Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

IJBMS

Mechanistic insights into the protective effects of vitamin E against olanzapine-induced testicular toxicity: The role of microRNAs in oxidative stress and apoptosis

Afraa Habeeb Qayssar [1], Ali Louei Monfared [1]*

[1] Department of Histology, Faculty of Veterinary Sciences, Ilam University, Ilam, Iran

ARTICLE INFO

Article type:
Original

Article history:
Received: Sep 8, 2025
Accepted: Apr 28, 2026

Keywords:
Histology
MicroRNA modulatory
Olanzapine
Rat
Vitamin E

ABSTRACT

Objective(s): Research on the male gonadotoxic effects of olanzapine (OLZ) and the potential protective role of vitamin E (Vit E) remains limited. This investigation aimed to assess the toxic impact of OLZ on the testes' integrity and the protective role of dietary Vit E.

Materials and Methods: In this experimental study, twenty-eight rats were divided into four groups: a control group, a group administered 5 mg/kg of OLZ orally, a group receiving both OLZ and 100 mg/kg of Vit E, and a group treated with Vit E alone. We assessed testes dimensions, structural alterations, serum concentrations of testosterone and prolactin, sperm parameters, oxidative stress, and apoptosis. Additionally, the expression levels of microRNA (miR)-122 and miR-202-5p were quantified.

Results: Administration of OLZ resulted in significant structural damages to the testes characterized by apoptosis, cellular stress, and disruption of miR-122 and miR-202-5p expression. Otherwise, Vit E restores the histological structure, apoptosis, and sperm quality affected by OLZ. Notably, hormonal profiles and indices of cellular stress showed significant improvement in rats receiving a combination of Vit E and OLZ. Furthermore, the expressions of miR-122 and miR-202-5p were normalized in the OLZ+Vit E treated group.

Conclusion: This study highlights the protective role of dietary Vit E against OLZ-induced testicular toxicity by enhancing testicular histo-architecture, reducing stress markers, and modulating of microRNA expressions.

►Please cite this article as:
Habeeb Qayssar A, Louei Monfared A. Mechanistic insights into the protective effects of vitamin E against olanzapine-induced testicular toxicity: The role of microRNAs in oxidative stress and apoptosis. Iran J Basic Med Sci 2026; 29: 883-892. doi: https://dx.doi.org/10.22038/ijbms.2026.91101.19649

Introduction

Olanzapine (OLZ) is an atypical antipsychotic medicine, used with high efficacy in the treatment of mental illnesses such as schizophrenia, bipolar disorder and other mood conditions (1, 2). However, the adverse effects and toxicity of atypical antipsychotic agents have proven in the past studies. For example, consistent evidence demonstrates that the use of OLZ in adults is associated with metabolic laboratory abnormalities like increased weight gain, risk of diabetes mellitus, dyslipidemia, and testicular damages (3, 4). Furthermore, in recent years concerns have been raised worldwide over the potential for OLZ to cause male sexual dysfunction (4-6). Antipsychotic medications can directly impact hormonal regulation or indirectly interfere with sexual function, spermatogenesis, and the maturation of sperm in the epididymis, leading to reproductive toxicity (6). OLZ is a famous contributor to male reproductive dysfunction by inducing oxidative stress, distorted histoarchitecture, and spermatogenic damage in rodent models. Notably, recent studies demonstrated that co-administration of zinc as well as umbelliferone effectively mitigated these detrimental effects (7, 8).

Vitamin E (Vit E) refers to a collective term encompassing a group of compounds that can be categorized into two primary subclasses: tocopherols and tocotrienols (9). Among these, alpha-tocopherol stands out as the most prevalent and biologically active form of Vit E. Due to its regulatory functions in numerous signaling pathways, alpha-tocopherol is recognized as a protective agent against various male reproductive challenges, including exposure to deltamethrin, valproic acid, and nicotine (10-12). Furthermore, Vit E promotes sperm production in sheep mainly due to its antioxidant properties, while also influencing non-antioxidant genes. A study identified 115 differentially expressed proteins in prepubertal ovine testicular cells treated with Vit E, revealing significant enrichment in biological processes related to membrane and enzyme activity, ultimately supporting improved spermatogenesis (13). Another study found that Vit E mitigates mancozeb-induced testis damage in first-generation male pups reversing adverse effects on sperm parameters and testis structure (14).

Micro-ribonucleic acids (miRNAs) are endogenous short non-coding RNAs that play vital roles in many biological

**Corresponding author:* Ali Louei Monfared. Department of Histology, Faculty of Veterinary Sciences, Ilam University, Ilam, Iran. Tel/ Fax: +98-8432224308, Email: a.loueimonfared@ilam.ac.ir

© 2026. This work is openly licensed via CC BY 4.0.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

functions and disease development. Abnormal expression of miRNAs has been linked to various illnesses such as cancers (15) and male infertility including idiopathic male infertility and non-obstructive azoospermia (16). Furthermore, several studies have demonstrated the essential role of miRNAs in regulating mammalian reproductive functions. MiR-202-5p is gonad-specific miRNA that is abundantly expressed across different species. For example, in mouse and bull, miR-202 is specifically expressed in Sertoli cells and at varying levels, in different developmental stages of germ cells (17, 18). Furthermore, miR-202 regulates spermatogonial differentiation and meiotic initiation by controlling key gene expressions, preventing early differentiation and supporting fertility in mice (17). Given the established role of miR-122 in regulating steroidogenesis and its hormonal modulation in testicular cells, it is crucial to investigate its specific functions and targets within the testis (19). This could enhance our understanding of male reproductive health and the molecular mechanisms underlying fertility. Furthermore, it has been demonstrated that miR-122-5p regulates tight junction integrity in Sertoli cells by negatively modulating occludin expression through the Sp1-miR-122-5p-occludin axis, impacting spermatogenic epithelium function (20). A critical review of the literature reveals a complete absence of data on the involvement of miR-122 and miR-202-5p in antipsychotic-induced testicular changes and also on the ability of Vit E to modulate these events. Therefore, the study of possible function of special miRNA in the toxicity associated with OLZ, as well as the protective effects of Vit E, holds significant promise. Addressing these gaps could enhance our understanding of the molecular mechanisms underlying antipsychotic-related reproductive dysfunction and help identify new biomarker targets. We hypothesized that dietary Vit E supplementation protects against OLZ-induced testicular toxicity and that this protection involves the amelioration of oxidative stress and the correction of OLZ-induced dysregulation of miR-122 and miR-202-5p. Thus, the current study was conducted to elucidate the potential protective role of Vit E supplementation against OLZ-induced reproductive toxicity in male rats and to clarify the related changes in the expression of miR-122 and miR-202-5p as a new potential mechanism.

Materials and Methods

Reagents

Vit E (97% pure α-Tocopherol acetate) was sourced from Sigma-Aldrich Company (USA). The antipsychotic medication OLZ (Zyprexa® powder for solution, Lilly USA, LLC, Indianapolis, USA) was administered daily via oral gavage between 07:00 and 09:00. Prior to administration, Vit E was dissolved in corn oil (21). OLZ was delivered at a dosage of 1 mL per 100 g body weight, prepared by dissolving the powder in distilled water (2021)(6).

Experimental animals

The study was conducted at Ilam University (Ilam, Iran). The animal ethics committee of the Ilam University approved the entire experimental work (IR.ILAM.REC.1402.016). Twenty-eight male Wistar rats weighing 210-220 grams and aged between 8 and 9 weeks were obtained from the animal house at the college of veterinary medicine sciences, Ilam (Ilam, Iran). All animals were kept in a standard room and maintained under standard laboratory condition (12:12 hr light/dark cycle and temperature 28-29 °C) and had free access to food and water. Following a one-week acclimatization period, the rats were randomly assigned to four experimental groups (N=7) as detailed below:

Group 1 (Control): This group served as the control and received normal saline solution orally for a duration of 60 consecutive days. Group 2 (OLZ): Animals in this group were OLZ at a dosage of 5 mg/kg orally. Group 3 (OLZ + Vit E): Rats in this group received a combination treatment of OLZ (5 mg/kg orally) and Vit E at a dosage of 100 mg/kg body weight. Group 4 (Vit E): Animals received Vit E alone using the same procedure. The doses of the OLZ and Vit E were selected according to previous research (4, 12).

Histology

At the end of the experimental period, all animals underwent a fasting procedure for approximately 18 hr prior to anesthesia induction. The rats were then euthanized under general anesthesia, achieved through intramuscular injection of a ketamine-xylazine combination (90 mg/kg and 9 mg/kg, respectively), (Alfasan Chemical Co., Woerden-Netherlands). The testes were excised and cleared of adherent connective tissue. The specimens were then fixed in buffered formalin for a period of 48 hr. Following fixation, tissues were processed using a standard histological protocol. Tissues were then dehydrated through a graded ethanol series and cleared in xylene. The samples were embedded in paraffin blocks, and the paraffin-embedded tissues were sectioned to a thickness of five microns using a rotary microtome. Serial sections were prepared and stained according to the Hematoxylin and Eosin (H&E) staining procedure. Finally, the stained slides were compared between various groups and captured using an optical microscope (Leica DM 750, Switzerland).

Tissue micrometry

To evaluate the average height of the germinal epithelium and the diameters of the seminiferous tubules; ten randomly selected round-shaped seminiferous tubules were examined at a magnification of ×10 in a single cross-section, following the methodologies outlined by Cheraghi Abajlou *et al.* (2025)(22) and Erfani Majd *et al.* (2021)(23). Additionally, the interstitial tissue area was determined by subtracting the area occupied by the seminiferous tubules from the total area of the microscopic fields (24). Leydig cells were also counted in 20 randomly selected inter-tubular spaces following the methodology established by Ujah *et al.* (2021) (25). All measurements were conducted using a light microscope (Olympus Optical Co., Japan) in conjunction with digital camera and image analysis software.

Immunofluorescence assay

In order to determine the expressions of apoptosis-related proteins, immunohistological analysis was performed. Paraffin-embedded sections were deparaffinized with xylene and alcohol. Then, the antigen unmasking of tissue sections was performed by sodium citrate buffer (pH 6.0) at 95-100 °C for 20 min. Sections were then blocked with 5% normal goat serum in phosphate-buffered saline (PBS) for 1 hour at room temperature. The sections were incubated overnight (4 °C) with mouse anti-bax (1:100; Cat Number # sc-7480; Santa Cruz Biotechnology), anti-caspase-3(1:100; Cat Number # sc-56053; Santa Cruz Biotechnology)

and anti-bcl2 (1:100; Cat Number #sc-7382; Santa Cruz Biotechnology) antibodies. The manufacturer validated the assay for application in rat tissues using western blot. Then, the slides were incubated for two hours in the dark at room temperature with a secondary antibody (Goat anti mouse IgG, 1:500; Alexa Fluor® 488 Conjugate, #89853). To demonstrate histological localization, the cell nuclei were counterstained with the nuclear counterstain DAPI (DAPI: 4'-6-Diamidino-2-phenylindole; 1 µg/ml for 5 min). PBS was used for rinsing between steps. As negative control; the tissue sections incubated with PBS instead of the primary antibody showed no specific immunoreactivity. As positive control; staining was initially optimized and confirmed for each antibody using rat spleen tissue sections known to express the target protein. Fluorescence was visualized in the five different areas of each stained tissue section with a fluorescence microscope (Olympus BX50) connected to a digital camera (Olympus DP72). Cell nuclei were counterstained with DAPI in blue color and in the case of bax, caspase-3 and bcl-2 expression; the green coloration of cell was recorded. In each sample the mean fluorescent area intensity of apoptosis-related proteins was measured using Microbin 5 software.

Serum testosterone measurement

Blood samples were promptly collected via cardiac puncture to obtain serum for subsequent biochemical analyses. Serum testosterone levels were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Chemicon, Temecula, California) following the manufacturer's instructions and the method certified by Li *et al.* (2018)(26) and Ma *et al.* (2019)(27).

Serum prolactin measurement

Serum prolactin (PRL) levels were quantified using an ELISA method as described previously (28).

Oxidative stress markers

For the assessment of oxidative stress biomarkers, 50 mg samples of testis tissue were homogenized in lysis buffer. The homogenates were centrifuged at 15,000 × g for 10 min. The resulting supernatant was utilized for the determination of glutathione peroxidase (GPx) activity and malondialdehyde (MDA) thiobarbituric acid reactive substances (TBARS) levels. In the obtained supernatants, total protein level was determined using the Bradford method to normalize GPx activity and TBARS levels. The quantification of GPx was conducted following the methodology described in previous study (29). The assay conjugates GPx-mediated reduction of tert-butyl hydroperoxide to the oxidation of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) by glutathione reductase. The decrease in absorbance at 340 nm due to NADPH consumption was recorded spectrophotometrically for three minutes. For TBARS-MDA quantification, 100 µL of the supernatant was combined with an equal volume of perchloric acid. The supernatant was then retrieved, and 300 µL of thiobarbituric acid -was added. The mixture was incubated at 100 °C for 60 min. Finally, the absorbance was measured at 532 nm using a spectrophotometer (30).

Testicular index

Rats were weighed before and after the study's completion. The testes were surgically removed and weighed and the relative weight ratio of the testes (both right and left) was calculated by using the formula established by Erfani Majd *et al.* (2021) (23) as: Gonadosomatic index=(testes weight in grams/body weight in grams)×100. Additionally, the relative weight of the epididymis was assessed. The measurements of length and width for both testes were also recorded (23).

Sperm examination procedures

To determine sperm concentration, sperm samples from epididymis were taken according to standardized methods adapted for rodents. The samples were diluted to a final volume of 500 µl using a 10% formalin solution in PBS. A total of 10 µl aliquot of the diluted sample was placed on a haemocytometer and allowed to settle for seven minutes. The number of spermatozoa within 250 small squares of the haemocytometer was then counted (31). For assessment of sperm motility, a drop of fresh semen was placed on a glass slide maintained at 37 °C. Two drops of warm 0.9% sodium chloride solution were added and a cover slip was applied immediately. The sample was examined under high magnification (400×) to assess both individual motility and progressive motility percentages (31). To assess sperm vitality, 40 µl of liquefied semen was mixed thoroughly with 10 µl of eosin Y (1% solution). A drop of this mixture was then placed on a clean slide for microscopic examination. A minimum of 200 spermatozoa were analyzed against a nigrosin background; those staining pink or red were classified as dead, while those remaining unstained were deemed viable. The percentage of viable sperm was calculated accordingly. Additionally, the proportion of morphologically abnormal sperm characterized by defects in the head and tail was also evaluated (31).

Real-time PCR

Real-time PCR was carried out to examine the tissue expression of miR-122 and miR-202-5p in the different groups. Total RNA was extracted from testis tissues using Tripure Isolation Reagent, and its optical densities at 260 nm and 280 nm were measured to determine the concentration and purity. First-stranded complementary DNA (cDNA) was synthesized using stem-loop reverse transcription primers provided in the kit and miRcute miRNA First-strand cDNA Synthesis Kit (Tiangen Biotech Co., Beijing, China; Cat. No. KR211). Real-time PCR was used to evaluate miR-122 and miR-202-5p expressions using the 2X SYBR Green Real-Time PCR kit (Pars Tous, Iran) and Lava 96 Real-time PCR Detection System (DaAnGene Co Ltd). Primer design was done using sRNAPrimerDB software. Then, quantitative PCR was performed using miRNA-specific forward primers and a universal reverse primer. The reverse transcription cycling conditions were 42 °C for 60 min, followed by 70 °C for 10 min to inactivate the enzyme. U6 was amplified using conventional primers and applied as the endogenous control. The expected amplicon sizes were approximately 75 bp for miR-122, 72 bp for miR-202-5p, and 85 bp for U6. The primer sequences were as follows:

miR122-Rat-Forward: GGAGTGTGACAATGGTGTTTG, (Accession No: NR_031864.1)

miR202-Rat-Forward: CATATACTTCTTTGTGGAT, (Accession No: NR_032736.1)

U6-Forward: TGCTTCGGCAGCACATATAC, (Accession No: K00784.1)

U6-Reverse: AGGGGCCATGCTAATCTTCT, (Accession

No: K00784.1)
Universal Reverse Primer: GAACATGTCTGCGTATCTC, (Accession No: K00784.1).

The amplification efficiencies for examined microRNAs and U6 primers were determined by using standard curves and were confirmed to be between 90% and 110%. A melting curve analysis was performed at the end of each run (from 65 ˚C to 95 ˚C) to confirm the specificity of amplification and the absence of primer-dimers. The number of reaction cycles for each gene was considered 50 cycles. The reaction steps were as follows: initial denaturation for 5 min at 94 ˚C, denaturation for 15 sec at 94 ˚C, primer connection for 15 sec at 60 ˚C, and prolongation for 30 sec at 72 ˚C. The reaction was performed on the samples repeating two times for each sample and for each gene, and the average cycle threshold (Ct) values of different dilutions were calculated in two repetitions. The results were evaluated using the ΔΔCt comparative method.

Statistical analysis

The selective miRs were considered up- or down-regulated if the *P*-value was <0.05 and logarithmic fold change ($\log2FC \geq 1$) for up-regulated or $\log2FC \leq -1$ for down-regulated. Statistical significance between vehicle control and each of the treatment groups was calculated using Statistical Package for Social Sciences version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to determine the normality of variables. The homogeneity of variances was verified using Levene's test. For multiple comparisons, one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was applied. Data are presented as mean±95% confidence interval (CI). Significance was set at $P<0.05$.

Results

Vit E ameliorates histological and micrometric features in the testis of rats induced by OLZ

The control group and the rats treated with Vit E exhibited normal seminiferous tubules filled with numerous germ cells and a clearly defined interstitial area. In contrast, the OLZ group showed signs of testicular atrophy and irregularities in the seminiferous tubules, along with the detachment of the spermatogenic epithelium. Also, the interstitial areas were significantly enlarged, characterized by hyalinized interstitial tissue. Conversely, in the OLZ+Vit E group, there was a noticeable preservation of both the seminiferous tubules and interstitial areas compared to the OLZ-only group (Figure 1). OLZ administration significantly reduced epithelial height, tubular diameter, and Leydig cell number. However, co administration of Vit E and OLZ significantly mitigated these changes (Table 1). The micrometric measurements in both the control and Vit E groups were comparable.

Vit E reduces OLZ-induced expression of apoptotic and anti-apoptotic proteins in testes

The control and OLZ+Vit E groups showed decreased immunoreactivity for bax and caspase-3 within the testicular architecture. In contrast, immunofluorescence analysis revealed that apoptosis was present in the testicular cells of the OLZ-treated group. Importantly, the levels of bax and caspase-3 were significantly elevated in the OLZ group in comparison to both the control and Vit E groups. In contrast, the combination of OLZ and Vit E led to a significant decrease in these levels when assessed against the OLZ group alone. Thus, Vit E supplementation significantly reduced apoptosis compared to the OLZ group. Furthermore, a significant down-regulation of bcl-2 protein was seen in the testes of rats treated with OLZ. Conversely, an increase in bcl-2 immunoreactivity was observed in the testes of the OLZ+Vit E group compared to the OLZ-only group (Figures 2, 3, 4).

Vit E improves serum testosterone and prolactin levels in OLZ-treated rats

In the OLZ group, there was a marked increase in serum prolactin level and a significant decrease in serum testosterone levels compared to the control group. Conversely, the combined treatment of OLZ and Vit E resulted in a significant increase in serum testosterone levels and a significant decrease in serum prolactin levels when assessed against the OLZ-only group ($P<0.05$)(Figure 5).

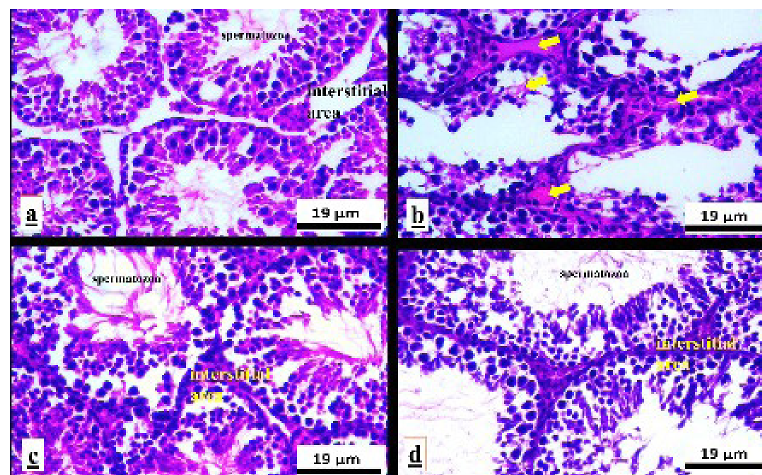

Figure 1. The protective effects of vitamin E on testicular histology of olanzapine-treated rats for 60 days (n=7)
Control group (a) shows the regular testicular seminiferous tubules alongside a well defined interstitial area (IA) and Leydig cells. Olanzapine group (b) depicts deformity and distortion of the seminiferous tubules accompanied by hyalinized interstitial tissue (yellow arrows) with apoptotic Leydig cells. The seminiferous tubules were irregular in shape and contained few numbers of spermatozoa (SP). Olanzapine+vitamin E group (c) demonstrated that the testicular tissue was restored to normal. It was also formed of regular seminiferous tubules filled with mature sperms separated by normal interstitial area. Vitamin E group (d) showed the normal architecture of the testis. (H&E, 400×), scale bar=19 μm

Vit E attenuates the oxidative stress markers of rats induced by OLZ

Significant differences in oxidative stress markers were observed among groups. The administration of OLZ led to increased levels of MDA and a reduction in GPx activity in the testicular tissues compared to the control group. In contrast, dietary supplementation with Vit E was significantly decreases the MDA levels and enhances the GPx activity in rats that underwent OLZ treatment (Figure 5).

Table 1. Effect of vitamin E treatment on micrometric indices of testes, the relative testes weight, relative epididymis weight, and sperm parameters in olanzapine caused testicular alterations in rats

Parameters	Control Mean (95% CI)	OLZ Mean (95% CI)	OLZ+Vit E Mean (95% CI)	Vit E Mean (95% CI)
Epithelial height (μm)	92.29 (87.92 to 96.65)	36.84 (33.67 to 40.02) a	87 (84.28 to 89.72) b	88 (81.64 to 94.36) b
Tubular diameter (μm)	307.7 (296.4 to 319.1)	116.3 (106.5 to 126.0) a	264.4 (240.1 to 288.8) a,b	315.3 (312.6 to 351.1) c
Leydig cell count (×10^3 mm area of the testis)	233 (229.7 to 236.3)	110.7 (109.7 to 111.7) a	225.6 (221.5 to 229.6) a,b	229 (226.7 to 231.3) b
Interstitial tissue area (%)	10.86 (9.868 to 11.85)	10.29 (9.257 to 11.31) ns	10.43 (9.526 to 11.33) ns	11.14 (9.388 to 12.95) ns
Relative weight of right testis (%)	1.8 (1.64 to 1.96)	0.67 (0.53 to 0.8) a	1.54 (1.299 to 1.787) b	1.72 (1.535 to 1.905) b
Relative weight of left testis (%)	1.64 (1.512 to 1.782)	0.63 (0.544 to 0.733) a	1.5 (1.22 to 1.78) b	1.62 (1.52 to 1.73) b
Relative weight of right epididymis (%)	0.37 (0.328 to 0.412) ns	0.33 (0.275 to 0.396) ns	0.29 (0.24 to 0.347) ns	0.35 (0.304 to 0.406) ns
Relative weight of left epididymis (%)	0.38 (0.311 to 0.456) ns	0.33 (0.262 to 0.403) ns	0.3 (0.251 to 0.348) ns	0.34 (0.293 to 0.403) ns
Length of right testis (mm)	21.56 (19.77 to 23.35)	17.3 (16.46 to 18.25) a	22.3 (21.11 to 23.66) b	22.27 (21.24 to 23.31) b
Length of left testis (mm)	19.97 (18.76 to 21.18)	16.6 (15.61 to 17.70) a	20.51 (19.01 to 22.02) b	21.9 (20.45 to 23.46) b
Width of right testis (mm)	9.34 (8.77 to 9.91)	5.07 (4.56 to 5.58) a	8.14 (7.72 to 8.56) a,b	8.6 (7.9 to 9.35) b
Width of left testis (mm)	9.2 (8.64 to 9.78)	4.9 (4.45 to 5.4) a	8.08 (7.68 to 8.48) a,b	8.4 (7.63 to 9.16) b
Sperm count (×10^6 ml)	115.8 (113. 4 to 118.2)	57.57 (51.87 to 63.27) a	71.67 (62.75 to 80.59) a,b	111.9 (109 to 114.8) b,c
Live sperm (%)	83.07 (80.5 to 85.6) ns	79.57 (71.3 to 87.8) ns	74.43 (68 to 80.8) ns	78.6 (76.4 to 80.8) ns
Waving sperm motility (%)	87.86 (83.74 to 91.97)	54.57 (47.8 to 61.34) a	76.99 (70.95 to 83.02) a,b	84.4 (77.41 to 91.45) b
Progressive sperm motility (%)	91.57 (87.7 to 95.3)	7.28 (4.05 to 10.5) a	60.57 (55.2 to 65.8) a,b	91.4 (89.4 to 93.4) b,c
Non-progressive sperm motility (%)	4.57 (2.37 to 6.76)	43.8 (40.1 to 47.61) a	18.5 (9.62 to 27.5) a,b	7 (4.22 to 9.77)b,c
Abnormal sperm (%)	7 (3.04 to 11)	13.3 (11.31 to 15.26) a	6.7 (5.05 to 8.37) b	9.57 (4.31 to 14.8)

Results are expressed as mean (95% confidence interval: CI)(n=7). Different letters (a, b, c) within a row indicate statistically significant differences between groups (P<0.05, one-way ANOVA with Tukey's *post hoc* test). Shared letters represent no significant difference. ns:not significant

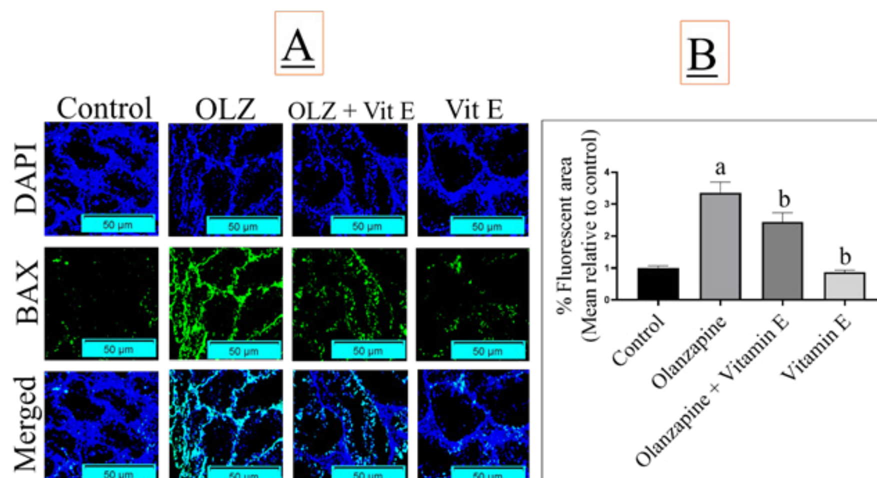

Figure 2. The protective effects of vitamin E on apoptotic proteins expression (A, B) depicted by immunofluorescence in the olanzapine-treated rats for 60 days
Results are expressed as Mean (95% confidence interval)(n=7). Different letters (a, b, c) indicate statistically significant differences between groups (P<0.05, one-way ANOVA with Tukey's *post hoc* test). (100 ×), scale bar=50 μm

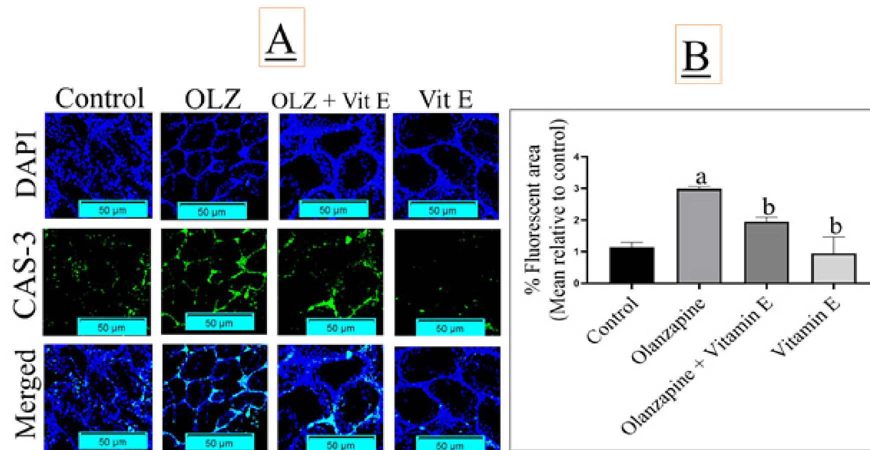

Figure 3. The protective effects of vitamin E on apoptotic proteins expression (A, B) depicted by immunofluorescence in the olanzapine-treated rats for 60 days
Results are expressed as Mean (95% confidence interval) (n=7). Different letters (a, b, c) indicate statistically significant differences between groups (P<0.05, one-way ANOVA with Tukey's *post hoc* test). (100 ×), scale bar=50 μm

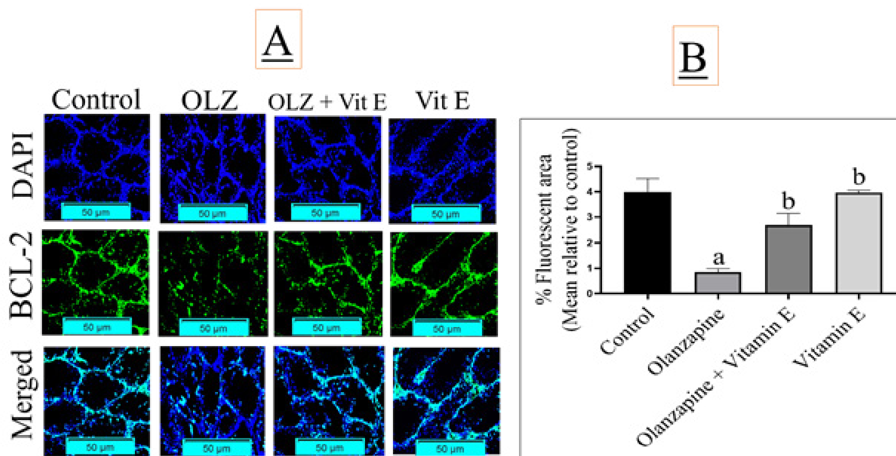

Figure 4. The protective effects of vitamin E on anti-apoptotic proteins expression (A, B) depicted by immunofluorescence in the olanzapine-treated rats for 60 days
Results are expressed as Mean (95% confidence interval)(n=7). Different letters (a, b, c) indicate statistically significant differences between groups (P<0.05, one-way ANOVA with Tukey's *post hoc* test). (100 ×), scale bar=50 μm

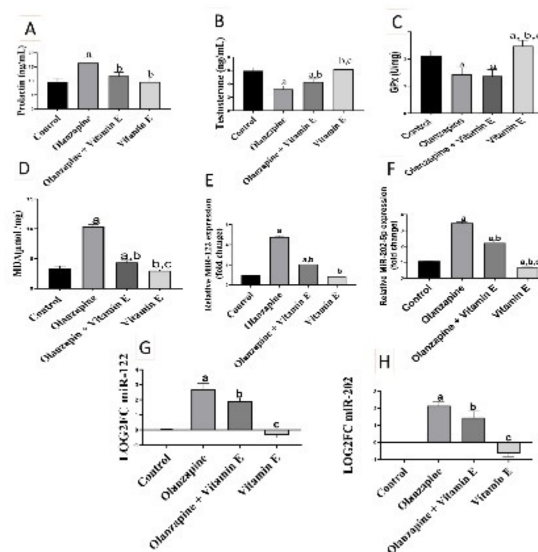

Figure 5. Effect of vitamin E on serum testosterone and prolactin hormones (A, B), oxidative stress markers (C, D), and testicular expression of mir-122 and mir-202-5p (E-H) of rats suffer from olanzapine-induced testicular dysfunction for 60 days (n=7)
Results are expressed as Mean (95% confidence interval) (n=7). Different letters (a, b, c) indicate statistically significant differences between groups (P<0.05, one-way ANOVA with Tukey's *post hoc* test)

Vit E attenuates testicular expression of miR-122 and miR-202-5p of rats induced by OLZ

In rats treated with OLZ, a significant increase in the levels of miR-122 and miR-202-5p was observed compared to the control group (>two-fold and ≥two-fold, *P*<0.05, respectively). In contrast, the administration of Vit E significantly (*P*<0.05) decreased the heightened expressions of miR-122 and miR-202-5p caused by OLZ (>one-fold and ≥one-fold, *P*<0.05, respectively). These results confirmed that the levels of miR-122 and miR-202-5p were significantly reduced after Vit E treatment (Figure 5).

Vit E ameliorates testicular weight and sperm quality of rats induced by OLZ

The relative weight of the testes and their dimensions were highest in the control and Vit E groups, while the OLZ group exhibited lower values. Although there was a numerical reduction in epididymis weight within the OLZ group, this change did not reach statistical significance. The combined administration of Vit E and OLZ resulted in a significant reduction in the relative weight, length, and width of both the right and left testes when compared to the OLZ group. Table 1 also presents a comparison of sperm parameters across the different groups. In the OLZ group, there was a significant decline in sperm count and motility relative to the control group, alongside an increase in the percentage of sperm with abnormal morphology (Figure 6). Conversely, the administration of Vit E in conjunction with OLZ led to a significant improvement in the sperm parameters compared to the OLZ group (Table 1, Figure 6).

Discussion

This study demonstrates the reproductive toxicity of the commonly prescribed antipsychotic OLZ and the protective efficacy of Vit E against its gonadotoxic effects. The histological examinations reveal that OLZ administration induces significant testicular degeneration concomitant with marked activation of the intrinsic apoptotic pathway as evidenced by upregulated bax and caspase-3 expression alongside significant bcl-2 suppression. Collectively, these alterations indicate OLZ-mediated initiation of apoptosis in germ cells, a finding consistent with previous report (4). The observed reproductive toxicity may be linked to OLZ's dopaminergic activity within germ cells, which has been shown to disrupt endocrine homeostasis and induce structural testicular abnormalities and impairing spermatogenesis (4). Vit E intervention effectively restored these pathological changes, suggesting its potential as an adjunct therapy for antipsychotic-induced reproductive dysfunction. The testicular degeneration evident in the OLZ-treated animals may be associated with a decline in plasma testosterone levels and an elevation in prolactin hormone. It has been noted that OLZ induced hyperprolactinemia likely by suppressing hypothalamic-pituitary-gonadal axis activity and reducing testosterone secretion (32). Otherwise, the anti-apoptotic effect of Vit E involves decreased expression of bax and caspase-3 and inversely increased expression of bcl-2 proteins in the rats' testis. These findings correlate with the previous reports regarding the potential protective effects of Vit E against different toxicants-induced chronic tissue injuries (10, 13, 33). The anti-apoptotic properties of Vit E, which are in accordance with its recognized role in maintaining tissue integrity (33), correspond with its documented protective effects against various toxic agents such as cadmium (34). This supports our findings regarding the reduction of OLZ-induced testicular injury.

In the current work the coadministration of Vit E mitigated OLZ-induced testicular toxicity through hormonal modulation, as evidenced by significant testosterone elevation and prolactin reduction. These endocrine improvements were correlated with preserved testicular histoarchitecture, demonstrating Vit E's multifaceted protective capacity. Our findings align with established literature documenting the efficacy of Vit E

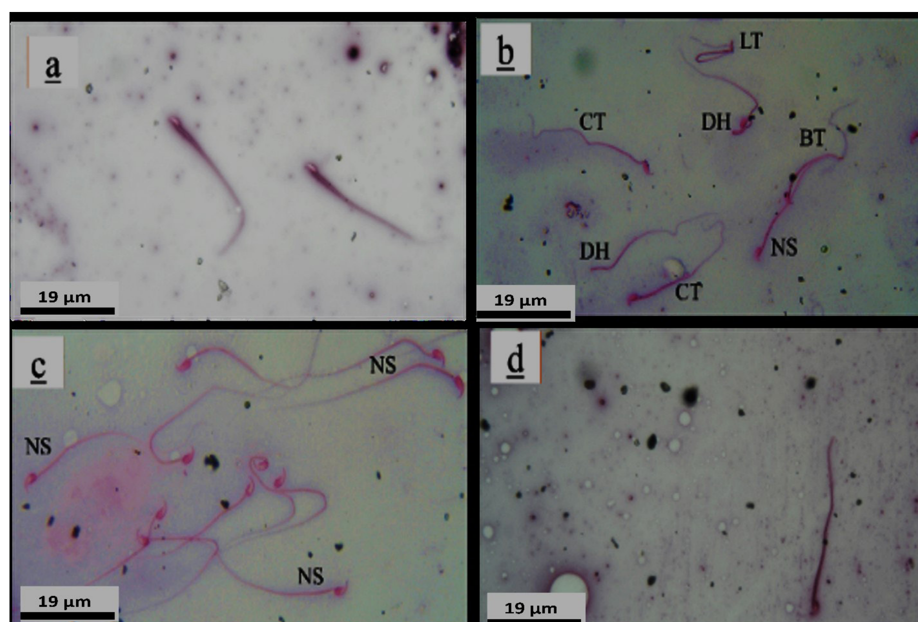

Figure 6. Effect of vitamin E on the morphological sperm features in olanzapine-induced dysfunction in rats
Control group (a) shows normal sperm morphology. Olanzapine group (b) depicts various sperm defects including curved tail (CT), detached head with curved tail (DH), looped tail (LT), and bent tail (BT). Olanzapine+vitamin E group (c) demonstrated a near complete restoration of sperm abnormalities to normal levels. Vitamin E group (d) showed the normal sperm morphology. (Eosin-Nigrosin staining, 1000 ×), scale bar=19 µm

in counteracting drug-induced testicular damage and testosterone production (35).

Biochemical analyses revealed hyperprolactinemia in OLZ-treated subjects, consistent with clinical observations of OLZ-induced prolactin elevation in psychiatric patients (32). The pathophysiological consequences of antipsychotic-mediated hyperprolactinemia are through dopamine D2 receptor blockade in the pituitary (32). Also, OLZ could disrupts the hypothalamic-pituitary-gonadal axis and suppressing pulsatile gonadotropin-releasing hormone secretion. This effect ultimately impairs gonadotropin releasing and hormone secretion that manifesting as testicular atrophy and immature germ cell abnormality (32, 36).

The present results provide experimental evidence for cellular stress caused by OLZ illustrated by increased MDA and reduced GPx levels. These findings are in accordance with a previous study (7). Otherwise, a significant increase in the tissue level of GPx along with a decline in the level of MDA was observed in the OLZ+Vit E group. These findings could be interpreted as the antioxidant effect of Vit E against OLZ toxicity. The current study aligns with recent published results that zinc counteracts OLZ-induced testicular toxicity by the principle of antioxidant intervention (7). Many other studies have also established these effects of Vit E in the case of various agent cytotoxicity (12, 37). Generally, the antioxidant capabilities of Vit E are significant (38, 39). Furthermore, the supplementation of Vit E has been shown to have a positive effect on spermatogenesis. Moreover, it enhances the presence of androgen receptors in the somatic cells of both Sertoli and Leydig cells, while also elevating the activity of superoxide dismutase within the testes (38).

Changes in organ weight have long been recognized as sensitive indicators of organ-specific injury. In studies focused on reproductive toxicity, the weights of the testes and epididymis are commonly assessed in male subjects (6). In our study, OLZ exposure resulted in a significant reduction in testicular weight. The probable cause of this reduced weight is the damage inflicted by OLZ which includes a significant decrease in sperm production as well as reducing epithelial height, tubular diameter, and Leydig cell numbers. Similar results were reported previously (4). The parameters of semen quality are examined in relation to male infertility, with abnormal semen analysis serving as a primary diagnostic criterion for infertility. Sperm morphology is recognized as a reliable indicator of spermatogenesis process. Furthermore, sperm exhibiting morphological defects may also indicate genetic damage in the germ cells (6). In the current study there was a significant decrease in the sperm profile in the OLZ group. These findings align with previous studies (4, 6). Additionally, sperm morphology was significantly impaired in the OLZ group which corroborates earlier study (6). In this study, the observed oxidative stress and elevated apoptosis may provide a plausible explanation for the compromised sperm quality. So that these conditions are known to disrupt spermatogenesis and sperm integrity. In accordance with this finding, the positive effects of Vit E have been extensively documented across various health conditions (10-12,39).

Oxidative stress and the dysregulation of specific microRNAs like miR-21, miR-34a, and miR-155 are established key contributors to the etiology of human infertility, where they form a damaging effect in reproductive tissues (40). This study provides new insight into the association of specific microRNAs with OLZ-induced testicular toxicity and the protective effects of Vit E. The current findings showed that miR-122 and miR-202-5p are significantly dysregulated by OLZ administration. Similarly, clinical cohorts demonstrate the functional significance of miR-122-5p dysregulation during testicular insufficiency in the human spermatogonial stem cells (41). Previous research indicates that Vit E status influences the expression of specific miRNAs which might have a important role in the bone homeostasis (42). In this study, Vit E exposure effectively regulated the expression of miR-122 and miR-202-5p, demonstrating its protective capacity that extends beyond conventional antioxidant activity. It has been shown that the elevated miR-122-5p expression in the human spermatogonial stem cells stimulates proliferation and inhibits apoptosis (41). Therefore, the normalization of miR-122-5p by Vit E may represent a crucial mechanism for preserving the testicular functionality.

Conclusion

This study provides the new experimental evidences for Vit E's miRNA-mediated protective effects against OLZ-induced gonadotoxicity in males. The results suggest that Vit E significantly restored OLZ cytotoxicity by its anti-oxidant, anti-apoptosis, and miRNAs modulatory properties. Furthermore, this research has involved a link between Vit E supplementation and the regulation of circulating miRNA-122 and miRNA-202-5p levels in individuals experiencing OLZ toxicity. This implies the need for dietary antioxidants in patients on long-term antipsychotics. Many limitations of this study point to essential need for future research. New research must confirm miR-122-bcl2 targeting, optimize Vit E dosing, and translate findings to patients via miRNA biomarkers for OLZ-induced toxicity. Also, while the current model uses a consistent and clinically relevant dose; future works should determine the optimal therapeutic dose and duration of Vit E co-administration.

Acknowledgment

This study formed a component of the MSc thesis completed by Afraa Habeeb Qayssar at Ilam University. The authors would like to thank Dr. Saleh Azizian, Dr. Salman Soltani, and Dr. Mohammad Reza Tabandeh for their great assistance during tissue slide preparations and biochemical analysis, respectively. There are no conflicts of interest regarding the publication of this article.

Ethical Approval

The animal ethics committee of the Ilam University (Ilam, Iran) approved the entire experimental work (IR.ILAM.REC.1402.016)

Funding

The authors declare that no funds were received during the preparation of this manuscript.

Authors' Contributions

AH Q conceived the study, performed validation, software analysis, investigation, methodology, writing the original draft, and project administration. AL M performed formal analysis, data curation, writing the original draft, supervision, review and editing, and acquired resources. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no competing interests.

Declaration

We acknowledge the use of DeepSeek to write the introduction section.

References

1. Huhn M, Nikolakopoulou A, Schneider-Thoma J, Krause M, Samara M, Peter N, *et al.* Comparative efficacy and tolerability of 32 oral antipsychotics for the acute treatment of adults with multi-episode schizophrenia: A systematic review and network meta-analysis. Lancet 2019; 394: 939-951.

2. Kishi T, Ikuta T, Matsuda Y, Sakuma K, Okuya M, Nomura I, *et al.* Pharmacological treatment for bipolar mania: a systematic review and network meta-analysis of double-blind randomized controlled trials. Mol psychiatr 2022; 27: 1136-1144.

3. Menard ML, Thümmler S, Giannitelli M, Cruzel C, Bonnot O, Cohen D, *et al.* Incidence of adverse events in antipsychotic-naïve children and adolescents treated with antipsychotic drugs: Results of a multicenter naturalistic study (ETAPE). Eur Neuropsychopharmacol 2019; 29: 1397-1407.

4. Samadi A, Isikhan SY, Ansari MHK, Samadi M, Sabuncuoglu S. Effects of clozapine and haloperidol treatment on plasma concentrations of androgen hormones and androgendependent organ changes in rats. Indian J Pharmacol 2019; 51: 269-275.

5. Ilgin S. The adverse effects of psychotropic drugs as an endocrine disrupting chemicals on the hypothalamic-pituitary regulation in male. Life Sci 2020; 253: 117704.

6. Ardıç CM, Ilgın S, Baysal M, Karaduman AB, Kılıç V, Aydoğan-Kılıç G, *et al.* Olanzapine induced reproductive toxicity in male rats. Sci Rep 2021; 11: 4739.

7. Ebaid H, Bashandy SAE, Hassan I, Al-Tamimi J, Haredy SA, Imbabi T, *et al.* The preventive effect of zinc sulfate against olanzapine-induced testicular toxicity in male rats. Biol Trace Elem Res 2025; 203: 3764-3778.

8. Khattab MA, Ahmed SM, Salama HG, Mekawy NH. Effect of olanzapine on testes of adult albino rats and the possible role of granulocyte colony stimulating factor versus umbelliferone light and electron microscopic study. Ultrastruct Pathol 2025; 49: 265-287.

9. Szewczyk K, Chojnacka A, Górnicka M. Tocopherols and tocotrienols—bioactive dietary compounds; what is certain, what is doubt? Int J Mol Sci 2021; 22: 6222.

10. Nady Ouais G, Kamar SA, Mousa AA, Sonbol MM. The protective effects of vitamin E against alterations of rat testis structure induced by deltamethrin; histological, ultrastructure, and biochemical study. Ultrastruct Pathol 2024; 48: 108-120.

11. Ourique GM, Saccol EM, Pês TS, Glanzner WG, Schiefelbein SH, Woehl VM, *et al.* Protective effect of vitamin E on sperm motility and oxidative stress in valproic acid treated rats. Food Chem Toxicol 2016; 95: 159-167.

12. Bisong SA, Ukoh IE, Nna VU, Ebong PE. Vitamin E attenuates nicotine- and noise-induced reproductive impairment in male albino Wistar rats. Andrologia 2018; 50: e13050.

13. Gao Y, Jian L, Lu W, Xue Y, Machaty Z, Luo H. Vitamin E can promote spermatogenesis by regulating the expression of proteins associated with the plasma membranes and protamine biosynthesis. Gene 2021; 773: 145364.

14. Saddein E, Haghpanah T, Nematollahi-Mahani SN, Seyedi F, Ezzatabadipour M. Preventative effects of vitamin E on testicular damage and sperm parameters in the first generation mice pups due to pre and postnatal mancozeb exposure. J Toxicol 2019; 2019: 4763684.

15. Faramin Lashkarian M, Hashemipour N, Niaraki N, Soghala S, Moradi A, Sarhangi S, *et al.* MicroRNA-122 in human cancers: from mechanistic to clinical perspectives. Cancer Cell Int 2023; 23: 29.

16. Shi Z, Yu M, Guo T, Sui Y, Tian Z, Ni X, *et al.* MicroRNAs in spermatogenesis dysfunction and male infertility: clinical phenotypes, mechanisms and potential diagnostic biomarkers. Front Endocrinol (Lausanne) 2024; 15: 1293368.

17. Chen J, Gao C, Lin X, Ning Y, He W, Zheng C, *et al.* The microRNA miR-202 prevents precocious spermatogonial differentiation and meiotic initiation during mouse spermatogenesis. Development 2021; 148: dev199799.

18. Mohammed BT, Donadeu FX. Localization and *in silico*-based functional analysis of miR-202 in bull testis. Reprod Domest Anim 2022; 57: 1082-1087.

19. Klees C, Alexandri C, Demeestere I, Lybaert P. The role of microRNA in spermatogenesis: is there a place for fertility preservation innovation? Int J Mol Sci 2024; 25: 460.

20. Liu L, Zhu M, Liu X, Fei L, Shen J, Chen D. miR-122-5p regulates the tight junction of the blood-testis barrier of mice via occludin: miR-122-5p can regulate the tight junction. Basic Clin Androl 2021; 31: 1-10.

21. Boroun R, Dehagi BF, Ahmadizadeh M. Protective effect of vitamin E on nickel sulfate-induced renal dysfunction in rats. J Renal Inj Prev 2020; 13: e28835- e28835.

22. Cheraghi Abajlou S, Tofighi A, Tolouei Azar J, Khaki AA, Razi M. Combined effects of chrysin supplementation and exercise training on diabetes-induced oxidative stress and apoptosis in rat testicular tissue. Int J Fertil Steril 2025; 19: 88-95.

23. Erfani Majd N, Tabandeh MR, Hosseinifar SH, Sadeghi M. Protective effect of aloe vera gel against cisplatin-induced testicular damage, sperm alteration and oxidative stress in rats. Int J Fertil Steril 2021; 15: 210-218.

24. Sziva RE, Ács J, Tőkés AM, Korsós-Novák Á, Nádasy GL, Ács N, *et al.* Accurate quantitative histomorphometric-mathematical image analysis methodology of rodent testicular tissue and its possible future research perspectives in andrology and reproductive medicine. Life (Basel) 2022; 12: 189.

25. Ujah GA, Nna VU, Suleiman JB, Eleazu C, Nwokocha C, Rebene JA, *et al.* Tert-butylhydroquinone attenuates doxorubicin-induced dysregulation of testicular cytoprotective and steroidogenic genes, and improves spermatogenesis in rats. Sci Rep 2021; 11: 5522.

26. Li L, Xie L, Ma L, Chen Y, Chen X, Ge F, *et al.* Triphenyltin chloride delays Leydig cell maturation during puberty in rats. Front Pharmacol 2018; 10: 833.

27. Ma D, Luo N, Xue G. Trimethyltin (TMT) Reduces testosterone production in adult leydig cells in rats. Int J Toxicol 2019; 38: 493-500.

28. Adel RM, Lotfy RA, Darwish AS, Amer AS. Destructive effect of iron overload in brain tissue of albino rats: Ameliorative role of silver immobilized organo-modified casein nanocomposite as co-treating agent with Deferasirox. J Trace Elem Med Biol 2021; 67: 126794.

29. Sattar AA, Matin AA, Hadwan MH, Hadwan AM, Mohammed RM. Rapid and effective protocol to measure glutathione peroxidase activity. Bull Natl Res Cent 2024; 48: 100.

30. Fekri K, Hooshangi F, Parvizpur A, Charkhpour M, Hamedeyazdan S. Serum malondialdehyde levels decreased along with the alleviating effects of marrubium parviflorum on morphine withdrawal syndrome in rats. Arch Razi Inst 2024; 79: 833-842.

31. Saleh DO, Baraka SM, Jaleel GA, Hassan A, Ahmed-Farid OA. Eugenol alleviates acrylamide-induced rat testicular toxicity by modulating AMPK/p-AKT/mTOR signaling pathway and blood–testis barrier remodeling. Sci Rep 2024; 14: 1910.

32. Haidenberg-David F, Sidauy-Adissi J, Moscona-Nissan A, Jonguitud-Zumaya E, Fugarolas-Morinelli M, Martinez-Mendoza F, *et al.* Overview of hyperprolactinemia: General approach and reproductive health implications. Arch Med Res 2024; 55: 103102.

33. Shams AA, Vesal S, Karoii DH, Vesali S, Alizadeh A, Shahhoseini M. Paternal trans fatty acid and vitamin E diet affect the expression pattern of androgen signaling pathway genes in the testis of rat offspring. Theriogenol 2025; 231: 1-10.

34. Amanpour P, Khodarahmi P, Salehipour M. Protective effects of vitamin E on cadmium-induced apoptosis in rat testes. Naunyn-Schmiedeberg's Arch Pharmacol 2020; 393: 349-358.

35. Alsemeh AE, Ahmed MM, Fawzy A, Samy W, Tharwat M, Rezq S. Vitamin E rescues valproic acid-induced testicular injury in rats: Role of autophagy. Life Sci 2022; 296: 120434.
36. Nikolić-Kokić A, Tatalović N, Brkljačić J, Mijović M, Nestorović V, Mijušković A, *et al*. Antipsychotic drug-mediated adverse effects on rat testicles may be caused by altered redox and hormonal homeostasis. Int J Mol Sci 2022; 23: 13698.
37. Fang J, Xie S, Chen Z, Wang F, Chen K, Zuo Z, et al. Protective effect of vitamin E on cadmium-induced renal oxidative damage and apoptosis in rats. Biol Trace Elem Res 2021; 199: 4675-4687.
38. Daeihamed M, Fallah Kochakam N, Taghizadeh Valdi A, Faghani M, Farzanegan A, Mohammadghasemi F. Tocopherol alleviates oxidative stress and increases androgen receptors in mice testicular tissues under forced swimming in warm water. Andrologia 2024; 1: 8190937.
39. Xu M, Ye J, Wang Y, Chu K, Péré M, Xu M, et al.Vitamin E performs antioxidant effect via PAP retrograde signaling pathway in *Nile tilapia* (*Oreochromis niloticus*). Fish Shellfish Immunol 2022; 127: 918-924.
40. Vassilaki I, Potiris A, Domali E, Karampitsakos T, Mavrogianni D, Grigoriadis T, *et al*. MicroRNAs regulating oxidative stress in human fertility: a narrative review of mechanistic insights and clinical potential. Med Sci 2025; 13: 254.
41. Zhou F, Chen W, Cui Y, Liu B, Yuan Q, Li Z, He Z. miRNA-122-5p stimulates the proliferation and DNA synthesis and inhibits the early apoptosis of human spermatogonial stem cells by targeting CBL and competing with lncRNA CASC7. Aging (Albany NY) 2020; 12: 25528-25546.
42. Wong SK, Mohamad NV, Ibrahim N', Chin KY, Shuid AN, Ima-Nirwana S. The molecular mechanism of vitamin E as a bone-protecting agent: A review on current evidence. Int J Mol Sci 2019; 20: 1453.