



The Potential of Passion Fruit (*Passiflora edulis*) Extract in Enhancing Reproductive Performance in Mice (*Mus musculus*)

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ABSTRACT

Passion fruit (*Passiflora edulis*) contains bioactive antioxidants such as flavonoids, polyphenols, and vitamin C, which are believed to support testicular function and protect germ cells from oxidative stress. This study aimed to examine the effects of *P. edulis* extract on reproductive parameters in male mice, including sperm quality, testicular morphology, testosterone levels, and histological features related to spermatogenesis. Twenty-four adult male mice were randomly divided into four groups: a control group and three treatment groups receiving 100, 200, and 400mg/kg body weight of *P. edulis* extract for 28 days. Sperm concentration, motility, and morphology were evaluated, followed by measurements of testicular weight, diameter, and volume. Serum testosterone levels were analyzed, and testicular tissues were processed for histology using the Johnsen scoring system and a spermatogenic index. The findings showed that the extract produced a general improvement across several reproductive parameters. Although not all differences were statistically significant, mice in the treatment groups tended to exhibit higher sperm concentration and motility compared to controls. A marked reduction in sperm abnormalities was observed at the highest dosage. Histological evaluation revealed more organized seminiferous tubules, thicker germinal epithelium, and a greater presence of mature spermatozoa in the treated groups. These observations were consistent with increased Johnsen scores and spermatogenic indices, suggesting enhanced germ cell development. The overall pattern indicates that the antioxidant components of *P. edulis* help maintain testicular architecture and support spermatogenesis. In conclusion, the *Passiflora edulis* extract shows potential as a natural supplement to improve male reproductive performance, although further studies are needed to clarify its molecular mechanisms.

Keywords: *Passiflora edulis*; Spermatogenesis; Sperm quality; Testicular histology; Male fertility.

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INTRODUCTION

Malino, located in Gowa Regency, South Sulawesi, is known for its cool climate and fertile soil, making it an ideal location for cultivating a range of horticultural crops, including passion fruit (*Passiflora edulis*). Passion fruit cultivated in Malino contains high levels of bioactive compounds such as flavonoids, alkaloids, and vitamin C, which are known to possess antioxidant properties and have the potential to enhance reproductive performance

(Abarikwu, 2017). Agro-environmental conditions, including temperature, can modulate the biosynthesis and accumulation of phytochemicals in plant-derived materials, thereby influencing the biological efficacy of their extracts in enhancing male reproductive performance (Sephefar et al., 2023). Optimizing reproductive performance through dietary strategies and the incorporation of natural bioactive compounds continues to represent a significant scientific and practical challenge in animal reproduction systems (BenSouf et al., 2025).

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Passion fruit (*Passiflora edulis*) is known to be rich in bioactive compounds such as flavonoids, alkaloids, and vitamin C, which have antioxidant effects (Zhang et al., 2023; Pereira et al., 2023). These compounds have the potential to protect germ cells from oxidative damage and improve sperm quality (Hungerford et al., 2024; Wang et al., 2025). Previous studies have shown that supplementation with natural antioxidants can improve reproductive parameters in test animals. For example, flavonoids have been proven to increase spermatozoa quality and protect against oxidative damage in animal models (Abarikwu, 2017). Flavonoids have been reported to modulate androgen synthesis and support spermatogenesis by regulating steroidogenic pathways in Leydig cells and interacting with the reproductive endocrine system, including the upregulation of key steroidogenic proteins such as steroidogenic acute regulatory (StAR) protein that facilitates testosterone biosynthesis in the testes (Martin & Touaibia, 2024). Enhanced testosterone availability subsequently contributes to the maintenance of spermatogenic activity and androgen-dependent Sertoli cell function, highlighting the role of flavonoid-rich plant sources in improving male reproductive performance (Mishra et al., 2024).

The use of passion fruit extract originating from Malino is an important aspect in exploring local potential as a natural solution in the field of animal reproduction. The content and yield of bioactive compounds in *Passiflora edulis* are closely associated with plant origin and production conditions, which determine the antioxidant potential of materials derived from passion fruit processing, including seeds and by-products (dos Santos et al., 2023). Passion fruit containing flavonoids has the potential to increase testosterone levels, support the spermatogenesis process, and maintain healthy testicular histology. Therefore, this research not only contributes to the development of animal reproductive science, but also supports the sustainable utilization of local natural resources.

This study aims to evaluate the effects of passion fruit extract on the reproductive performance of mice (*Mus musculus*) as an animal model. In addition, this study also aims to determine the optimal extract dose that provides the best effect on male reproductive parameters, as well as to assess the potential mechanisms of action of active compounds in passion fruit extract on the male reproductive system. The urgency of this research lies in its contribution to developing alternative natural supplements that have the potential to improve male fertility. With increasing awareness of the use of natural ingredients in the livestock industry, the results of this study may serve as a basis for developing functional feeds or herbal supplements that are safer and more effective.

MATERIALS & METHODS

Animals and Ethical Approval

Twenty-four healthy adult male mice (*Mus musculus*), aged 8–10 weeks and weighing 30–40g, were used in this study. The animals were obtained from the Animal Laboratory, Animal Hospital, Hasanuddin University. All

experimental procedures were approved by the Animal Ethics Committee, Hasanuddin University (Ethical Clearance No. 028/UN4.1.RSHUH/B/PP36/2025; October 21, 2025), and conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Mice were housed under controlled environmental conditions (25±2°C; 12 h light/dark cycle; relative humidity 50–60%) with ad libitum access to a standard pellet diet (PT Charoen Pokphand Indonesia, Sidoarjo, Indonesia) and water. Before the treatment period began, all mice were acclimatized for one week to minimize stress-related effects during the experiment.

Treatments

The mice were randomly assigned to four groups (n = 6 per group), P0 (Control): received aquadest orally (vehicle), P1: received *Passiflora edulis* extract at 100mg/kg body weight (BW), P2: received *P. edulis* extract at 200mg/kg BW, P3: received *P. edulis* extract at 400mg/kg BW. The extract was administered once daily via oral gavage for 28 consecutive days. The extract was administered once daily via oral gavage for 28 consecutive days. The dosages were selected based on preliminary studies and relevant literature on the pharmacological activity of *Passiflora* species.

The doses of *Passiflora edulis* extract (100, 200, and 400mg/kg BW) were selected based on previous experimental studies evaluating the reproductive and antioxidant effects of flavonoid-rich plant extracts in rodents. Moderate doses (100–200mg/kg BW) have been reported to improve sperm quality, testicular histology, and hormonal balance through antioxidant mechanisms (Ahmadi et al., 2016). The higher dose (400mg/kg BW) was included to assess potential dose-dependent responses and to evaluate the possibility of an antioxidant paradox effect at elevated concentrations, as reported in antioxidant-related reproductive studies (Moazamian et al., 2025).

Preparation of *Passiflora edulis*

Extract *Passiflora edulis* was thoroughly washed, then cut into pieces and dried using an oven. The dried passion fruit is then blended into a fine powder. This powder is subsequently macerated in 70% ethanol solution for 3×24 hours. The resulting filtrate is collected and then evaporated using a rotary vacuum evaporator to obtain the pure extract of passion fruit. The maceration process followed the modified method of Gonzalez et al. (2019), which reported high recovery of polyphenols and flavonoids from *P. edulis* peel using 70% ethanol.

Body Weight Measurement

Body weight (BW) of all animals was recorded at baseline and weekly during the treatment period. At the end of the study, body weight was measured prior to sacrifice to assess whether the treatments had any effect on general growth and metabolic status.

Necropsy and Testicular Sampling

On day 29, mice were anesthetized using ketamine

(80mg/kg BW) and xylazine (10mg/kg BW) administered intramuscular. Blood samples were collected via retro-orbital sinus puncture and centrifuged at 3,000rpm for 15min to separate serum. Serum aliquots were stored at -20°C until hormonal analysis. Animals were then euthanized by cervical dislocation under anesthesia. Both testes were carefully dissected, freed from adherent tissues, and weighed individually using a digital balance ($\pm 0.001g$). The testes were then measured for length and diameter using a digital caliper. Testicular volume was determined using the water displacement method, expressed in milliliters (mL). For each animal, mean values of the right and left testes were used for statistical analysis of testis weight (TW), testis diameter (TD), and testis volume (TV).

Hormonal Assay

Testosterone levels were measured using the ELISA (enzyme-linked immunosorbent assay) method based on a competitive enzyme immunoassay Testosterone EIA-1559, DRG Instruments GmbH, Frauenbergstrasse 18, 35039 Marburg, Germany). Microplates were coated with mouse monoclonal antibodies specific to the testosterone molecule. Samples were added to the wells and competed with enzyme-conjugated testosterone for antibody binding. Following incubation, unbound hormones were removed by washing, and a substrate solution was added, resulting in a blue-colored reaction. The color intensity was inversely proportional to testosterone concentration, with darker blue indicating lower levels and lighter blue indicating higher levels. The enzymatic reaction was terminated by adding a stop solution, resulting in a yellow color. Absorbance was measured at 450nm using an ELISA reader, and testosterone concentrations (Jangid et al., 2024).

Preparation of Testicular Histology Slides

Necropsy was performed on the mice beginning with the euthanasia process. An incision was made in the abdomen to remove the testicular organs, which were then placed into 10% formalin to prevent tissue damage. The preparation of histological slides followed the standard operating procedure of the Integrated Laboratory of the Hasanuddin University Veterinary Teaching Hospital, beginning with transverse sectioning of the testes. The dehydration process was carried out using graded alcohols (70, 80, 90, and 95%), each for one day. The testicular tissue was then placed in 100% alcohol 1 for 1 hour, followed by 100% alcohol 2 for 1 hour. The tissue was then placed into the clearing process using xylol 1 for 30min, and then xylol 2 for 30min. The tissue was subsequently placed into molten paraffin for infiltration and then placed in an incubator at 56°C for 2hours. Embedding of the organ in paraffin was carried out. Sectioning was then performed using a microtome at a thickness of 4 μ m and placed on a glass slide, followed by incubation. Staining was performed using Hematoxylin-Eosin to evaluate seminiferous tubule architecture and spermatogenic cell layers, following established histopathological protocols for testicular tissue analysis (Bacha & Wood, 2012).

Observation and Data Collection

The mean testicular biopsy score (MTBS) was

determined by the Johnsen's scoring system as an index of spermatogenesis (Table 1) and spermatogenic index was determined as earlier reported (Table 2) (Ogundola et al., 2021; Afolabi et al., 2022).

Table 1: Johnsen's scoring system for assessing mean testicular biopsy score (MTBS)

Grade	Features
10	Complete spermatogenesis with many spermatozoa
9	Many spermatozoa present but disorganized germinal epithelium
8	Only a few spermatozoa (<5-10) present
7	No spermatozoa but many spermatids present
6	No spermatozoa and only a few spermatids (<5-10) present
5	No spermatozoa or spermatids but several or many spermatocytes present
4	Only a few spermatocytes (<5) and no spermatids or spermatozoa present
3	Spermatogonia are the only germ cells present
2	No germ cells but Sertoli cells are present
1	No cells (either germ cell or Sertoli cell) in the tubular section

Table 2: Assessment of spermatogenic index

Grade	Features
4	Only spermatogonia present
3	Spermatogonia and spermatocytes present
2	Spermatogonia, spermatocytes, and round (early) spermatids present with <5 late spermatids per tubule
1	Spermatogonia, spermatocytes, and round spermatids present with up to 25 late spermatids per tubule

Observation of Sperm Quality Samples Sperm Motility

To determine sperm motility, one drop of sperm suspension was taken using a dropper pipette and placed on a glass slide, then covered with a cover slip. The wet preparation was then observed under a microscope at 400 \times magnification. In each field of view, the movement of individual spermatozoa was examined.

Sperm Concentration

For sperm concentration, the sperm suspension was taken using a dropper pipette and 0.005mL of the sample was aspirated. The suspension was dropped from the pipette at the edge of the cover glass until it spread evenly. The counting chamber of the Improved Neubauer hemocytometer was placed under a microscope at 400 \times magnification.

Sperm Morphology

Sperm morphology was observed using a glass slide, and a smear preparation was made. The evaluation was done by examining abnormal sperm morphology among 100 randomly selected spermatozoa stained with eosin-nigrosine. Counting was performed under a microscope at 400 \times magnification, and observations included normal sperm morphology and abnormalities such as primary abnormalities, secondary abnormalities and sperm agglutination.

Statistical Analysis

Data in this study were analyzed descriptively and qualitatively to explain the effect of passion fruit extract administration. In addition, a one-way ANOVA was performed to test each variable among the four treatments, followed by Tukey's post hoc test for multiple comparisons. Data analysis was conducted using SPSS for Windows version 26.

RESULTS & DISCUSSION

Body Weight

At the end of the experimental period, no significant differences in body weight (BW) were observed among the groups ($P > 0.05$). This finding indicates that administration of *Passiflora edulis* extract did not influence general growth or metabolic status of the mice.

Testicular Morphometry

Testicular morphometric analysis revealed significant group differences ($P < 0.05$). The testis weight (TW), diameter (TD), and volume (TV) were highest in the P1 group (100mg/kg BW), followed by P2 (200mg/kg BW), while P3 (400mg/kg BW) showed values comparable to the control group (P0). Testis weight (TW): P1 exhibited significantly higher TW compared to P0 and P3 ($P < 0.05$), whereas P2 showed moderate improvement without statistical significance.

Testis diameter (TD): P1 displayed the greatest TD, significantly different from P0 and P3 ($P < 0.05$). Testis volume (TV): Similar to TD and TW, TV was highest in P1 and significantly different from P0 ($P < 0.05$). These results suggest that the 100mg/kg BW dose of *P. edulis* extract produced the most beneficial effect on testicular morphometry (Fig. 1).

Testicular morphometry, including testis weight, seminiferous tubular diameter, and testis volume, serves as a reliable indicator of spermatogenic activity and overall testicular health. An increase in these parameters is commonly associated with enhanced spermatogenesis and steroidogenesis (Kaltsas, 2023). In this study, mice treated with 100mg/kg BW *P. edulis* extract (P1) exhibited significant improvements in all morphometric parameters. These findings suggest that *P. edulis* supplementation may have enhanced germ cell proliferation and survival, as well as improved the seminiferous tubule microenvironment. The observed improvements can be attributed to the antioxidant properties of *P. edulis*. The fruit is rich in flavonoids such as quercetin, luteolin, and apigenin, as well as vitamin C and polyphenolic compounds, which are known to

neutralize ROS and protect cellular structures from oxidative damage (Qureshi & Shaikh, 2025). By reducing oxidative stress in testicular tissue, *P. edulis* extract may help preserve the integrity of Sertoli and Leydig cells, both of which play critical roles in spermatogenesis and testicular morphometry.

Notably, the lack of improvement at higher doses (200 and 400mg/kg BW) suggests the presence of hormesis, where moderate doses confer benefits but excessive doses exert neutral or even adverse effects. Similar observations were reported by Lopes-Ferreira et al. (2025), who noted that comparable pattern has been described for phenolic phytochemicals, in which moderate exposure supports spermatogenic activity and male reproductive function, while excessive intake may attenuate these benefits due to redox imbalance and interference with cellular signaling pathways involved in spermatogenesis. The enhancement of testicular morphometric parameters in the P1 group corresponds with evidence that antioxidant polyphenols can protect the seminiferous epithelium from oxidative damage and support the maintenance of tubular structure and germ cell viability under stress conditions (Koohkan et al., 2025). These bioactive compounds mitigate reactive oxygen species accumulation and help preserve redox homeostasis, which is critical for supporting spermatogenic cell proliferation and differentiation within the seminiferous tubules (Zaki et al., 2024).

Serum Testosterone Levels

The results showed clear differences in testosterone levels among the treatment groups. The highest testosterone concentration was observed in the P1 group (2.63 ± 0.57 ng/mL), which was significantly ($P < 0.05$) higher than in P2 (1.11 ± 0.35 ng/mL), P0 (0.2 ± 0.03 ng/mL), and P3 (0.18 ± 0.04 ng/mL). No significant differences were found among the P2, P3, and P0 groups (Fig. 2). Overall, administration of *P. edulis* extract at a dose of 100mg/kg BW (P1) significantly improved testicular morphometric parameters and serum testosterone levels compared to the control group. In contrast, higher doses (200–400mg/kg BW) did not produce meaningful improvements, illustrating the presence of a hormetic effect.

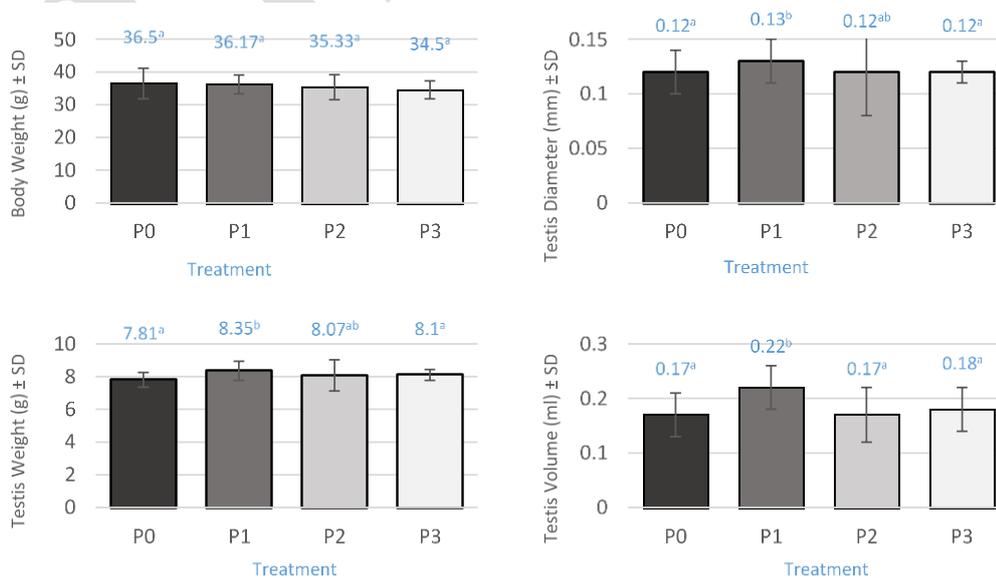


Fig. 1: Effect of *Passiflora edulis* extract on body weight and testicular morphometry in male mice (mean \pm SD, n=6). Different superscripts within the same column indicate significant differences ($P < 0.05$, Tukey HSD).

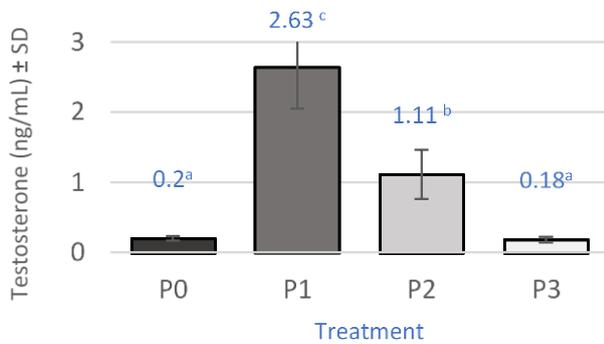


Fig. 2: Effect of *Passiflora edulis* extract on serum testosterone levels in male mice (mean±SD, n=6). Different superscripts within the same column indicate significant differences ($P < 0.05$, Tukey HSD).

The most striking finding of this study was the significant increase in serum testosterone levels in P1 (100mg/kg BW) compared to all other groups. Testosterone synthesis is primarily regulated by the activity of Leydig cells, which are highly sensitive to oxidative damage. ROS accumulation within Leydig cells impairs the steroidogenic acute regulatory protein (StAR), a key transporter of cholesterol into mitochondria for testosterone biosynthesis (Martin & Touaibia, 2024). Antioxidants have been shown to restore StAR expression, protect mitochondrial function, and enhance testosterone synthesis. The enhanced testosterone levels observed in P1 can therefore be explained by the antioxidant and anti-inflammatory activities of *P. edulis* bioactive compounds. Vitamin C, abundant in *P. edulis*, also contributes to steroidogenesis by protecting Leydig cells from oxidative apoptosis and maintaining luteinizing hormone (LH) responsiveness. Interestingly, serum testosterone did not improve in P2 and P3 groups despite higher extract doses.

This supports the hypothesis that excessive antioxidant supplementation may paradoxically lead to pro-oxidant effects, impairing steroidogenesis rather than enhancing it. Thus, the optimal dose of *P. edulis* for improving testosterone appears to be 100 mg/kg BW in this experimental model. Comparable dose-dependent elevations in testosterone have also been documented following dietary flavonoid intake, where compounds such as quercetin- and catechin-rich flavonoids were shown to modulate androgen profiles through antioxidant and steroidogenic regulatory mechanisms (Hu et al., 2023). According to Martin and Touaibia (2024), physiological levels of polyphenolic antioxidants support steroidogenic function by maintaining StAR-mediated cholesterol transport, whereas excessive antioxidant intake may interfere with redox-sensitive signaling pathways essential for normal androgen biosynthesis, ultimately leading to reduced testosterone production.

Spermatozoa Quality Observation

The spermatozoa quality parameters observed were concentration, motility, and abnormalities. The highest spermatozoa concentration was observed in P3 (400mg/kg BW) at 102.92×10^6 /mL, while the lowest was found in P0 group (56×10^6 /mL). However, the differences among groups were not significant. Sperm motility also showed an increase in the treatment groups, with the highest value in

P1 (32.50%) and the lowest in P0 group (19.00%). Unlike concentration and motility, the percentage of sperm abnormalities showed a significant difference ($P < 0.05$), with the control group displaying the highest abnormality rate (31.17%) and P3 showing the lowest (22.00%). These findings indicate that passion fruit extract has a significant effect in reducing sperm abnormalities (Fig. 3).

The results showed a tendency for increased spermatozoa concentration in treatment groups (P1–P3) compared with the control, although the differences were not statistically significant. This improvement may be attributed to the antioxidant compounds present in passion fruit extract, including flavonoids, polyphenols, and vitamin C. These bioactive molecules protect germ cells from oxidative stress and support spermatogenesis (Ahmadi et al., 2016; Mishra et al., 2024). Flavonoids have also been reported to enhance reproductive hormones such as FSH, LH, and testosterone, reduce sperm apoptosis, and improve mitochondrial integrity, which contributes to better sperm motility and concentration (Zhao et al., 2023).

Although the statistical analysis did not reveal significant differences, increases in progressive motility across treatment groups suggest that passion fruit extract may enhance sperm motility. Previous studies likewise demonstrate that flavonoids, polyphenols, and vitamin C enhance sperm quality by strengthening membrane integrity, mitigating oxidative damage, and improving ATP supply for flagellar movement (Ahmadi et al., 2016; Mishra et al., 2024). This aligns with the findings of Shahedi et al. (2021), which show that antioxidant compounds can improve mouse sperm motility.

However, some literature also highlights the "antioxidant paradox," in which excessive antioxidant intake may disrupt the redox balance required for spermatogenesis, ultimately impairing testicular function (Moazamian et al., 2025). Despite this, the present study observed a reduction in abnormal sperm morphology in the treatment groups, likely due to the protective actions of flavonoids, polyphenols, and vitamin C on plasma membranes, sperm head–tail formation, and DNA integrity (Ahmadi et al., 2016; Zhao et al., 2023).

Vitamin C acts as a reducing agent that supports cellular redox homeostasis and optimizes spermatogenesis, contributing to the production of morphologically normal spermatozoa. It also plays a role in repairing sperm DNA damage, reducing the occurrence of abnormalities such as bent tails, detached heads, or irregular head shapes, which were observed in both control and treatment groups (Mishra et al., 2024).

Histological Findings

Histological observations in this study revealed that *Passiflora edulis* extract improved the organization and integrity of the seminiferous tubules, supporting normal progression of spermatogenesis. In the treatment groups, particularly P3, P2 and P1, the germinal epithelium appeared thicker and more organized, displaying complete layers of spermatogenic cells from basal spermatogonia, primary and secondary spermatocytes, to round and elongated spermatids near the lumen (Fig. 4).

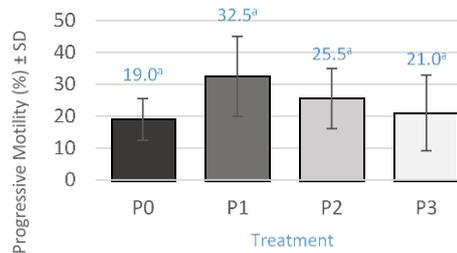
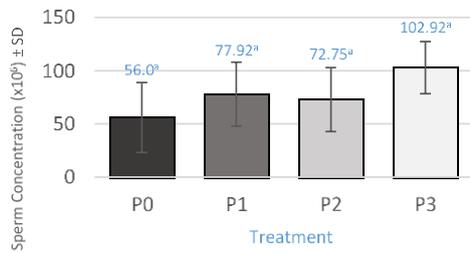


Fig. 3: Effect of *Passiflora edulis* extract on concentration, motility, and abnormalities sperm in male mice (mean±SD, n=6). Different superscripts within the same column indicate significant differences ($P < 0.05$, Tukey HSD).

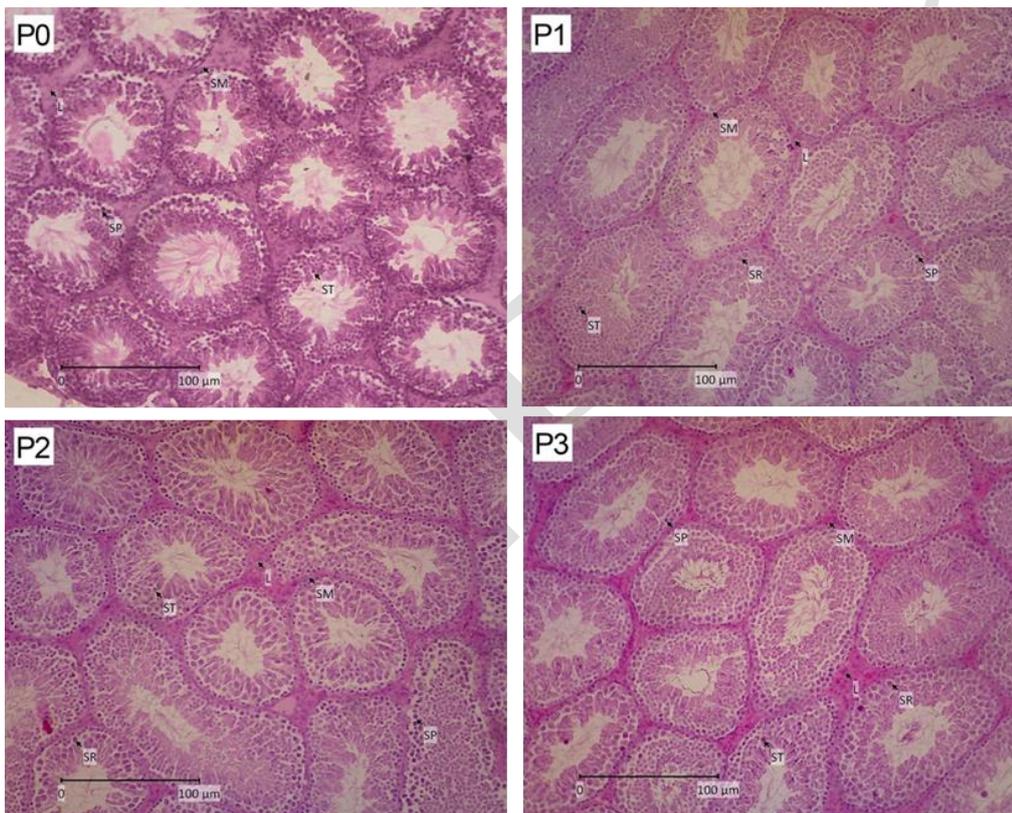
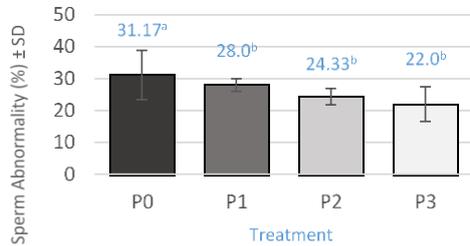


Fig. 4: Representative histological cross-sections of seminiferous tubules in male mice from the control group (P0) and *Passiflora edulis* extract-treated groups (P1, P2, and P3). SM = smooth muscle; SR = sertoli; SP = spermatogonium primer; ST = spermatid; L = leydig. Testicular tissues were stained with hematoxylin and eosin (H&E) and observed under light microscopy.

This indicates that the stages of proliferation, meiosis, and spermiogenesis proceeded efficiently. In contrast, the control group showed thinning of the germinal epithelium, sloughed germ cells, and fewer mature spermatozoa, suggesting disrupted spermatogenesis.

These microscopic features are consistent with higher qualitative spermatogenic maturity that is commonly summarized by histopathological scoring systems such as the Johnsen score and complementary spermatogenesis indices, which integrate the completeness of germ-cell layers, the presence of late spermatids/spermatozoa in the lumen, and preservation of tubular integrity (Dehdari Ebrahimi et al., 2023). The histological findings also support a dose-related interpretation in which higher extract doses tend to coincide with stronger structural

preservation of the seminiferous epithelium and improved germ-cell survival, reflected by fewer signs of epithelial thinning, cellular sloughing, or reduced luminal maturation compared with the control. Such tissue-level protection is biologically plausible in antioxidant-based interventions, where mitigation of oxidative injury in the testes can translate into better maintenance of spermatogenic cell populations and more favorable histology-derived indices (Dehdari Ebrahimi et al., 2023). Likewise, experimental evidence from toxicant-induced testicular injury models shows that antioxidant-related protection can improve sperm parameters while simultaneously yielding higher Johnsen-type histological scores and better overall spermatogenic organization (Ebaid et al., 2025).

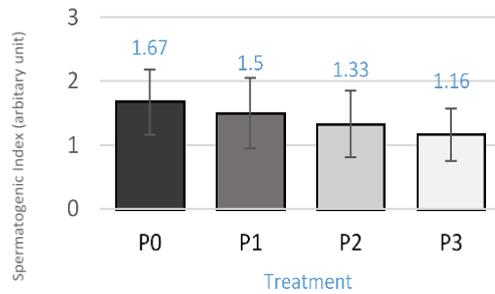
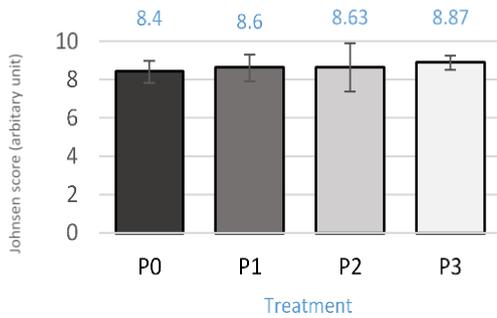


Fig. 5: Effect of *Passiflora edulis* extract on Johnsen score's and Spermatogenic index in male mice (mean±SD, n=6). Different superscripts within the same column indicate significant differences (P<0.05, Tukey HSD).

These qualitative improvements were consistent with the higher Johnsen scores and spermatogenic index observed in the treatment groups, which reflect better Sertoli cell function and enhanced germ cell maturation (Fig. 5). The protective effect of *P. edulis* is likely attributable to its antioxidant components, particularly flavonoids and vitamin C (Antinozzi et al., 2025), which protect testicular cells from oxidative stress and cellular damage, factors known to impair spermatogenesis (Bouhadana et al., 2025; Ogunleye et al., 2025). Likewise, Abarikwu (2017) found that flavonoid-rich plant extracts improve spermatogenic cell survival, while Agarwal et al. (2014) emphasized the essential role of oxidative balance in maintaining spermatogenic efficiency. Furthermore, Xu et al. (2020) reported that genipin improves testicular morphology and spermatogenic cell integrity in mice with circadian disruption, supporting the role of natural bioactive compounds in maintaining testicular histoarchitecture. These collective findings support the interpretation that *P. edulis* enhances spermatogenesis by structurally protecting the seminiferous tubules and reducing oxidative damage.

The highest dose group exhibited superior preservation of tubular architecture. The P3 group exhibited the most prominent tubular organization, aligning with the highest Johnsen score (8.87) observed among all experimental groups, characterized by a compact and well-arranged germinal epithelium and the presence of a complete spermatogenic sequence extending from basal spermatogonia through spermatocytes to abundant round and elongated spermatids near the lumen. Intermediate dose groups displayed histological features similarly organized but slightly less pronounced, Tubules in P2 (8.63) and P1 (8.60) displayed slightly lower yet comparable completeness of germ cell lineage progression, reflecting effective progression through meiotic and post-meiotic stages, whereas the control group showed comparatively reduced epithelial thickness and less advanced germ-cell maturation. The control group (P0) had the lowest Johnsen score (8.40) with histological features of thinner germinal epithelium, less orderly cellular arrangement, and reduced numbers of mature spermatids in the lumen.

Interestingly, in contrast to the upward trend in Johnsen's score with increasing extract dose, the spermatogenic index decreased from P0 (1.67) to P3 (1.16), indicating higher early spermatogenic activity in control and low-dose groups and a relative stabilization of mature cell populations in higher dose groups. These observations

align with previous reports indicating that histology-driven spermatogenic indices, including Johnsen scoring, are sensitive markers of subtle alterations in germ-cell layering and differentiation status (He et al., 2022). Furthermore, accumulating evidence suggests that antioxidant- and polyphenol-rich interventions support seminiferous tubule integrity and germ-cell survival by mitigating oxidative stress and inflammatory signaling within the testicular microenvironment (Guo et al., 2024; Mobasher et al., 2024). Such protective effects are also consistent with broader findings that dietary antioxidants and micronutrients modulate spermatogenic efficiency and tubular morphology through redox-sensitive regulation of mitochondrial function and sperm energy metabolism, leading to differential responses across reproductive endpoints (Ferramosca & Zara, 2022; Asghar & Wan, 2025).

Biological Mechanisms and Hormetic Response

The reproductive responses observed in this study reflect a complex dose-dependent pattern in which different parameters reached their optimal biological effect at different extract concentrations. Testicular morphometry did not differ significantly across groups, although the P3 dose showed a slight tendency toward better structural preservation. This suggests that at lower doses, *Passiflora edulis* extract may provide mild protective effects on testicular tissue without producing measurable morphological changes. A clearer dose response was seen in endocrine function. Testosterone levels increased significantly only in P1, which differed markedly from P2, P3, and P0 groups. This indicates that the 100mg/kg BW dose was the most effective in supporting Leydig cell steroidogenesis. Moderate antioxidant exposure may enhance mitochondrial function and promote the activity of steroidogenic enzymes (Martin & Touaibia, 2024; Chen et al., 2025), whereas higher doses may begin to interfere with physiological ROS signaling needed for hormonal regulation—a pattern consistent with hormetic dynamics reported in earlier literature (Calabrese & Mattson, 2017).

In contrast to hormonal findings, several sperm parameters showed optimal responses at higher doses. Sperm concentration followed the pattern P3>P2>P1>P0, suggesting that the antioxidant activity of *P. edulis* at higher doses was more effective in maintaining germ-cell viability during spermatogenesis. A similar trend was observed in sperm morphology, where abnormality rates were lowest in P3 and progressively higher toward the control group. The improved morphology at higher doses

is consistent with the ability of flavonoids and vitamin C to stabilize sperm plasma membranes, reduce lipid peroxidation, and preserve DNA integrity (Wang et al., 2025; Signorini et al., 2025).

Motility, however, showed a different pattern, with P1 demonstrating the highest value, followed by P2, P3, and P0 group. This suggests that although higher doses supported structural and numerical sperm parameters, moderate doses may better enhance mitochondrial efficiency and ATP production required for progressive motility (Vahedi Raad et al., 2024). Histological indices further supported these findings. Both the spermatogenesis index and Johnsen score followed the pattern P3 >P2>P1>P0, indicating that higher doses provided greater protection to seminiferous tubules and improved germ-cell layering and maturation. These improvements in cellular organization correspond with the reduced sperm abnormalities observed and reflect enhanced germ-cell survival under higher antioxidant availability (Bouhadana et al., 2025).

Taken together, the findings reveal that *P. edulis* extract does not exert uniform effects across all reproductive parameters. Instead, hormonal enhancement peaked at the lowest dose (P1), whereas structural and spermatogenic improvements were strongest at the highest dose (P3). This divergence underscores the hormetic nature of phytochemical exposure, where distinct physiological systems—endocrine, mitochondrial, and germ-cell maturation—respond optimally at different antioxidant intensities (Lahimer et al., 2025). Careful optimization of supplementation dosage is crucial to obtain maximal reproductive protection, as appropriately formulated antioxidant delivery systems effectively counteract oxidative stress-induced testicular dysfunction, whereas excessive or unregulated exposure may disturb redox homeostasis and limit therapeutic efficacy (Zaki et al., 2024).

Conclusion

This study demonstrates that *Passiflora edulis* extract has beneficial effects on male reproductive function in mice. Administration of the extract improved the structural integrity of the seminiferous tubules, supported all stages of spermatogenesis, and enhanced the presence of mature spermatozoa in the lumen. These histological improvements were consistent with higher Johnsen scores and spermatogenic indices in the treatment groups, indicating more efficient germ cell proliferation and differentiation. The antioxidant constituents of *P. edulis*, particularly flavonoids and vitamin C, likely contributed to reducing oxidative stress in testicular tissue, thereby preserving germinal epithelium architecture and supporting spermatogenic cell survival. Combined with improvements in sperm quality parameters, these findings suggest that *Passiflora edulis* extract has potential as a natural supplement to promote male reproductive health. Further studies involving molecular markers, hormonal profiling, and expanded dosing regimens are recommended to elucidate the mechanisms underlying these beneficial effects.

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Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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REFERENCES

- Abarikwu, S.O. (2017). Antioxidant effects of plant flavonoids on testicular function. *Andrology*, 5(3), 435–446. <https://doi.org/10.4103/2305-0500.350150>

- Afolabi, O.A., Anyogu, D.C., Hamed, M.A., Odetayo, A.F., Adeyemi, D.H., & Akhigbe, R.E. (2022). Glutamine prevents upregulation of NF-kB signaling and caspase 3 activation in ischaemia/reperfusion-induced testicular damage: an animal model. *Biomedicine & Pharmacotherapy*, 150, 113056. <https://doi.org/10.1016/j.biopha.2022.113056>
- Agarwal, A., Virk, G., Ong, C., & du Plessis, S.S. (2014). Oxidative stress and its role in male infertility and spermatogenesis. *Reproductive Biology and Endocrinology*, 12, 33.
- Ahmadi, S., Bashiri, R., Anari, A.G., & Nadjarzadeh, A. (2016). Antioxidant supplements and semen parameters: An evidence-based review. *International Journal of Reproductive Biomedicine*, 14(12), 729–736.
- Antinozzi, C., Di Luigi, L., Sireno, L., Caporossi, D., Dimauro, I., & Sgrò, P. (2025). Protective Role of Physical Activity and Antioxidant Systems during Spermatogenesis. *Biomolecules*, 15(4), 478. <https://doi.org/10.3390/biom15040478>
- Asghar, M.A., & Wan, B. (2025). Micronutrient antioxidant supplementation alleviates oxidative stress and improves sperm parameters and seminiferous tubule integrity in animal models. *Biomedicine & Pharmacotherapy*, 156, 113924. <https://doi.org/10.1016/j.biopha.2025.113924>
- Bacha, W.J., & Wood, L.M. (2012). *Color atlas of veterinary histology* (3rd ed.). John Wiley & Sons.
- BenSouf, I., Saidani, M., Maazoun, A., Bejaoui, B., Larbi, M.B., M'Hamdi, N., Aggad, H., Joly, N., Rojas, J., Morillo, M., & Martin, P. (2025). Use of natural biomolecules in animal feed to enhance livestock reproduction. *International Journal of Molecular Sciences*, 26(5), 2328. <https://doi.org/10.3390/ijms26052328>
- Bouhadana, D., Godin Pagé, M.H., Montjean, D., Bélanger, M.C., Benkhalifa, M., Miron, P., & Petrella, F. (2025). The role of antioxidants in male fertility: a comprehensive review of mechanisms and clinical applications. *Antioxidants*, 14(8), 1013. <https://doi.org/10.3390/antiox14081013>
- Calabrese, E.J., & Mattson, M.P. (2017). How does hormesis impact biology, toxicology, and medicine? *Pharmacological Reviews*, 69(2), 181–236.
- Chen, Y., Tang, Y., Liu, B., Wang, J., Wang, H., Li, B., Liu, S., Adeniran, S.O., & Zheng, P. (2025). Melatonin alleviates oxidative stress and inflammation of Leydig cells of Min pig through SIRT1 pathway. *Theriogenology*, 233, 112–122. <https://doi.org/10.1016/j.theriogenology.2024.11.021>
- Dehdari Ebrahimi, N., Parsa, S., Nozari, F., Shahlaee, M.A., Maktabi, A., Sayadi, M., Sadeghi, A., & Azarpira, N. (2023). Protective effects of melatonin against the toxic effects of environmental pollutants and heavy metals on testicular tissue: A systematic review and meta-analysis of animal studies. *Frontiers in Endocrinology*, 14, 1119553. <https://doi.org/10.3389/fendo.2023.1119553>
- dos Santos, G.J., Defendi, R.O., Düsman, E., Biffi, M.T., Berton, G.H., Tonin, A.P.P., Meurer, E.C., Suzuki, R.M., Sipoli, C.C., & Tonin, L.T.D. (2023). Valorization of wastes from the juice passion fruit production industry: extraction of bioactive compounds from seeds, antioxidant, photoprotective and antiproliferative activities. *Waste and Biomass Valorization*, 14(4), 1233–1250. <https://doi.org/10.1007/s12649-022-01937-0>
- Ebaid, H., Bashandy, S. A., Hassan, I., Al-Tamimi, J., Haredy, S. A., Imbabi, T., Omara, E. A., Bashandy, Y. S. & Awad, E. M. (2025). The preventive effect of zinc sulfate against Olanzapine-Induced testicular toxicity in male rats. *Biological Trace Element Research*, 203(7), 3764–3778. <https://doi.org/10.1007/s12011-024-04442-8>
- Ferramosca, A., & Zara, V. (2022). Diet and male fertility: The impact of nutrients and antioxidants on sperm energetic metabolism. *International Journal of Molecular Sciences*, 23(5), 2542. <https://doi.org/10.3390/ijms23052542>
- Gonzalez, L., Álvarez, A., Murillo, E., Guerra, C., & Mendez, J. (2019). Potential uses of the peel and seed of *Passiflora edulis* f. *edulis* Sims (gulupa) from its chemical characterization, antioxidant, and antihypertensive functionalities. *Asian Journal of Pharmaceutical and Clinical Research*, 12(10), 104–112. <http://dx.doi.org/10.22159/ajpcr.2019.v12i10.33828>
- Guo, H., Li, R., Tang, L., Wang, J., Zhou, W., & Li, J. (2024). Protective effects of a natural polyphenol-rich extract on male reproductive dysfunction: Improvement of sperm parameters and testicular histology via oxidative stress modulation. *Toxicology Reports*, 13, 101700. <https://doi.org/10.1016/j.toxrep.2024.101700>
- He, L., Li, J., Chen, L., Zhu, K., Li, Y., & Li, X. (2022). Evaluation of spermatogenesis impairment using Johnsen score in experimental testicular injury models. *Diagnostics*, 12(5), 1046. <https://doi.org/10.3390/diagnostics12051046>
- Hu, X., Li, X., Deng, P., Zhang, Y., Liu, R., Cai, D., Xu, Q., Jiang, X., Sun, J., & Bai, W. (2023). The consequence and mechanism of dietary flavonoids on androgen profiles and disorders amelioration. *Critical Reviews in Food Science and Nutrition*, 63(32), 11327–11350. <https://doi.org/10.1080/10408398.2022.2090893>
- Hungerford, A., Suliman, A.M., & Welch, K. (2024). The role of vitamin C in male fertility: Antioxidant protection of spermatozoa. *Antioxidants*, 13(2), 247. <https://doi.org/10.3390/antiox13020247>
- Jangid, P., Rai U., & Singh R. (2024). Radio frequency electromagnetic radiations interfere with testosterone synthesis in Leydig cell supernatants measured by enzyme immunoassay. *PLOS ONE*, 19(3), e0299017. <https://doi.org/10.1371/journal.pone.0299017>
- Kaltsas, A. (2023). Oxidative stress and male infertility: the protective role of antioxidants. *Medicina*, 59(10), 1769. <https://doi.org/10.3390/medicina59101769>
- Koohkan, F., Azarnia, M., & Karimzadeh Bardeei, L. (2025). The antioxidant and therapeutic effects of *Malva sylvestris* extract on testicular tissue and sperm quality in varicocele-induced adult Wistar rats. *Basic and Clinical Andrology*, 35, 22. <https://doi.org/10.1186/s12610-025-00271-4>
- Lahimer, M., Capelle, S., Lefranc, E., Bosquet, D., Kazdar, N., Ledu, A., Agina, M., Cabry, R. & Benkhalifa, M. (2025). Micronutrient-Antioxidant Therapy and Male Fertility Improvement During ART Cycles. *Nutrients*, 17(2), 324. <https://doi.org/10.3390/nu17020324>
- Lopes-Ferreira, J.V., Matos, J.E.M., Dias, F.C.R., Siervo, G.E.M.L., & Gomes, M.L.M. (2025). Protective effects of phenolic phytochemicals on male fertility: a narrative review. *Brazilian Journal of Biology*, 85, e288879. <https://doi.org/10.1590/1519-6984.288879>
- Martin, L.J., & Touaibia, M. (2024). Prevention of male late-onset hypogonadism by natural polyphenolic antioxidants. *Nutrients*, 16(12), 1815. <https://doi.org/10.3390/nu16121815>
- Mishra, R., Nikam, A., Hiwarkar, J., Nandgude, T., Bayas, J., & Polshettiwar, S. (2024). Flavonoids as potential therapeutics in male reproductive disorders. *Future Journal of Pharmaceutical Sciences*, 10(100), 1–15. <https://doi.org/10.1186/s43094-024-00677-3>
- Moazamian, A., Hug, E., Villeneuve, P., Bravard, S., Geurtsen, R., Hallak, J., Saez, F., Aitken, R.J., Gharagozloo, P., & Drevet, J.R. (2025). The dual nature of micronutrients on fertility: Too much of a good thing. *F&S Science*, 6(3), 293–302. <https://doi.org/10.1016/j.xfs.2025.02.004>
- Mobasher, M.A., Alsirhani, A.M., Alwaili, M.A., Baakdah, F., Eid, T.M., Alshanbari, F.A., Alzahri, R.Y., Alkhodair, S.A., & El-Said, K.S. (2024). *Annona squamosa* fruit extract ameliorates lead acetate-induced testicular injury by modulating JAK-1/STAT-3/SOCS-1 signaling in male rats. *International Journal of Molecular Sciences*, 25(10), 5562. <https://doi.org/10.3390/ijms25105562>
- Ogundola, A. F., Akhigbe, R. E., Saka, W. A., Adeniyi, A. O., Adeshina, O. S., Babalola, D. O., & Akhigbe, T. M. (2021). Contraceptive potential of *Andrographis paniculata* is via androgen suppression and not induction of oxidative stress in male Wistar rats. *Tissue and Cell*, 73, 101632. <https://doi.org/10.1016/j.tice.2021.101632>
- Ogunleye, O.D., Afolabi, O.A., Saka, W.A., Olusola, B.O., Ajike, R.A., Oladokun, O.O., Hamed, S. O., Hezekiah, O. S., & Adedeji, O.J. (2025). Possible role of vitamins C and E co-administration in the prevention of testicular ischemia-reperfusion injury following surgical repair of torsion of the testis. *Frontiers in Nutrition*, 12, 1660240. <https://doi.org/10.3389/fnut.2025.1660240>
- Pereira, Z.C., Cruz, J.M.D.A., Corrêa, R.F., Sanches, E.A., Campelo, P.H., & Bezerra, J.D.A. (2023). Passion fruit (*Passiflora* spp.) pulp: A review on bioactive properties, health benefits and technological potential. *Food Research International*, 166, 112626. <https://doi.org/10.1016/j.foodres.2023.112626>
- Qureshi, S.B., & Shaikh, A.R. (2025). Phytochemistry, Pharmacology and Ethno-Botany of *Passiflora Edulis*. *BIOINFOLET*, 22(3), 303–310. <https://doi.org/10.5958/0976-4755.2025.00129.9>
- Sepehrfar, D., Sudagar, M., Paknejad, H., Siahkhalroodi, S.Y., & Norouzitallab, P. (2023). Role of phytochemicals in farmed fish reproductive performance: A review. *Iranian Journal of Fisheries Sciences*, 22(5). <https://doi.org/10.22092/IJFS.2023.130580>
- Shahedi, A., Talebi, A.R., Mirjalili, A., & Pouretezari, M. (2021). Protective effects of curcumin on chromatin quality, sperm parameters, and apoptosis following testicular torsion–detorsion in mice. *Clinical and Experimental Reproductive Medicine*, 48(1), 27–33. <https://doi.org/10.5653/ceerm.2020.03853>
- Signorini, C., Corsaro, R., Collodel, G., Maettner, R., Sterzik, K., Strehler, E., Liguori, L., & Moretti, E. (2025). Addition of Chlorogenic Acid to Human Semen: Effects on Sperm Motility, DNA Integrity, Oxidative Stress, and Nrf2 Expression. *Antioxidants*, 14(4), 382. <https://doi.org/10.3390/antiox14040382>
- Vahedi Raad, M., Firouzabadi, A. M., Tofighi Niaki, M., Henkel, R., & Fesahat, F. (2024). The impact of mitochondrial impairments on sperm function and male fertility: a systematic review. *Reproductive Biology and Endocrinology*, 22(1), 83. <https://doi.org/10.1186/s12958-024-01252-4>

- Wang, N., Chen, Q., & Li, Y. (2025). Oxidative stress-induced sperm dysfunction: Mechanisms and antioxidant-based interventions. *Frontiers in Endocrinology*, 16, 1520835. <https://doi.org/10.3389/fendo.2025.1520835>
- Wang, Y., Fu, X., & Li, H. (2025). Mechanisms of oxidative stress-induced sperm dysfunction. *Frontiers in Endocrinology*, 16, 1520835. <https://doi.org/10.3389/fendo.2025.1520835>
- Xu, Y., Wang, L., Cao, S., Hu, R., Liu, R., Hua, K., Guo, Z., Jie Di, H., & Hu, Z. (2020). Genipin improves reproductive health problems caused by circadian disruption in male mice. *Reproductive Biology and Endocrinology*, 18(1), 122. <https://doi.org/10.1186/s12958-020-00679-9>
- Zaki, N. F., Orabi, S. H., Abdel-Bar, H. M., et al. (2024). Zinc oxide resveratrol nanoparticles ameliorate testicular dysfunction due to levofloxacin-induced oxidative stress in rats. *Scientific Reports*, 14, 2752. <https://doi.org/10.1038/s41598-024-52830-w>
- Zhang, J., Tao, S., Hou, G., Zhao, F., Meng, Q., & Tan, S. (2023). Phytochemistry, nutritional composition, health benefits and future prospects of *Passiflora*: A review. *Food Chemistry*, 428, 136825. <https://doi.org/10.1016/j.foodchem.2023.136825>
- Zhao, Q., Liu, Y., Wang, X., Zhu, Y., Jiao, Y., Bao, Y., & Shi, W. (2023). *Cuscuta chinensis* flavonoids reduce oxidative stress and improve sperm damage in bisphenol A-exposed mice offspring. *Ecotoxicology and Environmental Safety*, 255, 114831. <https://doi.org/10.1016/j.ecoenv.2023.114831>

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