Original Article

Evaluating the Antioxidant Potential of *Epimedium grandiflorum* in a Rat Model of Cryptorchidism: Reducing Malondialdehyde and Enhancing Antioxidant Enzymes

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ABSTRACT

Background: Cryptorchidism is one of the most common congenital malformations of the male genital organs.

Objectives: This study investigated the effects of *Epimedium grandiflorum* on the testes of rats with cryptorchidism.

Methods: Wistar rats were divided into healthy control, sham, cryptorchidism, and cryptorchidism treated with 100, 200, and 400 mg/kg of *E. grandiflorum*. Unilateral cryptorchidism was induced in rats through surgery. The hydro-ethanolic extract of *E. grandiflorum* was prepared by drying the leaves of *E. grandiflorum* at a temperature of 24 ˚C. The obtained powder was mixed with 80% ethanol. Treatment groups received *E. grandiflorum* daily through oral gavage for 7, 14, and 28 days. The expression levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) were examined. Hematoxylin and eosin (H&E) staining were used to study pathological changes.

Results: In rats treated with *E. grandiflorum* (400 mg/kg for 28 days), the highest decrease in MDA ($P<0.0001$) and increase in SOD and GPx ($P<0.0001$) were observed compared to the cryptorchidism group. Testis were seen with high normal spermatocytes in seminiferous tubules, and the highest spermatocyte count in rats treated with 400 mg/kg of *E. grandiflorum*. By reducing MDA and increasing SOD and GPx, the high antioxidant properties of *E. grandiflorum* lead to the control of oxidative stress in the testes of cryptorchid rats.

Conclusion: *E. grandiflorum* can control tissue level, severe destruction of sperm tubes, reduction of spermatogonia, spermatocytes, and ultimately infertility, and increase of spermatocytes. Therefore, it can be an essential therapeutic intervention as an antioxidant compound in cryptorchidism.

Keywords: Cryptorchidism, *Epimedium grandiflorum*, Histopathology, Testis

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Introduction

A defect in the descent of the testis into the scrotum at birth causes cryptorchidism (or undescended testis). It is one of the causes of infertility in humans and many animal species (Koch et al., 2020). In different mammalian species, relatively low temperatures are required for normal spermatogenesis and sperm fertility (Varuzhanyan & Chan, 2020). The scrotum temperature in most mammals is 4 ˚C to 5 ˚C lower than the temperature inside the abdomen (Varuzhanyan & Chan, 2020). Induction of experimental cryptorchidism causes complete failure of spermatogenesis in adult rats, chronic testicular inflammation manifested as edema and fibrosis, and progressive reduction in Sertoli and Leydig cell expression and function (Aldahhan & Hedger, 2021).

If the testicular temperature rises, the serum testosterone level decreases, and this temperature difference causes a change in the protein structure of the epididymal epithelial cells (Ilkhani et al., 2020). Changes in the thickness, the diameter of the tubes, and epididymal weight occur in cryptorchidism. Storing sperm in the epididymis and its ability to maintain sperm survival are highly dependent on the temperature of the scrotum and are impaired by increasing temperature (Hensel et al., 2020). Cryptorchidism is usually treated with surgery and hormone therapy, which involves injecting human chorionic gonadotropin. Hormone therapy is usually not recommended because it is less effective than surgery (Braga & Lorenzo, 2017). These days, many studies have been conducted concerning natural compounds and their use to treat diseases (Agarwal et al., 2021; Moghaddam et al., 2016; Moghaddam et al., 2020; Penson et al., 2013).

Epimedium with 52 species in the Berberidaceae family is known as Rowdy Lamb Herb, barrenwort, bishop's hat, fairy wings, horny goat weed, or yin yang huo (Munir et al., 2020). This plant contains countless biological and chemical compounds. Flavonoids are the predominant compounds in this plant with their health-promoting activities (Wang et al., 2020). Epimedium is also rich in lignans, phenol glycosides, sesquiterpenes, and other biochemicals with potentially beneficial properties (Munir et al., 2020). Modern pharmacological studies and clinical practice have shown that Epimedium and its active ingredients have broad applications in medicine, such as atherosclerosis, hormone regulation, anti-osteoporosis, anti-aging, anti-oxidation, and anti-depressant (Munir et al., 2020). The antioxidant potential of extracts and fractions from the leaves and stems of *Epimedium koreanum* Nakai was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, lipid peroxidation inhibition, and ferric-reducing power assays (Zhang et al., 2013). The total phenolic, flavonoid, and icariin contents were also measured. Results showed varying antioxidant activity, with the leaf extract and n-BuOH fraction exhibiting the highest potency (Zhang et al., 2013). The order of antioxidant activity was n-BuOH > ethyl acetate > ethanol > petroleum ether > water. Strong correlations were found between antioxidant activity and phenolics, flavonoids, and icariin levels. Icariin was identified as a key contributor to the antioxidant effects, and the n-BuOH leaf fraction may be a valuable natural antioxidant source (Zhang et al., 2013).

In people with cryptorchidism, the intracellular antioxidant system's capacity is insufficient to eliminate intracellular free radicals. This plant enhances sexual potency, anti-rheumatism, osteoporosis, and anti-cancer, and can also improve functions such as bone protection, nerve protection, heart protection, and anti-inflammatory (Munir et al., 2020). Correcting endothelial dysfunction, inhibiting the proliferation of smooth muscle cells, suppressing the formation of macrophages and inflammatory cells, and inhibiting platelets are other activities of this plant (He et al., 2020).

Since urogenital diseases are among the most common mammalian diseases and can cause irreversible complications, this study evaluated the effects of *Epimedium grandiflorum* on cryptorchidism in Wistar adult rats. If the therapeutic response of *E. grandiflorum* extracts with positive anti-inflammatory and antioxidant impact is positive, it can be a suitable alternative in urogenital diseases.

Materials and Methods

Extraction of epimedium grandiflorum

E. grandiflorum leaves were purchased from the local market of Tehran City, Iran. The Department of Plant Science, Science and Research Branch, Islamic Azad University, Tehran, Iran, taxonomically identified and confirmed them. It was reported that ethanol and methanol are effective solvents for extracting antioxidant phenolic compounds (Munir et al., 2020). *E. grandiflorum* leaves were dried at 24 ˚C and then powdered to prepare a hydro-ethanolic extract. In the next step, 300 g of powdered leaves were mixed with 2500 mL of 80% ethanol to prepare the hydroethanolic extract and then kept in a dark place for 72 hours in a closed container (Yang et al., 2020; Zhang et al., 2013). Then, the solution was filtered by Whatman filter paper number 1. The extract

was placed in a bain-marie at 50 ˚C for complete drying. After full drying, it was kept in a closed container at 4 ˚C until the experiments. The structure and investigation of the components of the *E. grandiflorum* have been done in previous studies through HPLC (high-performance liquid chromatography) (Munir et al., 2020).

Experimental animals

Wistar adult male rats (280-300 g) were purchased from the Pasteur Institute (Tehran, Iran). The number of rats was minimized as far as possible. Rats were housed separately in cages and had free access to water and food. They were also kept under light/dark cycles (12/12 hours), constant temperature (23 ˚C), and humidity (55%). After seven days of acclimatization, the rats were randomly divided into 16 groups (n = 5 per group).

Treatment period and study design

Seven, fourteen, and twenty-eight days were considered for treating rats. Treatment groups received *E. grandiflorum* daily through oral gavage.

Group 1: Served as the healthy control and received physiologic saline orally for 28 days.

Group 2: Served as a sham and received 100 mg/kg of *E. grandiflorum* for 28 days.

Group 3: Served as a sham and received 200 mg/kg of *E. grandiflorum* for 28 days.

Group 4: Served as a sham and received 400 mg/kg of *E. grandiflorum* for 28 days (Munir et al., 2020).

Group 5: Served as cryptorchidism, without treatment for 7 days.

Group 6: Served as cryptorchidism, without treatment for 14 days.

Group 7: Served as cryptorchidism, without treatment for 28 days.

Group 8: Served as cryptorchidism 100, received 100 mg/kg of *E. grandiflorum* for 7 days.

Group 9: Served as cryptorchidism 100, received 100 mg/kg of *E. grandiflorum* for 14 days.

Group 10: Served as cryptorchidism 100, received 100 mg/kg of *E. grandiflorum* for 28 days.

Group 11: Served as cryptorchidism 200, received 200 mg/kg of *E. grandiflorum* for 7 days.

Group 12: Served as cryptorchidism 200, received 200 mg/kg of *E. grandiflorum* for 14 days.

Group 13: Served as cryptorchidism 200, received 200 mg/kg of *E. grandiflorum* for 28 days.

Group 14: Served as cryptorchidism 400, received 400 mg/kg of *E. grandiflorum* for 7 days.

Group 15: Served as cryptorchidism 400, received 400 mg/kg of *E. grandiflorum* for 14 days.

Group 16: Served as cryptorchidism 400, received 400 mg/kg of *E. grandiflorum* for 28 days.

Surgical procedures

A surgical procedure for the induction of unilateral cryptorchidism was performed. All surgical procedures were performed under anesthesia by intraperitoneal injection of ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (10 mg/kg) (Zhou et al., 2021). After anesthesia, the surgical area was shaved and prepared with a povidone-iodine solution. A midline abdominal incision was made, and the right testis was manipulated into the abdomen and sutured to the abdominal wall with a suture. The right testis was treated as the experimental organ for each animal. In contrast, the left testis remained untouched throughout the procedure and acted as a control after the laparotomy incision was closed in layers with a suture. Silk 0-3 sutures were used to fix the testicles to the abdomen, absorbent sutures were used to sew the abdomen, and nylon sutures were used for skin sutures. After surgery, the surgical site was regularly checked for infection (redness, swelling, discharge) and kept clean and dry. The mice's appetite, hydration, and activity levels were also monitored. Swelling and infection were assessed in the rats.

Sampling and tissue preparation

After 7, 14, and 28 days, cryptorchid and normal testes were collected and cut in half post-surgery. One half was fixed in Bouin solution for 24 h for histopathological analysis via hematoxylin and eosin (H&E) staining. It used the other half to assess the malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) oxidative stress enzymes analysis.

Oxidative stress analysis

The tissue MDA level was determined based on the reaction with thiobarbituric acid (TBA). In the TBA test reaction, MDA or MDA-like substances and TBA react by producing a pink pigment with maximum absorption at 532 nm. The SOD activity was expressed as nmol/g tissue. The GPX catalyzes the oxidation of glutathione, and in the presence of glutathione reductase and NADPH (nicotinamide adenine dinucleotide phosphate), oxidized glutathione converts to the reduced form by changes in the oxidation of NADPH to NADP+. The GPX level was measured in absorbance at 340 nm. The GPX activity was expressed as U/mg tissue. Tissue SOD activity was measured according to the method of Paoletti and Mocali (1990). In brief, the superoxide anions were generated from manganese (II) chloride and mercaptoethanol in the presence of ethylenediaminetetraacetic acid. The SOD level was determined based on its ability to inhibit nicotinamide adenine dinucleotide oxidation in the reaction mixture after the addition of tissue homogenate. Nicotinamide adenine dinucleotide oxidation was measured at 340 nm. The SOD activity was expressed as U/mg tissue. Assay kits for MDA, SOD, and GPX were purchased from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom) and evaluated using an ELISA reader (DANA, Iran).

Histopathological examination

The tissue was fixed in Bouin solution and embedded in paraffin blocks. A tissue section (5 µm) was obtained, deparaffinized, and stained with H&E. The testicular tissue was evaluated randomly with standard light microscopy (LM) by an observer unaware of which group the rat belonged to. The testis sections were graded numerically to assess the degree of histological changes associated with seminiferous tubule injury as previously described by Johnsen, scoring as below (Johnsen, 1970):

Score 10: Complete spermatogenesis with many spermatozoa present

Score 9: Slightly impaired spermatogenesis with many late spermatids, disorganized epithelium

Score 8: Less than five spermatozoa per tubule, few late spermatids

Score 7: No spermatozoa, no late spermatids, many early spermatids

Score 6: No spermatozoa, no late spermatids, few early spermatids

Score 5: No spermatozoa or spermatids, many spermatocytes

Score 4: No spermatozoa or spermatids, few spermatocytes

Score 3: Spermatogonia only

Score 2: No germinal cells, Sertoli cells only

Score 1: No seminiferous epithelium

Statistical analysis

Data were reported as Mean±SD, and the graphs were plotted using GraphPad Prism software, version 8. Data were analyzed using analysis of variance (ANOVA) followed by a Tukey post hoc test, and a $P<0.05$ was considered a significant difference.

Results

The results are presented below after the induction of unilateral cryptorchidism (Figure 1) through surgery and treatment with different doses of *E. grandiflorum*.

Disinfecting and preparing the animal for auction (A), abdominal skin and muscle incision (B), taking out the testicle from the sac and sewing it to the abdominal wall with surgical silk thread (C and D), suturing the abdominal muscle with absorbable surgical thread (E), and suturing the skin with non-absorbable thread for surgery and recovery (F).

Oxidative stress markers

After 7 days, the cryptorchidism, cryptorchidism 100, cryptorchidism 200, and cryptorchidism 400, compared to the healthy control group, showed a significant increase in MDA level ($P<0.0001$) (Figure 2A) and a decrease in SOD and GPX level ($P<0.0001$) (Figures 2B and 2C). Compared to cryptorchidism, groups treated with cryptorchidism 100, 200, and 400 showed a decrease in MDA ($P<0.0001$) (Figure 2A). The increase of SOD and GPX was demonstrated in the cryptorchidism 400 group compared to cryptorchidism ($P<0.05$ and $P<0.001$) (Figures 2B and 2C), respectively.

After 14 days, the cryptorchidism, cryptorchidism 100, cryptorchidism 200, and cryptorchidism 400, compared to the healthy control group, showed a significant in-

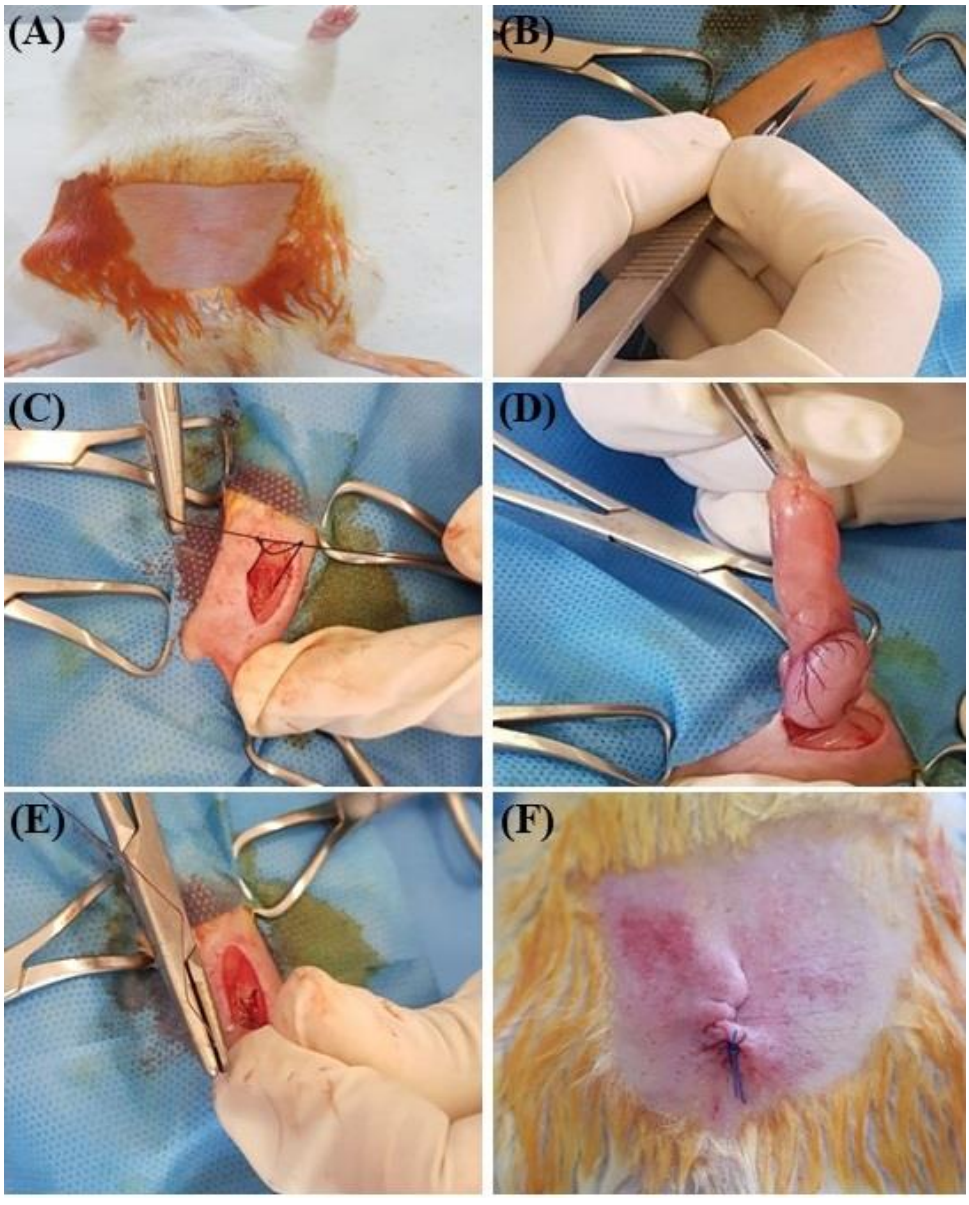

Figure 1. Stages of induction of unilateral cryptorchidism in Wistar adult male rats

crease in MDA level (P<0.0001) (Figure 3A) and a decrease in SOD and GPX level (P<0.0001) (Figures 3B and 3C). Compared to cryptorchidism, groups treated with cryptorchidism 100, 200, and 400 showed a decrease in MDA (P<0.0001) (Figure 3A). The increase of SOD and GPX was demonstrated in the cryptorchidism 400 group compared to cryptorchidism (P<0.05 and P<0.0001) (Figures 3B and 3C), respectively. Also, compared to cryptorchidism, cryptorchidism 200 showed an increase in GPX rate, too (P<0.001) (Figure 3C).

After 28 days, the cryptorchidism, cryptorchidism 100, cryptorchidism 200, and cryptorchidism 400, compared to the healthy control group, showed a significant increase in MDA level (P<0.0001) (Figure 4A) and a decrease in SOD and GPX level (P<0.0001) (Figures 4B and 4C). Compared to cryptorchidism, groups treated with cryptorchidism 100, 200, and 400 showed a decrease in MDA (P<0.0001) (Figure 4A). The increase of SOD and GPX was demonstrated in the cryptorchidism 400 group compared to cryptorchidism (P<0.0001) (Figures 4B and 4C), respectively. Also, compared to cryptorchidism, the cryptorchidism 200 showed an increase in SOD and GPX rate (P<0.05 and P<0.0001) (Figures 4B and 4C).

Histopathological findings

The results obtained from examining the testicular tissue section in the study groups are presented in Figure 5. As

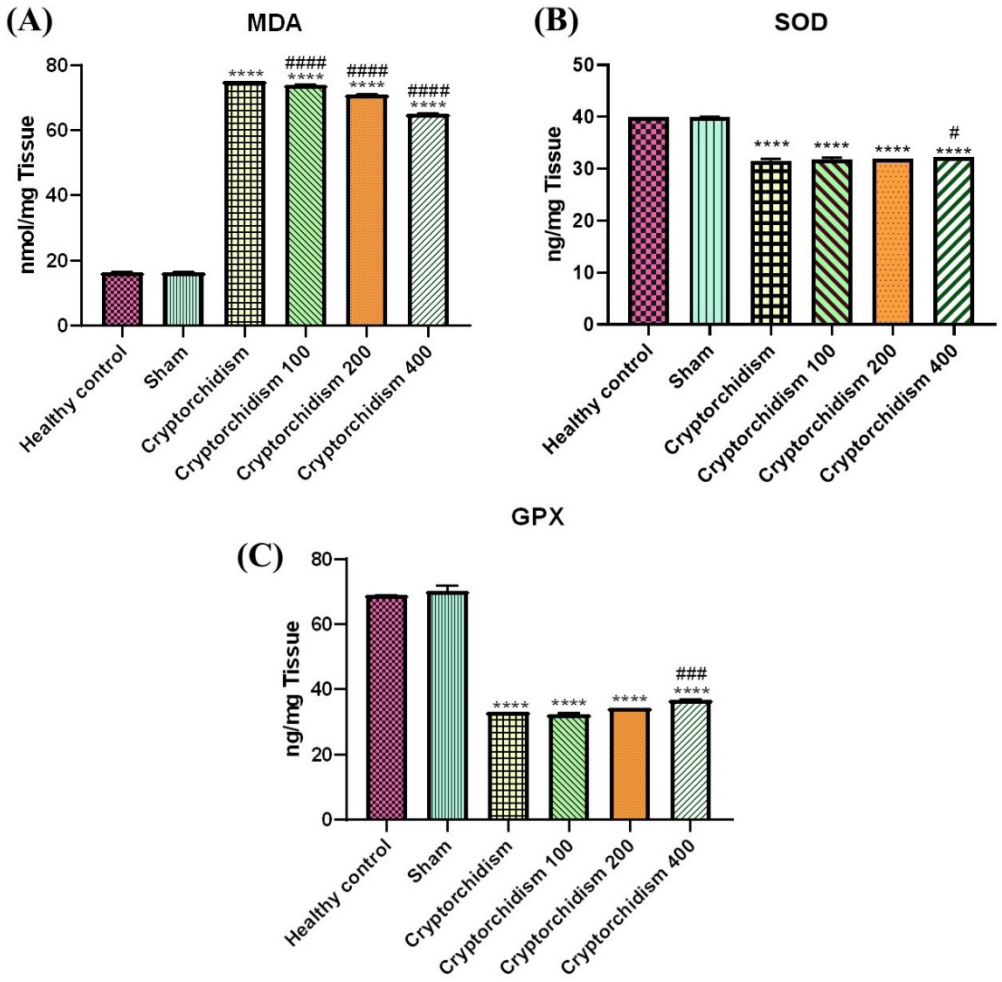

Figure 2. Effect of different levels of *E. grandiflorum* on tissue values of MDA (A), SOD (B), and GPX (C) in healthy control, sham, cryptorchidism, cryptorchidism 100, cryptorchidism 200, and cryptorchidism 400 mg/kg groups after 7 days

Note: Comparison of treated groups with healthy control $^{****}P<0.0001$; Comparison of groups treated with cryptorchidism $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$.

can be seen, rats with cryptorchidism treated with 400 mg/kg of *E. grandiflorum* for 7, 14, and 28 days, compared to cryptorchidism without treatment, showed a statistically significant increase in Johnsen scoring ($P<0.0001$).

Figure 6 shows the effect of *E. grandiflorum* on testis histopathology. The testis section of healthy control (Figure 6A) and sham 100, 200, and 400 mg/kg (Figures 6B, 6C, and 6D) had shown normal spermatogenesis, seminiferous tubules with spermatocytes, spermatids, and spermatozoa after 28 days. Figures 6E, 6F, and 6G show the cryptorchidism group without treatment after 7, 14, and 28 days, respectively. High seminiferous tubules degeneration (after 7 days), spermatogenesis with few spermatocytes (after 14 days), and loss of spermatogonia (after 28 days) were observed. Figures 6H, 6I, and 6J show the cryptorchidism groups treated with 100, 200, and 400 mg/kg of *E. grandiflorum*, respectively (after 7 days). Few spermatocytes are in groups treated with 100 and 200 mg/kg of *E. grandiflorum*, and improved testis characteristics with high normal spermatocytes in seminiferous tubules in rats treated with 400 mg/kg of *E. grandiflorum*. Figures 6K, 6L, and 6M show the cryptorchidism groups treated with 100, 200, and 400 mg/kg of *E. grandiflorum*, respectively (after 14 days). The results in these groups were similar to those of the treatment groups for 7 days. The highest spermatocyte count was seen in the groups treated with cryptorchidism, treated with 100, 200, and 400 mg/kg of *E. grandiflorum* after 7, 14, and 28 days (Figures 6N, 6O, and 6P).

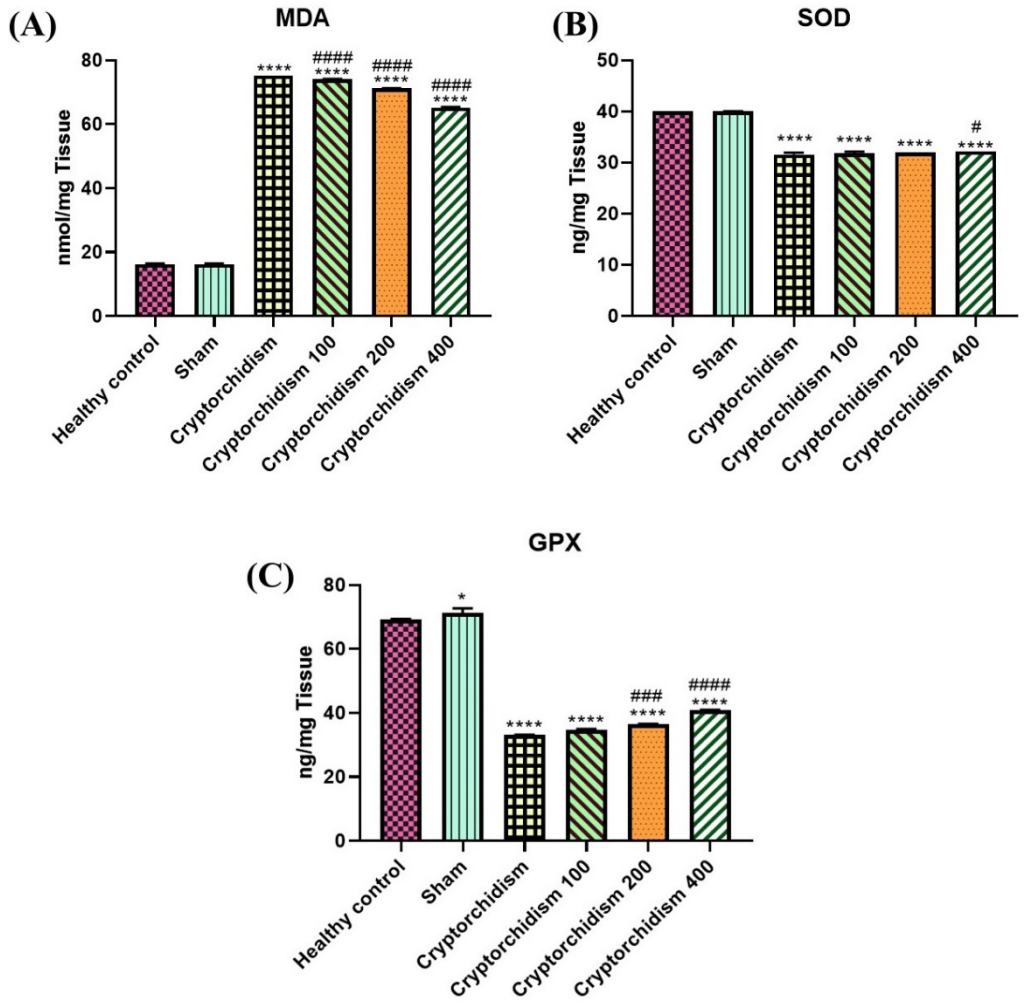

Figure 3. Effect of different levels of *E. grandiflorum* on tissue values of MDA (A), SOD (B), and GPX (C) in healthy control, sham, cryptorchidism, cryptorchidism 100, cryptorchidism 200, and cryptorchidism 400 mg/kg groups after 14 days

Note: Comparison of treated groups with healthy control $^{*}P<0.05$ and $^{****}P<0.0001$; Comparison of groups treated with cryptorchidism $^{\#}P<0.05$, $^{\#\#\#}P<0.001$, and $^{\#\#\#\#}P<0.0001$.

Discussion

Testicular descent or cryptorchidism is a complex process regulated by genetic, hormonal, and anatomical factors (Virtanen & Toppari, 2008). Unsuccessful testicular descent is one of the most common congenital anomalies (Virtanen & Toppari, 2008). Since there are limited studies regarding the role of *E. grandiflorum* in the treatment of cryptorchidism disease, in this study, we investigated the antioxidant and therapeutic effects of *E. grandiflorum* on rats with cryptorchidism.

Epimedium contains nutrients and phytochemicals such as proteins, essential elements, polysaccharides, flavonoids, alkaloids, lignans, and terpenoids and is used to prevent and treat diseases of the reproductive system, nervous system, endocrine system, and immune system (Yang et al., 2020). Flavonoids have essential effects on the hematopoietic, cardiovascular, cerebrovascular, endocrine, immune, reproductive, nervous, and anti-inflammatory systems. On the other hand, these flavonoids lack any acute toxicity or long-term health side effects (Yang et al., 2020).

The free radicals' production over the antioxidants' defensive capacity causes oxidative stress and irreversible responses such as apoptosis or necrosis in living cells (Ikeda et al., 1999). Oxidative stress is a condition in which there is an imbalance between the production of oxidants and free radicals and the body's antioxidant defense (Makvandi et al., 2021). Oxidizing agents include reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide (Moghaddam et al., 2021).

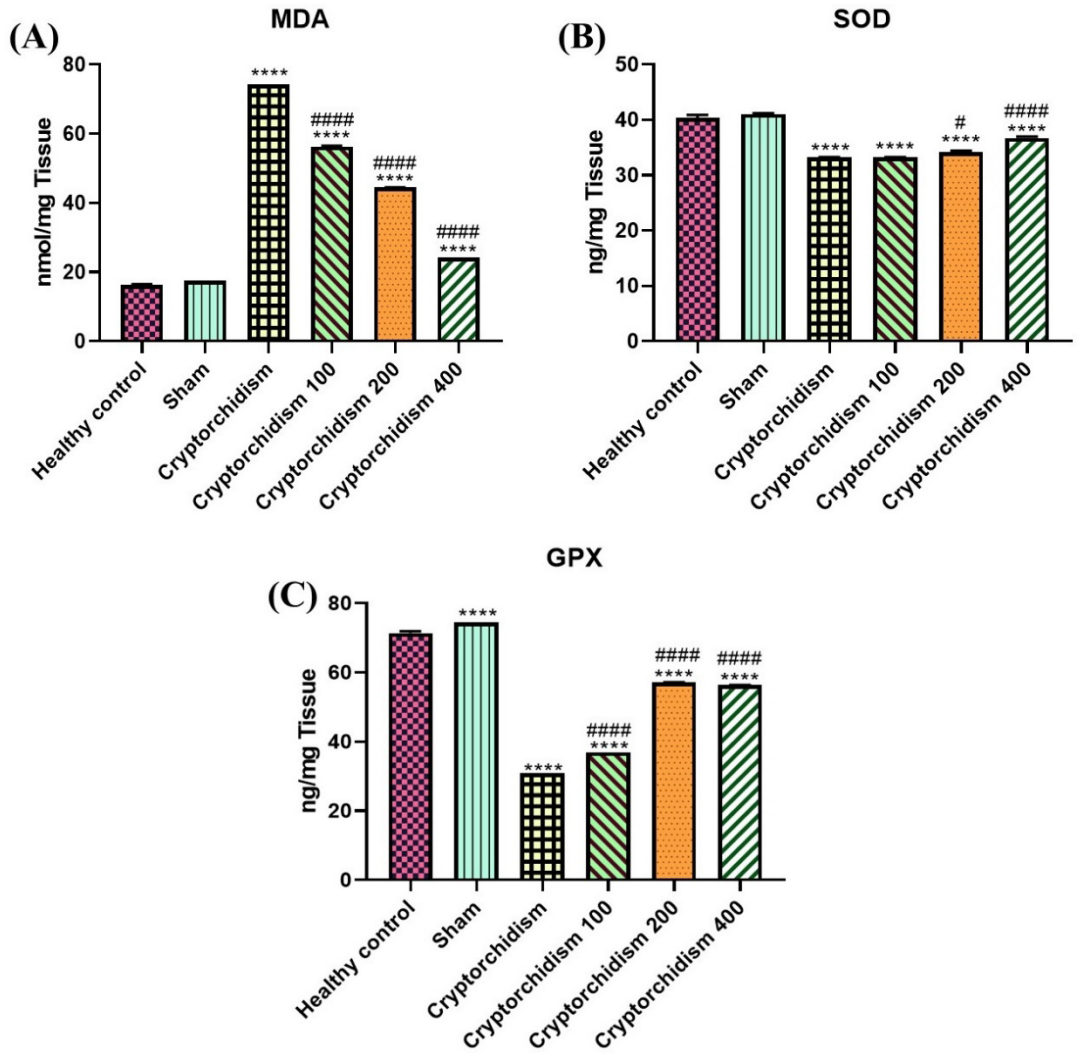

Figure 4. Effect of different levels of *E. grandiflorum* on tissue values of MDA (A), SOD (B), and GPX (C) in the healthy control, sham, cryptorchidism, cryptorchidism 100, cryptorchidism 200, and cryptorchidism groups after 28 days

Note: Comparison of treated groups with healthy control $^{****}P<0.0001$; Comparison of groups treated with cryptorchidism $^{\#}P<0.05$ and $^{\#\#\#\#}P<0.0001$.

Mammalian sperm are rich in unsaturated fatty acids, so they are susceptible to ROS invasion, which results in reduced fertility in males. High levels of MDA are essential for forming free radicals in tissues (Fakouri et al., 2017). On the other hand, SOD and GPX are the main enzymes to ROS eliminated in the male genitalia (Fakouri et al., 2017). Mice with cryptorchidism showed higher concentrations of SOD and MDA than sham mice (Afolabi et al., 2013). Increased MDA concentration and testicular temperature in these patients indicate peroxidation of sperm and lipid membranes, which leads to increased ROS production and ultimately leads to germ cell death (Munyali et al., 2020).

The researchers' findings showed a link between low antioxidant activity and severe DNA damage in the semen plasma of infertile men (Munyali et al., 2020). As a result of ROS overproduction, a decrease in testicular protein levels occurs (Munyali et al., 2020). Therapeutic responses to different doses of *E. grandiflorum* for recovery of antioxidants and reproductive hormones were evaluated in male rats, and the results showed positive therapeutic effects on impotence (Munyali et al., 2020). Consistent with the results of other researchers, our results in this study showed that high antioxidant properties of *E. grandiflorum* by reducing MDA and increasing SOD and GPX lead to the control of oxidative stress in the testes of cryptorchidism rats.

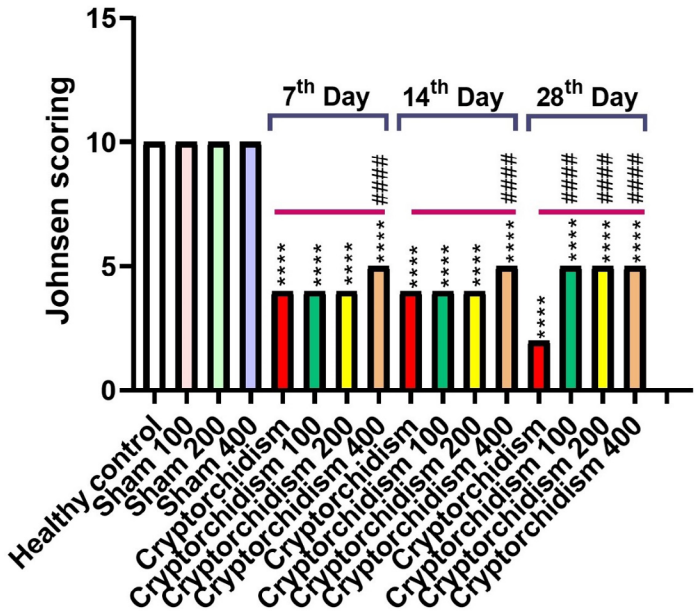

Figure 5. Effect of different levels of *E. grandiflorum* on testis tissue histopathology Johnsen score in the healthy control, sham, cryptorchidism, cryptorchidism 100, cryptorchidism 200, and cryptorchidism 400 mg/kg rats after 7, 14, and 28 days

Note: Comparison of treated groups with healthy control ****P<0.0001; Comparison of groups treated with cryptorchidism ####P<0.0001.

Different doses of the plant extract *E. grandiflorum* increase testosterone levels (Ibama et al., 2021). Consistent with these results, other reports were presented that indicate an increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH) secreted from the anterior part of pituitary in response to gonadotropin-releasing hormone (GnRH) (secreted from the hypothalamus) and led to an increase in gonadal hormones including testosterone from Leydig cells in the testes (Ibama et al., 2021). Epimedium is a sexual enhancer, anti-rheumatism, and anti-cancer, and a traditional formulation of herbal medicines in Asian countries such as China, Japan, and Korea is used. The active ingredient of this plant includes icariin and icariside D2 (Zhang et al., 2020). *Epimedium brevicornum* exhibits a promising effect on the enhancement of male anti-infertility effects (Zhang et al., 2020).

Consistent with the results of previous researchers, in this study, our results also showed high antioxidant properties of *E. grandiflorum* are associated with decreased MDA and increased SOD and GPX, leading to the control of oxidative stress in the testes of rats with cryptorchidism. A study investigated the effects of a new combined herbal formula, KH-465, consisting of *E. koreanum* Nakai and *Angelica gigas* Nakai, on sperm function in a rat model of infertility induced by LH-releasing hormone (LHRH) agonists (Park et al., 2017). Treatment with KH-465 restored sperm count, motility, and spermatogenic cell density, while reducing 8-OHdG levels and increasing SOD activity. These results suggest that KH-465 helps recover spermatogenesis and maintain normal sperm function. Rats treated with LHRH agonists showed decreased sperm count, motility, testosterone levels, and spermatogenic cell density, as well as lower levels of antioxidant enzymes like SOD. The study highlights the potential of KH-465 in reversing LHRH agonist-induced infertility by improving sperm function and antioxidant status (Park et al., 2017).

Oxidative stress plays a significant role in male infertility, often leading to sperm damage, reduced motility, and even testicular degeneration (Yuan et al., 2014). TFE acts as a potent antioxidant, mitigating oxidative damage by upregulating key antioxidant enzymes, such as SOD and GPX. These enzymes are crucial for neutralizing harmful ROS, which, if left unchecked, can lead to cellular damage and dysfunction in the testes (Yuan et al., 2014). By enhancing the expression of these antioxidant enzymes, TFE helps protect spermatogenic cells from oxidative injury, reduces apoptosis (programmed cell death), and preserves the integrity of the male reproductive system. This antioxidative action supports spermatogenesis (the production of sperm) and overall sperm health, ensuring normal reproductive function (Yuan et al., 2014).

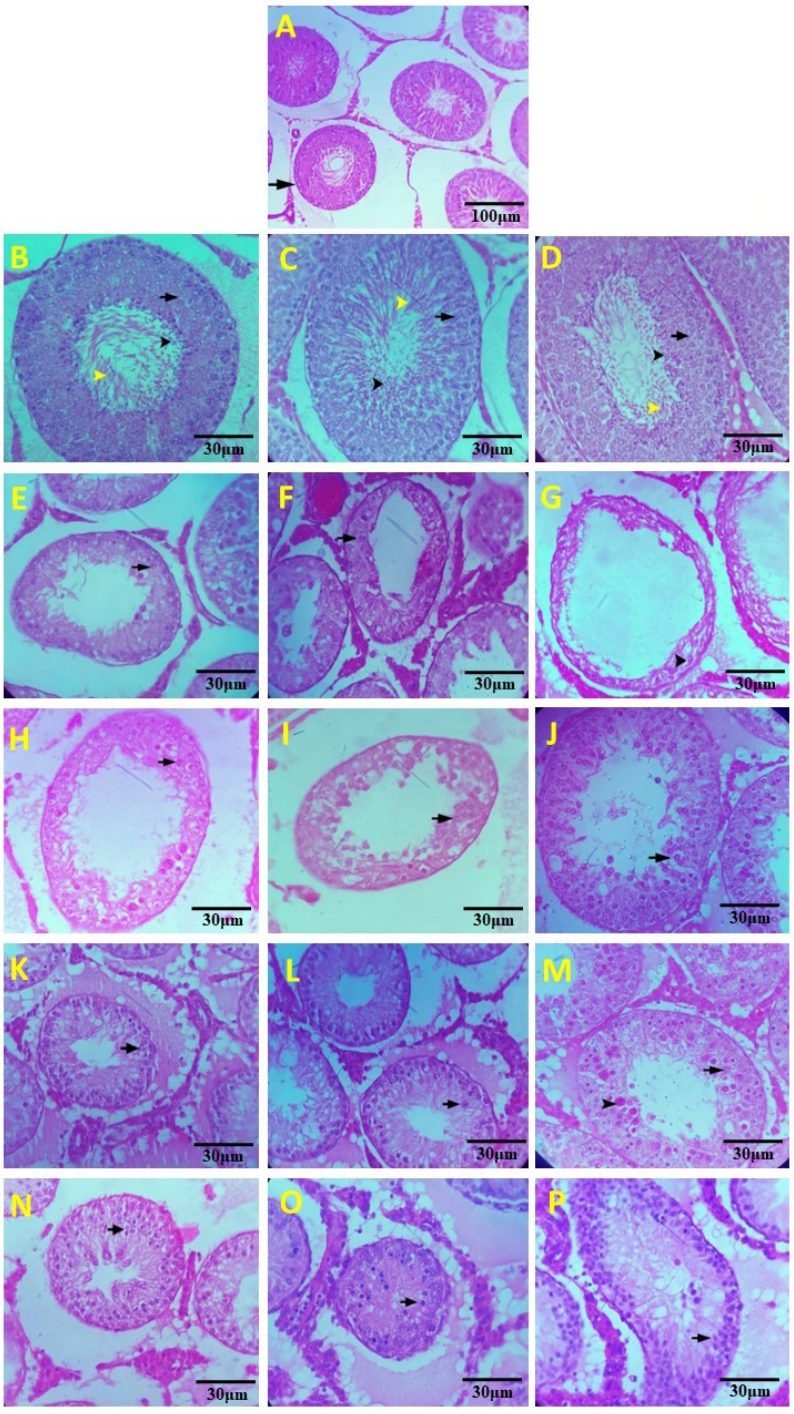

Figure 6. LM images of testis sections, H&E

A) Healthy control; B) Sham 100, treated with 100 mg/kg of *E. grandiflorum* for 28 days; C) Sham 200, treated with 200 mg/kg of *E. grandiflorum* for 28 days; D) Sham 400, treated with 400 mg/kg of *E. grandiflorum* for 28 days; E) Cryptorchidism, without treatment for 7 days; F) Cryptorchidism, without treatment for 14 days; G) Cryptorchidism, without treatment for 28 days; H) Cryptorchidism 100, treated with 100 mg/kg of *E. grandiflorum* for 7 days; I) Cryptorchidism 100, treated with 100 mg/kg of *E. grandiflorum* for 14 days; J) Cryptorchidism 100, treated with 100 mg/kg of *E. grandiflorum* for 28 days; K) Cryptorchidism 200, treated with 200 mg/kg of *E. grandiflorum* for 7 days; L) Cryptorchidism 200, treated with 200 mg/kg of *E. grandiflorum* for 14 days; M) Cryptorchidism 200, treated with 200 mg/kg of *E. grandiflorum* for 28 days; N) Cryptorchidism 400, treated with 400 mg/kg of *E. grandiflorum* for 7 days; O) Cryptorchidism 400, treated with 400 mg/kg of *E. grandiflorum* for 14 days; P) Cryptorchidism 400, treated with 400 mg/kg of *E. grandiflorum* for 28 days

Note: Arrowhead: Spermatid; Arrow: Spermatocyte; Yellow arrowhead: Spermatozoa.

Cryptorchidism also has adverse effects on the tissue surface, severe destruction of spermatic tubes, reduced spermatogonia, spermatocytes, and ultimately infertility. In the groups treated with *E. grandiflorum*, control of these symptoms and increased spermatocytes were observed.

E. grandiflorum in rats with cryptorchidism was able to prevent the progression of infertility by reducing the levels of MDA, and increasing SOD and GPX as an antioxidant compound by inhibiting free radicals. Also observed was less tissue damage in the groups treated with *E. grandiflorum*. Our findings strongly suggest that *E. grandiflorum* holds significant potential as a therapeutic intervention for cryptorchidism, a condition that often leads to male infertility if left untreated. The study demonstrates that *E. grandiflorum* can effectively improve testicular function, enhance spermatogenesis, and mitigate oxidative damage, which is crucial in treating cryptorchidism. These results imply that *E. grandiflorum* could be developed as a natural adjunctive treatment for individuals with cryptorchidism, offering a safer and potentially more effective alternative to current therapies. Given its antioxidant properties and ability to support testicular function, *E. grandiflorum* may provide a valuable option for improving fertility outcomes in males with this condition.

Future research should focus on conducting clinical trials to assess the long-term efficacy and safety of *E. grandiflorum* in human populations. Additionally, exploring the specific molecular mechanisms underlying its therapeutic effects could reveal new insights into its broader applications in reproductive medicine. Further studies should also investigate optimal dosages, potential side effects, and interactions with other treatments, which will be essential for translating these promising preclinical findings into clinical practice.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Medical Sciences Ethics Committee of the Science and Research Branch, Islamic Azad University, Tehran, Iran (Code: IR.IAU.SRB.REC.1399.051). Rat care and the experimental steps were performed per the criteria of care and use of institutional animals.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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