

Phytotherapeutic potential of herbal extracts on male reproductive organs in rat models

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*[Potencial fitoterápico de extratos de ervas nos órgãos reprodutores masculinos em modelos de ratos]*A.A. Ammari , A.R. Alhimaidi , R.A. Amran , A. Rady, H. aljawdah

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ABSTRACT

This study investigates the phytotherapeutic potential of herbal extracts on male reproductive organs using rat models. Herbal extracts, recognized for their bioactive properties such as antioxidation and hormone modulation, were evaluated for their effects on testicular function, spermatogenesis, and reproductive hormone levels. A polyherbal formulation consisting of *Ziziphus spina-christi*, *Trigonella foenum-graecum*, and *Nigella sativa* (ZTN) was prepared and administered to male rats at two doses (100mg and 250mg) for 14 days. Biochemical analysis revealed a dose-dependent impact on testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels. Histological examination demonstrated mild to severe disruptions in spermatogenesis and testicular architecture, particularly at the higher dose. While the 100mg dose exhibited mild positive effects on sperm quality and testicular weight, the 250mg dose resulted in testicular degeneration and reduced spermatozoa, indicating potential toxicity. The findings suggest that while herbal extracts hold promise for male reproductive health, appropriate dosing is crucial to avoid adverse outcomes.

Keywords: phytotherapy, herbal extracts, male reproductive health, testosterone, testicular function, rat models, *Ziziphus spina-christi*, *Trigonella foenum-graecum*, *Nigella sativa*

RESUMO

Este estudo investigou o potencial fitoterápico de extratos de ervas em órgãos reprodutivos masculinos usando modelos de ratos. Os extratos de ervas, reconhecidos por suas propriedades bioativas, como a antioxidação e a modulação hormonal, foram avaliados quanto aos seus efeitos sobre a função testicular, a espermatogênese e os níveis de hormônios reprodutivos. Uma formulação poli-herbal composta por *Ziziphus spina-christi*, *Trigonella foenum-graecum* e *Nigella sativa* (ZTN) foi preparada e administrada a ratos machos em duas doses (100 mg e 250 mg) por 14 dias. A análise bioquímica revelou um impacto dependente da dose nos níveis de testosterona, hormônio luteinizante (LH) e hormônio folículo-estimulante (FSH). O exame histológico demonstrou interrupções leves a graves na espermatogênese e na arquitetura testicular, principalmente na dose mais alta. Embora a dose de 100 mg tenha apresentado efeitos positivos leves na qualidade do esperma e no peso testicular, a dose de 250 mg resultou em degeneração testicular e redução dos espermatozoides, indicando possível toxicidade. Os resultados sugerem que, embora os extratos de ervas sejam promissores para a saúde reprodutiva masculina, a dosagem adequada é crucial para evitar resultados adversos.

Palavras-chave: fitoterapia, extratos de ervas, saúde reprodutiva masculina, testosterona, função testicular, modelos de ratos, *Ziziphus spina-christi*, *Trigonella foenum-graecum*, *Nigella sativa*

INTRODUCTION

The use of herbal extracts in traditional medicine has gained significant scientific attention due to their diverse therapeutic properties (Li and Weng, 2017). Among the various health applications, herbal extracts are increasingly being studied for their potential role in

modulating reproductive function (Qazi *et al.*, 2019). Phytotherapy, the use of plant-based compounds for therapeutic purposes, offers a natural and often less invasive alternative to synthetic drugs, particularly in reproductive health, where there is a growing need for safer interventions (Devi and Kumar, 2024; Harikrishnan and Balasundaram, 2020). Numerous plants possess bioactive compounds,

such as flavonoids, alkaloids, and phenolic acids, which have been reported to exhibit antioxidative, anti-inflammatory, and hormone-modulating properties—factors critical to maintaining reproductive health (Onukwuli *et al.*, 2024). Male infertility, characterized by reduced sperm count, motility, or abnormal morphology, is a significant global health concern. Various factors, including oxidative stress, hormonal imbalances, and environmental toxins, contribute to male reproductive dysfunction. Rodent models, especially rats, are widely used in preclinical studies to explore the underlying mechanisms of male infertility and assess the efficacy of potential treatments (Sharma, 2017; Gharagozloo *et al.*, 2016). The male reproductive organs, including the testes, epididymis, prostate, and seminal vesicles, are particularly sensitive to oxidative damage and hormonal disruptions. Recent research has focused on the potential of herbal extracts to protect and restore the function of these organs (Alahmar, 2019). Several studies have explored the efficacy of different herbal extracts on male reproductive health in rodent models. For instance (Chen *et al.*, 2019).

Several herbal medicines, including those traditionally used in Chinese, Indian, and other indigenous medical systems, show positive effects on male fertility. These herbs improve sperm quality, quantity, motility, and overall reproductive health (Nguyen *et al.*, 2024). Similarly, *Tribulus terrestris*, commonly used in traditional medicine for its reproductive-enhancing effects, has been studied extensively in animal models. In a study by Malekzadeh *et al.* (2024), the administration of *Tribulus terrestris* extract to male rats significantly improved libido, sperm count, and testosterone levels, indicating the herb's capacity to modulate androgen production and sperm parameters. Additionally, *Ginkgo biloba* extract has been shown to possess antioxidative properties that protect the testicular tissue from damage caused by environmental toxins. A study by Essawy *et al.* (2024) demonstrated that *Ginkgo biloba* reduced the detrimental effects of reactive oxygen species (ROS) on sperm motility and morphology in rats exposed to toxic agents, highlighting its protective role in preserving male fertility.

Other herbal extracts, such as *Nigella sativa* (black seed) and *Panax ginseng*, have also been investigated for their effects on male reproductive function. For example, *Nigella sativa* was found to enhance sperm count and motility in rats by modulating the expression of reproductive hormones and reducing oxidative stress (Nozad *et al.*, 2024). *Panax ginseng* has shown promising results in increasing sperm production and quality in rodent studies, likely due to its ability to enhance testicular blood flow and reduce apoptosis in reproductive cells (Khan *et al.*, 2024).

MATERIALS AND METHODS

The polyherbal formulation was created by combining equal amounts of each plant in the mixture. The selected plants were ground into a fine powder, thoroughly mixed, and then extracted using water. The herbal formula consisted of *Ziziphus spina-christi*, *Trigonella foenum-graecum*, and *Nigella sativa* (ZTN). The plants were sourced from various locations, with 500 grams of each plant collected. After collection the plants were ground into a fine powder and extracted following the method used in our previous study (Alhimaidi *et al.*, 2021). Two concentrations of the herbal mixture were prepared: 100mg and 250mg.

The study involved eighteen healthy rats, consisting of nine males and nine females, with weights ranging from 200 to 230 grams and ages between 12 to 14 weeks. These rats were obtained from the animal facility at the Zoology Department of the Science College, King Saud University (KSU). They were acclimatized to a well-ventilated environment at a room temperature of $25 \pm 2^\circ\text{C}$, with a standard 12-hour light/dark cycle. The rats were provided with a standard diet and had unrestricted access to water. All experimental procedures followed the guidelines of the ethics committee and Institutional Animal Care at KSU (Approval no: KSU-SE-24-3). The rats were divided into three groups, each containing three males and three females. The first group, serving as the control, received water. The second group was given 100 mg of the ZTN herbal formula, and the third group was administered 250 mg/kg of the ZTN herbal formula for 14 days.

On day 21, blood samples were collected from the animals' hearts into non-heparinized tubes, cooled at approximately 4 °C overnight, and then centrifuged at 1000x g for 15 minutes to separate the serum, which was subsequently frozen at -20 °C for hormonal analysis. A series of biochemical analyses were performed on the serum samples to investigate various mechanistic aspects, including the assessment of biological markers and oxidative stress. Fertility markers were specifically measured using enzyme-linked immunosorbent assay (ELISA) kits for rats (AFG Bioscience, Skokie Boulevard, Northbrook, IL 60062 USA). These markers included: Follicle-stimulating hormone (FSH) (Catalog No. EK720702), Luteinizing hormone (LH) (Catalog No. MBS2018978), and Testosterone (Catalog No. EK720991).

After anesthetizing the animals with CO₂, the testes were collected and immersed in 10% neutral formalin for 48 hours to achieve fixation. The fixed tissues were then dehydrated through a series of alcohol solutions, followed by infiltration with xylene for 4-6 hours. The testes were embedded in paraffin wax blocks, sectioned with a microtome, mounted on slides, and stained with hematoxylin and eosin (H&E). Histomorphological and histomorphometric assessments were performed using a light microscope.

All statistical analyses for this experiment were conducted using GraphPad Prism software (version 10.1.1). The Shapiro-Wilk test was applied to confirm the normal distribution of the data. Animal body weights were analyzed with two-way ANOVA, accounting for both the day and extract factors, followed by Tukey's multiple comparisons test. Hormone levels and reproductive organ weights were evaluated using one-way ANOVA, also followed by Tukey's test. Results are presented as mean values with standard deviations, and a P value of 0.05 or lower was considered statistically significant.

RESULTS

Control Group: Normal, healthy testicular tissue with active spermatogenesis. group 100 mg Mild disruption in spermatogenesis with a slight reduction in spermatozoa, possibly indicating early signs of toxicity. group 250mg Severe disruption in testicular architecture, with significant reduction in sperm production and increased signs of cellular degeneration, suggesting a strong toxic effect at this dosage. These findings suggest a dose-dependent effect of the herbal extract on testicular function, with higher doses leading to greater impairment of spermatogenesis and potential testicular damage Fig.1.

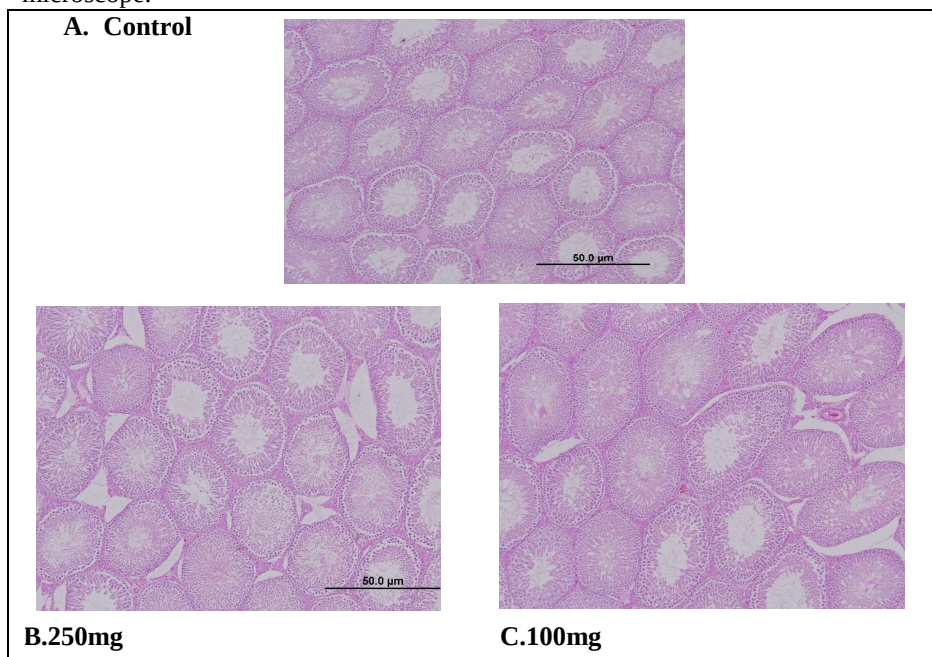


Figure 1. Dose-dependent histological changes in testicular tissue following herbal extract administration in rat models.

The study investigated the effect of various concentrations of herbal extracts on the male reproductive organs of rat models. Testicular weights were measured across three groups: the control group, a group receiving 100 mg of the herbal extract, and a group receiving 250mg of the herbal extract Fig. 2.

In the control group, the average testis weight was 2.4g. Rats administered with 100 mg of the herbal extract exhibited a slight increase in testicular weight, with an average of 2.72g, compared to the control group. At the 250mg dosage, the average testis weight increased a slight to 2.47g. The data indicates that higher concentrations of the herbal extract might be associated with increased testis weight,

suggesting a dose-dependent positive effect on testicular growth Fig.2.

Similarly, the epididymis weight was measured, and the graph illustrates the following: Control group: The average epididymis weight was around 0.36g. 100 mg group: The 100 mg treatment resulted in a moderate increase, with an average epididymis weight of 0.71. 250mg group: Rats receiving 250 mg of the extract exhibited a more average epididymis weight of 0.61g. This trend shows a positive correlation between the dosage of the herbal extract and epididymis weight, reinforcing the potential influence of the extract on male reproductive organs Fig. 2.

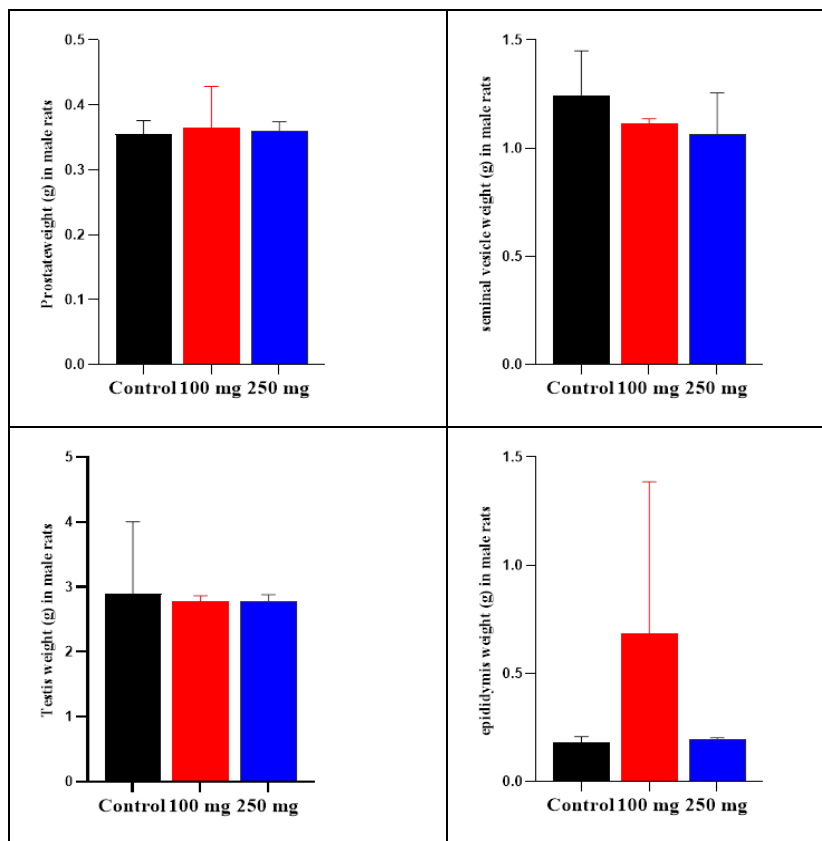


Figure 2. Effects of herbal extract doses on reproductive organ weights in male rats: a comparative analysis of control, 100mg, and 250mg treatments.

Control group the control group had an average seminal vesicle weight of around 1.3g. 100 mg group: Rats treated with 100 mg of the herbal extract exhibited a slight decrease in seminal vesicle weight, averaging 1.07g. 250mg group: The seminal vesicle weight in rats treated with

250mg a slight decrease to an average of 1.05g, compare with control group demonstrating increase in weight Fig. 2.

Control group: The prostate weight in the control group averaged 0.3g. 100 mg group: Rats receiving 100mg of the extract exhibited a slight

increase in prostate weight, with an average of 0.32g. 250mg group: The 250mg group showed a further increase, with an average prostate weight of 0.32g. Across all the examined reproductive organs, a dose-dependent increase in organ weights was observed with the administration of the herbal extract Fig. 2.

The study assessed the effect of different dosages of herbal extracts on testosterone hormone levels in male rats. The results were divided into three groups: control, 100 mg herbal extract, and 250mg herbal extract.

Control group in the control group, the average testosterone hormone level was approximately 14.29pg/mL. 100mg group: Rats treated with 100mg of the herbal extract showed decrease in

testosterone levels, with an average of approximately 10.39pg/mL. This suggests that the administration of the herbal extract at 100mg may have negative effect on testosterone production. 250mg group: Testosterone levels were significantly higher in the group receiving 250mg of the herbal extract compared to the control group, with an average of 16.45pg/mL. This further indicates a dose-dependent effect, where the highest dose led to an increase in testosterone levels. The findings indicate a dose-dependent effect of the herbal extract on testosterone levels, where the 100mg dose resulted in a reduction, while the 250mg dose led to a notable increase in testosterone production Fig. 3.

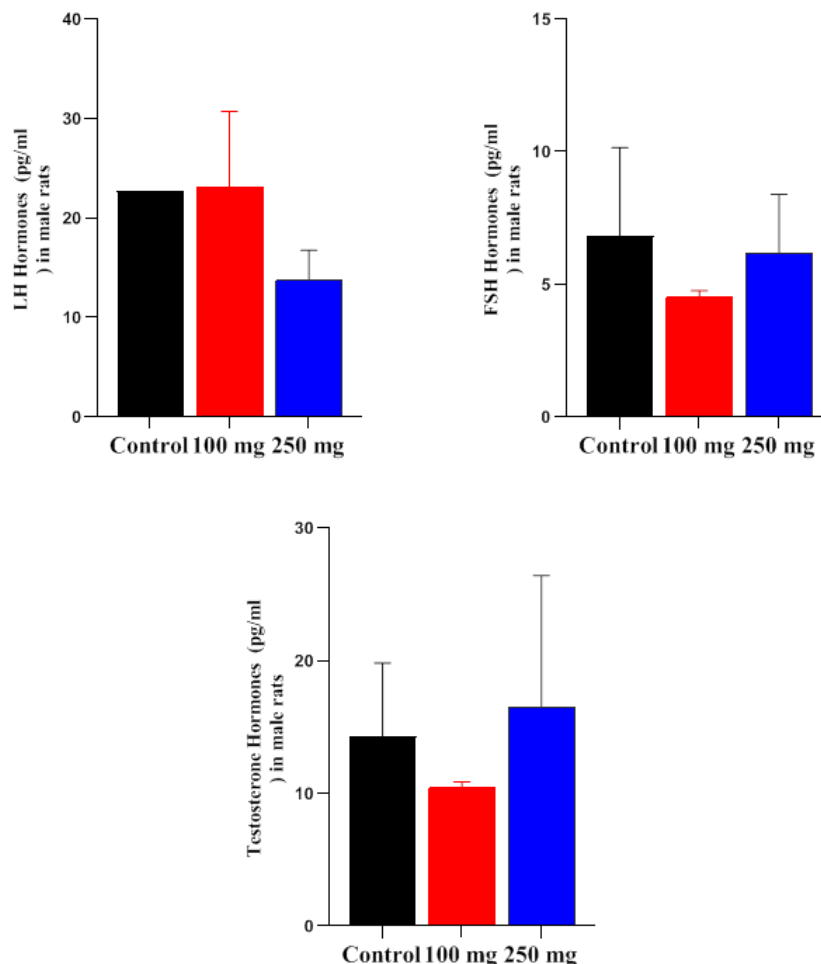


Figure 3. Dose-dependent effects of herbal extract on FSH, LH, and testosterone levels in male rats.

Control Group: The FSH levels in the control group averaged approximately 6.835pg/mL. 100mg Group: In the group treated with 100mg of herbal extract, there was a slight decrease in FSH levels, averaging around 4.505 pg/mL. This indicates that the lower dose of herbal extract may have a mild effect on FSH production. 250 mg Group: Rats treated with 250mg of herbal extract exhibited a slight decrease in FSH levels, with an average of 6.18 pg/mL. This suggests a dose-dependent effect, where the higher concentration of the extract had a stronger influence on FSH hormone decrease. The findings suggest that the herbal extract may have a dose-dependent effect on FSH production, with both the 100 mg and 250 mg doses causing a reduction in FSH levels, though the decrease was more pronounced at the lower dose Fig.3.

LH plays a critical role in regulating testosterone production and overall reproductive function in males. The study evaluated LH levels in three groups: a control group, a group treated with 100 mg of herbal extract, and a group treated with 250 mg of the herbal extract. Control group: The control group had an average LH level of approximately 21.405pg/mL. 100mg group: Rats treated with 100mg of herbal extract showed a slight increase in LH levels, averaging 22.835 pg/mL. This suggests that the herbal extract may stimulate LH production at this dosage. 250mg group: Rats treated with 250 mg of the herbal extract exhibited decrease in LH levels, with an average of 13.73 pg/ml. Overall, the herbal extract showed a dose-dependent effect, where a low dose slightly increased LH levels, while a higher dose led to a decrease in LH levels, which could impact testosterone regulation and reproductive function Fig.3.

DISCUSSION

In our study, histological analysis of the testis revealed dose-dependent effects of the herbal extract, with 100mg showing mild disruption in spermatogenesis and 250mg showing severe disruption and cellular degeneration. The high-dose treatment led to significant testicular damage, resulting in fewer spermatozoa and disrupted cellular architecture, highlighting the potential toxic effects of the herbal extract at higher doses. Alahmadi (2020) similarly notes that high doses of certain herbal medicines can lead to testicular degeneration and impaired spermatogenesis in experimental models. This

aligns with our study, where the 250mg group showed significant disruption. Alahmadi (2020) also emphasizes that the mechanism of damage in these cases often involves oxidative stress, apoptosis of germ cells, and direct toxic effects on Leydig and Sertoli cells. Our results, which show cellular degeneration and reduced sperm production at higher doses, reflect these mechanisms. In our study, testosterone levels exhibited a dose-dependent effect, with 100mg causing a reduction in testosterone compared to the control, and 250 mg causing an increase in testosterone levels. Additionally, observed reductions in FSH levels in both treatment groups and a mixed response in LH levels, with an increase in the 100 mg group and a significant decrease in the 250mg group. Alahmadi (2020) similarly reports that herbal medicines can modulate testosterone and gonadotropin levels in experimental animals.

Our study demonstrated a dose-dependent effect of the herbal extract on testicular tissue, where the 100 mg dose showed mild disruption in spermatogenesis, and the 250mg dose led to severe cellular degeneration and significant reduction in spermatozoa. This histological damage mirrors the findings in Bhardwaj *et al.* (2021)) concerning cadmium toxicity, where cadmium exposure causes similar disruption in spermatogenesis, testicular atrophy, and germ cell apoptosis.

In the research by Yu *et al.* (2020), cadmium-induced testicular toxicity is characterized by oxidative stress, inflammation, and apoptosis, leading to disruption of the seminiferous tubules, germ cell death, and spermatogenic failure. Cadmium, like the herbal extract in our study, caused significant testicular degeneration. However, Yu *et al.* demonstrated that wogonin, a flavonoid, mitigated these effects through its antioxidant, anti-inflammatory, and anti-apoptotic properties, preserving testicular structure and function.

Wal *et al.* (2022) discuss several herbal remedies known to improve testicular function and spermatogenesis, particularly herbs like Tribulus terrestris, Withania somnifera (Ashwagandha), and Ginseng, which are frequently used in traditional medicine to support male fertility. These herbs have demonstrated protective effects on testicular tissue and the ability to stimulate spermatogenesis, primarily through their

antioxidant and anti-inflammatory properties. For example, *Tribulus terrestris* has been shown to increase sperm count and motility by enhancing testosterone production and reducing oxidative stress.

The 100mg dose in our study, which showed milder effects, might hint at potential beneficial properties if used at the right dosage, similar to the herbs discussed by Wal *et al.* (2022). However, the 250mg dose clearly reflects toxicity, which aligns more with the notion that overdosing on herbal supplements can lead to adverse effects, including impaired reproductive function. This suggests that, while herbal remedies hold potential for improving fertility, dosing is critical, and excessive intake can lead to detrimental outcomes, as seen in our study.

ACKNOWLEDGMENTS

The authors sincerely acknowledge the Researcher Support Project (RSP-2024R232) for funding this work at King Saud University, Riyadh, Saudi Arabia.

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