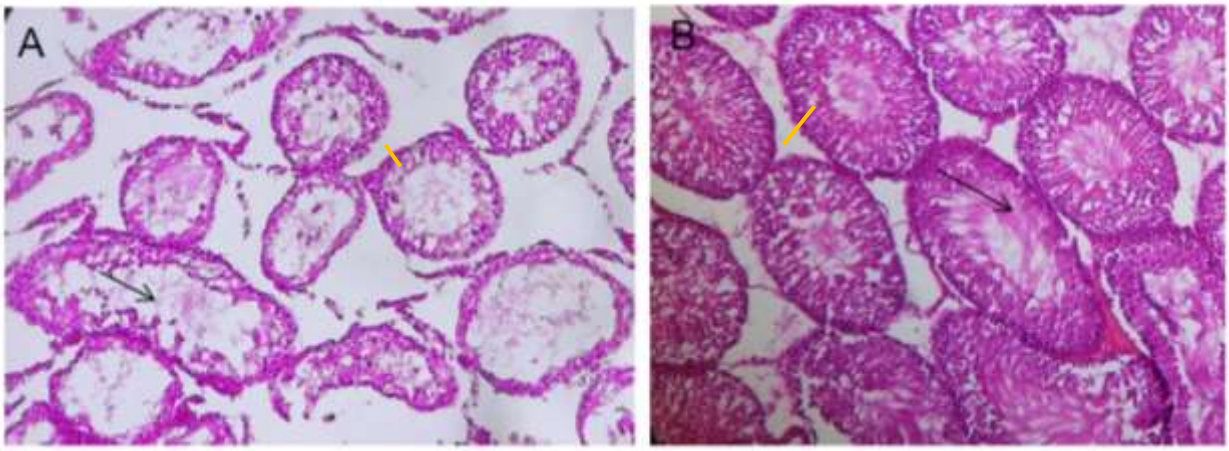

Figure 2. Histopathological evaluation of testicular tissue in the low and high dose groups.
A: Low dose group – Partial improvement in seminiferous tubule density with moderate restoration of spermatogenic cells, loss of seminiferous tubule density and irregular distribution; reduced sperm presence within the lumens (black arrow); and thinning of the germinal epithelium layer (yellow line). **B:** High dose group – Near-complete structural recovery, approximating control conditions, High density of seminiferous tubules (yellow line) and the presence of various spermatogenic cell layers and mature spermatozoa (black arrow).

3.2. Reproductive parameters

Quantitative assessments further substantiated these morphological observations. Table 1 presents the mean ± standard error values for key parameters including seminiferous tubules, spermatogonia, spermatocytes, spermatids, spermatozoa, and serum testosterone. Ethanol exposure (10 mg/kg) significantly reduced all these parameters compared to the control group. The differences among groups were statistically confirmed using one-way ANOVA followed by Tukey's post-hoc test ($P<0.001$). Groups annotated with different lowercase letters in Table 1 demonstrated significant differences ($P<0.05$), highlighting both the detrimental effects of ethanol and the dose-dependent restorative action of the extract.

Table 1. Comparison of reproductive parameters across different groups.

Groups / Variables	Ethanol Group	Control Group	Low Dose Group	High Dose Group
Seminiferous tubules	2.167 ± 0.401 [a]	21.833± 10.078 [b]	4.500 ± 0.885 [a]	20.500 ± 0.563 [b]
Spermatogonia	1.000 ± 0.365 [a]	14.500 ± 0.244 [c]	5.167 ± 1.014 [b]	13.333 ± 0.211 [c]
Spermatocyte	1.167 ± 0.307 [a]	12.167 ± 0.307 [c]	3.333 ± 0.882 [b]	10.000 ± 0.258 [d]
Spermatid	1.167 ± 0.307 [a]	12.167 ± 0.307 [c]	3.333 ± 0.882 [b]	10.000 ± 0.258 [d]
spermatozoa	0.333 ± 0.211 [a]	6.500 ± 1.250 [c]	20.500 ± 0.342 [b]	54.167 ± 1.579 [d]
Testosterone	0.018 ± 0.005 [a]	1.072 ± 0.032 [c]	0.040 ± 0.006 [a]	1.007 ± 0.003 [b]

Groups marked with different lowercase letters (a, b, c, d) exhibit statistically significant differences ($P<0.05$).

The combined histopathological and statistical data indicated that ethanol-induced testicular damage involves disruption of the seminiferous tubule integrity, depletion of spermatogenic cells, and a reduction in serum testosterone levels. These findings align closely with previous reports that documented ethanol's adverse effects on reproductive function, including testosterone depletion and impaired spermatogenesis

(Eid *et al.*, 2002; La Vignera *et al.*, 2013). Moreover, the role of ethanol in enhancing free radical generation (ROS) leads to oxidative injury, lipid peroxidation, and diminished cellular antioxidant defensesa process that ultimately depletes germ cells (Shaki *et al.*, 2019). Ethanol cytotoxicity is further mediated by acetaldehyde accumulation, which interferes with proteins and DNA by interacting with sulfhydryl groups (Dosumu *et al.*, 2012; Zakhari, 2006). Complementary to these observations, structural deterioration in the testicular tissue was accompanied by reduced sperm quality, as evidenced by lower counts of spermatogonia, spermatocytes, spermatids, and spermatozoa, along with decreased testosterone levels.

In contrast, administration of the *P. lentiscus* extract, particularly at 200 mg/kg, significantly mitigated ethanol-induced damage. The restorative effects of extract are likely attributed to its rich antioxidant components such as flavonoids, terpenes, oleic acids, and tocopherols which neutralize free radicals, inhibit lipid peroxidation, and support key enzymatic antioxidant activities (e.g., SOD, CAT, GPX). These biochemical actions help to preserve the structural integrity of testicular tissue and maintain testosterone levels, thereby safeguarding overall reproductive function. The present results confirm that *P. lentiscus* extract exerts a dose-dependent protective effect against ethanol-induced testicular damage. So, the high-dose extract not only restore structural integrity and organization within the seminiferous tubules, but it also significantly normalizes cellular populations and testosterone levels. Therefore, *P. lentiscus* extract holds potential as a natural therapeutic agent to mitigate reproductive toxicity caused by ethanol exposure. Similar beneficial outcomes have been observed in earlier studies, where natural agents with antioxidant properties improved both testicular architecture and reproductive indices (El-Sokkary, 2001; Nassab *et al.*, 2018). Moreover, the improvement in sperm morphology and function following extract administration also suggests a reduction in ethanol-mediated apoptosis, aligning with previous observations that document the cytotoxic effects of alcohol on spermatogenesis. (Donnelly, McClure, 1999; Martinez, Macera, 2009).

4. Conclusion

The findings of this study indicate that ethanol consumption induces severe histopathological damage and disrupts fertility markers in the testicular tissue of male rats. These adverse effects include a significant reduction in the number of spermatogenic cells, structural abnormalities in seminiferous tubules, and a decrease in serum testosterone levels. However, the concurrent administration of hydroalcoholic extract of *P. lentiscus*, particularly at a dose of 200 mg/kg, substantially mitigated these negative impacts. The protective effects of this plant extract are likely attributed to its potent antioxidant and anti-inflammatory properties, such as flavonoids, terpenes, and tocopherols. These bioactive molecules help neutralize free radicals, enhance the activity of antioxidant enzymes, and prevent lipid peroxidation, thereby preserving the structural integrity and function of testicular germ cells. Based on these findings, *P. lentiscus* extract may serve as a promising natural therapeutic option for reducing ethanol-induced oxidative damage. However, further molecular and clinical investigations are required to validate these results conclusively.

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