*Int. J. Morphol.*,
*43(5)*:1822-1832, 2025.

# Protective Role of *Ocimum tenuiflorum* L. Leaf Extract Against Testicular Damage Induced by Nandrolone: Histopathological, Molecular, Oxidative Stress, and Hormonal Insights

**Papel Protector del Extracto de Hojas de *Ocimum tenuiflorum* L. Contra el Daño Testicular Inducido por Nandrolona: Perspectivas Histopatológicas, Moleculares, de Estrés Oxidativo y Hormonales**

**Wenbo Mao[1] & Ruirui Huangfu[2]**

**MAO, W. & HUANGFU, R.** Protective role of *Ocimum tenuiflorum* L. leaf extract against testicular damage induced by nandrolone: Histopathological, molecular, oxidative stress, and hormonal insights. *Int. J. Morphol., 43(5)*:1822-1832, 2025.

**SUMMARY:** This study aimed to evaluate the protective effects of *Ocimum tenuiflorum* L. (*O. tenuiflorum*) extract against testicular damage induced by nandrolone (ND) in rats. A total of sixty adult male rats were divided into six groups, each consisting of ten animals: Group 1 served as the control; Group 2 received ND (3 mg/kg orally); Groups 3, 4, and 5 were administered *O. tenuiflorum* extract at doses of 50, 100, and 200 mg/kg/day, respectively, alongside ND (3 mg/kg/day); and Group 6 received *O. tenuiflorum* extract at 200 mg/kg/day for 30 days. The study assessed serum levels of testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH), inflammatory cytokines (IL-6, TNF-α, IL-1β), oxidative stress indicators (superoxide dismutase, catalase, glutathione peroxidase, and nitric oxide), and apoptotic markers (Bcl-2, p53, caspase-3, and Bax). Administration of ND significantly elevated serum levels of T, pro-inflammatory cytokines, and pro-apoptotic gene expression while reducing LH and FSH levels, as well as sperm viability, count, and motility. In contrast, *O. tenuiflorum* extract notably normalized serum levels of LH, FSH, and T, restored antioxidant enzyme activity, and reduced pro-inflammatory cytokines. Furthermore, it inhibited germ cell apoptosis by down-regulating p53, caspase-3, and Bax, while up-regulating Bcl-2. The findings suggest that *O. tenuiflorum* extract effectively protects testicular structure and function from ND-induced toxicity through its antioxidant and anti-inflammatory properties.

**KEY WORDS: Apoptosis; Male fertility; Nandrolone; *Ocimum tenuiflorum* L.; Sperm parameters.**

## INTRODUCTION

Infertility is a significant clinical condition defined as the inability of couples to achieve conception after 24 months of unprotected intercourse, affecting approximately 20 % of couples worldwide (Agarwal *et al*., 2021). Beyond the physical implications, infertility can lead to profound emotional, social, and familial challenges. Male infertility is a multifaceted issue, categorized into five primary factors: testicular injuries (such as trauma, varicocele, and torsion), exposure to gonadotoxins (including radiation and certain medications), systemic health conditions (like alcoholism and obesity), hereditary disorders (such as cryptorchidism and Klinefelter syndrome), and idiopathic causes (Mannucci *et al*., 2022). Among these, anabolic androgenic steroids (AAS), commonly used by athletes to enhance muscle mass, are notable gonadotoxins that can adversely affect male fertility.

Nandrolone (ND), a specific type of AAS and a derivative of testosterone, is associated with various side effects, including hirsutism, gynecomastia, erectile dysfunction, and alterations in libido (Bond *et al*., 2022). Research indicates that non-therapeutic doses or prolonged use of ND can lead to significant damage to the seminiferous tubules, increased apoptosis in sustentacular cells (Sertoli cells) and spermatogenic cells, and abnormalities in sperm morphology and motility. Such changes can progress from oligozoospermia to azoospermia, alongside testicular atrophy and altered male hormonal secretions, including elevated testosterone and free radicals (Alves *et al*., 2024). Notably, ND also suppresses the hypothalamic-pituitary-gonadal axis by reducing luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, further complicating male reproductive health (Sretenovic *et al*., 2021).

[1] Urology Department, Norinco General Hospital, Xi'an City, Shaanxi Province, China.
[2] Department of Pharmacy, Xi'an Daxing Hospital, Xi'an, Shaanxi Province, 710000, China.

Received: 2025-07-23 Accepted: 2025-08-18

The reactive oxygen species (ROS) generated during sperm metabolism play a dual role; while they are essential for processes such as acrosome reaction and sperm maturation, excessive ROS can lead to oxidative stress, damaging cellular components and impairing fertility (Wang *et al*., 2025). Sperm possess endogenous antioxidant systems, including enzymes like superoxide dismutase and glutathione peroxidase, which help mitigate oxidative damage and maintain sperm quality (Asadi *et al*., 2021). However, factors such as AAS use, environmental toxins, and systemic diseases can disrupt this balance, leading to increased oxidative stress and subsequent apoptosis of sperm cells (Shi *et al*., 2024). Enhancing the antioxidant capacity of sperm through dietary supplements or natural compounds may restore balance and protect testicular health (Gualtieri *et al*., 2021).

*Ocimum tenuiflorum L.* (*O. tenuiflorum*), a member of the Lamiaceae family, is recognized for its potential health benefits, including its use in traditional medicine to enhance sexual potency and treat various ailments (Bhattarai *et al*., 2024). This study aims to explore the protective effects of *O. tenuiflorum* against ND-induced testicular injury, focusing on sperm parameters, biochemical markers, antioxidant properties, and cellular health in male rats.

## MATERIAL AND METHOD

**Preparation *O. tenuiflorum* extract.** The leaves of *O. tenuiflorum* were harvested from rivers in Xi'an city. After the fresh leaves were dried, a total of 1200 g was ground into a fine powder and subsequently mixed with 2000 mL of 70 % ethanol. The mixture was allowed to extract for 72 hours, after which the solution was filtered using Whatman filter paper no. 42 (Millipore, United States) at a temperature of 25 °C. The resulting extract was then concentrated using a Buchi rotary evaporator (Buchi, rotavapor R-100, Flawil, Switzerland), yielding 145 g of concentrated extract, which was stored at 4 °C for further use (Sharma *et al*., 2022).

**Animals and experimental design.** Sixty Wistar rats, each weighing approximately 220 ± 15 g, were divided into six groups of ten for the study. All rats were housed in polypropylene cages maintained at a relative humidity of 50-60 %, with a 12-hour light/dark cycle at a temperature of 22 ± 2 °C. They had unrestricted access to food (standard rat pellets) and water, adhering to standard laboratory animal care protocols. The study complied with all institutional and national animal welfare regulations as outlined by the Institutional Animal Care and Ethical Committee of Daxing Hospital.

The treatment regimen included ND and extracts of *O. tenuiflorum* as follows:

- Normal Control (NC): Received 1 ml of distilled water (DW) daily for 30 days via gavage.
- Positive Control (ND): Administered 3 mg/kg of ND dissolved in 1 ml of DW daily via gavage.

*O. tenuiflorum*-treated Groups (ND + 50, 100, and 200 mg/kg): Each group received 3 mg/kg of ND along with 50, 100, or 200 mg/kg of *O. tenuiflorum* extract daily via gavage.

*O. tenuiflorum* Group: Received 200 mg/kg of *O. tenuiflorum* extract daily via gavage.

Both *O. tenuiflorum* extracts and ND were dissolved in 1 ml of DW and administered daily at the same time, with a 2-hour interval, over a period of 30 days (Yuniarti *et al*., 2021; Sonpol *et al*., 2022).

**Serum hormones assay.** On day 31, the rats were weighed and then pre-anesthetized with an intraperitoneal (i.p.) injection of 2 % xylazine at a dosage of 100 mg/kg, followed by anesthesia using an i.p. injection of 10 % ketamine at 10 mg/kg, in accordance with standard laboratory animal protocols. Blood samples were collected via cardiac puncture and subsequently centrifuged at 2500 g for 20 min. The serum samples were stored at -70 °C until the measurement of serum factors. Finally, the serum concentrations of testosterone (T), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (Crystal Chem Biotech Company, United States) were determined using enzyme-linked immunosorbent assay (ELISA) kits, with readings taken using an ELISA reader (Biotek Instruments, United States) (Villarraza *et al*., 2024).

**Sperm parameters**

**Sperm count.** To prepare the primary sperm suspension (PSS), the tail of the epididymis was first weighed using a digital scale and then rinsed in normal saline at 37 °C. It was subsequently gently crushed with a razor blade in 2 ml of Ham's F10 medium. From the resulting PSS, 200 µl was mixed with a 10 % formaldehyde solution, and 10 µl of this mixture was carefully placed into a Neobar counting chamber. The sperm count was then conducted at 400x magnification using a Makler Chamber and using a light microscope (Olympus Corporation, Tokyo), counting the sperm in five Neobar squares (comprising four small corner squares, each measuring 0.2 $mm^2$, and one large center square measuring 1 $mm^2$). After five repetitions, the average sperm count (P) was calculated using the following formula:

$$P \times DV/N \times D$$

In this equation, (P) represents the mean number of sperm counts, (N) denotes the number of counted sperm per $mm^2$, (D) is the depth of the chamber, and (DV) is the dilution volume. Ultimately, the sperm concentration in 1 ml of the sperm suspension was reported as the number of sperm per $10^6$ (Wang *et al*., 2021).

**Sperm motility.** Sperm motility was assessed according to the criteria set by the World Health Organization (WHO). The classification included Grade 1 (immotile sperm, which exhibit no tail movement), Grade 2 (*in situ* sperm, which show motility in the tail and trunk but lack forward movement), and Grade 3 (forward sperm, which move in a straight line). To evaluate and categorize sperm based on motility, 10 µl of the primary sperm suspension (PSS) was carefully placed on a glass slide and observed at 400x magnification using a Makler Chamber and using a light microscope (Olympus Corporation, Tokyo). The assessment was conducted across 30 fields of view under the light microscope, following the established protocol (Cooper *et al*., 2010).

**Sperm viability.** To evaluate sperm viability, a 0.5 % eosin Y staining technique was employed. In this method, sperm with damaged plasma membranes (dead sperm) appear pink, while sperm with intact plasma membranes (live sperm) remain colorless. For the procedure, 10 µl of eosin Y was mixed with 40 µl of the primary sperm suspension on a glass slide, which was then covered with a cover slip. Sperm viability was assessed by examining 30 fields of view under at 400x magnification using a Makler Chamber and using a light microscope (Olympus Corporation, Tokyo) (Aboutalebi *et al*., 2025).

**Serum measurements of IL-6, TNF-α, IL-1β cytokines.** Serum levels of cytokines IL-6, TNF-α, IL-1β, and IL-10 were quantified using ELISA kits from Bio Legend. The assay utilized bovine serum albumin (BSA) as a blocking solution and phosphate-buffered saline (PBS) as the wash buffer. Following the manufacturer's protocol, primary antibodies were pre-coated on a 96-well plate and incubated at 37 °C for 24 h, after which the plate was washed three times with the wash buffer. A blocking solution was then added and incubated for one hour. Serum samples were diluted and added to the wells, followed by the addition of secondary antibodies (200X) for another hour of incubation. The resulting yellow color was measured at wavelengths of 450 and 570 nm using an ELISA reader (Biotek Instruments, United States). The serum cytokine levels were reported in picograms per deciliter (pg/dl) (Wang *et al*., 2021).

**Serum and testis tissue antioxidant levels**

**Nitric Oxide (NO) Assay.** The serum nitric oxide level was measured using the Griess colorimetric method. Initially, 6 mg of zinc sulfate was added to 400 µl of serum for deproteinization, followed by centrifugation at 12,000 g for 12 min at 4°C. Standard nitrite dilutions from 0 to 200 mM were prepared and 100 µl of serum samples were added to each well. A mixture of 50 µl sulfanilamide and 100 µl vanadium chloride was incorporated into each sample. After a 20 min incubation at 4°C, absorbance was measured at 450 and 630 nm using a Statfax 100 ELISA reader, with results reported in µM/ml (Mohanty *et al*., 2025).

**FRAP Assay.** The total antioxidant capacity (TAC) of testicular tissue was assessed using the FRAP method. Testicular tissue was homogenized in cold PBS with EDTA, and 200 µl of this homogenate was mixed with FRAP solution (containing acetate buffer, TPTZ, and ferric chloride). After a 15 min incubation, the mixture was centrifuged at 15,000 g for 10 min. The absorbance was measured at 593 nm using a Mettler-Toledo spectrophotometer, with results reported as µmol/mg protein (Mohammadi *et al*., 2023).

**Lipid Peroxidation Levels (TBARS).** Lipid peroxidation in testicular tissue was measured using the TBARS method. A 50 µl sample of the homogenized tissue was combined with phosphoric acid, BHT, and TBA solution. This mixture was centrifuged at 10,000 g for 4 min at 60 °C, and the absorbance of the supernatant was read at 532 nm with a Mettler-Toledo spectrophotometer, reported in nmol/mg (Fariello *et al*., 2021).

**Serum Antioxidant Enzyme Activity**

**SOD Activity:** Serum superoxide dismutase (SOD) activity was measured using a commercial kit from Abcam, with absorbance read at 450 nm using a Statfax ELISA reader, reported in U/ml.

**GPx Activity:** Glutathione peroxidase (GPx) activity was evaluated with an Abcam kit, based on NADPH oxidation, with readings taken at 340 nm via a Biotek ELISA reader, reported in U/ml.

**CAT Activity:** Catalase (CAT) activity was determined using a Cayman Chemical kit, where absorbance was recorded at 405 nm after reacting $H_2O_2$ with a chromogen, reported in U/ml (Abedini Bajgiran *et al*., 2023; Sundaresan *et al*., 2023).

**RNA extraction from testicular tissue**. RNA was extracted from frozen testicular tissue using the Qiagen RNA extraction kit (QIAGEN Ltd, Manchester, UK). Following the manufacturer's instructions, 50 mg of frozen right testicular tissue was homogenized on ice and mixed with 500 µl of

Trizol solution. The mixture was then centrifuged at 12,000 g for 10 min, and the supernatant was transferred to an RNA separation column, which was subsequently washed with wash buffers 1 (500 µl) and 2 (700 µl). For cDNA synthesis, the final mixture was centrifuged again at 17,000 g for 10 min, and 50 µl of distilled water (DW) was added before storing at -70 °C. The quality and purity of the RNA were assessed using a 2 % agarose gel and a NanoDrop spectrophotometer (Thermo Science 2000c, United States) (Vasisth *et al*., 2023).

**cDNA Synthesis and Quantitative Real-Time PCR.** For cDNA synthesis, the BioFact cDNA synthesis kit (BioFact™ RT Series, Korea) was utilized with 1 µg of RNA. The reaction mixture included 0.5 µl of Random Hexamer primer, 0.5 µl of oligo primer, and 10 µl of SYBR Green I Master Mix (TaKaRa, Japan), along with 1000 ng of RNA, bringing the total volume to 20 µl with RNase-free water. The reverse transcription (RT) temperature protocol consisted of an initial step at 95 °C for 2 min, followed by 60 °C for 30 s. The cDNA was stored at -70 °C. Gene sequences were designed using Gene Runner software version 6.5.52 (Hastings Software, Hudson, NY, United States) and were verified through NCBI, as listed in Table I.

α

Table I. Primer sequences

| Gene | Sequences |
|---|---|
| β-actin | F: 5'-GGCTGTATTCCCCTCCATCG-3' |
| Caspase 3 | F: 5'-CAGCAGTGTGCTGCTGAGAC-3' |
| Bcl-2 | F: 5'-TGGGATGACCTGGTGATGAG-3' |
| p53 | F: 5'-GCCAGCAGTCTTCTCTGCTG-3' |
| Bax | F: 5'-GCCGAGGATGCGTGTCTT-3' |

The expression levels of pro-apoptotic genes (p53, caspase-3 (c3), and Bax) and the anti-apoptotic gene (Bcl-2) were quantified using β-actin as an internal control (housekeeping gene). The real-time PCR mixture comprised 10 µl of SYBR Premix master mix, 1 µl of cDNA template, and 1 µl each of forward and reverse primers, using a Real-Time PCR system (StepOne™, U.S.) as per the manufacturer's guidelines. Each cycle involved 30 s at 95 °C for denaturation, followed by 1 min at 60 °C for annealing, and 1 min at 72 °C for extension, totaling 42 cycles. A pre-synthesis phase of 2 minutes at 50 °C was conducted, followed by 4 minu at 95 °C. The melting curve analysis was performed by gradually increasing the temperature from 60 °C to 95 °C at a rate of 1 °C every 5 s. All qRT-PCR reactions were performed in duplicate for each sample, and the fold change in mRNA expression levels was calculated using the formula:

$$\text{Fold change}=2^{-\Delta\Delta CT},\ \Delta\Delta Ct = [(T\text{-}R) - (t\text{-}r)]$$

where $\Delta\Delta CT = [(T\text{-}R) - (t\text{-}r)]$. Here, T and R represent the target and reference Ct values in the test sample, while t and r denote the target and reference Ct values in the control sample, respectively (Changizi *et al*., 2021).

**Immunohistochemical (IHC) assay for p53 and Bcl-2.** Immunohistochemical (IHC) staining for Bcl-2 (an anti-apoptotic marker) and p53 (a pro-apoptotic marker) was conducted on germinal and testicular parenchymal cells to assess apoptotic changes among the groups. Initially, right testicular specimens were carefully excised from surrounding tissues and fixed in a 10 % buffered formalin solution for 72 h. Paraffin blocks were then prepared into 5 µm sections using a Leica RM2125 RTS microtome (Leica Microsystems Inc, United States), which were subsequently placed onto glass slides. The samples were incubated at 95 °C for 2 h to facilitate antibody retrieval, followed by immersion in a Tris buffer solution (comprising 2 g of tris and 0.4 g of EDTA dissolved in 1 liter of distilled water, pH 9) for 20 min. To block endogenous peroxidase activity, the slides were treated with 5 % bovine serum albumin (BSA) and 3 % hydrogen peroxide for 40 min. Next, the slides were incubated with biotinylated rabbit anti-mouse IgG antibodies: Bcl-2 (Cell Signaling Technology, Inc, Danvers, MA, United States; dilution 1:1000) and p53 Rabbit mAb (Rodent Specific) (Cell Signaling Technology, Inc, Danvers, MA, United States; dilution 1:1000) as primary antibodies for 45 minutes at room temperature. After this, the slides were treated with a chromogen solution of 3,3'-diaminobenzidine (DAB) for 10 min, followed by a 30-min incubation with streptavidin-horseradish peroxidase. Following hematoxylin counterstaining, all slides were examined using an Olympus IX71 light microscope (Olympus, Japan) at 400X magnification, connected to a Moticam camera system (Moticam Technologies, Japan). For each sample, 50 fields were selected, and within each field, the ratio of Bcl-2 and p53-positive cells to the total number of cells was calculated. The mean values for each group were reported as "mean ± SD" (Abdelmonem *et al*., 2024).

**Testis histopathology.** Following the fixation of right testicular samples, paraffin blocks were created, and 5 µm sections were prepared using a method similar to immunohistochemistry (IHC). Routine tissue processing was then carried out, along with hematoxylin and eosin (H&E) staining. Three key proliferation indices in the seminiferous tubules (ST) were assessed, calculated using the following formulas:

- Tubular differentiation index (TDI) = Number of STs with three or more differentiated germ cells with SPA/ Total STs × 100.
- Spermiogenesis index (SPI) = Number of STs containing sperm in the lumen/ Total STs × 100.

- Repopulation index (RI) = Number of STs containing active form (type A) spermatogonia / /Total STs × 100.

All sections were examined at 400X magnification using an Olympus IX71 light microscope (Olympus, Japan) connected to a Moticam camera system (Moticam Technologies, Japan) (Kavak *et al*., 2015; Rad *et al*., 2021).

**Statistical Analysis.** Statistical analysis was conducted using SPSS version 16, while GraphPad Prism version 8 was utilized for designing graphs. The Kolmogorov-Smirnov test ($p > 0.05$) was employed to assess the normality and homogeneity of the data. Significant differences among the groups were determined using Tukey's *post hoc* descriptive test and one-way analysis of variance (ANOVA). All data are presented as "mean ± standard error of the mean (SEM)," with a p-value of less than 0.05 indicating statistical significance.

## RESULTS

**Impact of *O. tenuiflorum* extract on rat's body (BW), epididymis weights (EW), and testicular (TW).** Rats intoxicated with ND showed a significant decrease in BW, EW, and TW ($p < 0.05$) compared to the NC group. However, in rats treated with 200 mg/kg of *O. tenuiflorum* extract, all three weight parameters were significantly increased ($p < 0.05$) compared to the ND group. Additionally, a dosage of 100 mg/kg of this plant also significantly enhanced ($p < 0.05$) both TW and EW when compared to the ND group (Fig. 1a).

**Hormones analyzes.** The ND group showed a significant increase ($p < 0.05$) in T levels while decreasing serum levels of FSH and LH compared to the NC group. Conversely, administration of 100 mg/kg of *O. tenuiflorum* resulted in a significant increase ($p < 0.05$) in serum LH and FSH levels when compared to the ND group. Additionally, the 200 mg/kg dose of *O. tenuiflorum* extract not only significantly elevated ($p < 0.05$) serum LH and FSH levels but also led to a significant reduction ($p < 0.05$) in serum T levels relative to the ND group (Fig. 1b).

**Sperm parameters.** The ND group demonstrated a significant reduction ($p < 0.05$) in sperm viability, count, and forward motility, along with an increase in the number of immotile sperm. In contrast, the groups treated with *O. tenuiflorum* exhibited a dose-dependent enhancement in all sperm parameters compared to the ND group. Both the 100 mg/kg and 200 mg/kg doses of *O. tenuiflorum* extract resulted in a significant increase in sperm viability and count ($p < 0.05$), as well as forward motility ($p < 0.01$), while also significantly decreasing the number of immotile sperm ($p < 0.05$) (Table II).

**Serum measurements of TNF-α, IL-1β, and IL-6 cytokines.** The ND group showed a significant increase ($p < 0.05$) in serum levels of TNF-α, IL-1β, and IL-6 cytokines compared to the NC group. In contrast, the administration of 100 and 200 mg/kg of *O. tenuiflorum* extracts resulted in a significant decrease ($p < 0.05$) in serum levels of TNF-α, IL-1β, and IL-6 when compared to the ND group (Fig. 2).

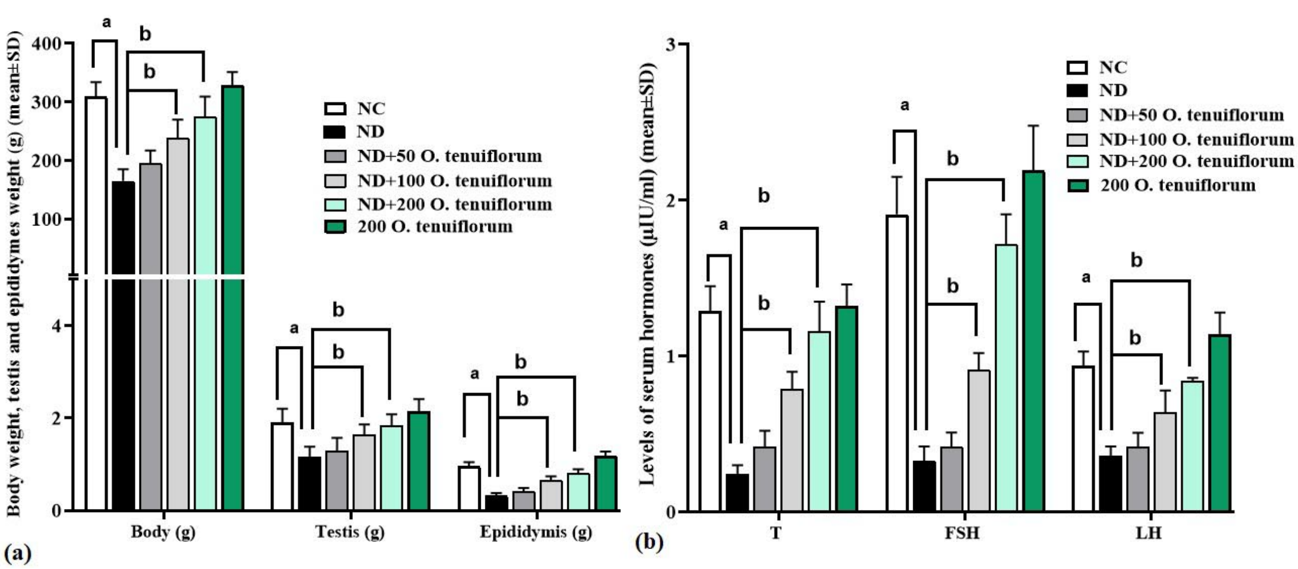


Fig. 1. (a) Impact of *O. tenuiflorum* extract on rat's body (BW), epididymis (EW), and testicular (TW) weights (g) and (b) Impact of *O. tenuiflorum* extract on rat's T, LH and FSH serum levels in NC, ND, 50, 100 and 200 *O. tenuiflorum* extract plus ND and 200 *O. tenuiflorum* extract groups. Data's are statistically significant at a ($p < 0.05$) *versus* ND and NC groups, and *O. tenuiflorum* treatment at b ($p < 0.05$) *versus* ND groups (means± SEM, n=10/group).

Table II. Sperm parameters in NC, ND, 50, 100 and 200 *O. tenuiflorum* extract plus ND and 200 *O. tenuiflorum* extract groups (mean±SD).

| Groups | Sperm count (x$10^6$/ml) | Motility (%) | | | Viability (%) |
|---|---|---|---|---|---|
| | | Forward | Insitu | Immotile | |
| NC | 5.60±1.06 | 72.11±9.63 | 22.74±3.14 | 11.29±2.94 | 74.17±8.91 |
| ND | 2.39±0.79[a] | 32.1±4.12[a] | 18.61±3.19 | 46.17±7.17[a] | 16.17±4.11[a] |
| ND + *O. tenuiflorum (*50 mg/kg) | 2.65±0.66 | 35.10±5.19 | 20.14±4.21 | 36.17±6.19 | 24.07±5.29 |
| ND + *O. tenuiflorum (*100 mg/kg) | 3.29±0.90[b] | 50.17±6.16 | 22.19±6.12 | 24.71±6.21 | 45.14±6.19[b] |
| ND + *O. tenuiflorum (*200 mg/kg) | 5.10±0.92[b] | 65.11±7.11[b] | 24.44±4.90 | 16.21±4.11[b] | 63.11±9.91[b] |
| *O. tenuiflorum (*200 mg/kg) | 5.42±0.82 | 69.23±6.14 | 25.14 ±5.61 | 11.70±2.16 | 78.11±10.14 |

Data's are statistically significant at a ($p < 0.05$) *versus* ND and NC groups, and *O. tenuiflorum* treatment at b ($p < 0.05$) *versus* ND groups.

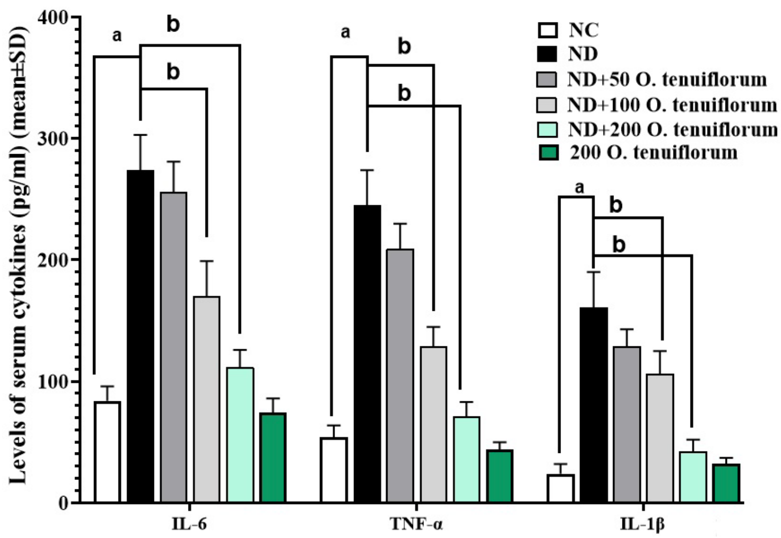


Fig. 2. Impact of *O. tenuiflorum* extract on TNF-, IL-1β, and IL-6 (pg/ml) serum levels in NC, ND, 50, 100 and 200 *O. tenuiflorum* extract plus ND and 200 *O. tenuiflorum* extract groups. Data's are statistically significant at a ($p < 0.05$) *versus* ND and NC groups, and *O. tenuiflorum* treatment at b ($p < 0.05$) *versus* ND groups (means± SEM, n=10/group).

**Serum activity of antioxidant enzymes (SOD, CAT and GPx) and serum NO levels.** At the conclusion of the study, serum analysis of antioxidant parameters revealed that the ND group had a significant increase ($p < 0.05$) in serum NO levels compared to the NC group, along with a significant decrease ($p < 0.05$) in the activity of oxidative stress enzymes, including SOD, CAT, and GPx. In comparison to the ND group, the 200 mg/kg dose of *O. tenuiflorum* significantly reduced ($p < 0.05$) serum NO levels, while the activities of GPx, SOD, and CAT significantly increased ($p < 0.05$). Similarly, the 100 mg/kg dose of *O. tenuiflorum* also significantly decreased ($p < 0.05$) serum NO levels, with notable increases in the activities of SOD and CAT ($p < 0.05$) compared to the ND group (Fig. 3a).

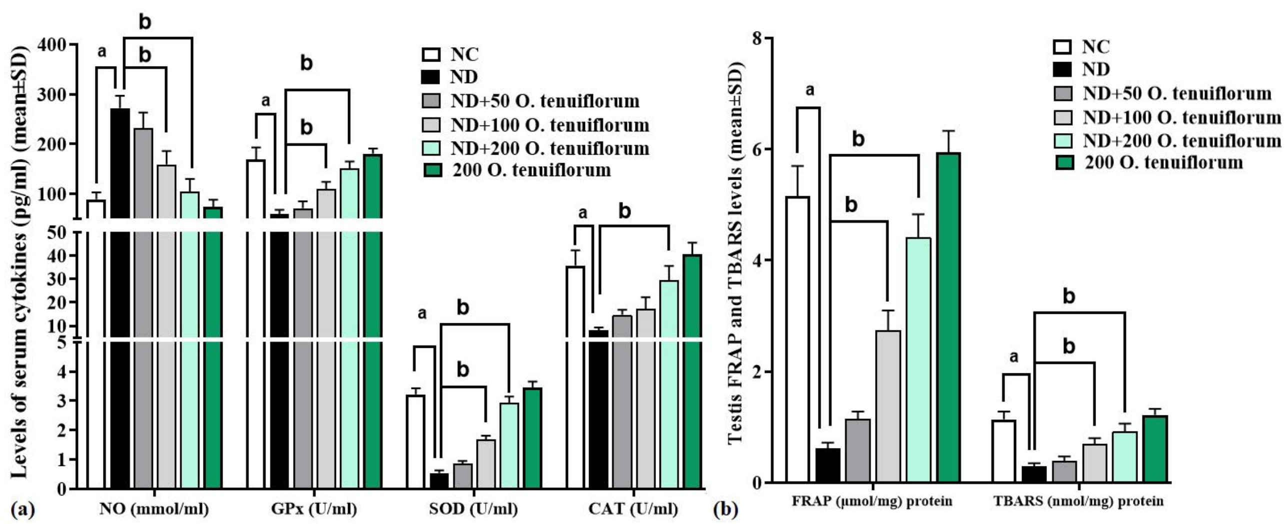


Fig. 3. (a) Impact of *O. tenuiflorum* extract on NO (mmol/ml) serum levels and also GPx, CAT and SOD (U/ml) activity and (b) testis tissue FRAP (µmol/mg protein) and TBARS (nmol/mg proteins) levels in NC, ND, 50, 100 and 200 *O. tenuiflorum* extract plus ND and 200 *O. tenuiflorum* extract groups. Data's are statistically significant at a ($p < 0.05$) *versus* ND and NC groups, and *O. tenuiflorum* treatment at b ($p < 0.05$) *versus* ND groups (means± SEM, n=10/group).

**Testis tissue antioxidant status.** At the conclusion of the study, analysis of antioxidant parameters in testis tissue indicated that the ND group exhibited a significant decrease ($p < 0.05$) in testis levels of FRAP and TBARS compared to the NC group. In contrast, both the 100 mg/kg and 200 mg/kg doses of *O. tenuiflorum* significantly increased ($p < 0.05$) the testis levels of FRAP and TBARS when compared to the ND group (Fig. 3b).

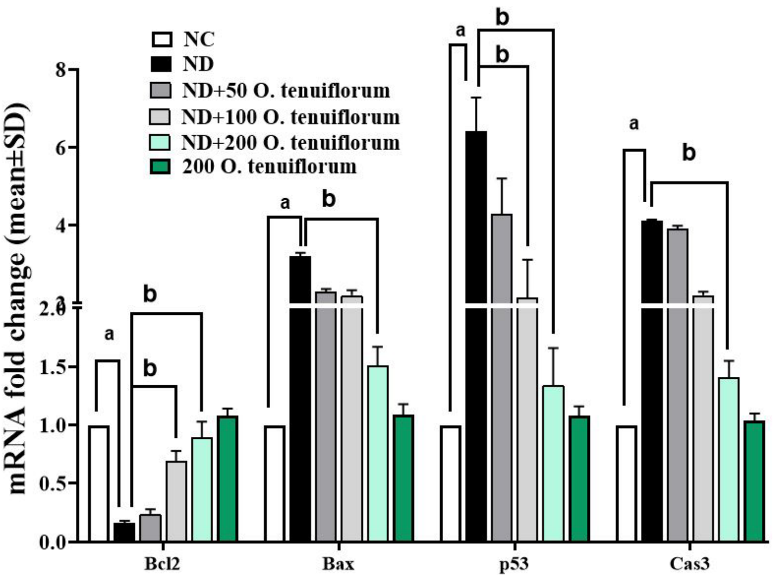


Fig. 4. Impact of *O. tenuiflorum* extract on expression of p53, c3, Bcl-2, and Bax genes in NC, ND, 50, 100 and 200 *O. tenuiflorum* extract plus ND and 200 *O. tenuiflorum* extract groups. Data's are statistically significant at a ($p < 0.05$) *versus* ND and NC groups, and *O. tenuiflorum* treatment at b ($p < 0.05$) *versus* ND groups (means± SEM, n=10/group).

**P53, Bax, Bcl-2 and c3 genes expression levels.** The ND-intoxicated rats exhibited a significant up-regulation ($p < 0.05$) of the Bax, p-53, and Cas3 genes, along with a significant down-regulation ($p < 0.05$) of the Bcl-2 gene compared to the NC group. This resulted in a significantly increased ($p < 0.05$) Bax/Bcl-2 ratio in the ND group relative to the NC group. In comparison to the ND group, the 100 mg/kg dose of *O. tenuiflorum* significantly increased the expression of Bcl-2 ($p < 0.05$) and decreased p-53 gene expression ($p < 0.05$). The 200 mg/kg dose of *O. tenuiflorum* extract had an even greater effect in reducing testicular cell apoptosis, significantly down-regulating ($p < 0.05$) the expression of Cas3, p-53, and Bax genes, while significantly up-regulating ($p < 0.05$) Bcl-2 gene expression. This dose also significantly decreased the Bax/Bcl-2 ratio ($p < 0.05$) compared to the ND group (Fig. 4).

**Histomorphometric parameters.** After assessing spermatogenesis indices, it was observed that the ND group significantly reduced all three indices—RI, SPI, and TDL—compared to the NC group ($p < 0.05$). In contrast, both the 100 mg/kg and 200 mg/kg doses of *O. tenuiflorum* significantly increased ($p < 0.05$) all three indices (RI, SPI, and TDL) in the treatment groups compared to the ND group (Fig. 5a).

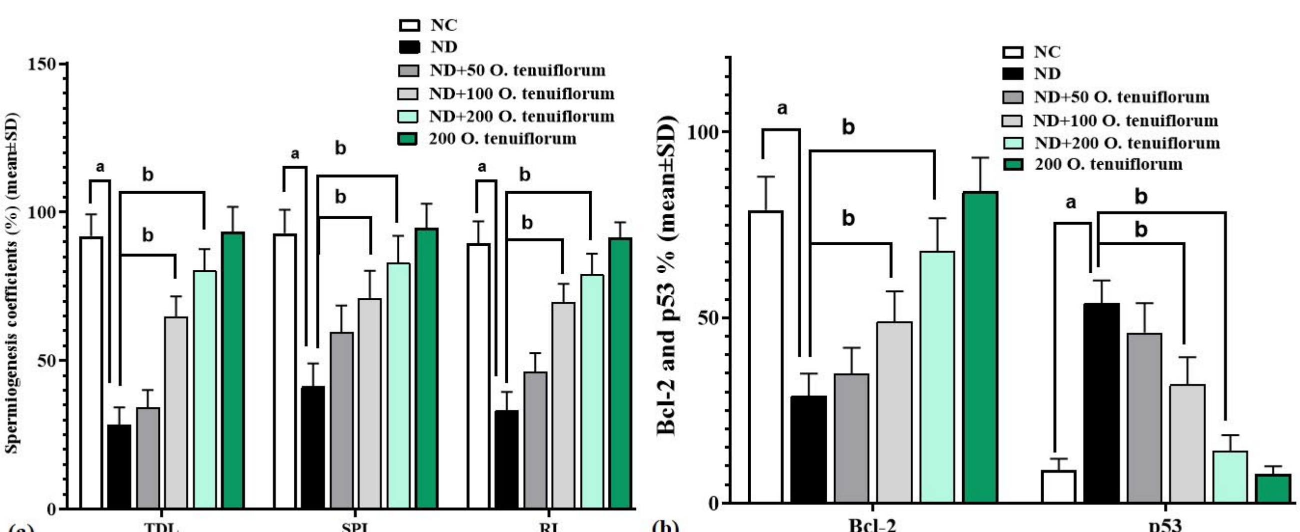


Fig. 5. (a) Impact of *O. tenuiflorum* extract on testis histomorphometric parameters (TDL, SPI and RI) and (b) Impact of *O. tenuiflorum* extract on Bcl-2 and p53 positive cells (%) in NC, ND, 50, 100 and 200 *O. tenuiflorum* extract plus ND and 200 *O. tenuiflorum* extract groups. Data's are statistically significant at a ($p < 0.05$) *versus* ND and NC groups, and *O. tenuiflorum* treatment at b ($p < 0.05$) *versus* ND groups (means± SEM, n=10/group).

**Histological and histomorphometrical findings.** Histopathological examination of the spermatogenic lineage and supporting cells in the seminiferous tubules (H&E staining × 100) revealed that in the NC group, spermatogonia type A (SpA) cells were attached to the integrated walls of the tubules and sustentacular cells, filling the base of the tubule with dense nuclei (heterochromatin). Spermatogonia type B (SpB), primary spermatocytes (PrS), secondary spermatocytes (SrS), and spermatids were arranged toward the lumen. No pyknotic nuclei, vacuolation, irregularities, or destruction of tubular walls were observed, nor were there any cytoplasmic or residual bodies (Rb) or detached spermatozoa tufts (DSTs) in the lumen space or at the level of the germinal epithelium. Conversely, the ND group, through the production of reactive oxygen species (ROS), caused destruction of the germinal epithelium, leading to shrinkage of tubular walls, vacuolization in the germinal epithelium—especially in SpA cells—and degeneration of PrS, SrS, and elongated spermatids (ES). Pyknotic nuclei, along with the presence of cytoplasmic or Rb in the lumen of the tubules, and degeneration of Sustentacular cells (SC) and interstitial cells (Leydig cells) (IC) in the walls of the tubules and the spaces between them were also observed. *O. tenuiflorum* extract, with its antioxidant, aphrodisiac, and anti-inflammatory properties, protects the germinal epithelium and supporting cells (sustentacular cells and interstitial cells) in a dose-dependent manner against the destructive effects of ND. This protection resulted in an increased density of germ cells, normalization of the walls and lumen of the seminiferous tubules, and the absence of vacuolated and degenerated cells in both the lumen and germinal epithelium. Additionally, no apoptotic cells or residual bodies (Rb) were observed in the tubular space, and the SpA cells were arranged normally in their positions within the tubular wall, characterized by heterochromatin nuclei and cytoplasm attached to sustentacular cells (Fig. 6).

**Effect of *O. tenuiflorum* on expression of apoptosis index (Bcl-2 and p53) in STs.** After evaluating the apoptotic indices via IHC staining, it was found that compared to the NC group, the ND group significantly increased the percentage of p53-positive cells and decreased the percentage of Bcl-2-positive cells ($p < 0.05$). However, the *O. tenuiflorum* extract, with its protective effects at doses of 100 and 200 mg/kg, significantly reduced the percentage of p53 immunopositive cells and increased the percentage of Bcl-2 immunopositive cells compared to the ND group ($p < 0.05$) (Figs. 5b and 7).

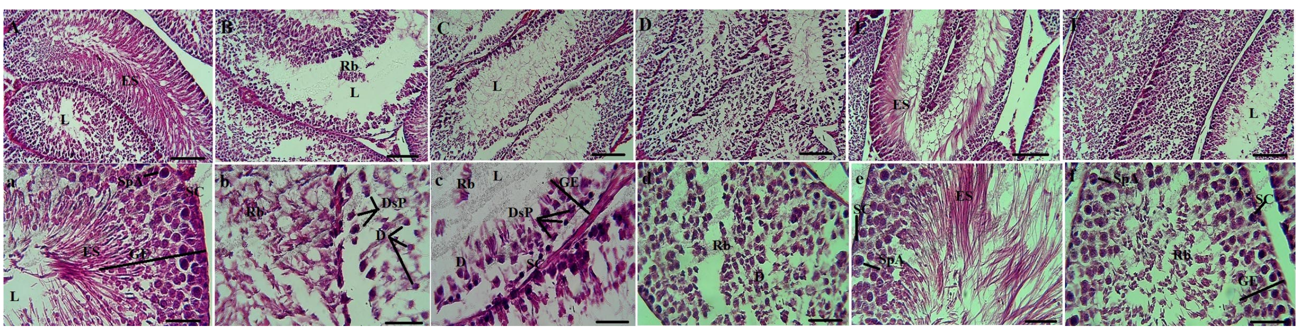


Fig. 6. Impact of *O. tenuiflorum* extract on STs histopathology (top row scale bar = 200 mm, H&E staining × 100; bottom row scale bar = 50 µm, H&E staining × 400) in NC (A, a), ND (B, b) and the *O. tenuiflorum* extract plus ND [*O. tenuiflorum* 50 (C, c), 100 (D, d) and 200 (E, e) mg/kg] and *O. tenuiflorum* 200 mg/kg (F, f) groups. Elongate spermatids (ES), sustentacular cells (SC), residual bodies (Rb), germinal epithelium (GE), tubular lumen (L), type A spermatogonia (SpA), degenerated spermatids (D) and degenerated spermatogonial cell (DsP).

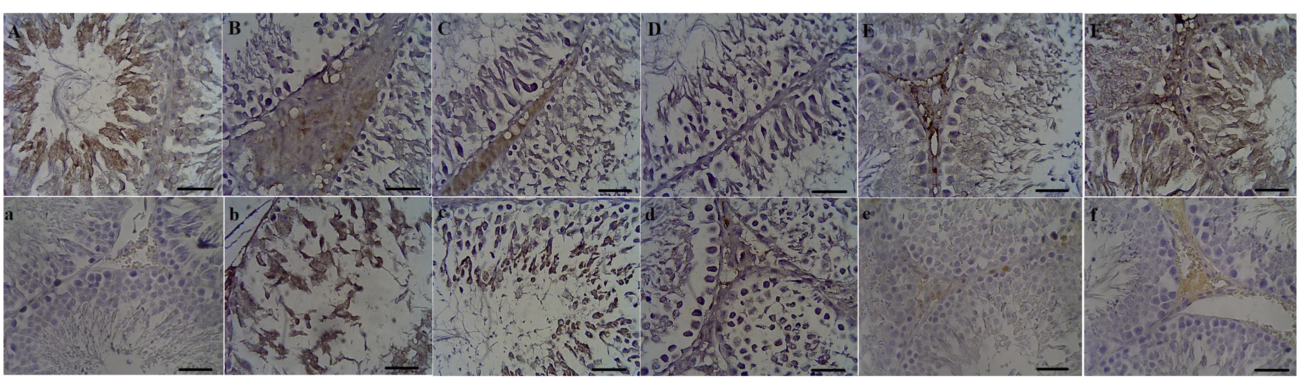


Fig. 7 Impact of *O. tenuiflorum* extract on Bcl-2 (bottom row) and p53 (top row) positive cells in STs in NC (A, a), ND (B, b), *O. tenuiflorum* extract treatment groups [*O. tenuiflorum* 50 (C, c), 100 (D, d) and 200 (E, e) mg/kg] 200 mg/kg *O. tenuiflorum* extract (F, f) (Scale bar = 50 µm, DAB staining × 400). In the top row, p53 negative cells, p53 positive cells, and in the bottom row, Bcl-2 positive cells and Bcl- 2 negative cells are marked.

## DISCUSSION

**OM-induced testicular injury**. Based on biochemical, molecular, immunohistochemical, histomorphometric, and antioxidant findings, the present study demonstrated the protective effects of *O. tenuiflorum* extract against structural and functional defects in the testes caused by ND toxicity in rats.

Following a 31-day gavage of ND, serum levels of LH and FSH decreased while T levels increased, indicating that, similar to findings in other studies, ND suppresses the HPGA and leads to increased endogenous T levels. Shahraki *et al*. (2015), showed that due to HPGA suppression and the negative feedback mechanism induced by ND, testicular stimulating hormones are reduced, ultimately affecting spermatogenesis, puberty, and the function of Sustentacular cells. Various studies have also reported that a dose of 3 mg/kg ND administered by gavage suppresses LH and FSH secretion while increasing testosterone secretion in rats and mice (Patanè *et al*., 2020; Santos *et al*., 2021). FSH is crucial for the proliferation of Sustentacular cells; by binding to these cells, it enhances water secretion and maintains ionic balance around spermatogenic cells, particularly spermatogonia (SpA) cells (Muratori & Baldi, 2018). Ni *et al*. (2019), demonstrated a direct relationship between the number of Sustentacular cells and sperm parameters, suggesting that an increase in Sertoli cell numbers leads to a corresponding increase in sperm count and their survival (Ni *et al*., 2019).

In addition, this study revealed that ND increased ROS levels, as indicated by elevated serum NO levels and decreased testicular tissue FRAP and TBARS. This increase in ROS was associated with a reduction in the activity of the exogenous antioxidant system, specifically through decreased activities of GPx, CAT, and SOD. Elevated ROS levels contribute to apoptosis in spermatogenic lineage and testicular parenchymal cells by damaging cellular membranes and directly affecting ion channels in spermatid flagella. This leads to a reduction in the height of the germinal epithelium, an increase in degenerative cells within the luminal space, and ultimately diminished sperm parameters (Sharma *et al*., 2022). Furthermore, ROS have been shown to damage DNA, alter intracellular $Ca^{2+}$ concentrations in sperm, and induce sperm apoptosis (Liu *et al*., 2023). ROS also enhance the expression of caspase-3 by regulating apoptosis-related genes such as Bax and Bcl-2, which subsequently results in Cytochrome-c leakage from mitochondria and triggers cell apoptosis (Liu *et al*., 2015). Recent *in vivo* studies indicate that ND (3 mg/kg/day) suppresses the activity of endogenous antioxidant enzymes in rat sperm, leading to increased ROS levels and enhanced apoptosis through the regulation of cell cycle-related genes like p53 (Mohamed & Mohamed, 2015). In the present study, ND (3 mg/kg/day) was found to increase the expression of Bax, p53, and c3 while decreasing Bcl-2 expression in testicular tissue. Consequently, apoptosis of testicular cells increased (as shown by elevated p53 positive cells), and parameters of epididymal sperm decreased. Additionally, studies indicate that ND elevates inflammation in testicular tissue, increasing serum levels of pro-inflammatory cytokines TNF-α, IL-8, and IL-6, suggesting a link between ROS production and the enhancement of inflammatory pathways (Shirpoor & Naderi, 2024). The results of this study confirm that ND significantly increases serum levels of these pro-inflammatory cytokines. Various plant polyphenolic compounds, including isoflavones and flavonoids, along with certain aphrodisiac substances, exhibit steroidogenic, antioxidant, and anti-inflammatory properties that protect the spermatogenic lineage from ROS damage. They achieve this by enhancing the HPGA and promoting the proliferation and differentiation of Sertoli and Interstitial cells (Akbaribazm *et al*., 2024).

Numerous studies have demonstrated the protective and enhancing effects of compounds such as Apigenin, quercetin, myricetin, kaempferol, a-Tocopherol, daidzein, and other phenolic compounds against ROS-induced damage in the reproductive system (Akbaribazm *et al*., 2024). These compounds regulate testosterone secretion from Interstitial cells, enhance HPGA activity, increase the secretion of water and micronutrients from sustentacular cells to the spermatogenic lineage, and help cleanse the lumen and surrounding areas of sperm from ROS (Monageng *et al*., 2023). Our research demonstrated that *O. tenuiflorum* extract, particularly at doses of 100 and 200 mg/kg, strengthened the HPGA and increased the secretion of LH and FSH. Additionally, due to its rich content of polyphenolic compounds with potent antioxidant properties, the serum activities of antioxidant enzymes (CAT, GPx, and SOD) and tissue levels of FRAP increased, while testicular tissue levels of TBARS and serum levels of nitric oxide (NO) decreased.

In this study, *O. tenuiflorum* extract suppressed the secretion of pro-inflammatory cytokines TNF-α, IL-8, and IL-6 at doses of 100 and 200 mg/kg. However, a study by Wang *et al*. (2021) indicated that pro-inflammatory cytokines, through pathways involving IL-6 or TNF-α/caspase-3/STAT-3, increase the expression of p53 and activate the Cytochrome c-dependent apoptosis pathway by up-regulating Bax and down-regulating Bcl-2 gene expression. In the Cytochrome c-dependent apoptotic pathway, an increased Bax/Bcl-2 ratio activates the caspase effector (Cas3), leading to Cytochrome c leakage from mitochondria and subsequent apoptosis (Xu *et al*., 2016).

*O. tenuiflorum* extract decreased the Bax/Bcl-2 ratio by reducing Bax expression and increasing Bcl-2 expression, which ultimately led to decreased expression of c3 and p53, thereby diminishing apoptosis in testicular cells. Similar studies have shown that plants containing polyphenolic compounds, like those in *O. tenuiflorum* extract, not only suppress the secretion of TNF-a, IL-8, and IL-6 cytokines but also inhibit Cytochrome c-dependent apoptosis by reducing Bax and increasing Bcl-2 expression, which lowers the Bax/Bcl-2 ratio (Samie *et al*., 2018; Zhang *et al*., 2023). All these effects, as observed in our study, resulted in increased sperm count, viability, and motility of sperm extracted from the tail of the epididymis compared to the ND group.

## CONCLUSION

ND, when used as a drug in non-therapeutic doses, particularly among bodybuilders, can lead to loss of sexual function and may induce suppression of the HPGA along with testicular cell apoptosis, resulting in irreversible damage. However, *O. tenuiflorum* can serve as a protective agent against oxidative stress and inflammatory pathways that contribute to the destruction of testicular germ cells, helping athletes avoid impotence associated with ND. The extract of *O. tenuiflorum* contains various polyphenolic compounds (such as Kaempferol, Quercetin, Daidzein, Ferulic acid, and Harman) and aphrodisiac compounds (like Proanthocyanidin), which enable it to maintain the function and structure of the testes in treated rats. Additionally, it strengthens the HPGA, balances, and enhances the exogenous antioxidant system of sperm, thereby protecting Sustentacular cells, Interstitial cells, and the spermatogenic lineage from apoptosis and necrosis. Therefore, *O. tenuiflorum* or its purified effective compounds could be utilized in the pharmaceutical industry for the treatment of infertile men, especially those who are required to take AAS.

**Ethical Approval.** The experimental protocols of this study were approved by the Daxing Hospital ethics committee.

**MAO, W. & HUANGFU, R.** Papel protector del extracto de hojas de *Ocimum tenuiflorum L.* contra el daño testicular inducido por nandrolona: Perspectivas histopatológicas, moleculares, de estrés oxidativo y hormonales. *Int. J. Morphol., 43(5)*:1822-1832, 2025.

**RESUMEN:** Este estudio tuvo como objetivo evaluar los efectos protectores del extracto de *Ocimum tenuiflorum L.* (*O. tenuiflorum*) contra el daño testicular inducido por nandrolona (ND) en ratas. Un total de sesenta ratas macho adultas se dividieron en seis grupos, cada uno compuesto por diez animales: el Grupo 1 sirvió como control; el Grupo 2 recibió ND (3 mg/kg por vía oral); a los Grupos 3, 4 y 5 se les administró extracto de *O. tenuiflorum* a dosis de 50, 100 y 200 µg/kg/día, respectivamente, junto con ND (3 mg/kg/día); El grupo 6 recibió extracto de *O. tenuiflorum* a una dosis de 200 µ/kg/día durante 30 días. El estudio evaluó los niveles séricos de testosterona (T), hormona foliculoestimulante (FSH), hormona luteinizante (LH), citocinas proinflamatorias (IL-6, TNF-$\alpha$, IL-1$\beta$), indicadores de estrés oxidativo (superóxido dismutasa, catalasa, glutatión peroxidasa y óxido nítrico) y marcadores apoptóticos (Bcl-2, p53, caspasa-3 y Bax). La administración de ND elevó significativamente los niveles séricos de T, las citocinas proinflamatorias y la expresión de genes proapoptóticos, a la vez que redujo los niveles de LH y FSH, así como la viabilidad, el recuento y la motilidad espermática. En contraste, el extracto de *O. tenuiflorum* normalizó notablemente los niveles séricos de LH, FSH y T, restauró la actividad de las enzimas antioxidantes y redujo las citocinas proinflamatorias. Además, inhibió la apoptosis de las células germinales mediante la regulación negativa de p53, caspasa-3 y Bax, y la regulación positiva de Bcl-2. Estos hallazgos sugieren que el extracto de Ocimum tenuiflorum protege eficazmente la estructura y la función testicular de la toxicidad inducida por la nandrolona gracias a sus propiedades antioxidantes y antiinflamatorias.

**PALABRAS CLAVE: Apoptosis; Fertilidad masculina; Nandrolona; *Ocimum tenuiflorum L.*; Parámetros espermáticos.**

## REFERENCES

Abedini Bajgiran, F.; Khazaei Koohpar, Z. & Salehzadeh, A. Effects of N-acetylcysteine supplementation on oxidative stress and expression of apoptosis-related genes in testicular tissue of rats exposed to lead. *Biol. Trace Elem. Res., 201(5)*:2407-15, 2023.

Aboutalebi, H.; Alipour, F. & Ebrahimzadeh-Bideskan, A. Preventive and therapeutic effects of Nasturtium officinale hydroalcoholic extract on cyclophosphamide-induced testicular toxicity in rats. *Avicenna J. Phytomed., 15(3)*:1091-101, 2025.

Akbaribazm, M.; Khordad, E. & Rahimi, M. A review on male infertility and herbal medicine: Complementary and alternative therapies in animal models. *OBM Genet., 8(1)*:221, 2024.

Alves, F. L.; Oliveira, M. A. F.; de Morais, A. N. P.; Martins, S. D.; de Sá, N. A. R.; Ceccatto, V. M.; Watanabe, Y. F. & Araújo, V. R. Supraphysiological doses of nandrolone decanoate disrupt spermatogenesis but did not interfere on embryo rate. *Naunyn-Schmiedeberg's Arch. Pharmacol., 397(6)*:4025-34, 2024.

Bhattarai, K.; Bhattarai, R.; Pandey, R. D.; Paudel, B. & Bhattarai, H. D. A comprehensive review of the phytochemical constituents and bioactivities of Ocimum tenuiflorum. *ScientificWorldJournal, 2024*:8895039, 2024.

Bond, P.; Smit, D. L. & de Ronde, W. Anabolic-androgenic steroids: how do they work and what are the risks? *Front. Endocrinol. (Lausanne), 13*:1059473, 2022.

Changizi, V.; Azadbakht, O.; Ghanavati, R.; Behrouj, H.; Motevaseli, E. & Khanzadeh, P. Effect of Lactobacillus species on apoptosis-related genes BCL2, BAX, and caspase 3 in the testes of gamma-irradiated rats. *Rev. Assoc. Med. Bras. (1992), 67(11)*:1581-5, 2021.

Cooper, T. G.; Noonan, E.; Von Eckardstein, S.; Auger, J.; Baker, H. G.; Behre, H. M.; Haugen, T. B.; Kruger, T.; Wang, C.; Mbizvo, M. T.; *et al*. World Health Organization reference values for human semen characteristics. *Hum. Reprod. Update, 16(3)*:231-45, 2010.

Fariello, R. M.; de Carvalho, R. C.; Spaine, D. M.; Andretta, R. R.; Caetano Jr., E. M.; Sá, G. P.; Cedenho, A. P. & Fraietta, R. Analysis of the functional aspects of sperm and testicular oxidative stress in individuals undergoing metabolic surgery. Obes. Surg., 31(7):2887-95, 2021.

Gualtieri, R.; Kalthur, G.; Barbato, V.; Longobardi, S.; Di Rella, F.; Adiga, S. K. & Talevi, R. Sperm oxidative stress during in vitro manipulation and its effects on sperm function and embryo development. *Antioxidants (Basel), 10(7)*:1025, 2021.

Kavak, V.; Balsak, D.; Togrul, C.; Deveci, E.; Ekinci, A.; Ekinci, C. & Babacan, F. The effects of maternal low protein and estrogen on sexual behavior and testicular maturation in male rats. *Int. J. Morphol., 33(1)*:204-9, 2015.

Liu, H.; Wang, R.; OuYang, H.; Wang, Y.; Wu, J.; Li, M.; Hu, Y.; Yao, Y.; Liu, Y. & Ji, Y. Cadmium induced mouse spermatogonia apoptosis via mitochondrial calcium overload mediated by IP3R-MCU signal pathway. *Toxicology, 486*:153448, 2023.

Liu, Q.; Si, T.; Xu, X.; Liang, F.; Wang, L. & Pan, S. Electromagnetic radiation at 900 MHz induces sperm apoptosis through bcl-2, bax and caspase-3 signaling pathways in rats. *Reprod. Health, 12*:65, 2015.

Mannucci, A.; Argento, F. R.; Fini, E.; Coccia, M. E.; Taddei, N.; Becatti, M. & Fiorillo, C. The impact of oxidative stress in male infertility. *Front. Mol. Biosci., 8*:799294, 2022.

Mohamed, H. M. & Mohamed, M. A. H. Effect of different doses of nandrolone decanoate on lipid peroxidation, DNA fragmentation, sperm abnormality and histopathology of testes of male Wistar rats. *Exp. Toxicol. Pathol., 67(1)*:1-11, 2015.

Monageng, E.; Offor, U.; Takalani, N. B.; Mohlala, K. & Opuwari, C. S. A review on the impact of oxidative stress and medicinal plants on Leydig cells. *Antioxidants (Basel), 12(8)*:1559, 2023.

Muratori, M. & Baldi, E. Effects of FSH on sperm DNA fragmentation: review of clinical studies and possible mechanisms of action. *Front. Endocrinol., 9*:734, 2018.

Ni, F. D.; Hao, S. L. & Yang, W. X. Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis. *Cell Death Dis., 10(8)*:541, 2019.

Patanè, F. G.; Liberto, A.; Maria Maglitto, A. N.; Malandrino, P.; Esposito, M.; Amico, F.; Cocimano, G.; Rosi, G. L.; Condorelli, D.; Nunno, N. D.; *et al*. Nandrolone decanoate: use, abuse and side effects. *Medicina (Kaunas), 56(11)*:606, 2020.

Rad, I.; Saberi, A.; Koochakzadeh-Nematollahi, N. S.; Habibzadeh, V.; Salarkia, E.; Amanollahi, S. & Nikpour, S. The effects of folic acid on testicular histology, sperm quality, and spermatogenesis indices following 3, 4-methylenedioxymethamphetamine exposure in adult male rats. *Addict. Health, 13(1)*:36-44, 2021.

Santos, L. C. D. S.; Lapa Neto, C. J. C.; Santos, A. M. G. D.; Marinho, K. S. D. N.; Nascimento, B. J. D.; Alves, E. R.; Teixeir, Á. A. C. & Wanderley-Teixeira, V. Immunohistochemical and histophysiological study of prolonged use of nandrolone on reproductive organs and fertility. *Biotechnic Histochem., 96(6)*:468-86, 2021.

Samie, A.; Sedaghat, R.; Baluchnejadmojarad, T. & Roghani, M. Hesperetin, a citrus flavonoid, attenuates testicular damage in diabetic rats via inhibition of oxidative stress, inflammation, and apoptosis. *Life Sci., 210*:132-9, 2018.

Sharma, A. D.; Kaur, I.; Angish, S.; Thakur, A.; Sania, S. & Singh, A. Comparative phytochemistry, antioxidant, antidiabetic, and anti-inflammatory activities of traditionally used Ocimum basilicum L. Ocimum gratissimum L., and *Ocimum tenuiflorum L. Biotechnologia (Pozn.), 103(2)*:131-42, 2022.

Shi, H.; Li, Q. Y.; Li, H.; Wang, H. Y.; Fan, C. X.; Dong, Q. Y.; Pan, B. C.; Ji, Z. L. & Li, J. Y. ROS-induced oxidative stress is a major contributor to sperm cryoinjury. *Hum. Reprod., 39(2)*:310-25, 2024.

Shirpoor, A. & Naderi, R. Nandrolone decanoate induced kidney injury through miRNA-146a targeting IRAK1 and TRAF6 via activation of the NF-kB pathway: The effect of moderate exercise. *Steroids, 211*:109503, 2024.

Sretenovic, J.; Joksimovic Jovic, J.; Srejovic, I.; Zivkovic, V.; Mihajlovic, K.; Labudovic-Borovic, M.; Trifunovic, S.; Milosevic, V.; Lazic, D.; Bolevich, S.; *et al*. Morphometric analysis and redox state of the testicles in nandrolone decanoate and swimming treated adult male rats. *Basic Clin. Androl., 31(1)*:17, 2021.

Sundaresan, A.; Radhiga, T. & Pugalendi, K. V. Ursolic acid and rosiglitazone combination prevent low-grade inflammation and hepatic insulin resistance by modulating cytokine expression in mice fed a high-fat diet. *Int. J. Clin. Med. Res., 1(3)*:108-15, 2023.

Vasisth, R.; Gurao, A.; Kumari, N.; Kumar, G.; Kumar, A.; Sriranga, K. R.; Dige, M. S.; Mukesh, M.; Aggarwal, R. A. K.; Singh, P.; *et al*. Development and validation of most efficient RNA isolation method from buffalo bull spermatozoa. *Mol. Biol. Rep., 50(8)*:6717-27, 2023.

Villarraza, J.; Antuña, S.; Tardivo, M. B.; Rodríguez, M. C.; Díaz, P. U.; Notaro, U. S.; Ortega, H. H.; Prieto, C. & Ceaglio, N. Development of a biotechnology process for the production of a novel hyperglycosylated long-acting recombinant bovine follicle-stimulating hormone. *Biotechnol. J., 19(6)*:2400260, 2024.

Wang, Y.; Fu, X. & Li, H. Mechanisms of oxidative stress-induced sperm dysfunction. *Front. Endocrinol. (Lausanne), 16*:1520835, 2025.

Wang, Y.; Bai, L.; Li, H.; Yang, W. & Li, M. Protective effects of Lepidium draba L. leaves extract on testis histopathology, oxidative stress indicators, serum reproductive hormones and inflammatory signalling in oxymetholone-treated rat. *Andrologia, 53(11)*:e14239, 2021.

Yuniarti, W. M.; Krismaharani, N.; Ciptaningsih, P.; Celia, K.; Veteriananta, K. D.; Ma'ruf, A. & Lukiswanto, B. S. The protective effect of Ocimum sanctum leaf extract against lead acetate-induced nephrotoxicity and hepatotoxicity in mice (*Mus musculus*). *Vet. World, 14(1)*:250-8, 2021.

Zhang, X.; Tang, Y.; Lu, G. & Gu, J. Pharmacological activity of flavonoid quercetin and its therapeutic potential in testicular injury. *Nutrients, 15(9)*:2231, 2023.

Corresponding author:
Ruirui Huangfu
Department of Pharmacy
Xi'an Daxing Hospital
Xi'an
Shaanxi Province, 710000
CHINA

E-mail: huangfu20251@outlook.com

https://orcid.org/0009-0008-0138-7143

.