WILEY | Hindawi

Research Article

Antioxidant Properties of *Pistacia vera* against the Effects of Phenylhydrazine-Induced Hemolytic Anemia on Male Fertility in Mice

Soudeh Khanamani Falahati-pour,[1] Arezoo Khoradmehr,[2] Fatemeh Baghery,[1] Fatemeh Amin,[3,4] Najmeh Parvaz,[5] and Soheila Pourmasumi [6,7]

[1]*Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
[2]*The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Research Sciences Center Institute, Bushehr University of Medical Sciences, Bushehr, Iran*
[3]*Physiology-Pharmacology Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
[4]*Department of Physiology and Pharmacology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
[5]*Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
[6]*Social Determinants of Health Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
[7]*Clinical Research Development Unit (CRDU), Ali-Ibn Abi-Talib Hospital, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

Correspondence should be addressed to Soheila Pourmasumi; spourmasumi@yahoo.com

Objective. The reproductive system can be adversely affected by anemia, characterized by elevated free radical production and lipid peroxidation. Conversely, the pistachio is well-known for its antioxidant capabilities. In this study, a mouse model was employed to investigate the safeguarding effects of the aqueous extract of pistachio (*Pistacia vera*) against oxidative stress induced by phenylhydrazine (PHZ)-triggered hemolytic anemia. *Materials and methods.* Forty-eight male mice were distributed into six distinct experimental groups ($n = 8$): control, PHZ, PHZ + 20Pis, PHZ + 80Pis, PHZ + 160Pis, and 80Pis groups. The experimental groups were administered a PHZ dose of 80 mg/kg/48 hr, along with pistachio doses of 20, 80, and 160 mg/kg/day, delivered intraperitoneally. After a period of 35 days, an assessment of sperm count, motility, morphology, and viability was conducted. Additionally, sperm chromatin quality was evaluated through Chromomycin A3, aniline blue, and toluidine blue staining. Hematoxylin–eosin staining was performed to examine the total diameter of seminiferous tubes and spermatogenic cells. Hormonal levels (FSH, LH, T) and antioxidant levels were analyzed using standard kits. *Results.* The PHZ groups exhibited a noteworthy decrease in various sperm parameters, as well as diminished chromatin quality assays and reduced levels of sex hormones when compared to the control group. Moreover, the morphometric evaluation indicated a substantial reduction in spermatogonia cell number, spermatid count, tubule count, seminiferous diameters, and seminiferous area in the PHZ group. Notably, the Pis group demonstrated the highest concentration of antioxidant contents. *Conclusion.* The protective impact of *P. vera* extract against PHZ-induced anemia on the male reproductive system in mice may be attributed to its abundant antioxidant components, which possess therapeutic properties at elevated levels.

1. Introduction

Certain industrial chemicals have the potential to induce hemolysis and hemolytic anemia, a condition characterized by the breakdown of red blood cells (RBCs). This includes specific commercial pesticides, the improper use of certain medications, as well as exposure to natural agents such as animal toxins or parasites [1]. One of the prominent repercussions associated with this type of anemia is an elevation in tissue iron levels stemming from the lysis of RBCs. Concurrently, there is a reduction in oxygen levels (hypoxia) due to the anemic state. The intricate balance of intracellular iron levels is crucial, and

disrupting this equilibrium by increasing extracellular iron is believed to trigger oxidative stress (OS). This OS, in turn, can lead to damage in the lipid components of cell membranes and various organs [2]. The consequences of this chemical imbalance extend beyond the immediate impact on RBCs, affecting the overall physiological well-being by inducing hypoxia and subjecting cells to the detrimental effects of OS.

Various mechanisms have been proposed to explain male infertility in cases of hemolytic anemia. First, the sperm cell membrane, which is rich in unsaturated fatty acids, is particularly susceptible to oxidative damage. The OS present in hemolytic anemia poses a significant threat to sperm and adversely impacts male fertility potential [3]. Second, alterations in blood flow associated with hemolytic anemia represent a critical factor leading to cell degeneration [4]. Any degenerative factor can adversely affect the spermatogenesis process. Third, OS in anemic conditions contributes to tissue ischemia, particularly in the testis. This, in turn, triggers changes in the activation of leukocytes, ultimately resulting in the development of edema and apoptosis in the testicular tissue. Additionally, the increased presence of free iron in hemolytic anemia can lead to testicular failure, thereby halting the process of spermatogenesis [5]. Finally, chronic hypoxia has been observed to inhibit spermatogenesis in both rats and monkeys. Furthermore, hypoxia in male rodents hinders the synthesis and release of gonadotropins [3]. These multifaceted mechanisms underscore the intricate relationship between hemolytic anemia and male infertility, shedding light on the various pathways through which this hematological condition adversely affects reproductive processes in males [6].

In essence, sperm DNA carries fifty percent of the genetic material crucial for successful reproduction, and any anomalies within the sperm genome can result in failures in the reproductive process [7]. Phenylhydrazine (PHZ) proves to be a valuable element in experimental models studying hemolytic anemia due to its toxic effects on RBCs. The auto-oxidation of PHZ generates radicals and reactive oxygen species (ROS), contributing to various adverse responses, including the manifestation of hemolytic anemia [8].

Given their therapeutic efficacy, cost-effectiveness, accessibility, and minimal side effects, herbal medicine and natural substances find application as traditional treatments for a spectrum of ailments, particularly in developing nations [9]. Numerous studies indicate that natural medicinal herbs, endowed with high antioxidant activity and potential protective effects, can mitigate injuries stemming from OS-related disorders. This is achieved by inhibiting ROS production and enhancing antioxidant defense systems [10, 11]. This highlights the significance of exploring natural remedies with antioxidant properties as potential interventions for conditions associated with OS.

Pistachio (*Pistacia vera*) is considered a member of the Anacardiaceae family, extensively cultivated from Asia to the eastern Mediterranean owing to its medicinal purposes and nutritional benefits. Pistachios hold a distinguished status as one of the most significant nuts globally, celebrated for their nutritional richness and diverse array of compounds. The

pistachio fruit, in particular, is abundant in various elements, including phenolic compounds, flavonoids, fatty acids, and essential minerals. Notably, it boasts a repertoire of potent and widely recognized antioxidants, which harbor the potential to safeguard against diseases resulting from the excessive production of free radicals [12]. Within the pistachio, the pericarp emerges as a particularly valuable reservoir of antioxidants. Among these antioxidants are notable components such as epicatechin, catechin, eriodictyol-7-O-glucoside, cyanidin-3-O-galactoside, and gallic acid. These compounds play a pivotal role in combating the detrimental effects of free radicals, functioning as a robust defense mechanism against cellular defects and the restoration of damaged cells [13].

The phytochemical examination of *P. vera* has unveiled the existence of noteworthy polyphenolic compounds that holding medicinal promise. This includes a remarkable antioxidant capacity effective against ROS and the deterrence of free radical generation [14]. Past investigations have highlighted the pericarp aqueous extract's noteworthy activities, encompassing nephron-protective attributes [15, 16], antibacterial properties [17], and anticancer potential [18]. In the realm of fertility preservation, antioxidant therapy stands out as a natural and healthful approach. Consequently, the antioxidant prowess exhibited by *P. vera* suggests its potential role in safeguarding fertility against OS activity. The preventive influence of *P. vera* on fertility, as inferred from its antioxidant capabilities, underscores its significance in the broader context of promoting reproductive health. This multifaceted plant not only holds promise in countering ROS but also demonstrates therapeutic potential across various health dimensions, extending its utility beyond fertility preservation.

To the best of our understanding, there is a paucity of published studies delving into the intersection of anemia and male fertility. Additionally, the potential protective impacts of both natural and chemical antioxidants have yet to be thoroughly explored in the context of infertility arising from anemia. Therefore, the primary objective of this research is to fill these gaps in knowledge by investigating the safeguarding effects of the aqueous extract derived from pistachio pericarp. The study specifically aims to assess how Pis can counteract the consequences of hypoxia and the subsequent OS resulting from PHZ-induced hemolytic anemia. The experiment focuses on a mouse model subjected to PHZ-induced toxicity, with a comprehensive examination of sperm parameters, sex hormones, and selected OS markers. By undertaking this investigation, we aspire to shed light on the potential protective properties of Pis against the deleterious effects of anemia-induced hypoxia, unveiling novel insights into how antioxidants, both of natural and chemical origin, might play a role in mitigating male fertility issues associated with anemia. This research contributes to the broader understanding of the intricate relationship between anemia, OS, and male reproductive health.

2. Materials and Methods

2.1. Experimental Groups. Forty-eight male mice (NMRI strain) weighing about 25–30 g were organized in six
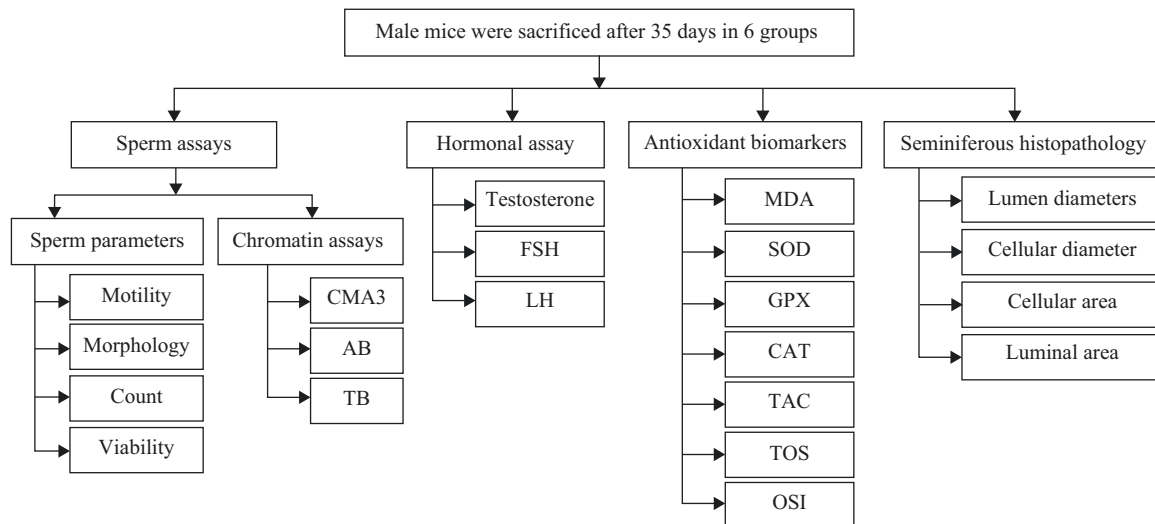
FIGURE 1: After 35 days, all groups were sacrificed, and sperm parametres, hormonal levels, antioxidant biomarkers, and seminiferous histopathology tests were performed. AB, aniline blue; CAT, catalase; CMA3, chromomycin A3; FSH, follicle-stimulating hormone; GPX, glutathione peroxidase; LH, luteinizing hormone; MDA, malondialdehyde; OSI, oxidative stress index; SOD, superoxide dismutase; TAC, total antioxidant capacity; TB, toluidine blue; TOS, total oxidant status.

experimental groups ($n = 8$), randomly: control group (normal diet), PHZ group (only PHZ 80 mg/kg/48 hr, IP), PHZ + 20Pis group (PHZ 80 mg/kg/48 hr, IP and Pis 20 mg/kg/day, IP), PHZ + 80Pis group (PHZ 80 mg/kg/48 hr, IP and Pis 80 mg/kg/day, IP), PHZ + 160Pis group (PHZ 80 mg/kg/48 hr, IP and Pis 160 mg/kg/day, IP) and 80Pis group (Pis 80 mg/kg /day, IP) [15]. The PHZ was prepared by Sigma-Aldrich (Cas No: P6926). The fresh fruit of the pistachio (*Pistachia vera* L. cv *Akbari*) was collected and then verified by an expert from the Department of Botany at the Rafsanjan Valiasr University, Iran (genetic code: M30). The aqueous extract is prepared by shaking 50 g of ground and sifting the pistachio pericarp in 200 ml of water for 48 hr at room temperature. The aqueous extract is filtered from the pistachio pericarp and water, then evaporated in a rotary at 40°C.

The methods were conducted according to the guidelines of the Ethics Committee of Rafsanjan University (IR.RUMS. REC.1399.039). Mice had free access to food and tap water during the experiment. Animals were maintained in plastic cages individually for 2 weeks before and during the entire experiment, with ambient temperatures of $23 \pm 2°C$ and $55\% \pm 5\%$ relative humidity and a natural cycle of day/night. Mice were sacrificed by cervical dislocation after 40 days (which is prolonger than what is required for mice to develop sperm), and the caudal epididymis was placed into a petri dish. Caudal epididymis suspension was incubated with 1,000 μl of Ham's F10 medium (Vitrolife, Gothenburg, Sweden) at 37°C in a humidified atmosphere of 5% CO_2 for 30 min [19]. Using standard procedures, the quality of sperm DNA and its chromatin was analyzed 30 min after the sperm parameters had been evaluated. Bouin's solution was used to fix the testis for histological examination. Figure 1 shows a summary of all assays.

2.2. *Sperm Parameters Assessment.* To assess sperm motility, the percentages of nonprogressive, progressive, and immotile sperm were classified into three categories. Makler's counting chamber was applied to determine the sperm count. The sperm viability was evaluated in all groups 30 min after the sperm motility assessment. White sperm heads (unstained) or pink (or red) were categorized as alive or expired, respectively. To examine sperm viability and morphology, eosin-nigrosin staining (Merck, Germany) and Papanicolaou (Merck, Germany), respectively, were applied [20]. The sperm morphological abnormalities were classified into three groups: heads (shape, size, acrosomal section, double heads), necks (cytoplasmic droplets, bent), and tails (cytoplasmic droplets, double tails, coiled, bent). Using a light microscope (Motic, Spain) at ×400 magnification, 200 sperm were evaluated for motility, morphology, and viability.

2.3. *Assessment of Sperm DNA Integrity and Chromatin Packaging Quality*

2.3.1. *Aniline Blue (AB) Staining.* As AB stains respond to lysine residues in histone proteins, it detects how sperm chromatin is remodeled [21]. After 30 min, the air-dried smears were fixed in glutaraldehyde buffered with 0.2 M phosphate buffer (pH: 7.2). Staining was done with 5% AB (Sigma, Germany) in 4% acetic acid (pH: 3.5) for 5 min. The slides were rinsed in distilled water and assessed under a magnification of ×400 with 200 sperm. AB-negative mature sperm display a clear head, while immature sperm exhibit a blue head (AB-positive).

2.3.2. *Toluidine Blue (TB) Staining.* TB staining was applied for DNA strands to define their phosphate groups and chromatin condensation. A mixture of ethanol and acetone (1 : 1) was applied to stabilize the slides at 4°C for 30 min; then, the slides were hydrolyzed in 0.1 N HCl for 5 min. After washing in distilled water three times, the slides were stained with 5% TB (Sigma, Germany) in 50% McIlvain's citrate phosphate buffer (pH: 3.5) [21]. Two hundred sperm were examined

under a light microscope (Olympus BX51, Japan) at a magnification of ×400 for each slide. Staining with TB determines whether the sperm chromatin is intact or not, resulting in light blue heads for sperm with good integrity and dark blue heads for sperm with low integrity.

2.3.3. Chromomycin A3 (CMA3) Staining.

Using the CMA3 test can detect sperm chromatin deficient in protamine. The air-dried slides were fixed in a refrigerator using methanol and glacial acetic acid (3 : 1). Afterward, the slides were stained with $100 \mu l$ of chromomycin A3 solution (Sigma, Germany) for 10 min before they were rinsed with McIlvain's buffer. Using a fluorescent microscope (Olympus BX51, Japan) at ×400 magnification, 200 sperm were evaluated per slide [19]. Spermatozoa that stain yellow-green are positive for CMA3, while those that stain dull yellow-green are negative for CMA3.

2.4. Plasma Sex Hormone Levels.

Based on the radioimmunoassay technique (DIA source Immuno-assays, S.A., Belgium), we determined the presence of sex hormones (estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) in serum samples [22].

2.5. Measurement of Antioxidant Contents

2.5.1. Preparation of Testicular Tissue Homogenate.

In 10 volumes (1 : 10, w/v) of ice-cold Tris–HCl buffer (50 mM, pH 7.4), testicular tissues were homogenized using an ultrasonic homogenizer after being rinsed with ice-cold 0.9% NaCl, transferred on ice, cut into small pieces and homogenized (IKA T18, Germany). For homogenization, $5,000\,g$ was applied for 2 min. Afterward, homogenates were centrifuged for 10 min at 2,795 at 4°C. To assess OS parameters, testis samples were stored at 80°C [23].

2.5.2. Determination of the Antioxidant Biomarkers in Testis.

For the assessment of testicular tissue malondialdehyde (MDA) level at 550 nm, the absorbance of the test was monitored immediately after all the reagents (according to the manufacturer) were added to a 1,000 ml reaction in which 200 ml of supernatant was added, and 800 ml of different reagents were added [24]. Measurement of superoxide dismutase (SOD) level was investigated based on an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and the method of xanthine oxidase to measure its absorbance at 450 nm [23]. At 340 and 550 nm, respectively, the absorbance of the testicular glutathione peroxidase (GPX) and catalase (CAT) reactions was monitored in 300 ml, including 40 ml of supernatant and 260 ml from the different reagents of the kit [24]. Testicular total antioxidant capacity (TAC) assuagement was achieved by incubating the reaction mixture for 5 min at room temperature. The mixture had $1,000 \mu l$ reaction buffer containing $400 \mu l$ distilled water, $100 \mu l$ supernatant, and $500 \mu l$ ABTS buffer, and monitoring absorbance at 414 nm after incubation was complete [24]. The total oxidant status (TOS) level analysis was done in 240 ml, including 30 ml of supernatant and 210 ml from several reagents from the kit, as instructed by the manufacturer. After all stages of the experiment, the absorbance

was checked at 530 nm after 10 min [24]. Using the TOS to TAC ratio, the OS index (OSI value was calculated to measure OS [23].

2.6. Histomorphometric Analysis and Cell Numbers of Seminiferous Tubules.

For each group, Bouin's solution was used to fix the five testis samples. Dehydrated tissues were clarified in xylene and immersed in paraffin wax. Eosin and hematoxylin were applied to treat glass slides after cutting the testis into 7 mm slices. It was calculated the mean diameter, numerical density area, and cross-sectional area of the seminiferous tubules [25]. To measure Sertoli, Leydig, primary spermatocytes, spermatogonia, and spermatid cell numbers on each slide, the average of 10 random fields was computed [26].

2.7. Statistical Analysis.

The normality of the data was assessed using the Shapiro–Wilk test. To compare the data across different groups, statistical analyses were conducted using SPSS version 20 (SPSS Inc., USA). A one-way analysis of variance (ANOVA) was performed, followed by the Tukey post hoc test for pairwise comparisons between groups. Statistical significance was set at $p < 0.05$. The results are presented as mean $\pm$ standard deviation (SD).

3. Results

3.1. Summary.

In the present study, a dose PHZ of 80 mg/kg/48 hr in male mice prompted a significant reduction in the sperm parameters (normal morphology, count, and motility), chromatin quality (AB, TB, CMA3), and sex hormones (FSH, LH, T) when compared with the control treatment. Also, in morphometrical assessment, there was a considerable decrease in spermatogonia cell number, spermatid, tubule count, seminiferous diameters, and seminiferous area in the PHZ group in contrast to the control group ($p < 0.001$).
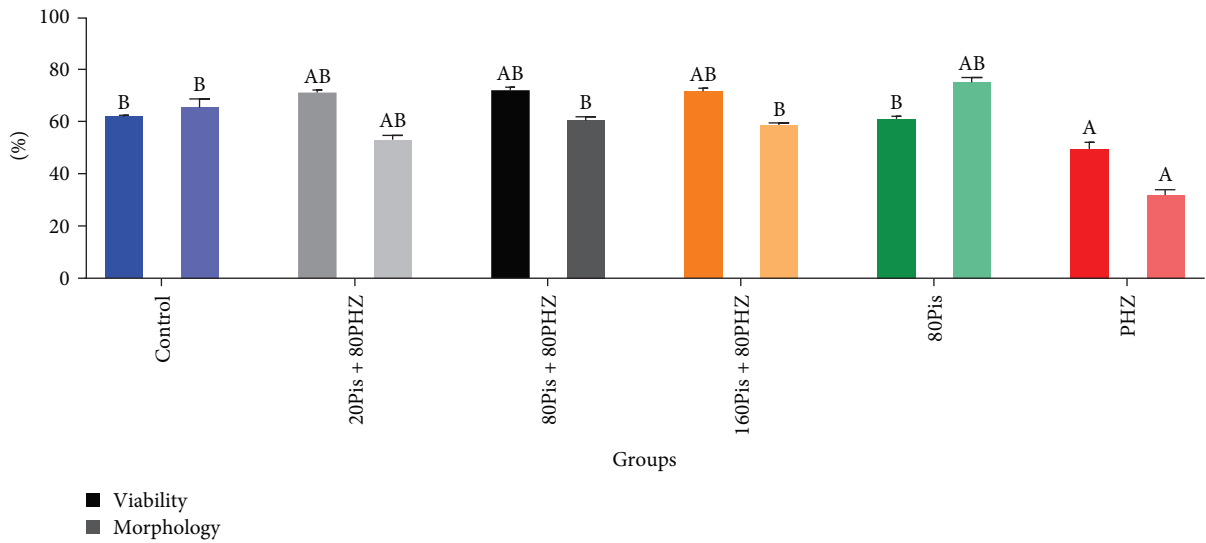
3.2. Sperm Parameters

3.2.1. Count.

The sperm count within the experimental groups revealed a significant reduction in the number of sperm in the PHZ group compared to the control group ($p < 0.001$). Conversely, the 80Pis group exhibited a notably higher sperm count than the control treatment ($p < 0.001$). Interestingly, no significant differences were observed between the PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups when compared to the control group. A comparative analysis between the PHZ group and the PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups highlighted a substantial increase in sperm count in the Pis-treated groups (see Figure 2(a) and Table 1).

3.2.2. Motility.

The proportion of progressive sperm in both the PHZ 20Pis and PHZ + 80Pis groups exhibited a significant decrease compared to the control treatment. Notably, there was an increase in progressive sperm in the PHZ + 80Pis and PHZ + 160Pis groups when compared to the PHZ-treated group, although the increase in the PHZ + 20Pis group was not higher than that observed in the PHZ-treated group. The percentage of nonprogressive sperm showed a considerable increase in all groups (except the PHZ group) compared to
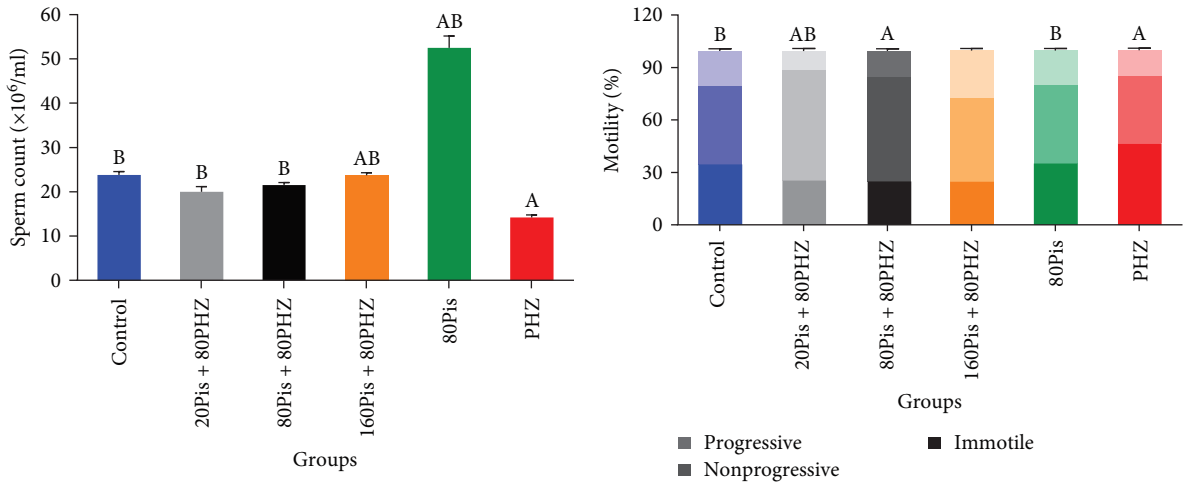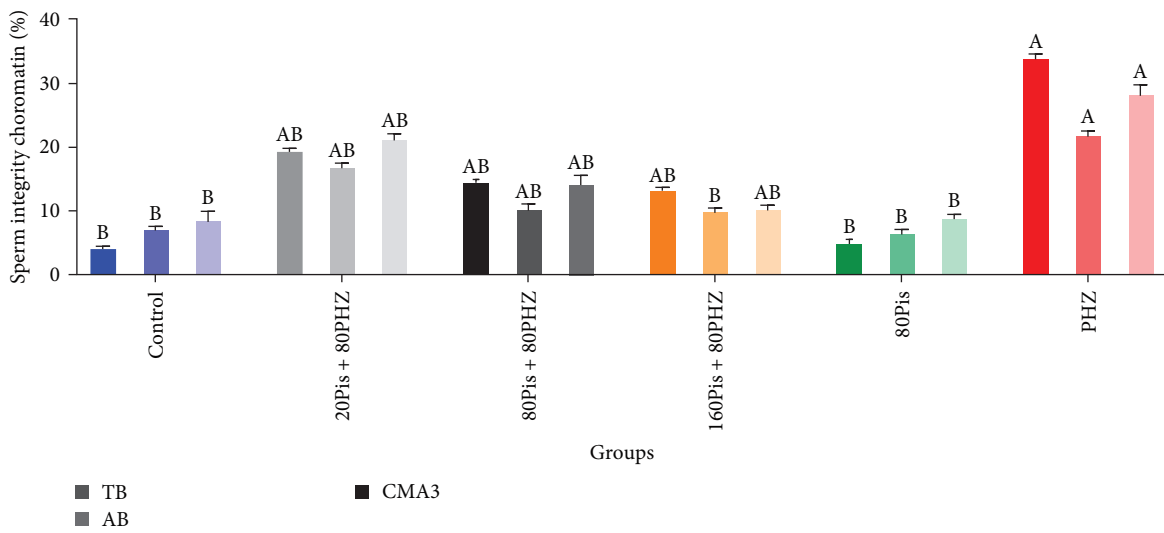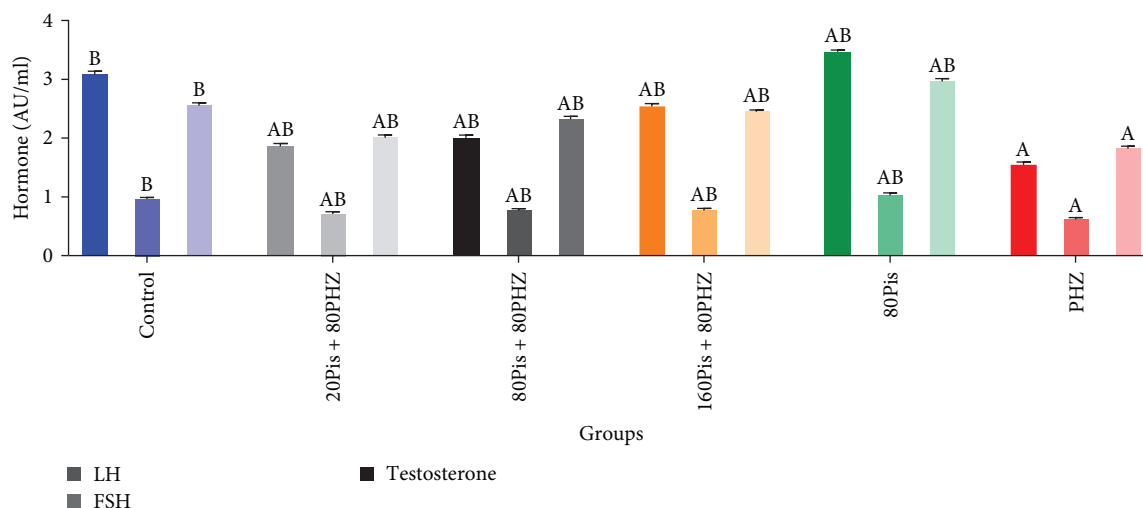
(a)

(b)

(c)

(d)

FIGURE 2: Continued.

(e)

FIGURE 2: Examined parameters including sperm parameters (a) sperm count, (b) motility, (c) viablity and dmorphology, (d) chromatin quality, and (e) sex hormones in experimental groups. One-way ANOVA was used to compare dependent variables. p-Value < 0.05 was considered statistically significant. Values are presented by mean $\pm$ SD. TB, toluidine blue staining; AB, aniline blue staining; CMA3, chromomycine A3 staining; LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, testosterone; PHZ, phenylhydrazine; Pis, aqueous extract of pistachio pericarp; A = when groups compared to control group, B = when groups compared to PHZ group.

TABLE 1: Comparison of sperm parameters, chromatin quality, and hormone level between groups.

| | Groups | | | | | | | |
| | p-Values between groups | | | | | | | |
Factors	Control and PHZ + 20Pis	Control and PHZ 80Pis	Control and PHZ + 160Pis	Control and 80Pis	Control and 80 PHZ	80 PHZ and PHZ + 20Pis	80PHZ and PHZ + 80Pis	80 PHZ and PHZ + 160Pis
Count ($\times 10^6$/ml)	0.245	0.770	1.00	0.001	0.001	0.034	0.003	0.001
Motility (%)								
Progressive	0.001	0.001	0.001	0.967	0.001	0.04	1.00	0.001
Nonprogressive	0.001	0.001	0.343	1.00	0.003	0.001	0.001	0.001
Immotile	0.001	0.001	0.001	0.999	0.001	0.001	0.001	0.001
Normal morphology (%)	0.001	0.426	0.179	0.009	0.001	0.001	0.001	0.001
Viability	0.001	0.001	0.001	0.999	0.001	0.001	0.001	0.001
AB	0.001	0.004	0.783	0.998	0.001	0.001	0.001	0.001
TB	0.001	0.002	0.006	0.997	0.001	0.001	0.001	0.001
CMA3	0.001	0.001	0.001	0.672	0.001	0.001	0.001	0.001
FSH (ng/ml)	0.001	0.001	0.009	0.001	0.001	0.001	0.001	0.001
LH (ng/ml)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
T (ng/ml)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Note: Values are presented by p-value. One-way analysis of variance (ANOVA) followed by a Tukey HSC post hoc test was performed to determine the statistical significance between different groups. p-Value < 0.05 is considered significant. AB, aniline blue staining; TB, toluidine blue staining; CMA3, chromomycine A3 staining; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; PHZ, phenylhydrazine; Pis, aqueous extract of pistachio pericarp.

the control group. Furthermore, progressive sperm saw a significant elevation in all groups in contrast to the PHZ group. Interestingly, the immotile sperm count in the PHZ + 80Pis and PHZ + 160Pis groups was observed to be lower than in the PHZ group (see Figure 2(b) and Table 1).

3.2.3. Sperm Normal Morphology. Administering Pis at doses of 80 and 160 mg/kg/day significantly increased sperm normal morphology in both the PHZ + 80Pis and PHZ + 160Pis groups compared to the PHZ group ($p < 0.001$) (Table 1). Interestingly, the PHZ + 20Pis group exhibited a notable

TABLE 2: Oxidative stress parameters in experimental groups.

Histology	Groups					
	Contol	PHZ + 20Pis	PHZ + 80Pis	PHZ + 160Pis	80Pis	80PHZ
MDA	130.12 ± 2.23	142.75 ± 2.45	139.71 ± 2.68	134.07 ± 2.85	129.61 ± 2.20	148.56 ± 3.24
SOD	142.12 ± 1.64	123.28 ± 2.64	134.20 ± 2.68	138.07 ± 3.23	143.38 ± 3.40	115.63 ± 2.91
GPX	13.00 ± 1.30	7.57 ± 1.17	9.25 ± 1.32	10.75 ± 1.81	14.47 ± 1.44	5.28 ± 1.10
CAT	14.25 ± 2.12	10.32 ± 1.49	12.21 ± 1.77	13.7 ± 1.40	19.92 ± 2.26	9.66 ± 1.46
TAC	65.12 ± 0.99	60.21 ± 2.61	60.46 ± 2.69	61.97 ± 2.86	66.32 ± 2.82	60.37 ± 4.52
TOS	12.62 ± 1.40	19.58 ± 2.52	19.65 ± 4.39	18.90 ± 3.39	14.11 ± 1.92	21.27 ± 2.14
OSI	0.19 ± 0.02	0.32 ± 0.02	0.32 ± 0.06	0.30 ± 0.04	0.21 ± 0.02	0.35 ± 0.02

Note: Values are presented by mean ± SD. One-way ANOVA was used to compare dependent variables. p-Value < 0.05 was considered statistically significant. MDA, malondialdehyde; SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index; PHZ, phenylhydrazine, Pis, aqueous extract of pistachio pericarp.

reduction in normal morphology compared to the control treatment ($p < 0.001$). However, there was no statistically significant decline in the PHZ + 80Pis and PHZ + 160Pis groups when compared to the control group (0.426 and 0.179, respectively) (Figure 2(c) and Table 1).

3.2.4. Sperm Viability. There was a notable elevation in the PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups compared to both the PHZ group and the control treatment. Additionally, these findings indicated a significant decrease in sperm viability in the PHZ group in contrast to the control group ($p < 0.001$) (Figure 2(c) and Table 1).

3.3. Chromatin Quality Assessment

3.3.1. Ab. The outcomes from Ab staining in the experimental groups revealed a reduction in the percentages of dark blue-stained sperm, indicating abnormal spermatozoa. This reduction was observed in the PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups when compared to the PHZ group. However, the presence of abnormal sperm decreased in the PHZ + 80Pis and PHZ + 160Pis groups in comparison to the control, although this difference was not statistically significant (0.783 and 0.998, respectively) (Figure 2(d) and Table 1).

3.3.2. TB. In the TB results, significant differences were observed between all groups when compared to both the control and PHZ groups ($p < 0.001$). However, the comparison between the control treatment and the group administered with 80Pis did not reveal any differences ($p = 0.997$) (Figure 2(d) and Table 1).

3.3.3. CMA3. The results from CMA3 demonstrated a significant increase in the PHZ group compared to all PHZ + Pis20, PHZ + Pis80, and PHZ + Pis160 groups. Additionally, there was a notable decrease in the PHZ + Pis20, PHZ + Pis80, and PHZ + Pis160 groups compared to the control ($p < 0.001$) (Figure 2(d) and Table 1).

3.4. Hormone Profile

3.4.1. FSH. A significant difference in FSH concentration was identified among the PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups compared to both the control and PHZ groups. Furthermore, the FSH level results indicated a statistically significant rise in hormone levels in the 80Pis

group compared to the control group ($p < 0.001$) (Figure 2(e) and Table 1).

3.4.2. LH. The LH concentration in various groups revealed a significant reduction in its levels across all groups compared to the control group ($p < 0.001$). Conversely, the 80Pis group exhibited a noteworthy increase in LH levels compared to the control group. Additionally, the comparison between the PHZ-administered group and the PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups showed a substantial elevation in LH levels in the groups injected with pistachio alongside PHZ injection ($p < 0.001$) (Figure 2(e) and Table 1).

3.4.3. Testosterone. A significantly elevated level of testosterone was noted in the 80Pis group compared to the control group ($p < 0.001$). Furthermore, the testosterone levels in the PHZ + Pis20, PHZ + 80Pis, and PHZ + 160P groups were significantly reduced compared to the control group ($p < 0.001$). The testosterone level in the PHZ group was notably lower than in the other experimental groups ($p < 0.001$) (Figure 2(e) and Table 1).

3.5. Antioxidant Contents

3.5.1. MDA. The comparison of MDA levels across different groups indicated a decrease in the 80Pis group compared to the control treatment; however, this increase was not statistically significant (129.61 ± 2.20 vs. 130.12 ± 2.23, respectively) ($p = 0.1$). The analysis of MDA levels revealed an increase in the PHZ, PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups compared to the control group, but no statistically significant differences were observed (Tables 2 and 3).

3.5.2. SOD. The activity of SOD was significantly lower in the PHZ + 20Pis and 80Pis groups compared to the control treatment ($p < 0.001$). However, SOD activity increased in the PHZ + Pis treated groups compared to the PHZ group ($p < 0.001$). The mean ± SD values of SOD in the control, PHZ + 160Pis, and 80Pis groups were not significantly different ($p = 0.064$ vs. $p = 0.945$, respectively) (Tables 2 and 3).

3.5.3. GPX. The GPX activity results revealed elevated levels in both the control and 80Pis groups. Conversely, the GPX activity was diminished in the PHZ, PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups compared to the control

TABLE 3: Comparison of oxidative stress parameters between groups.

| Factors | Groups | | | | | | | |
| | p-Values between groups | | | | | | | |
	Control and PHZ + 20Pis	Control and PHZ + 80Pis	Control and PHZ + 160Pis	Control and 80Pis	Control and 80 PHZ	80 PHZ and PHZ + 20Pis	80 PHZ and PHZ + 80Pis	80 PHZ and PHZ + 160Pis
MDA	0.492	0.757	0.993	1.00	0.959	0.934	0.995	1.00
SOD	0.001	0.001	0.064	0.945	0.001	0.001	0.001	0.001
GPX	0.001	0.001	0.025	0.290	0.001	0.022	0.001	0.001
CAT	0.001	0.225	0.776	0.001	0.001	0.975	0.068	0.005
TAC	0.021	0.032	0.289	0.963	0.051	0.999	1.00	0.958
TOS	0.001	0.001	0.001	0.895	0.001	0.835	0.855	0.570
OSI	0.001	0.001	0.001	0.922	0.001	0.760	0.729	0.200

Note: Values are presented by *p*-value. One-way analysis of variance (ANOVA) followed by a Tukey HSC post hoc test was performed to determine the statistical significance between different groups. *p*-Value < 0.05 is considered significant. MDA, malondialdehyde; SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index, PHZ, phenylhydrazine; Pis, aqueous extract of pistachio pericarp.

group ($p < 0.001$). Table 3 provides a comparison of GPX activity across different experimental groups (Tables 2 and 3).

3.5.4. CAT. The CAT activity was significantly lower in both the PHZ and PHZ + 20Pis groups compared to the control treatment ($p < 0.001$). Additionally, the CAT activities in the PHZ + 80Pis and PHZ + 160Pis groups were lower than in the control group, although this decrease was not statistically significant ($p = 0.225$ vs. $p = 0.776$, respectively). The comparison between the PHZ group and the PHZ + 20Pis and PHZ + 80Pis groups showed no significant increase in CAT function in the PHZ + 20Pis and PHZ + 80Pis groups compared to the PHZ group ($p = 0.934$ vs. $p = 0.995$, respectively) (Tables 2 and 3).

3.5.5. TAC. The TAC levels exhibited no significant variation in both the PHZ + 160Pis and 80Pis groups compared to the control group ($p = 0.289$ vs. $p = 0.963$, respectively). However, the TAC levels in the PHZ + 20Pis and PHZ + 80Pis groups were significantly lower than in the control group ($p = 0.021$ vs. $p = 0.032$, respectively). Additionally, there was no considerable difference in TAC levels between the PHZ group and the PHZ + 20Pis, PHZ + 80Pis, and PHZ + 160Pis groups (Tables 2 and 3).

3.5.6. TOS. The TOS level exhibited a significant increase in the PHZ + 20, 80, and 160Pis groups compared to the control ($p < 0.001$). These results also indicated a significant elevation of TOS in the PHZ group compared to the control group ($p < 0.001$). A comparison between the PHZ group and PHZ + 20, 80, and 160Pis groups revealed a considerable reduction in TOS levels in the PHZ + 160Pis group compared to the PHZ group. Furthermore, there was no statistically significant reduction in the level of TOS in the PHZ + 20 and PHZ + 80Pis groups compared to the PHZ group ($p = 0.835$ vs. $p = 0.855$, respectively) (Tables 2 and 3).

3.5.7. OSI. The comparison of OSI in various groups revealed a significantly higher value in the control and 80Pis groups compared to the experimental groups. A higher OSI level was observed in the PHZ group. However, there was no statistically

significant variation in the OSI level between the PHZ-treated group and the PHZ + 20, 80, and 160Pis groups (Tables 2 and 3).

3.6. Stereological Profile and Assessment of Different Testis Cell Types. Testis weight remained consistent across all experimental groups and controls (Figure 3(a)). The number of tubules significantly decreased in both the PHZ and PHZ + 20Pis groups compared to the control. However, in the 80Pis group, there was an increase in tubular numbers compared to other treated groups (Figure 3(b)). Lydig cells, spermatogonia, spermatids, and primary spermatocytes showed a significant reduction in the PHZ and PHZ + 20Pis groups. Conversely, in the PHZ + 80Pis, PHZ + 160Pis, and 80Pis groups, there was a statistically significant increase in Lydig cells, spermatogonia, primary spermatocytes, and spermatids compared to the control group (Figures 3(c) and 4). The cross-sectional area of seminiferous tubules exhibited a significant difference in the PHZ group compared to the control group. A notable increase in cross-sectional seminiferous area was observed in the 80Pis and PHZ + 160Pis groups compared to the PHZ and control groups (Figures 3(d) and 4). Cellular diameter, lumen diameter, and total diameter significantly decreased in the PHZ group compared to the control treatment. Seminiferous diameter increased in the PHZ + 80Pis, PHZ + 160Pis, and 80Pis groups compared to the PHZ group (Figure 5(a)). Additionally, in the PHZ + 20Pis and PHZ groups, the seminiferous area was smaller than in the control group. In the 80Pis group, the seminiferous area increased significantly compared to the control and 80PHZ groups (Figures 4 and 5(b)). The numerical density of seminiferous tubules exhibited a decrease in the PHZ, PHZ + 20Pis, and PHZ + 80Pis groups compared to the control treatments, although this decrease was not statistically significant (Figures 4 and 5(c)).

4. Discussion

As per the findings of this study, the injection of pistachio aqueous extract has been demonstrated to bring about noteworthy enhancements in various sperm parameters. These encompass motility, morphology, abnormal morphology,
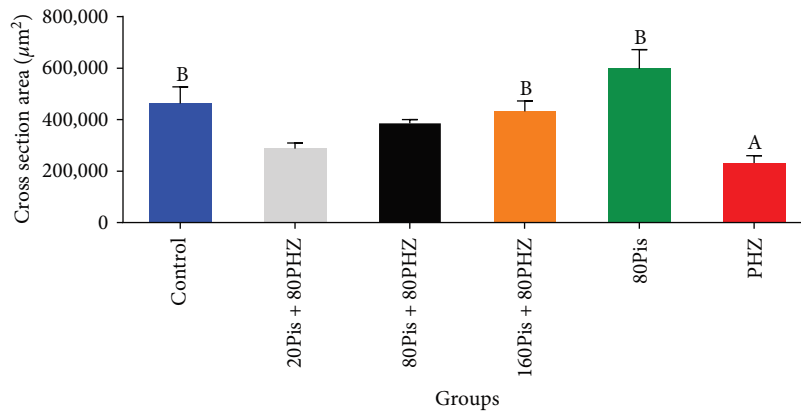
(a)

(b)

(c)

(d)
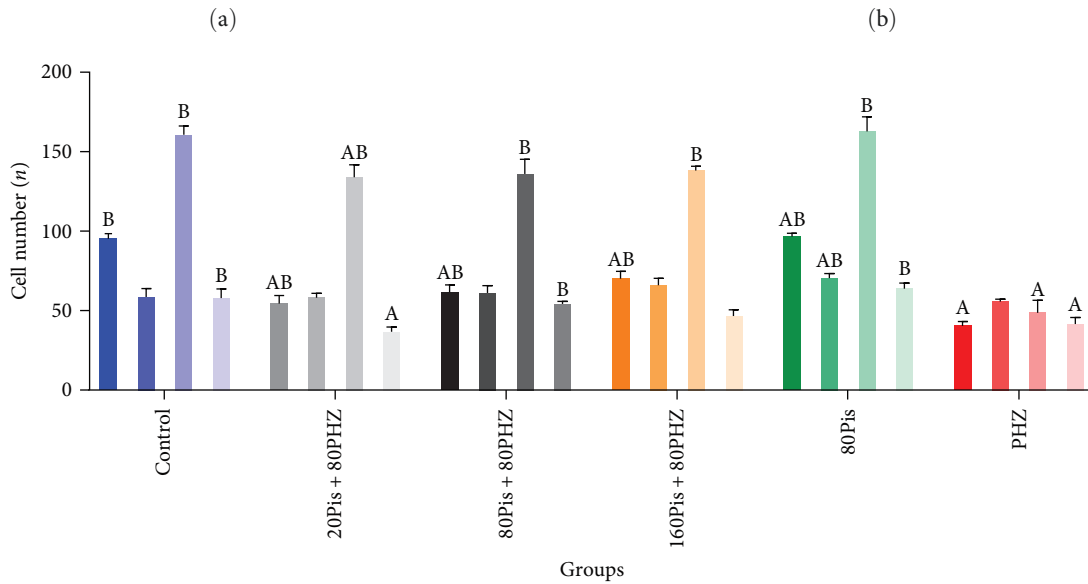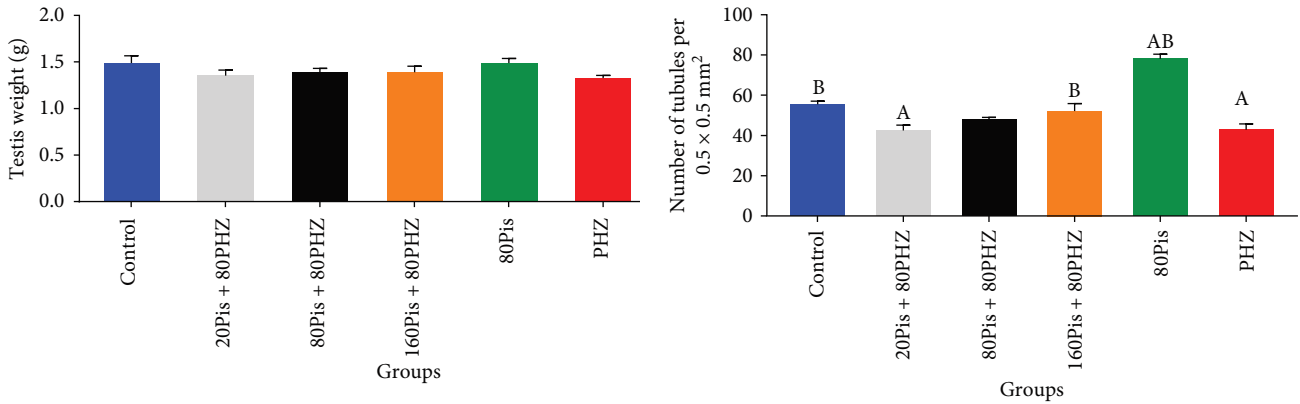
FIGURE 3: Mean ± SD of (a) testis weight, (b) a number of tubules, (c) cell numbers (Leydig cell, primary spermatocyte, spermatid, and spermatogonia), and (d) cross-section area of seminiferous tubules in PHZ + 20pis, PHZ + 80pis, PHZ + 160pis, and 80pis groups comparison with control and PHZ groups after 35 days. PHZ, phenylhydrazine; Pis, aqueous extract of pistachio pericarp; A = when groups compared to control group, B = when groups compared to PHZ group, significant difference between groups was p-value < 0.05.
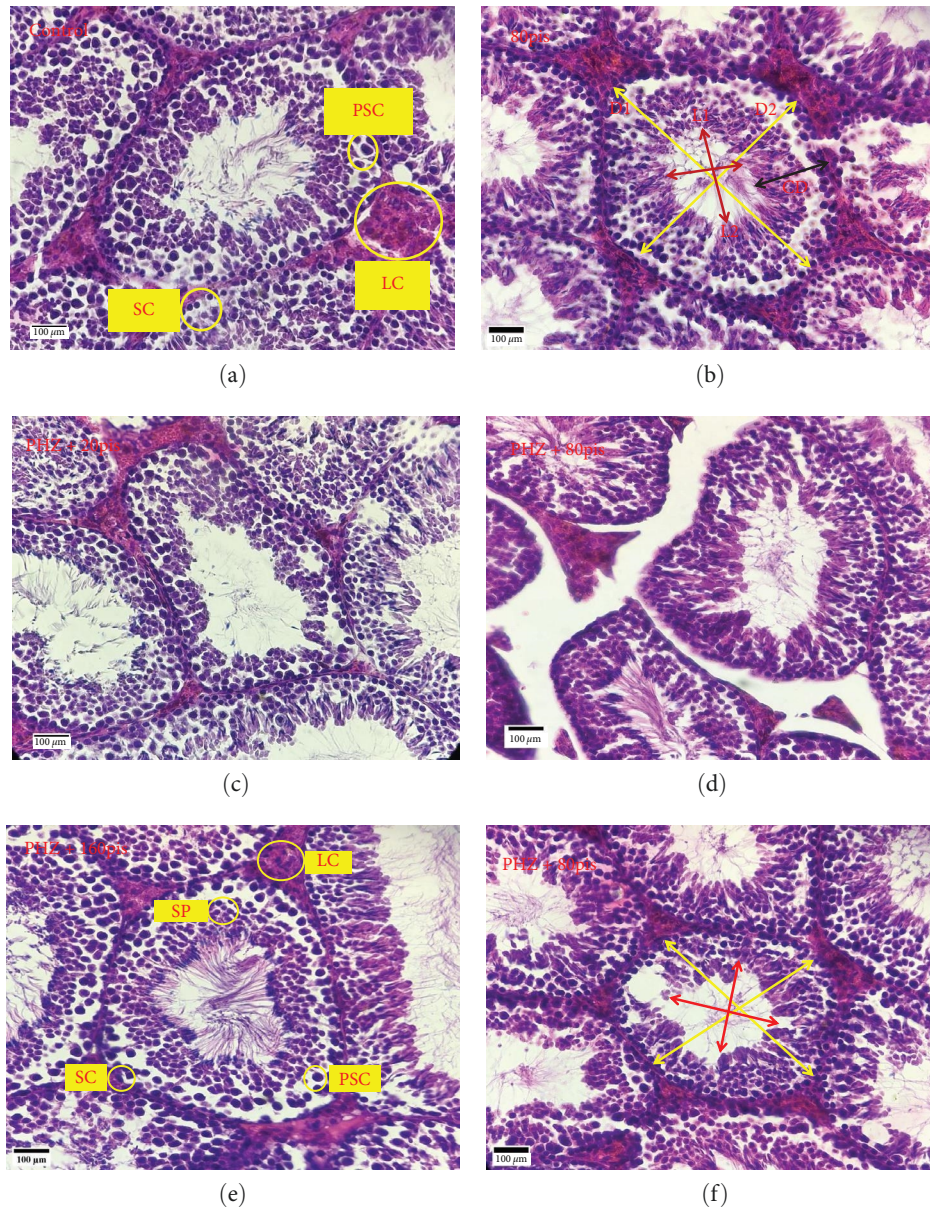
FIGURE 4: (a–f) Hematoxylin–eosin staining of seminiferous tubules for analyses of spermatogenesis and morphometry of testis. D1 and 2, total diameter (yellow arrows), L1 and 2, Lumen diameter (red arrows); CD, cellular diameter (black arrow); LC, Leydig cell; SC, spermatogonia cell; SPC, spermatid cell; PSC, primary spermatocyte cell; PHZ, phenylhydrazine; Pis, aqueous extract of pistachio pericarp. Scale bar = 100 μm.

count, as well as chromatin quality, which includes AB, TB, and CMA3. Additionally, the research indicates a substantial improvement in sex hormones such as FSH, LH, and T. The application of pistachio aqueous extract appears to be associated with a considerable amelioration in sperm abnormalities and pathological damage, suggesting its potential as a beneficial intervention for reproductive health.

The recent investigation revealed a notable decrease in sperm parameters within the PHZ group when compared to the control group. Conversely, there was a notable increase in these parameters within the PHZ + Pis groups as opposed to the PHZ group. The decline in the average sperm count is often attributed to disruptions in the spermatogenesis process and the elimination of sperm at various stages of development [27]. This aligns with the present study's findings, as Karimipour et al. [28] observed significantly lower quality and quantity of sperm parameters in mice induced with PHZ compared to the control group.

Moreover, supporting evidence comes from Mozafari et al. [29], who documented a substantial decrease in the average percentage of sperm count, motility, and normal morphology in mice administered with PHZ in comparison to the control group. These consistent findings across multiple studies underscore the impact of PHZ induction on sperm parameters, emphasizing the potential deleterious effects on spermatogenesis and overall reproductive health. Furthermore, the observed
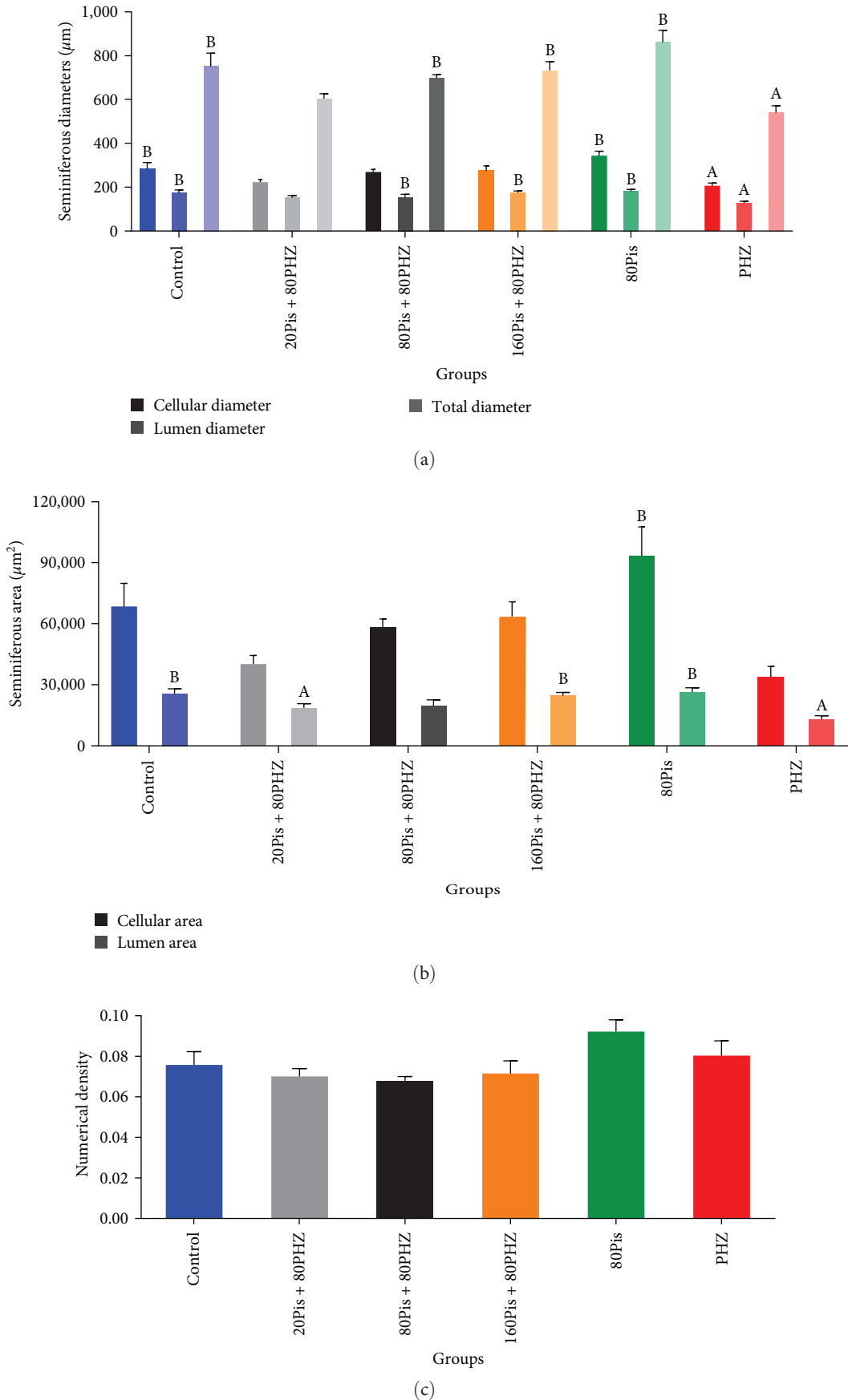
(a)

(b)

(c)

FIGURE 5: Mean ± SD of (a) cellular, Lumen, and total diameters, (b) cellular and luminal areas (μm^2), (c) numerical density of seminiferous tubules in PHZ + 20pis, PHZ + 80pis, PHZ + 160pis, and 80pis groups comparison with control and PHZ groups after 35 days. PHZ, phenylhydrazine; Pis, aqueous extract of pistachio pericap; A = when groups compared to control group, B = when groups compared to PHZ group, a significant difference between groups was p-value < 0.05.

improvements in the PHZ + Pis groups suggest a promising avenue for mitigating these detrimental effects through the administration of pistachio extract.

In a comprehensive research investigation, the impact of pistachio byproducts (PBP) on various parameters related to sperm quality and fatty acid utilization was thoroughly examined. The findings of the study conclusively demonstrated that the inclusion of 12.5% PBP in the diet did not adversely affect sperm parameters, as reported in a previous study [30]. Moreover, a separate investigation highlighted a significant enhancement in the normal morphology, viability, and motility of sperm in a mouse model with infertility issues. This improvement was observed following the administration of Bene powder, derived from *Pistacia atlantica*, at a dosage of 10 mg/kg over a period of 35 days [31]. These compelling results underscore the potential positive impact of pistachio byproducts on reproductive health, providing valuable insights into their influence on sperm quality and overall fertility.

The observed phenomenon can be linked to the heightened generation of free radicals following the administration of PHZ, leading to disruptions in the operational capacity of Leydig and Sertoli cells [32]. The heightened OS adversely affects the synthesis and release of testosterone in Leydig cells. The diminution of this crucial hormone emerges as a pivotal factor contributing to dysfunction in spermiogenesis and a subsequent decline in epididymal sperm count [33]. Consequently, an elevation in OS amplifies the presence of free radicals while concurrently diminishing the antioxidant levels within Leydig cells. However, it is noteworthy that the introduction of pistachio supplementation resulted in a substantial augmentation of sperm count. This suggests a potential mitigating effect of pistachio in counteracting the detrimental impact of OS on Leydig cells, thereby contributing to the improvement of spermiogenesis and an increase in epididymal sperm count.

The elevated generation of free radicals following PHZ administration may be responsible for disrupting the normal functioning of Leydig and Sertoli cells. This heightened OS hampers the synthesis and release of testosterone in Leydig cells. The reduction in testosterone levels plays a significant role in inducing dysfunction in spermiogenesis and lowering the epididymal sperm count. Consequently, an increase in OS contributes to a higher presence of free radicals and a decline in antioxidant levels within Leydig cells. However, it's noteworthy that the supplementation of pistachio resulted in a substantial improvement in sperm count.

In our investigation, we observed a significant decrease in the quantity of T within the group administered with PHZ compared to the control treatment. This decline in T levels suggests that PHZ-induced hypoxia may adversely impact testicular function. Anbara et al. [34] also reported a substantial reduction in T, LH, and FSH levels in response to PHZ administration. The co-occurrence of elevated serum levels of T, FSH, and LH, as indicated by Pis' antioxidant effect, implies that pistachios, with their inherent antioxidant properties, can effectively mitigate the tissue damage caused by PHZ.

Moreover, OS triggered by PHZ is implicated in the generation of free radicals, leading to processes such as sperm membrane formation, unsaturated lipid peroxidation, interstitial cell reduction, and testis tissue death. The decrease in T levels can be attributed to the decline in interstitial cells, as testosterone is a crucial androgen hormone that plays a pivotal role in maintaining the function and structure of male accessory sex glands. Furthermore, the disruption of spermatogenesis is a consequence of testosterone deficiency [35]. In summary, our findings suggest that PHZ-induced hypoxia, coupled with OS, adversely affects testosterone levels and overall testicular function, and the antioxidant properties of pistachios contribute to mitigating these deleterious effects.

Concerning the assessment of chromatin quality using AB, TB, and Chromomycin A3, it is plausible to hypothesize that PHZ induces an increase in DNA damage in sperm. Nuclear transformations transpire during the spermiogenesis phase, a crucial period in which chromatin undergoes concentration, and protamine is substituted by histone, underscoring the importance of fat biosynthesis. Our investigation suggests that OS resulting from PHZ administration has compromised fat biosynthesis, concurrently elevating the average percentage of sperm exhibiting DNA fragmentation [36].

Moreover, our study establishes a distinctive correlation between the generation of ROS and apoptosis, which instigates DNA damage in sperm. Elevated levels of ROS, in particular, directly undermine the DNA of both sperm and oocytes, culminating in sperm death [37]. ROS, in their heightened state, detrimentally impact sperm DNA integrity and contribute to lipid peroxidation in the male reproductive system [38]. Biological membranes, particularly those of sperm, are exceptionally susceptible to the effects of ROS. The escalated production of ROS in the reproductive tract has the potential to compromise both the integrity of DNA in the sperm nucleus and the fluidity of the sperm plasma membrane, thereby leading to spermatogenesis failure and deleterious consequences for sperm. This, in turn, results in lipid peroxidation of the sperm membrane, with significant pathological alterations and infertility being among the negative outcomes [39]. In essence, our findings suggest that PHZ-induced OS adversely affects sperm DNA integrity and membrane structure, potentially contributing to infertility and pathological changes in the male reproductive system.

Our study unveiled a significant reduction in the activities of SOD, GPX, and CAT in the testicular tissue of the PHZ group when compared to the control group. This decline in SOD activity may be linked to the oxidation of cysteine in the enzyme by superoxide anions during its conversion to hydrogen peroxide [40]. The administration of PHZ triggers the production of ROS and free radicals, encompassing hydrogen peroxide and superoxide anions [41]. Furthermore, the heightened lipid peroxidation induced by PHZ has been correlated with a reduction in SOD function, aligning with findings from recent investigations [3, 42]. The decrease in SOD activity could also be ascribed to a concurrent reduction in CAT activity, operating through a feedback inhibition mechanism. The diminished CAT levels lead to an accumulation of

hydrogen peroxide, consequently inhibiting SOD function [43]. Conversely, TOS and OSI levels showed an increase. This suggests that anemia induced by PHZ might contribute to OS, as indicated in our study, although the introduction of Pis appears to mitigate this issue [44].

In line with our findings, Norasteh et al. [31] demonstrated in their study that Bene administration increased the activities of SOD and CAT enzymes in an infertile mouse model. Another study examining the protective effects of bene extracts on hepatotoxicity in rats reported a decrease in the levels of ROS and prevented liver damage, functioning as antioxidants through an elevation in SOD and CAT enzyme activity. These collective results underscore the intricate relationships between PHZ-induced OS, the activities of antioxidant enzymes, and the potential ameliorative effects of interventions like Pis and Bene in counteracting the detrimental impacts on testicular function and overall health.

Anemia has demonstrated adverse effects on the male reproductive system in mice, primarily attributed to the deprivation of oxygen and the induction of OS. This OS stems from heightened production of free radicals, increased lipid peroxidation, and a reduction in glutathione levels [45]. It is crucial to highlight that OS ranks among the primary contributors to male infertility. The equilibrium between pro-oxidants and antioxidants plays a pivotal role in determining the overall oxidative state of cells. Pro-oxidants can be categorized into radicals and nonradicals. The overproduction of ROS in sperm is associated with nuclear DNA fragmentation and lipid peroxidation, ultimately leading to cell death [46]. Spermatozoa are particularly susceptible to an increase in ROS due to their high content of polyunsaturated fatty acids and a limited capacity for DNA repair. Consequently, elevated levels of ROS have been linked to both diminished sperm quality and quantity [47, 48].

Ultimately, the substantial decrease in the activity of antioxidant enzymes induced by PHZ resulted in significant OS and lipid peroxidation. Our study's findings highlight the antianemic potential of *P. vera* pericarp extract, showcasing a high degree of protection against the adverse effects of hemolytic anemia triggered by PHZ. Notably, Pis demonstrated excellent tolerance and safety, proving to be nontoxic, and concurrently exhibited benefits in reducing sperm DNA fragmentation. It is important to emphasize that the pistachio pericarp contains a higher quantity of phenolic compounds compared to its kernel. However, during processing, the green pericarp is separated from the pistachio and is often discarded as pistachio scrap, contributing to various environmental issues. The environmental impact of discarding pistachio pericarp during processing should be carefully considered in light of its rich phenolic content and potential health benefits. This underscores the need for sustainable and environmentally friendly practices in the utilization of pistachio byproducts to harness their valuable bioactive compounds and mitigate environmental concerns.

5. Conclusion

The safeguarding influence of *P. vera* pericarp extract against PHZ-induced impacts on the male reproductive system in mice can be attributed, at least in part, to the presence of abundant antioxidant components exhibiting therapeutic capabilities. The substantial protective effects observed suggest that the extract may play a crucial role in mitigating OS and associated damage in the male reproductive organs. However, to gain a comprehensive understanding of the underlying mechanisms, further research is imperative. Investigating specific cellular and molecular signaling pathways will be crucial in unraveling the intricate details of how *P. vera* pericarp extract exerts its protective effects on the male reproductive system. This deeper exploration will not only enhance our comprehension of the extract's therapeutic potential but also pave the way for the development of targeted interventions for male reproductive health.

Abbreviation

PHZ: Phenylhydrazine
ROS: Reactive oxygen species
Pis: Aqueous extract of pistachio pericarp
AB: Aniline blue staining
TB: Toluidine blue staining
CMA3: Chromomycin A3 staining
LH: Luteinizing hormone
FSH: Follicle-stimulating hormone
E2: Estradiol
T: Testosterone
MDA: Malondialdehyde
SOD: Superoxide dismutase
GPX: Glutathione peroxidase
CAT: Catalase
TAC: Total antioxidant capacity
TOS: Total oxidant status
OSI: Oxidative stress index
OS: Oxidative stress.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable institutional request.

Conflicts of Interest

The authors have no financial or nonfinancial conflicts of interest.

Acknowledgments

References

[1] S. Paul, S. Naaz, A. K. Ghosh, S. Mishra, A. Chattopadhyay, and D. Bandyopadhyay, "Melatonin chelates iron and binds directly with phenylhydrazine to provide protection against phenylhydrazine induced oxidative damage in red blood cells along with its antioxidant mechanisms: an in vitro study," *Melatonin Research*, vol. 1, no. 1, 2018.

[2] P. Sridhar, H. Padmaja, M. Shajina, V. Mirshad, and M. Fasalu, "Hematinic and antioxidant potential of aqueous extract of sesamum indicum seeds against phenylhydrazine-induced hemolytic anemia in albino rats," *National Journal of Physiology, Pharmacy and Pharmacology*, vol. 8, no. 8, Article ID 1092, 2018.

[3] H. Anbara, R. Shahrooz, M. Razi, H. Malekinejad, and G. Najafi, "The effect of vitamin C on mice hemolytic anemia induced by phenylhydrazine: an animal model study using histological changes in testis, pre-implantation embryo development and biochemical changes," *Iranian Journal of Basic Medical Sciences*, vol. 21, no. 7, pp. 668–677, 2018.

[4] U. Midha, J. Aggarwal, and J. Batra, "Oxidative stress and anemia. Review article: oxidative stress in anemia: an update," *Journal of Coastal Life Medicine*, vol. 10, no. 3, pp. 502–506, 2022.

[5] Y. Liu, X. Cao, C. He et al., "Effects of ferroptosis on male reproduction," *International Journal of Molecular Sciences*, vol. 23, no. 13, Article ID 7139, 2022.

[6] J. C. Macome, L. E. Costa, I. H. Martin, and A. C. Taquini, "Steroid biosynthesis by gonads of rats submitted to chronic hypobaric hypoxia," *Acta Physiologica Latino Americana*, vol. 27, no. 5, pp. 249–257, 1977.

[7] P. Sabeti, F. Amidi, S. M. Kalantar et al., "Evaluation of intracellular anion superoxide level, heat shock protein A2 and protamine positive spermatozoa percentages in teratoasthenozoospermia," *International Journal of Reproductive Bio-Medicine*, vol. 15, no. 5, pp. 279–286, 2017.

[8] Y.-M. Sung, S. R. Gayam, and S.-P. Wu, "The oxidation of phenylhydrazine by tyrosinase," *Applied Biochemistry and Biotechnology*, vol. 169, pp. 2420–2429, 2013.

[9] E.-O. J. Ozioma and O. A. N. Chinwe, "Herbal medicines in African traditional medicine," in *Herbal Medicine*.

[10] C. Forni, F. Facchiano, M. Bartoli et al., "Beneficial role of phytochemicals on oxidative stress and age-related diseases," *BioMed Research International*, vol. 2019, Article ID 8748253, 16 pages, 2019.

[11] J. Banerjee, A. Das, M. Sinha, and S. Saha, "Biological efficacy of medicinal plant extracts in preventing oxidative damage," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 7904349, 2 pages, 2018.

[12] A. Tomaino, M. Martorana, T. Arcoraci, D. Monteleone, C. Giovinazzo, and A. Saija, "Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins," *Biochimie*, vol. 92, no. 9, pp. 1115–1122, 2010.

[13] M. Seifaddinipour, R. Farghadani, F. Namvar, J. Bin Mohamad, and N. A. Muhamad, "In vitro and in vivo anticancer activity of the most cytotoxic fraction of pistachio hull extract in breast cancer," *Molecules*, vol. 25, no. 8, Article ID 1776, 2020.

[14] A. Kocyigit, A. A. Koylu, and H. Keles, "Effects of pistachio nuts consumption on plasma lipid profile and oxidative status in healthy volunteers," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 16, no. 3, pp. 202–209, 2006.

[15] N. Parvaz, F. Amin, N. Askari, M. Khademalhosseini, S. K. Falahati-Pour, and S. K. Falahati-Pour, "The protective effect of *Pistacia vera* pericarp on kidney function in rats with hemolytic anemia," *Research Journal of Pharmacognosy*, vol. 9, no. 1, pp. 17–28, 2022.

[16] B. H. Zahra, R. Mahsa, J. Elham et al., "Nephroprotective activity of pistachio kernels in STZ-induced diabetic rats," *Pistachio and Health Journal*, vol. 3, no. 4, pp. 4–20, 2020.

[17] S. Keivani, F. Salamat, M. Emami, P. Adimi, and G. Amin, "In vitro evaluation of the susceptibility of dermatophytic and saprophytic fungi to pistacia vera's pericarp extract," *Medical Science Journal of Islamic Azad Univesity Tehran Medical Branch*, vol. 16, no. 3, pp. 135–140, 2006.

[18] S. Khanamani Falahati-pour, S. Azh, and M. Noroozi, "The hydroalcoholic extract of pistachio kernel and pericarp has an inhibitory effect against prostate cancer cells," *Pistachio and Health Journal*, vol. 2, no. 4, pp. 4–15, 2019.

[19] M. Sabour, A. Khoradmehr, S. M. Kalantar et al., "Administration of high dose of methamphetamine has detrimental effects on sperm parameters and DNA integrity in mice," *International Journal of Reproductive BioMedicine*, vol. 15, no. 3, pp. 161–168, 2017.

[20] A. Danafar, A. Khoradmehr, M. H. Bondarabadi et al., "Impairment of sperm efficiency in mice following short-term nano-titanium dioxide exposure: an experimental study," *International Journal of Reproductive BioMedicine (IJRM)*, vol. 19, no. 12, pp. 1045–1058, 2021.

[21] M. Nazar, A. R. Talebi, M. H. Sharifabad, A. Abbasi, A. Khoradmehr, and A. H. Danafar, "Acute and chronic effects of gold nanoparticles on sperm parameters and chromatin structure in mice," *International Journal of Reproductive BioMedicine*, vol. 14, no. 10, pp. 637–642, 2016.

[22] A. Bahaodini, M. Owjfard, A. Tamadon, and S. M. Jafari, "Low frequency electromagnetic fields long-term exposure effects on testicular histology, sperm quality and testosterone levels of male rats," *Asian Pacific Journal of Reproduction*, vol. 4, no. 3, pp. 195–200, 2015.

[23] X.-X. Han, Y.-P. Jiang, N. Liu et al., "Protective effects of astragalin on spermatogenesis in streptozotocin-induced diabetes in male mice by improving antioxidant activity and inhibiting inflammation," *Biomedicine & Pharmacotherapy*, vol. 110, pp. 561–570, 2019.

[24] F. Radmanesh, M. Razi, and A. Shalizar-Jalali, "Curcumin nano-micelle induced testicular toxicity in healthy rats; evidence for oxidative stress and failed homeostatic response by heat shock proteins 70-2a and 90," *Biomedicine & Pharmacotherapy*, vol. 142, Article ID 111945, 2021.

[25] M. Panahi, S. Keshavarz, F. Rahmanifar et al., "Busulfan induced azoospermia: stereological evaluation of testes in rat," *Veterinary Research Forum*, vol. 6, no. 4, pp. 273–278, 2015.

[26] M. Panahi, N. Karimaghai, F. Rahmanifar et al., "Stereological evaluation of testes in busulfan-induced infertility of hamster," *Comparative Clinical Pathology*, vol. 24, pp. 1051–1056, 2015.

[27] A. Salas-Huetos, M. Bulló, and J. Salas-Salvadó, "Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies," *Human Reproduction Update*, vol. 23, no. 4, pp. 371–389, 2017.

[28] M. Karimipour, Z. Dibayi, A. Ahmadi, M. Z. Javanmard, and E. Hosseinalipour, "The protective effect of vitamin C on phenylhydrazine-induced hemolytic anemia on sperm quality and in-vitro embryo development in mice," *International Journal of Reproductive BioMedicine*, vol. 16, no. 12, 2018.

[29] A. A. Mozafari, R. Shahrooz, A. Ahmadi, H. Malekinjad, and K. Mardani, "Protective effect of ethyl pyruvate on mice sperm parameters in phenylhydrazine induced hemolytic anemia," *Veterinary Research Forum*, vol. 63, no. 8, 2016.

[30] M. Khodaei-Motlagh, M. Zhandi, M. Kazemi-Bonchenari, M. Moradi, and A. Mohamadi, "Sperm quality parameters and fatty acid composition in Farahani rams fed pistachio by-products," *Journal of Livestock Science and Technologies*, vol. 7, no. 1, pp. 39–44, 2019.

[31] H. Norasteh, S. Mohammadi, M. R. Nikravesh, S. Boroumand-Noughabi, and F. Beheshti, "Effects of bene (pistacia Atlantica) on histopathology of testis, sperm chromatin quality and stress oxidative in busulfan-induced infertile mice," *Pharmaceutical Sciences*, vol. 25, no. 1, pp. 24–30, 2019.

[32] E. Fibach and E. Rachmilewitz, "The role of oxidative stress in hemolytic anemia," *Current Molecular Medicine*, vol. 8, no. 7, pp. 609–619, 2008.

[33] L. Cao, S. Leers-Sucheta, and S. Azhar, "Aging alters the functional expression of enzymatic and non-enzymatic anti-oxidant defense systems in testicular rat Leydig cells," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 88, no. 1, pp. 61–67, 2004.

[34] H. Anbara, R. Shahrooz, H. Malekinejad, and S. Saadati, "Protective effects of royal jelly and vitamin C against experimental hemolytic anemia on sex hormones and histochemical testicle tissue histochemistry of adult mice," *Scientific Information Database*, vol. 23, no. 12, pp. 1140–1154, 2016.

[35] R. I. McLachlan, L. O'Donnell, S. J. Meachem et al., "Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man," *Recent Progress in Hormone Research*, vol. 57, no. 1, pp. 149–179, 2002.

[36] D. Komljenovic, R. Sandhoff, A. Teigler, H. Heid, W. W. Just, and K. Gorgas, "Disruption of blood-testis barrier dynamics in ether-lipid-deficient mice," *Cell and Tissue Research*, vol. 337, pp. 281–299, 2009.

[37] O. Adeoye, J. Olawumi, A. Opeyemi, and O. Christiania, "Review on the role of glutathione on oxidative stress and infertility," *JBRA Assisted Reproduction*, vol. 22, no. 1, pp. 61–66, 2018.

[38] Z.-Q. Wu, D.-L. Chen, F.-H. Lin et al., "Effect of bajijiasu isolated from *Morinda officinalis* F. C. how on sexual function in male mice and its antioxidant protection of human sperm," *Journal of Ethnopharmacology*, vol. 164, pp. 283–292, 2015.

[39] S. Zhou, Z. Weng, A. Liang, and S. Zhang, "Experimental research on therapeutic efficacy of traditional Chinese medicine Shengjing capsule extracts in treating spermatogenesis impairment induced by oxidative stress," *Medical Science Monitor*, vol. 22, pp. 50–56, 2016.

[40] I. Plestina-Borjan, D. Katusic, M. Medvidovic-Grubisic et al., "Association of age-related macular degeneration with erythrocyte antioxidant enzymes activity and serum total antioxidant status," *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 804054, 8 pages, 2015.

[41] E. Kocaoğlu, O. Talaz, H. Çavdar, M. Şentürk, C. T. Supuran, and D. Ekinci, "Determination of the inhibitory effects of N-methylpyrrole derivatives on glutathione reductase enzyme," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 34, no. 1, pp. 51–54, 2019.

[42] S. Maity, N. Nag, S. Chatterjee, S. Adhikari, and S. Mazumder, "Bilirubin clearance and antioxidant activities of ethanol extract of Phyllanthus amarus root in phenylhydrazine-induced neonatal jaundice in mice," *Journal of Physiology and Biochemistry*, vol. 69, pp. 467–476, 2013.

[43] Z. Zaka-Ur-Rab, M. Adnan, S. M. Ahmad, and N. Islam, "Effect of oral iron on markers of oxidative stress and antioxidant status in children with iron deficiency anaemia," *Journal of Clinical and Diagnostic Research: JCDR*, vol. 10, no. 10, pp. SC13–SC19, 2016.

[44] M. Tolooei and A. Mirzaei, "Effects of pistacia Atlantica extract on erythrocyte membrane rigidity, oxidative stress, and hepatotoxicity induced by CCl4 in rats," *Global Journal of Health Science*, vol. 7, no. 7, 2015.

[45] P. Kaur and M. P. Bansal, "Effect of experimental oxidative stress on steroidogenesis and DNA damage in mouse testis," *Journal of Biomedical Science*, vol. 11, pp. 391–397, 2004.

[46] W. Liu, F. Gong, K. Luo, and G. Lu, "Comparing the pregnancy rates of one versus two intrauterine inseminations (IUIs) in male factor and idiopathic infertility," *Journal of Assisted Reproduction and Genetics*, vol. 23, pp. 75–79, 2006.

[47] S. Bisht, M. Faiq, M. Tolahunase, and R. Dada, "Oxidative stress and male infertility," *Nature Reviews Urology*, vol. 14, pp. 470–485, 2017.

[48] S. Pourmasumi, P. Sabeti, T. Rahiminia, E. Mangoli, N. Tabibnejad, and A. R. Talebi, "The etiologies of DNA abnormalities in male infertility: an assessment and review," *International Journal of Reproductive Biomedicine*, vol. 15, no. 6, pp. 331–344, 2017.