Research Article

Evaluation of the Endocrine Effects of *Eruca sativa* on Male Reproductive System in a Rat Model

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Abstract | *Eruca sativa* possesses a diverse phytochemical profile, including antioxidants and bioactive constituents that are recognized for their potential reproductive health benefits. The present study aimed to investigate the modulatory effects of *E. sativa* extract on serum concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in adult male rats, and explore the histological alterations in their testes. A total of forty adult male rats were randomly allocated into four experimental groups (n = 10 per group): a control group and three treatment groups administered *E. sativa* extract orally at doses of 250 mg/kg (low), 375 mg/kg (medium), and 500 mg/kg (high) for 30 consecutive days. Hormonal levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits. Histological sections were routinely prepared from the testes after dissection of the rats, stained with Hematoxylin and Eosin, and examined under a light microscope. The results showed that treatment with *E. sativa* resulted in statistically significant ($p < 0.05$) dose dependent increases in serum testosterone, LH, and FSH levels. The high dose group showed the greatest hormonal elevations compared to control group ($p < 0.05$), whereas the medium dose group also showed significant elevation ($p < 0.05$). Although the low dose group showed high level but the changes were not statistically significant. Histological study showed multiple alterations in the treated group testes including, increase in the size, capsule thickness, diameter of seminal tubule, germinal cells, Sertoli cells, and Leydig cells compared to control group. In conclusion, the results indicate that *E. sativa* exerts stimulatory-effects on male-reproductive system potentially through activation of steroidogenic pathways and modulation of the hypothalamic-pituitary-gonadal-axis.

Keywords | *Eruca sativa*, Testosterone, Luteinizing hormone, Follicle-stimulating hormone, Male fertility, Reproductive endocrinology, Leydig cells, Hypothalamic-pituitary-gonadal axis

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INTRODUCTION

The testes are the primary anatomical structures in the male reproductive system. Their physiological roles include the production of both spermatozoa and sex steroid hormones (Li *et al*., 2024). Male-reproductive-health is regulated by a complex interaction of hormonal messages, cellular activity and extrinsic-influences (Al-Suhaimi *et al*., 2022). Central to this regulation is a hypothalamic-pituitary-gonadal (HPG) axis which controls the synthesis and the secretion of the primary-reproductive-hormones which including testosterone hormones, luteinizing hormone (LH) and follicle-stimulating-hormone (FSH) (Dwyer and Quinton, 2019). These hormones are crucial for spermatogenesis, sexual-function and the development of male secondary sexual characteristics. Disorders in this hormonal system can cause reduced fertility in both male or female (Li *et al*., 2024).

Testosterone hormone considers as the main androgen hormone in males is mostly synthesized by the Leydig-cells which located within the testes (Zirkin and Papadopoulos, 2018). It is responsible for male-sexual-development, muscle and bone density. It is stimulated by LH which is secreted by the anterior pituitary gland under the influence of gonadotropin-releasing hormone (GnRH) from the hypothalamus gland (Oduwole *et al*., 2021). FSH hormone also from the anterior-pituitary gland and acts on Sertoli-cells to stimulate spermatogenesis. Together, these hormones (LH and FSH) form a tightly regulated system which are fundamental to maintaining male-reproductive capacity and overall physiological health (Santi *et al*., 2020).

Multiple factors including advancing age, chronic-medical-conditions, exposure to environmental-toxins and insufficient-nutritional status can interfere with the hormonal-axis and leading to diminished levels of testosterone hormone and gonadotropins-hormones (Maqbool *et al*., 2016). The growing worldwide prevalence of male-infertility has driven efforts to identify therapeutic approaches that are both effective and safe. As part of these efforts, natural-products particularly those derived from medicinal-plants have emerged as promising candidates for promoting and sustaining male reproductive health (Adewoyin *et al*., 2017).

Medicinal plants had been utilized for centuries in traditional medicine systems to address infertility and sexual-dysfunction (Abed *et al*., 2019). These plants commonly contain several bioactive-compounds such as flavonoids, alkaloids, saponins and phenolic- compound which may enhance hormonal activity, mitigate oxidative-stress and support the integrity of reproductive-tissues (Abed *et al*., 2015). *E. sativa* plant commonly referred to as 'rocket or arugula' is one such plant with recognized-therapeutic potential. A member of the *Brassicaceae* family, *E. sativa* is a staple in Mediterranean and Middle Eastern diets and is widely valued for its health-enhancing properties (Barazani and Ziffer-Berger, 2014). *E. sativa* is rich in bioactive-constituents which including glucosinolates, flavonoids, phenolics, alkaloids and unsaturated fatty-acids (Pagnotta *et al*., 2022). These compounds are associated with antioxidant, anti-inflammatory, antimicrobial and also anticarcinogenic properties. The antioxidant-potential of *E. sativa* is particularly relevant to male-fertility as oxidative-stress can damage testicular-tissue, impair spermatogenesis and decreased testosterone hormone production (Grami *et al*., 2024). By attenuating oxidative injury, *E. sativa* may contribute to the preservation of testicular-function and endocrine-balance.

In addition to its antioxidant-capacity, different phytochemicals compounds present in *E. sativa* may exert direct effects on hormonal-regulations (Grami *et al*., 2024; Katsarou *et al*., 2016). Flavonoids may influence steroidogenesis-related-enzymes and therefore potentially accelerated testosterone hormone synthesis (Abed *et al*., 2022). Glucosinolate metabolites (such as isothiocyanates) may influence hormonal-pathways and alter gene-expression (Fuentes *et al*., 2015). These properties suggest that *E. sativa* may improve male-reproductive-health both by protecting against oxidative stress and by enhancing hormonal-activity.

Results from animal studies supports the hypothesis that *E. sativa* may stimulate the HPG axis, potentially through mechanisms such as increased GnRH secretion also enhanced release of pituitary hormones and activation of steroidogenic-enzymes within the testes (Egbuniwe *et al*., 2024). Although the specific mechanisms responsible for these effects have not yet been fully characterized, it is hypothesized that *E. sativa* may enhance the functional activity of the hypothalamus and pituitary-gland, ultimately facilitating steroid-hormone production within the testes (Hassan and Meligi, 2017). The nitrate content of the plant may also be beneficial by aiding in nitric-oxide production which causing an increase in blood flow and possibly assisting with hormone-production and transport (Achike and Kwan, 2003). Despite traditional usage, its precise effects on male reproductive endocrine function remain insufficiently characterized. Moreover, review of literature regarding its effects on male reproductive system revealed scarce publications. Consequently, this study intends to evaluate the effect of *E. sativa* plant extract on testosterone, LH, and FSH levels in experimental male rats, in addition to explore the testes histological alterations.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh *E. sativa* leaves were collected from a local market, thoroughly washed, shade-dried, and ground into a fine powder. The powdered material was macerated in 70% ethanol for 72 hours with occasional shaking. The extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The final crude extract was stored at 4°C until use.

Experimental animals

This study approved by Animal Ethical and Research Committee, College of Dentistry, Al-Iraqia University (Ethical approval Number: ESA and HER- 10-10-07-2025). Forty adult male Wistar rats (8–10 weeks old; 200–250 g) were obtained from an accredited animal facility. Animals were housed in polycarbonate cages under standard laboratory conditions (22 ± 2°C, 12-hour light/dark cycle, relative humidity 55 ± 5%) and were allowed

free access to commercial rat chow and water. The animals were randomly divided into four equal groups (n = 10 per group), viz.,

- Group I (Control): Received distilled water orally.
- Group II (Low Dose): Received *E. sativa* extract at 250 mg/kg body weight per day orally.
- Group III (Medium Dose): Received *E. sativa* extract at 375 mg/kg body weight per day orally.
- Group IV (High Dose): Received *E. sativa* extract at 500 mg/kg body weight per day orally.

The treatment was administered daily for 30 consecutive days using an oral gavage.

Blood collection and hormone analysis

At the end of the experimental period, animals were fasted overnight and anesthetized using ketamine/xylazine. Blood samples were collected via cardiac puncture, allowed to clot, and centrifuged at 3000 rpm for 15 minutes to obtain serum (Ali *et al.*, 2024). The serum was stored at −20°C until hormonal assays were performed. Levels of testosterone, LH, and FSH were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's protocols.

Histological investigation

The testes of each rat were extracted, washed carefully with normal saline and placed in 10% neutral buffered formalin for fixation for 48 hours. The testes were processed with routine histological procedures (Parhizkar *et al.*, 2014). The specimens hydrated in graded ethanol, cleared in xylene and embedded in paraffin wax as blocks. The 4-5 µm thick sections were cut with automatic microtome and mounted on glass slides for further processing. The sections stained with Hematoxylin and Eosin and examined by light microscope under 4 X, 10 X, 20X, and 40 X magnification. The qualitative histological features were examined and image were captured by Leica image analyzer.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant. GraphPad Prism software was used for data analysis and graphical representation (Abed *et al.*, 2023).

RESULTS AND DISCUSSION

This investigation examined the impact of *E. sativa* extract on serum concentrations of testosterone, LH, and FSH and histological alterations in adult male rats administered varying doses (250, 375, and 500 mg/kg) over a 30-day period.

Administration of *E. sativa* extract produced statistically significant, dose-dependent elevations in serum testosterone levels relative to the control group 2.45 ± 0.31, 0.75 ± 0.10, and 0.62 ± 0.08 for testosterone (ng/mL), LH (mIU/mL) and FSH (mIU/mL), respectively. The highest dosage elicited the most substantial increase (5.15 ± 0.47 ng/mL), significantly surpassing control values (2.45 ± 0.31 ng/mL) at a threshold of $p < 0.05$ as shown in Figure 1.

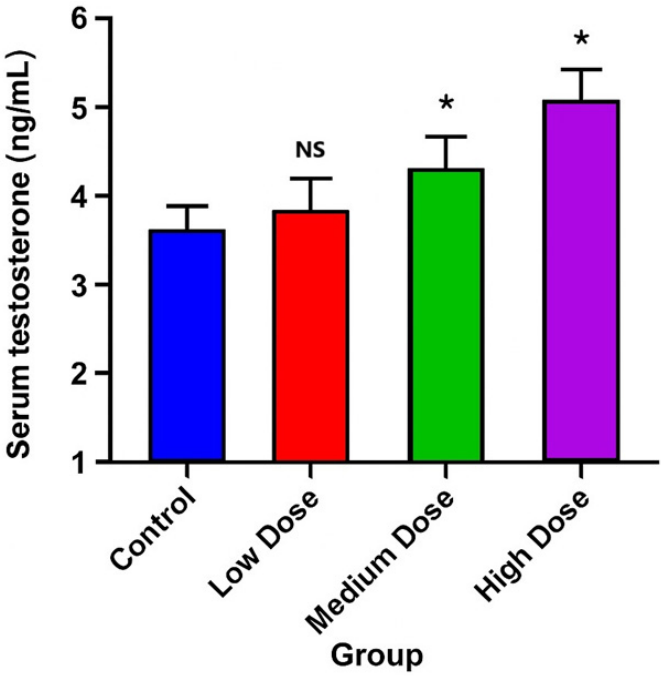

Figure 1: Effect of *E. sativa* extract on serum testosterone levels in male rats (P value ≤ 0.05).

Similarly, serum LH concentrations were markedly augmented in the medium and high-dose cohorts (1.12 ± 0.13 and 1.31 ± 0.15 mIU/mL, respectively) compared to controls (0.75 ± 0.10 mIU/mL), with significance achieved at $p < 0.05$. The low-dose group demonstrated an upward trend that did not reach statistical significance as shown in Figure 2.

FSH levels exhibited significant enhancement in both medium (0.84 ± 0.10 mIU/mL) and high-dose (0.95 ± 0.12 mIU/mL) groups versus controls (0.62 ± 0.08 mIU/mL), with the highest dose showing the greatest effect, again significant at $p < 0.05$ as illustrated in Figure 3.

The testes of the treated rats with *E. sativa* extract were larger in size than the control group. Various histological features were observed including dense regular interstitial connective tissue without inflammatory cells infiltration or fibrosis. The treated groups sections revealed well-organized seminiferous tubules and conserved architecture. Multiple layers of spermatogenic cell were appeared active

and in various stages of maturation like spermatogonia, primary spermatocytes and spermatids (Figure 4).

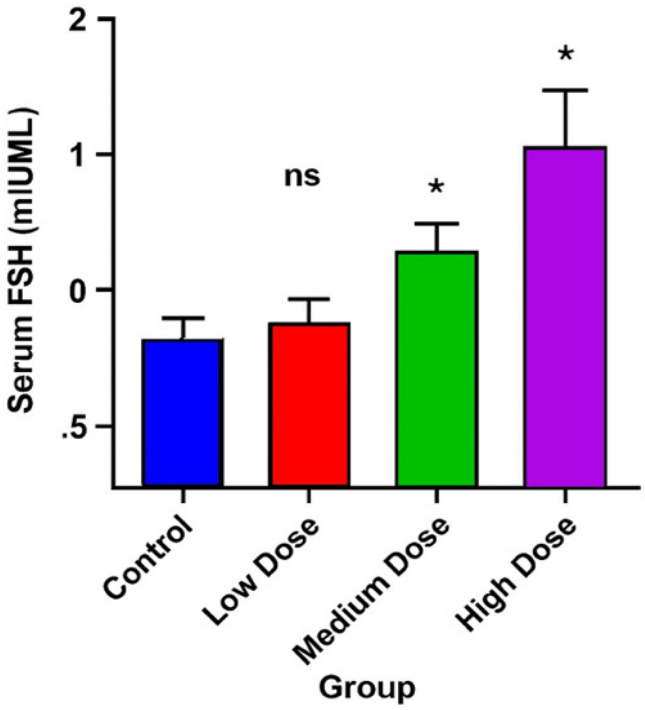

Figure 2: Effect of *E. sativa* Extract on Serum Luteinizing Hormone (LH) Levels in Male Rats (P value ≤ 0.05).

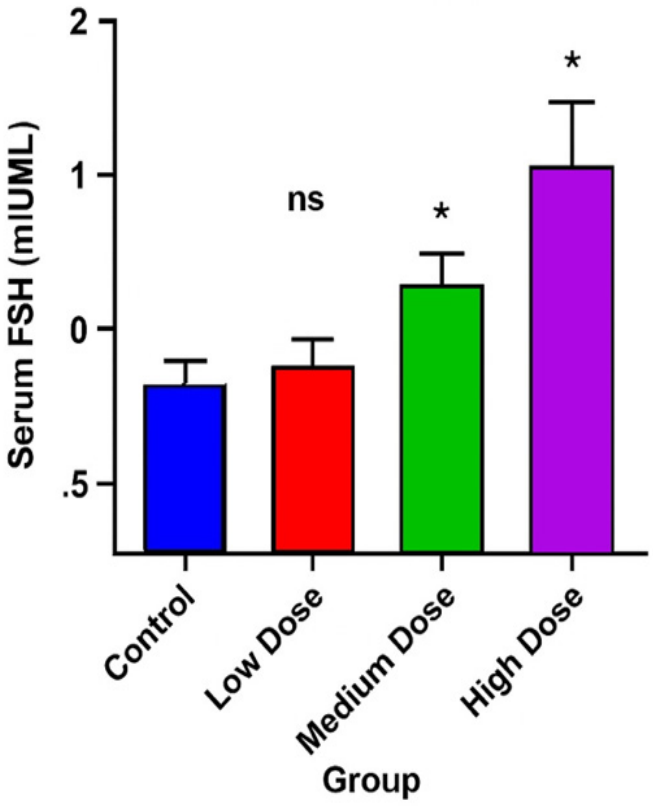

Figure 3: Effect of *E. sativa* extract on serum follicle-stimulating hormone (FSH) levels in male rats (P value ≤ 0.05).

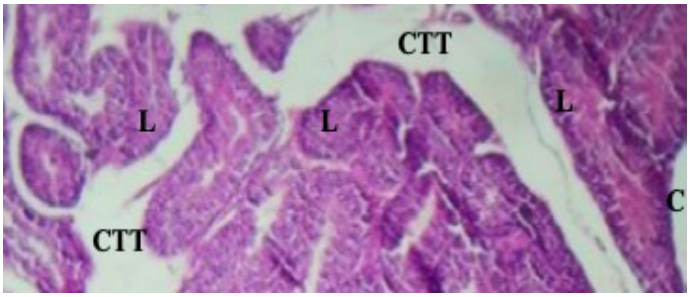

Figure 4: Histological section of testicular tissue from the treatment group, showing connective tissue trabeculae (CTT), capsule (C), and seminiferous tubules. Stained with H and E, magnification 100×.

There was also obvious increase in the density of spermatogenic cells compared to the control (untreated) group, with no vacuolation or damage in the germinal epithelium. These features reflex the protection effects of *E. sativa* against oxidation or toxic injuries. The present Sertoli cells supported the germ cells and preserve the tubular organization. The testes sections of the experimental groups also showed normal or minor increase in the leydig interstitial cell population that sustained the morphology and support the intact steroidogenic activities (Figures 5 and 6).

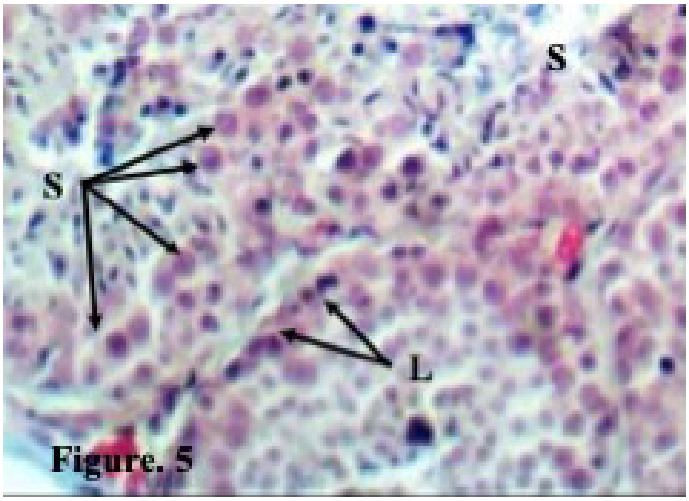

Figure 5: Histological section of testicular tissue from the treatment group showing Sertoli cells (S) and interstitial Leydig cells (L). Stained with H and E, magnification 100×.

These histological features approved the ameliorative effect of *E. sativa* on testicular tissue to keep the normal spermatogenesis, which might be related to the antioxidant and protective phytochemical constituents such as the flavonoids, glucosinolates, and isothiocyanates. These results are compatible with previously published studies (Sukmawati *et al.*, 2019), who approved the ameliorative effects of vitamin E against the toxic effects of allethrin by investigating the histological alterations and estimated the androgen binding protein (ABP). Moreover, these

results agree with Grami *et al.* (2024), who mentioned the potential preventive properties of *E. sativa* particularly the functional derangements of the male reproductive system. Multiple animal experimental studies had been done on the *E. sativa* effects on male reproductive system and fertility. Because *E. sativa* is a source of polyphenols with antioxidant properties, it is used extensively for treatment of diseases associated with oxidative stress, comprising male infertility. Animal histological studies approved a favorable effect on male reproductive disorders after using of *E.* sativa extract or oil (Aitken, 2017; Ansari and Ganaie, 2014; Hassan and Meligi, 2017; Hussein, 2013; Taher *et al.*, 2015).

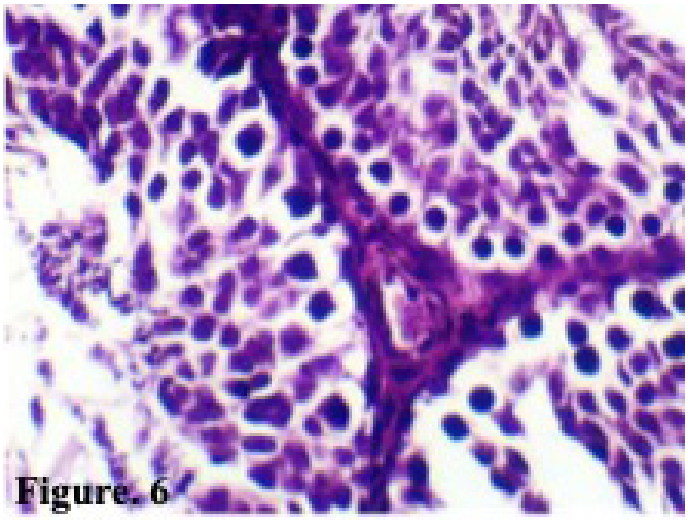

Figure 6: Higher magnification (400×) histological section of testicular tissue from the treatment group highlighting Sertoli cells (S) and interstitial Leydig cells (L). Stained with H and E.

The observed dose-dependent elevations in testosterone strongly suggest that *E. sativa* extract enhances steroidogenic activity within Leydig cells. This effect is likely mediated through multiple bioactive phytochemicals, predominantly flavonoids and isothiocyanates, known to upregulate enzymes critical for androgen biosynthesis such as 3β- and 17β-hydroxysteroid dehydrogenases, as well as cytochrome P450 side-chain cleavage enzyme (P450scc) (Martin and Touaibia, 2020). Such enzymatic enhancement facilitates the conversion of cholesterol to testosterone, thereby increasing circulating androgen levels. These findings align with previous studies reporting steroidogenic stimulation by plant-derived flavonoids and phenolics (Ohno *et al.*, 2002; Zand *et al.*, 2000).

Moreover, the potent antioxidant capacity of *E. sativa* likely contributes significantly to its protective effects on testicular tissue (Abd-Elsalam *et al.*, 2021). Oxidative stress, caused by excessive reactive oxygen species (ROS), is a well-documented cause of Leydig cell dysfunction and impaired testosterone synthesis (Xu *et al.*, 2023). By scavenging free radicals and reducing lipid peroxidation, the antioxidant constituents in *E. sativa* preserve mitochondrial function and cellular integrity in Leydig cells, sustaining their capacity for steroidogenesis (Dolatabadi *et al.*, 2025).

The significant increase in LH levels in the medium and high-dose groups suggests that *E. sativa* influences upstream regulation within the HPG axis. This may occur via enhanced secretion of GnRH from hypothalamic neurons or increased pituitary sensitivity to GnRH, leading to elevated LH secretion. The rise in LH is critical, as LH directly stimulates Leydig cells to produce testosterone, thus reinforcing the observed hormonal changes. These mechanisms are consistent with the regulatory feedback loops governing male reproductive endocrinology (Lei *et al.*, 2025).

Similarly, the increased serum FSH levels observed in this study indicate that *E. sativa* extract may promote Sertoli cell activity, essential for spermatogenesis and testicular function. FSH acts on Sertoli cells to facilitate the nourishment and development of germ cells, and its elevation suggests enhanced support for spermatogenic processes. Phytochemicals in *E. sativa* might modulate pituitary FSH release or exert direct paracrine effects on Sertoli cells, although this requires further investigation (Sadogh *et al.*, 2022).

Additionally, *E. sativa* contains naturally occurring nitrates, which can be metabolized into nitric oxide (NO) in vivo. NO is a critical signaling molecule that induces vasodilation, improving blood flow to the testes. Enhanced testicular perfusion may increase the delivery of hormones, nutrients, and oxygen, further supporting steroidogenesis and spermatogenesiss (Rosselli *et al.*, 1998).

The integration of these multifaceted actions antioxidant protection, steroidogenic enzyme activation, HPG axis stimulation, and improved testicular blood flow likely underlies the robust hormonal enhancements observed. These findings are supported by prior animal studies demonstrating that plants rich in similar bioactive compounds improve reproductive hormone profiles and fertility parameters (Niama *et al.*, 2025; Seetharaman *et al.*, 2025). However, this study focused exclusively on hormonal measurements; thus, it did not evaluate functional reproductive endpoints such as sperm quality, motility, morphology, or fertility rates. Future studies incorporating these parameters, along with histopathological examination of testicular tissue, are necessary to comprehensively determine the reproductive benefits and safety profile of *E. sativa.*

The results indicate promising potential for *E. sativa* as a phytotherapeutic agent to ameliorate male reproductive

dysfunction, particularly hypogonadism or infertility related to impaired steroidogenesis. Given the growing prevalence of male infertility and limitations of current pharmacological treatments including side effects and high costs natural plant-based interventions like *E. sativa* may offer safer, accessible alternatives. Nonetheless, rigorous clinical trials are imperative to validate efficacy, optimal dosing, and long-term safety in humans.

CONCLUSION

In conclusion, this study demonstrates that *E. sativa* extract exerts a beneficial, dose-dependent effect on male reproductive hormones in rats, notably increasing serum testosterone, LH, and FSH levels. Histological analysis revealed an increase in the density of spermatogenic cells, along with a slight increase in the population of interstitial Leydig cells. These changes maintain normal testicular morphology and support intact steroidogenic activity. These hormonal modulations and histological features approved the ameliorative effect of *E. sativa* on testes to keep the normal spermatogenesis and suggest the plant's potential to enhance testicular function through its phytochemical constituents and antioxidant mechanisms. The current findings provide experimental support for the traditional use of *E. sativa* in promoting male fertility and suggest its viability as a natural therapeutic agent for improving reproductive health.

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NOVELTY STATEMENT

This study showed the first integrated evidence of the hormonal and histological effects of Eruca sativa extract on the male reproductive system, revealing its dose-dependent stimulatory impact on testosterone and spermatogenesis, and highlighting its potential as a natural modulator of male fertility.

Author's Contribution

Jasim MF: Suggested the concept of the research, and writing the original draft of the manuscript.
Hameed KI: did the measurements and edited the final version of the manuscript.
Hindi MH: Did the histological processing of the samples and their interpretation and reported the results and revised the draft version.
Kadhim RS : Did the practical part of the experiment and the statistical analysis

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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