RESEARCH ARTICLE

Effects of hederagenin on spermatogenic function in a mouse model of surgical cryptorchidism

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Heat stress is a major contributor to testicular injury and spermatogenic dysfunction, ultimately leading to male infertility. Natural bioactive compounds have attracted considerable attention as potential therapeutic agents because of their favorable safety profiles and broad reproductive protective effects. This study investigated the protective effects of hederagenin (HG), a naturally occurring triterpenoid saponin, against heat stress-induced testicular damage and spermatogenic dysfunction and explored its potential endocrine regulatory mechanisms. A mouse model of surgical cryptorchidism was established to mimic heat stress-induced reproductive damage. Animals were randomly assigned to a sham surgery group, a cryptorchidism model group, and three HG treatment groups receiving low (20 mg/kg), medium (40 mg/kg), or high (80 mg/kg) doses. HG was administered daily by subcutaneous injection for five consecutive weeks. The results showed that HG treatment significantly improved testicular development, alleviated histopathological damage, and increased epididymal sperm counts in cryptorchid mice in a dose-dependent manner with the greatest efficacy observed in the high-dose group. Furthermore, HG markedly modulated reproductive hormone profiles, increasing serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2) levels while reducing testosterone (T) concentrations. These findings indicated that HG could effectively mitigate heat stress-induced testicular injury and spermatogenic dysfunction. The protective effects of HG might be mediated through regulation of the hypothalamic-pituitary-gonadal (HPG) axis. This study demonstrated the therapeutic potential of HG in ameliorating heat stress-associated reproductive damage and provided experimental evidence supporting its development as a natural candidate for the treatment of male infertility associated with impaired spermatogenesis.

Keywords: hederagenin; cryptorchidism; spermatogenic dysfunction; spermatogenesis; reproductive hormones; male infertility.

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Introduction

Male infertility is a major global public health concern, affecting approximately 15% of couples worldwide with male-related factors accounting for nearly half of all infertility cases [1]. The causes of male infertility are multifactorial and include genetic abnormalities, endocrine disorders, anatomical defects, infections, environmental exposures, and idiopathic factors. Impaired spermatogenesis, one of the most common pathological features of male infertility, is frequently manifested by abnormal semen parameters including oligospermia, asthenospermia, and azoospermia, ultimately compromising male fertility [2]. Consequently, the development of effective strategies to preserve or restore spermatogenic function remains a critical objective in the management of male reproductive disorders. While assisted reproductive technologies provide effective clinical options for male infertility, there remains a pressing need for pharmacological interventions capable of restoring the testicular microenvironment and improving sperm quality. However, current therapeutic strategies for idiopathic spermatogenic disorders are limited and often exhibit variable efficacy [3].

In recent years, natural bioactive compounds have attracted increasing attention as potential candidates for the prevention and treatment of reproductive dysfunction, owing to their multi-target regulatory properties and favorable safety profiles [4]. A growing body of evidence indicates that such compounds can protect male reproductive function by maintaining endocrine homeostasis, attenuating tissue damage, and promoting spermatogenesis. Resveratrol and curcumin have been documented to alleviate toxicant- and stress-induced testicular injury through modulation of reproductive hormone secretion [5, 6]. Normal spermatogenesis depends on the precise regulation of reproductive endocrine hormones. Endocrine imbalance is widely recognized as a central pathological feature of spermatogenic disorders as well as a key therapeutic target [7, 8], which provides a critical theoretical basis for the exploration of natural compounds with endocrine-regulating and reproductive-protective potential. Hederagenin (HG) is a naturally occurring pentacyclic triterpenoid aglycone widely distributed in medicinal plants including *Hedera helix*, *Lonicera japonica*, and *Sambucus williamsii* [9]. Pharmacological studies have demonstrated that HG possesses diverse biological activities including anti-inflammatory [10], antioxidant [11], antitumor [12], and neuroprotective properties [13]. Notably, HG can modulate multiple hormone-related signaling pathways. Nguyen *et al*. reported that an HG-containing formulation ameliorated atopic dermatitis in mice through the regulation of multiple hormones [14]. Zhou *et al*. demonstrated that HG exerted antidepressant effects in rats as evidenced by reduced plasma adrenocorticotropic hormone and serum corticosterone levels [15]. These findings indicate a potential role for HG in the regulation of endocrine homeostasis. Despite its broad pharmacological potential, the effects of HG on male reproductive function, particularly spermatogenesis, remain poorly understood. A previous study on a traditional Chinese medicine (TCM) formula for obesity-related oligoasthenospermia identified HG as a key bioactive constituent involved in improving sperm quality by mediating hormone-related signaling pathways [16]. However, the direct effects of HG on testicular function and spermatogenesis, as well as its underlying mechanisms, have yet to be experimentally validated.

Given the high safety and multi-target pharmacological properties of HG, this study explored its protective effects against heat stress-induced spermatogenic dysfunction and further investigated the underlying endocrine regulatory mechanisms to provide valuable insights into the development of HG as a natural therapeutic candidate for male infertility. The study employed an established mouse model of cryptorchidism to simulate heat stress-induced testicular injury by surgically fixing the animal

testis within the abdominal cavity to create a high-temperature microenvironment that caused damage characterized by seminiferous tubule degeneration, germ cell apoptosis, and spermatogenic arrest [17]. The effects of HG on general growth status, relative testicular weight, testicular histopathological alterations, spermatogenic function were evaluated, and serum concentrations of key reproductive hormones including testosterone (T), estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH) were measured to elucidate the endocrine mechanisms underlying the protective actions of HG. The results of this study provided experimental evidence supporting the development of HG as a potential natural therapeutic agent for male infertility associated with spermatogenic dysfunction.

Materials and methods

Experimental animals

A total of 25 specific pathogen-free (SPF) male Kunming mice, aged 10 – 15 days, weighed 10 – 15 g, were obtained from the Huaxing Experimental Animal Breeding Farm (Zhengzhou, Henan, China). Animals were housed in a controlled environment with 23 ± 2°C, 50 – 65% relative humidity, and a 12-h light/12-h dark cycle. All animals had free access to food and water throughout the experiment period. The procedures of this research were approved by the Animal Ethics Committee of Huanghuai University (Zhumadian, Henan, China).

Establishment of the cryptorchidism animal model and experimental grouping

Following a 3-day acclimation period, the animals were randomly allocated into five groups with 5 mice in each group, which included a sham surgery group (BO), a cryptorchidism model group (CO), and three HG treatment groups with the HG doses of 20 mg/kg as low (H20), 40 mg/kg as medium (H40), and 80 mg/kg as high (H80). Animals were housed individually according to their group assignments. After anesthesia by intraperitoneal injection of 1% pentobarbital sodium, a midline abdominal incision was performed. The testis was moved outside the body and gently pulled, then secured to the abdominal wall muscle with sutures to establish a cryptorchidism model, while the testis in BO group was only exteriorized gently without traction or fixation. Daily subcutaneous HG (Bide Pharmatech, Shanghai, China) administration was initiated immediately after surgery, while BO and CO groups received an equivalent volume of saline following the same schedule for five consecutive weeks.

Sample collection

Body weight was recorded weekly throughout the experimental period. 24 hours after the final treatment, blood samples were collected from the retro-orbital venous plexus and stored at 4°C overnight followed by centrifugation at 3,000 × g for 15 min. The serum samples were aliquoted and stored at –80°C until further analysis. Following blood collection, mice were euthanized by cervical dislocation. Both testes and epididymides were carefully excised. Adherent connective and adipose tissues were removed before weighing. The testicular coefficient was calculated as below.

Testicular coefficient (%) = (testis weight / body weight) × 100%.

Sperm count

Each epididymis was finely minced in 200 μL of pre-warmed phosphate-buffered saline (PBS). After brief incubation, a 10 μL aliquot of the resulting suspension was loaded onto a ML-900II hemocytometer (Mailang, Nanning, Guangxi, China). The spermatozoa were counted under a light microscope.

Histopathological examination

Testicular tissues were fixed in Bouin's solution for 10 h, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Paraffin blocks were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin (H&E) using standard procedures.

Histopathological alterations were evaluated under a light microscope at 200× magnification.

Serum hormone measurement

Serum concentrations of T, E2, LH, FSH, and GnRH were determined by using commercial enzyme-linked immunosorbent assay (ELISA) kits (Uping Bio, Hangzhou, Zhejiang, China) following the manufacturer's instructions.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 8.0.2 (GraphPad Software, Boston, MA, USA). Data were presented as mean ± standard deviation (SD). Differences among multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Comparisons between two groups were performed using Student's t-test. A *P* value less than 0.05 was defined as statistically significant difference, while *P* value less than 0.01 was defined as very significant difference.

Results

Effects of HG on body weight and testicular development

The results demonstrated that mice exhibited transient reductions in food intake and locomotor activity following surgery. These changes were resolved within 24 hours. No obvious signs of distress or treatment-related adverse effects were observed during the study. No significant differences in initial body weight were detected among groups. Body weight increased in all groups throughout the study period. Although mice in the CO group displayed a transient increase in body weight compared with those in the H80 group at week 3 ($P < 0.05$), this difference was not maintained at later time points. At the end of the experiment, body weights did not differ significantly among groups (Figure 1). The results indicated that neither cryptorchidism induction nor HG treatment adversely affected normal growth. Testicular development was further assessed by calculating the testicular coefficient. The results showed that the left testicular coefficient of CO group was significantly decreased compared to that of BO group ($P < 0.01$). HG intervention partially attenuated this effect with H80 group significantly increased the left testicular coefficient compared to that of CO group ($P < 0.01$), whereas no significant differences were observed in H20 or H40 groups. A similar pattern was observed for the right testicular coefficient (Table 1). These findings suggested that HG could alleviate cryptorchidism-induced testicular impairment in a dose-dependent manner.

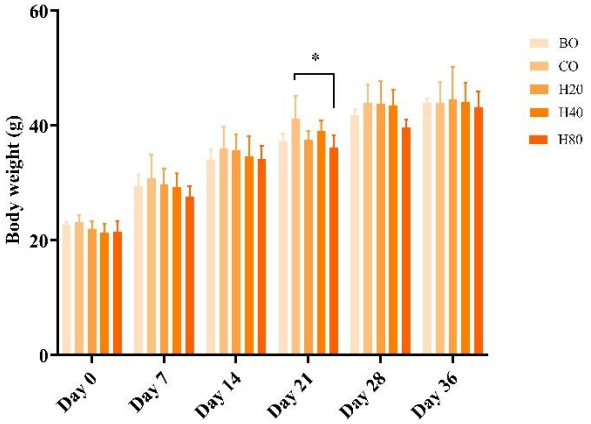

Figure 1. Effect of hederagenin on body weight in cryptorchid mice. *: $P < 0.05$.

Table 1. Effect of hederagenin on testicular coefficient in cryptorchid mice.

No.	Group	Left testis (mg/g)	Right testis (mg/g)
1	BO	3.46 ± 0.21$^{\#\#}$	3.44 ± 0.40$^{\#\#}$
2	CO	1.00 ± 0.04**	1.00 ± 0.05**
3	H20	0.96 ± 0.05**	1.03 ± 0.06**
4	H40	1.03 ± 0.23**	1.13 ± 0.08**
5	H80	2.11 ± 0.44$^{**\#\#}$	2.23 ± 0.08$^{**\#\#}$

Notes: **: $P < 0.01$ compared with BO group. $^{\#\#}$: $P < 0.01$ compared with CO group.

HG ameliorated testicular histopathology and promoted spermatogenesis

Testicular histopathological alterations demonstrated that, in BO group, seminiferous tubules exhibited normal architecture,

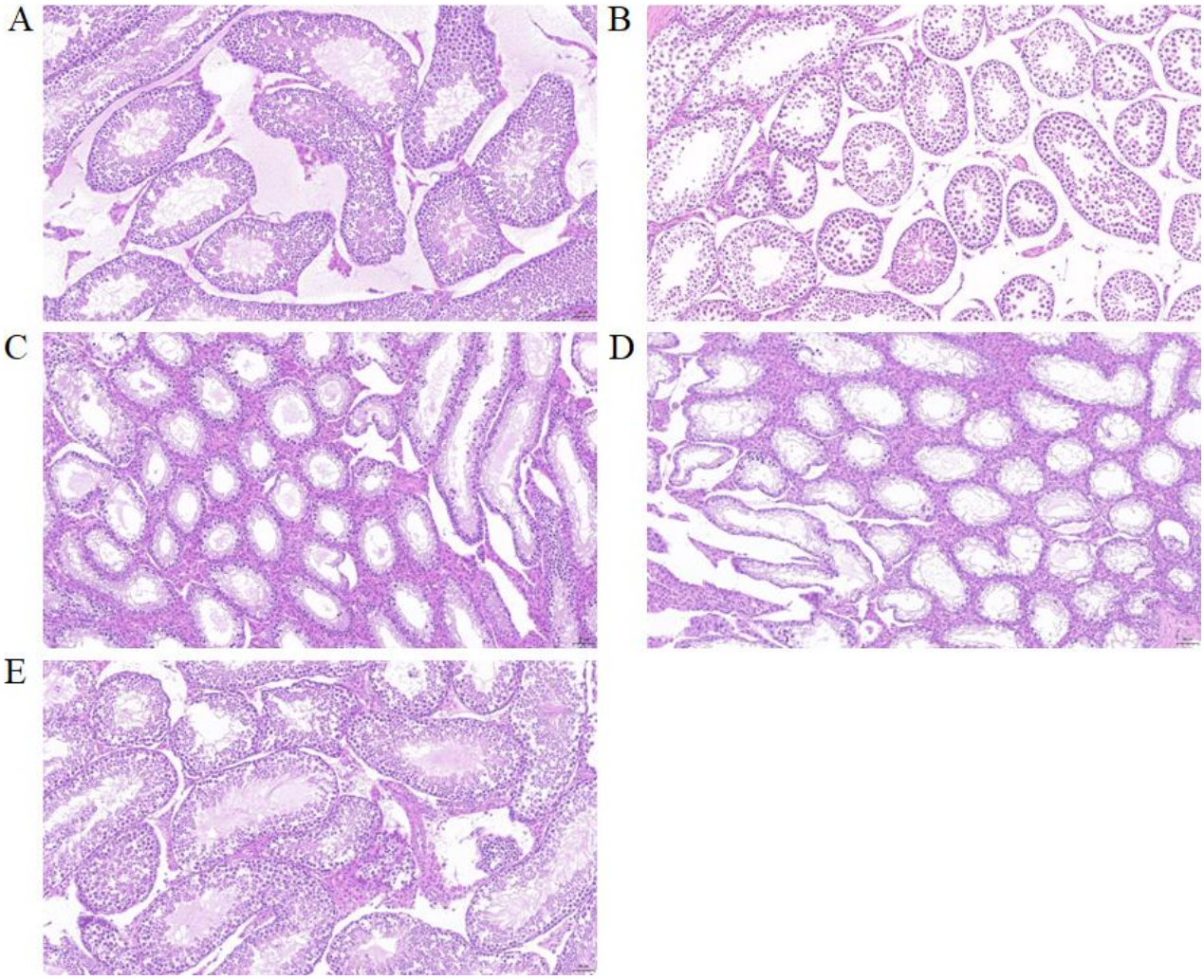

Figure 2. Effect of hederagenin on testicular histology induced by cryptorchid surgery. **A.** BO group. **B.** CO group. **C.** H20 group. **D.** H40 group. **E.** H80 group.

characterized by intact tubular structure, orderly stratification of germ cells, abundant mature spermatozoa in the lumen, and normal interstitial cell morphology (Figure 2A). In contrast, CO group showed severe testicular damage including seminiferous tubular atrophy, reduced luminal diameter, disorganization and depletion of germ cell layers, complete absence of mature sperm in the lumen, and degeneration of interstitial cells (Figure 2B). These findings confirmed the successful establishment of cryptorchidism-induced testicular injury and spermatogenic arrest. HG treatment significantly alleviated these histopathological abnormalities. H20 group showed minimal histological recovery (Figure 2C), while H40 group showed moderate improvements including improved tubular structure, thickened epithelium, and occasional presence of luminal spermatozoa (Figure 2D). H80 group exhibited the most pronounced improvement with partially restored seminiferous tubular architecture, increased epithelial thickness, elevated germ cells, visible mature sperm in the lumen, and reduced interstitial degeneration (Figure 2E). The results demonstrated that HG mitigated cryptorchidism-induced testicular damage in a dose-dependent manner. Consistent with the histopathological findings, epididymal sperm analysis further demonstrated the pro-spermatogenic effect of

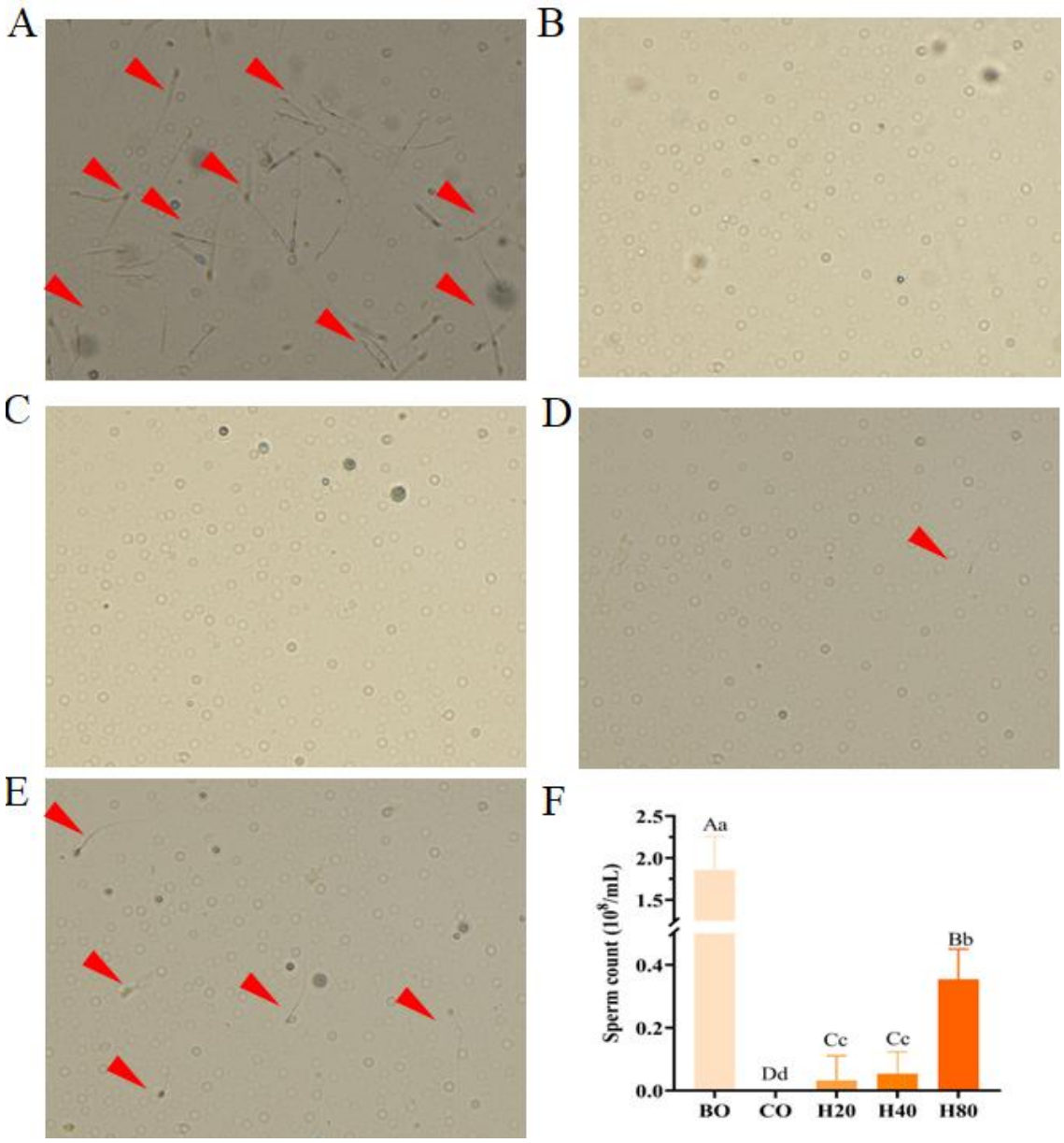

Figure 3. Sperm counts in different experimental groups. **A.** BO group. **B.** CO group. **C.** H20 group. **D.** H40 group. **E.** H80 group. **F.** quantification of A - E. Red arrow indicated spermatozoa.

HG. Compared to BO group (Figure 3A), spermatozoa were nearly absent in CO group (Figure 3B). However, HG treatment restored sperm presence in a dose-dependent manner with sparse sperm observed in H20 group (Figure 3C), a moderate increase in H40 group (Figure 3D), and a marked increase in H80 group (Figure 3E). Quantitative analysis confirmed that sperm density was significantly higher in all HG-treated groups than that in CO group ($P < 0.01$) (Figure 3F). The sperm concentrations were 0.03 ± 0.07, 0.05 ± 0.06, and 0.35 ± 0.09 (× 10^8/mL) in H20, H40, and H80 groups, respectively. However, all treatment groups remained significantly lower than BO group's 1.87 ± 0.36 × 10^8/mL. These findings confirmed successful model establishment and demonstrated a dose-dependent improvement in spermatogenic function following HG administration.

Table 2. Effect of hederagenin on hormone levels in cryptorchid mice.

No.	Group	T (ng/ mL)	E2 (pmol/L)	FSH (mIU/mL)	LH (mU/ mL)	GnRH (mIU/ mL)
1	BO	18.20 ± 2.28$^{\#\#}$	20.51 ± 1.78	26.82 ± 2.15	19.51 ± 0.76$^{\#}$	50.63 ± 5.11
2	CO	15.42 ± 1.49**	20.28 ± 1.13	25.75 ± 4.80	19.49 ± 1.91*	45.50 ± 1.45
3	H20	7.74 ± 0.67$^{**\#\#}$	35.53 ± 1.17$^{**\#\#}$	61.36 ± 5.44$^{**\#\#}$	26.19 ± 3.08$^{**\#\#}$	30.68 ± 5.54$^{**\#\#}$
4	H40	9.37 ± 0.57$^{**\#\#}$	33.18 ± 3.07$^{**\#\#}$	52.61 ± 2.97$^{**\#\#}$	23.96 ± 2.62$^{**\#\#}$	36.23 ± 1.64$^{**\#\#}$
5	H80	11.78 ± 1.15$^{**\#\#}$	27.20 ± 3.11$^{**\#\#}$	33.11 ± 2.63$^{**\#\#}$	22.79 ± 1.84$^{*\#}$	42.54 ± 4.37**

Notes: *: $P < 0.05$ compared to BO group. **: $P < 0.01$ compared to BO group. $^{\#}$: $P < 0.05$ compared to CO group. $^{\#\#}$: $P < 0.01$ compared to CO group.

Effects of HG on serum reproductive hormone profiles

Measurements of key serum hormone levels revealed that HG treatment induced significant alterations in the hormonal profiles of cryptorchid mice with most effects exhibiting a dose-dependent pattern. Serum testosterone (T) levels were significantly reduced in CO group compared to that in BO group ($P < 0.01$). HG administration in all different concentration groups further decreased T levels in a dose-dependent manner compared to that in CO group ($P < 0.01$). H20 group exhibited the lowest T concentration followed by H40 and H80 groups. Despite partial variation among doses, all HG-treated groups showed significantly lower T levels than that in CO group. Serum estradiol (E2) displayed a non-significant decrease in CO group compared to that in BO group. HG treatment significantly increased E2 levels compared to that in CO group ($P < 0.01$). This increase was dose-dependent. E2 levels in H20 and H40 groups were comparable and at the highest levels, whereas E2 levels in H80 group were significantly lower than those in H20 and H40 groups ($P < 0.01$). Notably, the E2 levels in all HG treated groups remained significantly higher than that in both BO and CO groups ($P < 0.01$). Serum follicle-stimulating hormone (FSH) levels were comparable between CO and BO groups. HG treatments significantly increased FSH levels compared to that in CO group ($P < 0.01$) in a dose-dependent manner. H20 group showed the highest FSH levels followed by a decline in H40 group, while H80 group exhibited intermediate levels that remained significantly higher than that in CO group but lower than that in H40 group ($P < 0.01$). The FSH level in H80 group did not differ significantly from BO group. Luteinizing hormone (LH) levels did not differ significantly between CO and BO groups. All HG treatment groups significantly increased serum LH level compared to that in CO group ($P < 0.01$). H20 group exhibited the highest LH level, whereas H40 and H80 groups showed comparatively lower but still elevated LH levels compared to that in both BO and CO groups. In addition, LH levels in H80 group were significantly lower than that in H20 group ($P < 0.05$). Gonadotropin-releasing hormone (GnRH) levels showed no significant difference between CO and BO groups. HG treatment produced a dose-dependent modulation of GnRH. GnRH levels were significantly reduced in H20 and H40 groups compared to CO group ($P < 0.01$) with no significant difference between H20 and H40. In contrast, H80 group exhibited higher GnRH levels than that in H20 group ($P < 0.01$) and no significant difference to CO group, indicating a dose-dependent regulatory effect with attenuation at higher dosage (Table 2).

Discussion

The results of this research demonstrated that HG treatment significantly improved testicular development, ameliorated histopathological damage, promoted spermatogenesis, and modulated key serum reproductive hormone profiles in a dose-dependent manner, which provided *in vivo* evidence supporting the protective effects of HG on male reproductive

function under heat stress conditions. Cryptorchidism-induced testicular heat stress is known to promote excessive reactive oxygen species (ROS) accumulation, leading to oxidative damage, inflammatory responses, and subsequent germ cell loss, which underlying spermatogenic failure. HG markedly attenuated structural damage to the seminiferous epithelium and restored spermatogenic activity. This study successfully established a mouse model of surgical cryptorchidism to mimic heat stress-induced testicular injury by relocating the testis into the abdominal cavity. Compared to control (BO) group, cryptorchid (CO) mice group exhibited reduced testicular coefficients, severe seminiferous tubule atrophy, disorganization of the seminiferous epithelium, and complete spermatogenic arrest. These findings were consistent with previous reports, confirming successful model establishment [5, 6, 17, 18]. HG administration, particularly at high dosage, significantly ameliorated these pathological alterations and partially restored spermatogenic function. Recovery from azoospermia to the presence of detectable epididymal sperm was observed following HG treatment, which indicated that HG protected the seminiferous epithelium, inhibited germ cell apoptosis, and promoted germ cell proliferation and differentiation, thereby helping to restore a testicular microenvironment conducive to spermatogenesis. HG exhibited potent anti-inflammatory [19], antitumor [11], and antioxidant [14] activities through the modulation of multiple signaling pathways. In the cryptorchidism model, elevated intratesticular temperature induced mitochondrial dysfunction and excessive generation of ROS, leading to oxidative stress, inflammatory responses, and germ cell apoptosis, all of which were key pathological processes of spermatogenic dysfunction [20]. The results of this study suggested that the protective effects of HG might be mediated through its antioxidant and anti-inflammatory properties, which could mitigate oxidative damage and inflammatory injury within the testicular microenvironment. Such effects might enhance germ cell survival and differentiation, thereby contributing to the observed histological recovery and restoration of sperm production. Future studies were warranted to elucidate the underlying mechanisms by examining the expression of oxidative stress-related markers, inflammatory cytokines, and apoptosis-associated proteins in testicular tissue.

This study explored the modulating effects of HG on serum reproductive hormone profiles. Precise regulation of the HPG axis is fundamental to normal spermatogenesis [8]. Cryptorchidism significantly reduced serum testosterone levels, reflecting impaired Leydig cell function. HG treatment further decreased testosterone levels while simultaneously increasing LH and FSH levels, indicating a deviation from the classical negative feedback regulation of the HPG axis [21]. Several non-mutually exclusive mechanisms might account for this endocrine profile. Cryptorchidism-induced Leydig cell dysfunction might be too severe to be fully compensated by gonadotropin stimulation within the experimental period [22]. Further, HG might influence steroidogenesis by modulating key enzymes involved in testosterone biosynthesis and steroid hormone metabolism [23]. Furthermore, HG might act at the level of the hypothalamus and/or anterior lobe of pituitary, enhancing GnRH releasing and directly stimulating gonadotrophs releasing, resulting in excessive LH/FSH secretion [24], which consequently caused reduced responsiveness to LH stimulation in the testes [25]. Besides its effects on androgen metabolism, HG also significantly increased circulating estradiol levels. Estradiol plays a bidirectional role in male reproductive physiology. The physiological concentrations of estradiol are essential for maintaining testicular homeostasis and spermatogenic function, whereas excessive estradiol may impair spermatogenesis [26]. In this study, the concomitant rise in E2 levels and the improvement in spermatogenesis outcomes might be associated with adaptive changes in testicular aromatase activity during tissue repair or with alterations in the hepatic metabolism and

clearance of estrogenic hormones under the influence of HG [27]. In addition, HG exerted dose-dependent effects on hypothalamic endocrine regulation. GnRH levels were reduced at low and medium doses of HG treatments but partially recovered at the high dose, which suggested that HG might act through a threshold- or bidirectional regulatory mechanism within the HPG axis. These endocrine alterations demonstrated that the reproductive protective effects of HG were not solely mediated at the gonadal level but might also involve systemic modulation of hormonal homeostasis.

This study also had several limitations. The research was primarily descriptive, relying largely on histopathological and hormonal endpoints. The precise molecular targets and intracellular signaling pathways mediating the effects of HG remained unclear. Although the tested HG dose range of 20 – 80 mg/kg demonstrated significant biological activity, it might not encompass the optimal therapeutic dose. Moreover, the 5-week treatment period primarily reflected short-term efficacy. The long-term effectiveness and safety of HG required further assessment. Further, this study was conducted exclusively *in vivo*. The direct cellular effects of HG and the underlying molecular mechanisms warranted further investigation using *in vitro* models. The present *in vivo* findings of this research demonstrated that HG effectively attenuated cryptorchidism-induced testicular injury and spermatogenic dysfunction through the regulation of reproductive hormone homeostasis within the HPG axis. HG, as a natural triterpenoid compound with a favorable safety profile, showed great promise as a candidate for developing novel therapies for heat-related male infertility. These findings provided a valuable experimental basis for future translational studies and the development of HG-derived compounds and structural analogs for the treatment of male reproductive disorders.

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References

1. Agarwal A, Baskaran S, Parekh N, Cho C, Henkel R, Vij S, *et al*. 2021. Male infertility. The Lancet. 397(10271):319-333.
2. Cannarella R, Condorelli RA, Mongioì LM, La Vignera S, Calogero AE. 2020. Molecular biology of spermatogenesis: Novel targets of apparently idiopathic male infertility. Int J Mol Sci. 21(5):1728.
3. Duca Y, Aversa A, Condorelli RA, Calogero AE, La Vignera S. 2019. Substance abuse and male hypogonadism. J Clin Med. 8(5):732.
4. Xie S, Zhan F, Zhu J, Xu S, Xu J. 2025. The latest advances with natural products in drug discovery and opportunities for the future: A 2025 update. Expert Opin Drug Discov. 20(7):827-843.
5. Li S, Li Y, Chen F, Yang Y, Song L, Liu C, *et al*. 2021. Metabolomics analysis reveals metabolic changes associated with trans-resveratrol treatment in experimental cryptorchidism mice. Reprod Fertil Dev. 33(5):328-337.
6. Wang Z, Chen F, Li Y, Liu C, Wang L, Shao W, *et al*. 2025. Curcumin promotes spermatogenesis in mice with cryptorchidism by regulating testicular protein O-GlcNAcylation. Front Endocrinol (Lausanne). 16:1555721.
7. Stavros S, Kathopoulis N, Moustakli E, Potiris A, Anagnostaki I, Topis S, *et al*. 2025. Endocrine-disrupting chemicals and male infertility: Mechanisms, risks, and regulatory challenges. J Xenobiot. 15(5):165.
8. Lahimer M, Abou Diwan M, Montjean D, Cabry R, Bach V, Ajina M, *et al*. 2023. Endocrine disrupting chemicals and male fertility: From physiological to molecular effects. Front Public Health. 11:1232646.
9. Zeng J, Huang T, Xue M, Chen J, Feng L, Du R, *et al*. 2018. Current knowledge and development of hederagenin as a promising

medicinal agent: A comprehensive review. RSC Adv. 8(43):24188-24202.
10. Mfotie Njoya E, Ndemangou B, Akinyelu J, Munvera AM, Chukwuma CI, Mkounga P, *et al*. 2023. *In vitro* antiproliferative, anti-inflammatory effects and molecular docking studies of natural compounds isolated from *Sarcocephalus pobeguinii* (Hua ex Pobég). Front Pharmacol. 14:1205414.
11. Kim EH, Baek S, Shin D, Lee J, Roh J. 2017. Hederagenin induces apoptosis in cisplatin-resistant head and neck cancer cells by inhibiting the Nrf2-ARE antioxidant pathway. Oxid Med Cell Longev. 2017:5498908.
12. Rodríguez-Hernández D, Demuner AJ, Barbosa LCA, Csuk R, Heller L. 2015. Hederagenin as a triterpene template for the development of new antitumor compounds. Eur J Med Chem. 105:57-62.
13. Yu H, Song L, Cao X, Li W, Zhao Y, Chen J, *et al*. 2020. Hederagenin attenuates cerebral ischaemia/reperfusion injury by regulating MLK3 signaling. Front Pharmacol. 11:1173.
14. Nguyen LTH, Oh T, Nguyen UT, Choi M, Yang I, Shin H. 2020. A natural compound mixture containing arctigenin, hederagenin, and baicalein alleviates atopic dermatitis in mice by regulating HPA axis and immune activity. Evid Based Complement Alternat Med. 2020:1-13.
15. Zhou D, Jin H, Lin H, Yang X, Cheng Y, Deng F, *et al*. 2010. Antidepressant effect of the extracts from *Fructus Akebiae*. Pharmacol Biochem Behav. 94(3):488-495.
16. Dong Y, Zheng Y, Zhu L, Li T, Guan Y, Zhao S, *et al*. 2022. Hua-Tan-Sheng-Jing decoction treats obesity with oligoasthenozoospermia by up-regulating the PI3K-AKT and down-regulating the JNK MAPK signaling pathways: At the crossroad of obesity and oligoasthenozoospermia. Front Pharmacol. 13:896434.
17. Li E, Guo Y, Wang G, Chen F, Li Q. 2015. Effect of resveratrol on restoring spermatogenesis in experimental cryptorchid mice and analysis of related differentially expressed proteins. Cell Biol Int. 39(6):733-740.
18. Li Y, Xin J, Li S, Chen F, Yang Y, Guo H, *et al*. 2024. Lycopene restores spermatogenesis in cryptorchidism mice through Wnt signaling pathway. J Biotech Res. 18:44-52.
19. Yang G, Yang W, Jiang H, Yi Q, Ma W. 2023. Hederagenin inhibits high glucose-induced fibrosis in human renal cells by suppression of NLRP3 inflammasome activation through reducing cathepsin B expression. Chem Biol Drug Des. 102(6):1409-1420.
20. Wagner H, Cheng JW, Ko EY. 2017. Role of reactive oxygen species in male infertility: An updated review of literature. Arab J Urol. 16(1):35-43.
21. Finkelstein JS, O'Dea LS, Whitcomb RW, Crowley WF Jr. 1991. Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab. 73(3):621-628.
22. Leiper AD, Grant DB, Chessells JM. 1986. Gonadal function after testicular radiation for acute lymphoblastic leukaemia. Arch Dis Child. 61(1):53-56.
23. Wang W, Mu Q, Feng X, Liu W, Xu H, Chen X, *et al*. 2023. Sweet taste receptor T1R3 expressed in Leydig cells is closely related to homeostasis of the steroid hormone metabolism profile. J Agric Food Chem. 71(20):7791-7802.
24. Sesti LA, Britt JH. 1993. Agonist-induced release of gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone and their associations with basal secretion of luteinizing hormone and follicle-stimulating hormone throughout lactation in sows. Biol Reprod. 49(2):332-339.
25. Bhasin S, Fielder TJ, Sod-Moriah UA, Swerdloff RS. 1987. Testicular modulation of luteinizing hormone response to gonadotropin-releasing hormone (GnRH)-agonist treatment. Biol Reprod. 36(2):309-313.
26. Carreau S, Bouraima-Lelong H, Delalande C. 2012. Estrogen, a female hormone involved in spermatogenesis. Adv Med Sci. 57(1):31-36.
27. Jawad M, Nasir S, Xu H, Wang Y, Qin S, Wang A, *et al*. 2026. Molecular and histopathological mechanisms underlying pesticide-induced disruption of the hypothalamic-pituitary-gonadal axis in Japanese medaka. J Hazard Mater. 503:141069.