

# Dose-Dependent Effects of Herbal Extract on Reproductive Health: Implications for Ovarian Structure, Organ Weight and Hormonal Balance in Female Rats

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## ABSTRACT

**Aim:** Investigate the impact of varying dosages (100 mg and 250 mg/kg) of a specific herbal extract on reproductive parameters in female albino rats. **Background:** Herbal extracts are extensively utilized in traditional medicine for their possible therapeutic advantages, including the promotion of reproductive health. The effects of different dosages on reproductive function are little comprehended. **Materials and Methods:** This research examines the dose-dependent impacts of a particular herbal extract on ovarian histology, reproductive organ weights and hormonal profiles in female rats. Female albino rats received varying dosages (100 mg and 250 mg/kg) of the extract, followed by an examination of ovarian morphology, reproductive organ weights (ovary, uterus and oviduct) and hormone concentrations (FSH, LH and estrogen). **Results:** The findings demonstrated a substantial dose-dependent response, where increased dosages resulted in marked ovarian follicular degeneration, decreased organ weights and modified hormone levels, suggesting possible detrimental impacts on reproductive health. These findings underscore the intricate equilibrium between therapeutic efficacy and toxicity, highlighting the necessity for meticulous dose consideration in the utilization of herbal extracts for reproductive health. **Conclusion:** The study offers essential insights into the safety and efficacy of herbal medicines, highlighting the necessity for more research to enhance their application.

**Keywords:** Dose-dependent effects, Female rats, Herbal extract, Hormone levels, Ovarian histopathology, Reproductive health.

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## INTRODUCTION

The reproductive health of females is regulated by a complex interaction of hormones, physiological mechanisms and environmental influences. Recent years have witnessed an increasing interest in natural therapies, especially polyherbal formulations, for their ability to increase reproductive health and fertility.<sup>1-3</sup> Polyherbal extracts, including many medicinal plants, are thought to provide synergistic effects that augment therapeutic efficacy relative to individual herbs. These formulations are frequently employed in traditional medical systems like Ayurveda and Traditional Chinese Medicine (TCM) to address reproductive problems, regulate menstrual cycles and enhance fertility results. Nonetheless, the scientific data substantiating the usefulness of these formulations, especially under controlled laboratory conditions, remains in development.<sup>4-7</sup> Polyherbal extracts, rich in bioactive compounds such as flavonoids, alkaloids

and terpenoids, are thought to exert beneficial effects on these hormonal pathways by promoting ovarian function, improving hormonal balance and providing antioxidant protection.<sup>8,9</sup> Numerous studies indicate that polyherbal formulations may provide a wider array of biological activity than individual herbs, rendering them especially advantageous for improving female reproductive health. Recent study indicates that oxidative stress, marked by an excess of Reactive Oxygen Species (ROS), significantly contributes to female infertility. Oxidative stress can cause the degradation of ovarian tissues, hinder egg maturation and obstruct embryo development. Polyherbal extracts, owing to their potent antioxidant properties, may mitigate these detrimental effects and enhance reproductive results. Formulations comprising herbs such as *Curcuma longa* (turmeric) and *Embllica officinalis* (Indian gooseberry) have exhibited protective benefits against oxidative damage in the ovaries of female rats, resulting in improved reproductive results.<sup>10-16</sup>

Recent scientific studies have provided evidence supporting the efficacy of polyherbal formulations in treating reproductive disorders. For example,<sup>17</sup> Formulated and assessed polyherbal capsules for the management of PCOS, showing substantial enhancements in ovarian function, hormonal balance



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and menstrual cycle consistency. The research shown that the polyherbal capsules significantly alleviated symptoms related to PCOS, including irregular menstrual periods and hyperandrogenism, while providing a safer alternative to traditional therapies. The combined benefits of many herbs in the formulation were shown to enhance insulin sensitivity and restore hormonal equilibrium, both essential for controlling PCOS.

In an *in vivo* study,<sup>18</sup> evaluated the efficacy of selected polyherbal products in improving reproductive performance in rabbit does. Their findings demonstrated that polyherbal formulations could significantly enhance reproductive parameters, including ovulation rates, fertility and overall reproductive health in rabbit models. This study underscores the potential of polyherbal remedies to improve reproductive performance not only in humans but also in animal models, making them promising candidates for broader reproductive health applications. In this study, we aim to explore the effects of a polyherbal extract on reproductive health and fertility in female rats. By examining key reproductive parameters such as hormonal changes, ovarian function, we seek to provide a deeper understanding of the therapeutic potential of polyherbal formulations for female reproductive health.

## MATERIALS AND METHODS

### Preparation of polyherbal extracts

The herbal ZTN comprises 33.3% *Ziziphus spina-christi*. 33.3% *Trigonella foenum-graecum*. 33.3% *Nigella sativa*. The herbs were let too steep in the shaker for 24 hr. The three-herb combination was filtered until the volume was halved. Following the filtration of the substantial components from the extract, the residual liquid was subjected to temperature-induced drying, yielding 0.5 g of solid material from 10 mL of liquid. The herbal combination was formulated in two concentrations: 100 mg and 250 mg/kg.<sup>19</sup>

### Experimental design

#### Methods

The experiment included fifteen healthy rats. The weight of rats ranged from 200 to 230 g and their ages were between 12 and 14 weeks, female albino rats were obtained from the animal facility of the Zoology Department, College of Sciences, King Saud University [KSU] Rats were kept in a well-ventilated room at 25±2°C on a 12/12-hr light/dark cycle as per their acclimatization. Rats were fed standard diet and tap water ad libitum. Ethical approval was obtained from the ethics committee at KSU and the experimental procedures were approved by the Institutional Animal Care (Approval no: KSU-SE-23-6). Rats were separated in three groups of 5 females each. The control group, nestled in between the others, got plain water only. The 2<sup>nd</sup> group was treated with 100 mg/kg daily ZTN herbal solution and the 3<sup>rd</sup> group was administered 250/mg/kg daily ZTN for 3 weeks orally.

### Biochemical examination

24 hr post the last injection (day 21) blood was collected from animals' hearts into non-heparinized tubes and left at 4°C for overnight to allow clot formation, then centrifuged at 1000xg for 15 min and serum separated and stored at -20°C until hormonal assay. Some biochemical analyses in serum sample were done to explore diverse mechanistic aspects and examine physiological markers. reproductive health markers were also assessed using Enzyme-Linked Immunosorbent Assay (ELISA) kits for rats purchased from AFG Bioscience, Skokie Boulevard, Northbrook, IL 60062 USA. These markers are: Follicle-Stimulating Hormone (FSH) (Catalog No. EK720702), Luteinizing Hormone (LH) (Catalog No. MBS2018978) and Estrogen (Catalog No. EK730734).

### Histomorphometric analysis

The ovaries were collected following the anesthesia of the animals with CO<sub>2</sub> and immersed in 10% neutral formalin for 48 hr for fixation. The preserved organs underwent dehydration through a series of alcohol solutions, were infiltrated with xylene for 4-6 hr, embedded in paraffin wax cubes, sectioned using a microtome, mounted on slides and stained with Hematoxylin and Eosin (H and E). Histomorphological and histomorphometric assessments were performed utilizing a light microscope.

### Statistical Analysis

All statistical analyses for this experiment were performed utilizing GraphPad Prism software (version 10.1.1). The Shapiro-Wilk test was utilized to verify the normal distribution of the data. Animal body weights were examined using 2-way ANOVA, using both the day and extract variables, then followed by Tukey's multiple comparisons test. Hormone levels and reproductive organ weights were assessed using one-way ANOVA, then followed by Tukey's test. Results are expressed as mean values accompanied by standard deviations, with a *p* value of 0.05 or less being statistically significant.

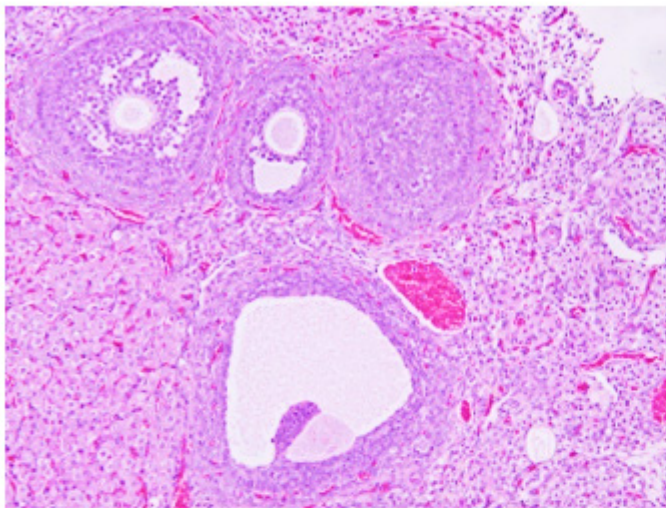
## RESULTS

### Histopathological examination

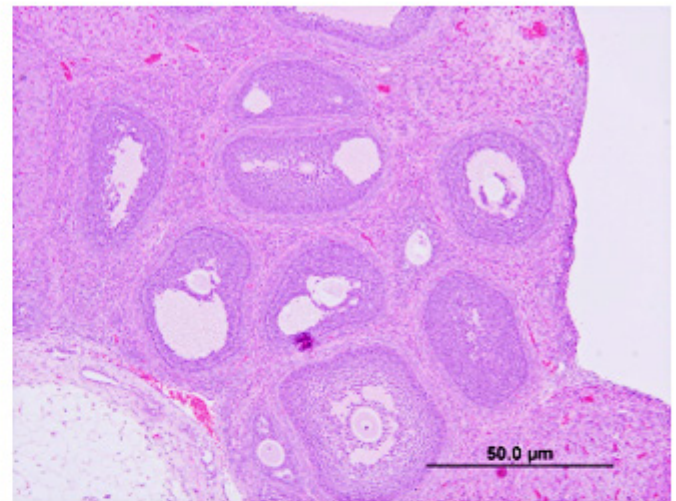
Control group the section displays a spectrum of follicles at different developmental phases, encompassing primordial, primary, secondary and antral follicles. The structure is clearly delineated, exhibiting distinct oocyte presence and numerous layers of granulosa cells in more advanced follicles. The stroma is uniformly distributed and exhibits no indications of fibrosis or atypical cell aggregation. The inter-follicular spacing is uniform and there is no apparent indication of bleeding or tissue disarray. The control sample has typical ovarian architecture, characterized by a healthy equilibrium of follicles and supporting stroma. In the group receiving 100 mg, there is a noticeable decrease in the quantity of developing follicles compared to the control group.

Certain follicles have uneven forms or disturbed cellular layers, indicating first symptoms of atresia (degeneration). Granulosa cells in certain follicles may exhibit increased condensation or pyknotic characteristics, suggesting cellular apoptosis. Stromal Modifications: The stromal tissue exhibits increased cellularity and potential density compared to the control, however lacks overt fibrosis. The connective tissue remains predominantly intact but may exhibit minor increases in cellularity or smaller cell clusters. The 100 mg treated slice exhibits mild abnormalities, including follicular degeneration and maybe early indications of stromal changes. The overall architecture is still identifiable; however, these modifications indicate a reaction to the treatment. Compared to both the control and the 100 mg groups, the 250 mg

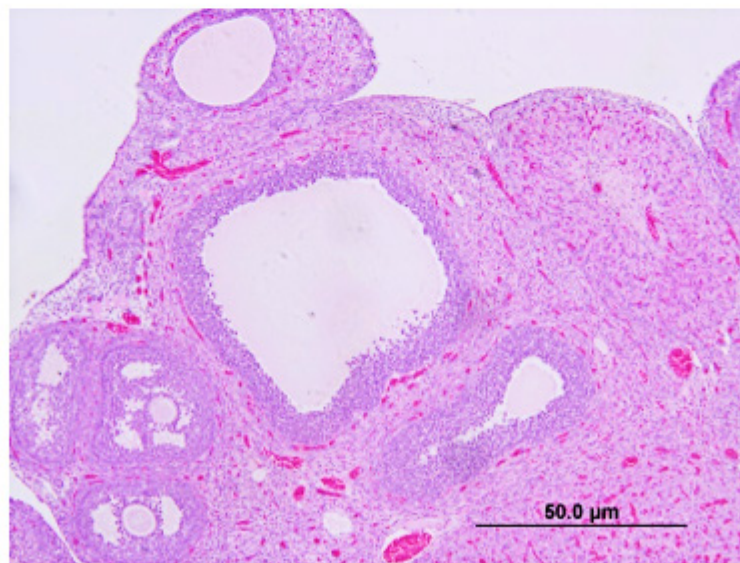
sample demonstrates a more significant decrease in the number of healthy follicles. The residual follicles exhibit significant atresia: the granulosa cells are markedly contracted and several follicles seem either collapsed or vacuolated. The stroma in this area exhibits greater density and cellularity compared to both the control and 100 mg samples. There may be an augmentation of fibrotic regions or indications of disarray within the connective tissue. The inter-follicular gaps are diminished and localized hemorrhagic regions may be present. The 250 mg group exhibits a more pronounced effect on ovarian architecture, characterized by increased follicular degeneration and more significant stromal alterations. The tissue exhibits more disruption, suggesting a more pronounced effect of therapy at this elevated dose. The



**A. 100 mg of herbal.**



**B. 250 mg of herbal.**



**c. control**

**Figure 1:** Histological Analysis of ovary in Female Rats Exposed to Herbal extract.

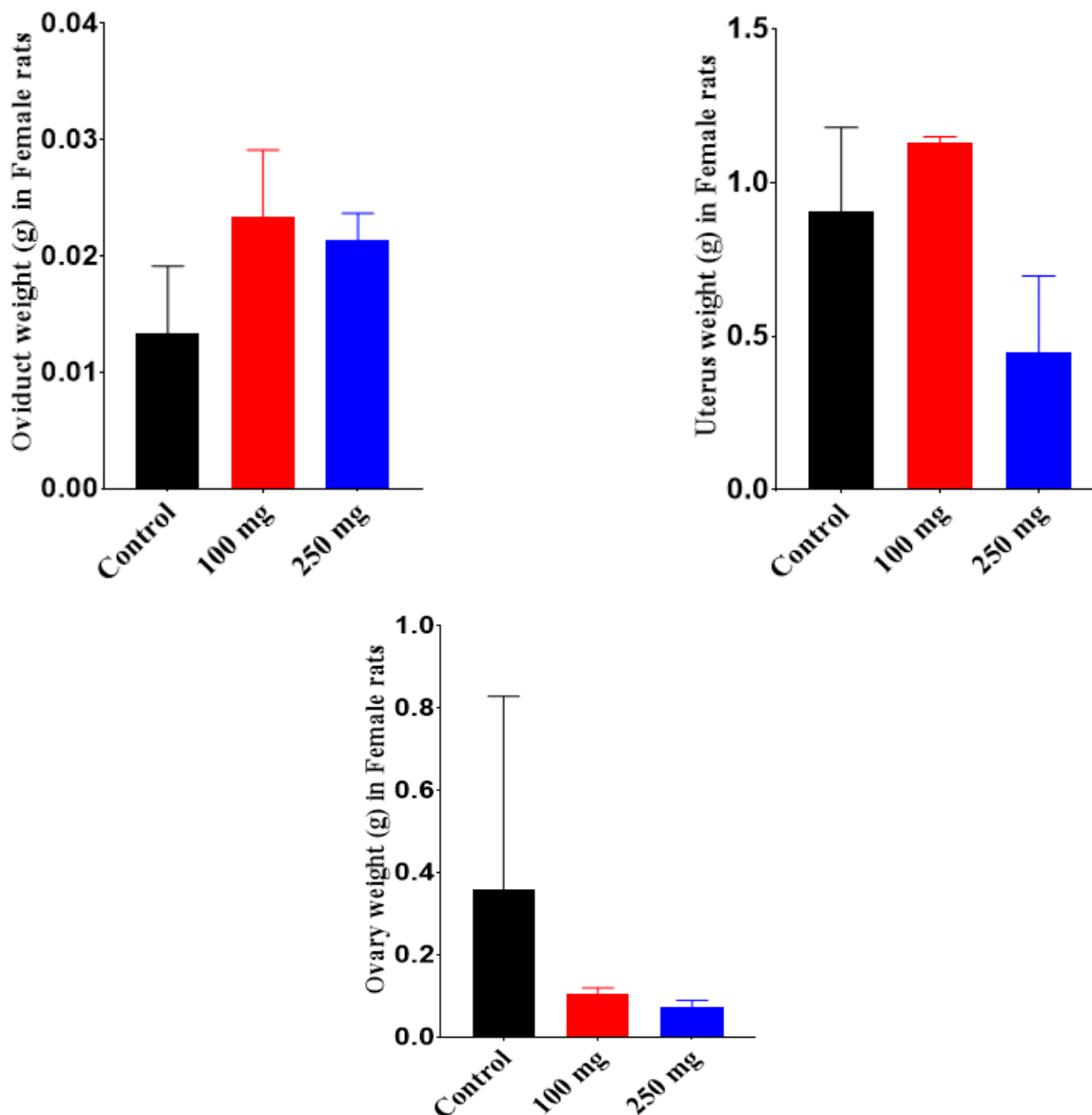
control group exhibits normal ovarian histology, characterized by well-organized follicles and stroma. The 100 mg treated group has modest to moderate alterations, predominantly in follicular health and stromal density. The 250 mg group has pronounced follicular degeneration and stromal modifications, suggesting a substantial impact of the therapy. These sections demonstrate a dose-dependent response in ovarian tissue, with more degeneration and alterations in tissue structure associated with higher treatment concentrations (Figure 1).

## Reproductive Organs

### Ovary

The control group indicates that the ovarian weight is around 0.36 g. The 100 mg treatment group exhibited around 0.10 g,

indicating a significant reduction in ovarian weight relative to the control group. The ovary weight in the 250 mg treatment group is 0.07 g lower than that in the 100 mg group. The administration of herbal extract results in a substantial drop in ovarian weight, with both the 100 mg and 250 mg groups exhibiting a significant reduction relative to the control group. A dose-dependent impact is evident, with the 250 mg group exhibiting a lower ovarian weight than the 100 mg group, however the difference between the two treated groups is not as pronounced in comparison to the control group. The findings indicate that the herbal extracts significantly reduced ovary weight, presumably reflecting an influence on ovarian development or tissue health in female rats. Additional context is necessary to ascertain the process or ramifications of this weight loss (Figure 2).



**Figure 2:** Impact of herbal extract on Reproductive Organ Weights in Female Rats.

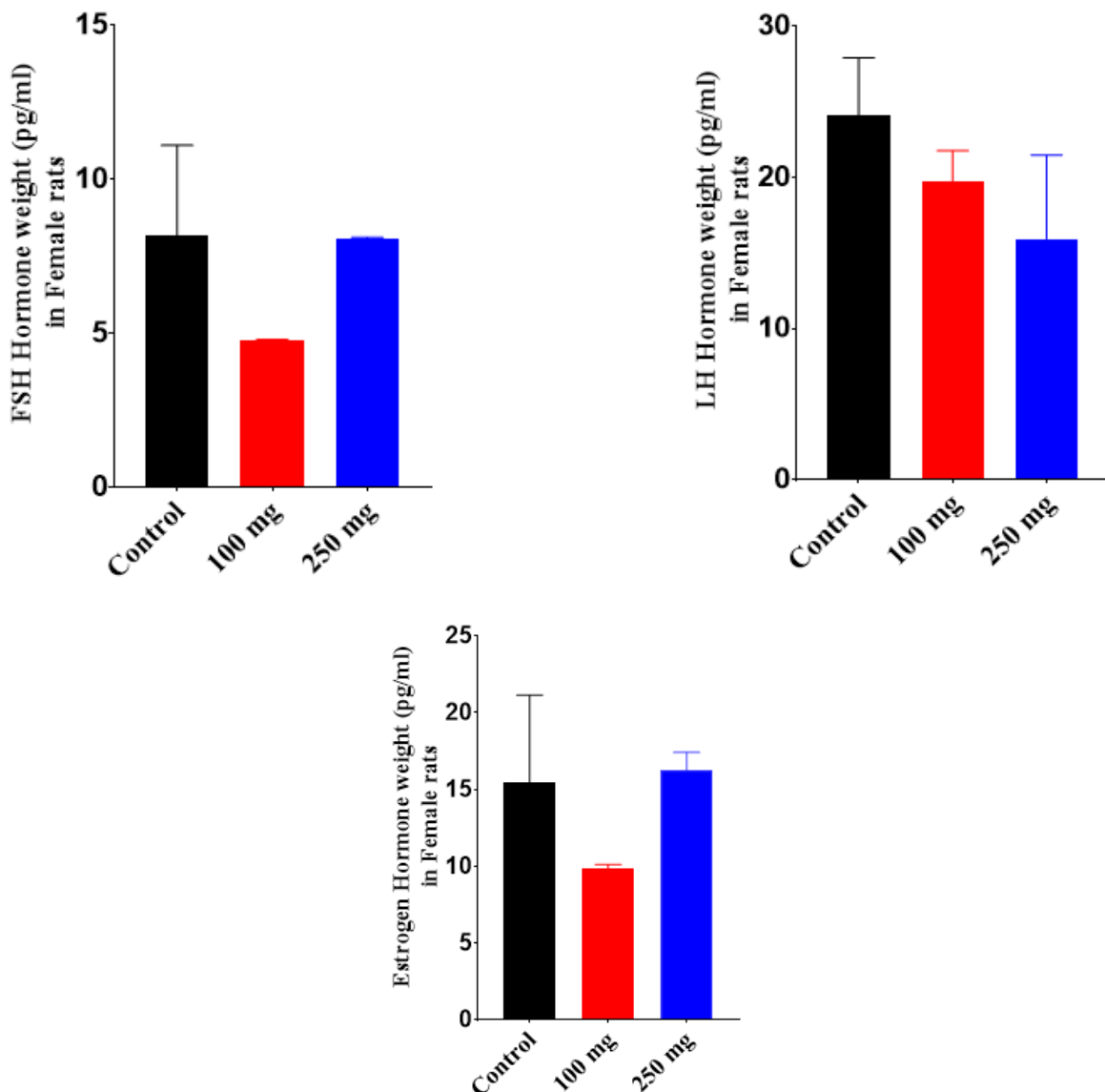
### Uterus: The control group

Uterine weight about 0.72 g. The 100 mg treatment group had a uterine weight about 0.9 g. 250 mg Treatment group: suggesting a uterine weight of about 0.44 g, reflecting a significant decrease relative to both the control and the 100 mg group. A notable reduction in uterine weight occurs with an increase in the dosage of the herbal extract. The 100 mg treatment group exhibits a little rise in uterine weight relative to the control, however maintains a value comparable to that of the control group. The 250 mg therapy leads to a significant reduction in uterine weight, indicating a dose-dependent influence of the herbal extract on diminishing uterine tissue mass. The research demonstrates that the herbal extract exerts a dose-dependent effect on decreasing uterine weight in female rats, with the higher dosage (250 mg) exhibiting

a more significant influence. This decrease may be associated with alterations in reproductive health or uterine development (Figure 2).

### Oviduct: control group

Suggesting an oviduct weight of around 0.012 g. The 100 mg treatment group had an oviduct weight of around 0.023 g. The 250 mg treatment group exhibited a weight of around 0.021 g as well. The treatment groups receiving 100 mg and 250 mg have elevated oviduct weight relative to the control group, indicating that the herbal extract may influence the augmentation of oviduct tissue weight. Negligible Dose-Dependent Variation: The oviduct weight of the 100 mg and 250 mg groups is comparable, suggesting that elevating the dosage from 100 mg to 250 mg does not substantially influence oviduct weight. The herbal extract



**Figure 3:** Effects of herbal extract on Hormone Levels (LH, FSH and Estrogen) in Female Rats.

therapy seems to elevate oviduct weight in female rats, while the impact does not appear to be significantly dose-dependent across the 2 treatment groups. The control group has a reduced and more stable oviduct weight, indicating that the extract's impact is apparent in both treated groups (Figure 2).

## Hormones levels

### FSH hormones: Control group

FSH level about 8.44 pg/mL. 100 mg treatment cohort: FSH concentration approximately 5 pg/mL. 250 mg Treatment Group: suggesting an elevated FSH level of approximately 8 pg/mL. Reduction in FSH with 100 mg the 100 mg herbal extract therapy demonstrates a marginal reduction in FSH levels relative to the control group. Elevation of FSH with 250 mg the 250 mg treatment group exhibits a substantial elevation in FSH levels relative to the 100 mg group, indicating that a greater dosage of the herbal extract enhances FSH levels. A dose-dependent effect on FSH levels is evident, with the 250 mg dosage resulting in a more significant increase in FSH compared to the 100 mg dosage. The herbal extract has varying effects on FSH levels contingent upon the dosage. The 100 mg therapy marginally decreases FSH levels relative to the control, indicating a minor suppressive impact. The 250 mg therapy markedly elevates FSH levels, indicating a stimulatory impact on FSH production or secretion at elevated concentrations. The elevation of FSH levels with the increased dosage may suggest an impact on ovarian function or follicle maturation, given FSH's essential role in governing the reproductive process in female rats (Figure 3).

### LH hormones

The control group has LH levels of around 25.63 pg/mL. The 100 mg treatment group has LH levels of 19 pg/mL, marginally lower than the control group. 250 mg Treatment Group: LH levels were 15.38 pg/mL compared to the control and 100 mg group. Marginal Reduction in LH Following Treatment: Both the 100 mg and 250 mg treatment cohorts exhibit a marginal reduction in LH levels relative to the control group. The reduction is negligible, suggesting that the herbal extract does not significantly influence LH levels at current dosages. In contrast to FSH, LH levels do not exhibit a notable dose-dependent impact across the 100 mg and 250 mg treatment groups. The herbal extract induces a modest decrease in LH levels in female rats relative to the control group. Nonetheless, this effect is not pronounced and the disparities are negligible. The increased variability in the 250 mg group may suggest individual variances in reaction to the elevated dosage of the herbal extract. The therapy appears to have no substantial effect on LH levels at the administered dosages (Figure 3).

### Estrogen hormones: control group

The estrogen levels approximate 16 pg/mL. 150 mg treatment group: reduction of estrogen levels to around 12 pg/mL. 250 mg treatment group: estrogen concentration of approximately

16.40 pg/mL. Reduction of Estrogen with 100 mg Treatment: In comparison to the control group, the 100 mg cohort exhibits reduced estrogen levels, suggesting that this dosage may exert a suppressive influence on estrogen production or secretion. Elevation of Estrogen with 250 mg Therapeutic Intervention: The 250 mg treatment group has elevated estrogen levels relative to both the control and 100 mg groups, indicating a stimulatory impact on estrogen at this dosage. A dosage-dependent tendency is evident, with a lower dose (100 mg) decreasing estrogen levels and a larger dose (250 mg) markedly increasing them. The 100 mg dosage marginally decreases estrogen levels relative to the control group. The 250 mg dosage significantly elevates estrogen levels, demonstrating a pronounced stimulatory impact at this elevated concentration. This pattern indicates that the herbal extract influences estrogen regulation in a dose-dependent way, perhaps exhibiting contrasting effects at varying dosages (Figure 3).

## DISCUSSION

The observed dose-dependent degeneration of follicles, alteration in organ weights and hormonal imbalances at higher doses highlight potential risks that contradict the protective effects seen at therapeutic or lower doses.<sup>20</sup> findings emphasize the protective and enhancing effects of herbs on fertility, particularly in the context of obesity-induced damage. our study, however, underlines the necessity for careful dosing when using herbal extracts for fertility and reproductive health.

The significant histopathological changes and reductions in reproductive organ weights at higher doses (250 mg) in our study align with concerns of toxicity raised by<sup>21</sup> suggesting that higher doses may induce toxic effects, particularly on ovarian tissue integrity and function and In contrast, Solanki *et al.*, might have found some safety at particular lower doses of their aphrodisiac formulation, showing that the margin between a therapeutic and toxic dose can be quite narrow for herbal formulations.

While<sup>22</sup> may have found protective or enhancing effects on ovarian and uterine tissues at certain doses; our study reveals that higher doses (250 mg) of herbal extracts lead to significant ovarian degeneration, including follicular atresia and stromal alterations. This suggests a dose-dependent effect where herbal extracts can either support or damage reproductive tissues. Similarly, our findings on the uterine weight reduction at higher doses align with<sup>22</sup> focus on how herbal extracts can influence uterine structure, demonstrating that extracts like *Dracaena cinnabari* and the ones used in our study can have substantial impacts on uterine health and mass.<sup>23</sup> Investigated how a traditional herbal tea affects lipid profiles and liver enzyme activities, aiming to understand its metabolic and liver-protective effects in dyslipidemic rats. our study shares the approach of using herbal extracts to explore their physiological impacts but focuses on reproductive health parameters, particularly ovarian and uterine

tissues, as well as hormonal levels in female rats. Both studies highlight the complexity of herbal treatments and the potential for these extracts to exert broad physiological effects. While Odia and Bekinbo's study emphasizes the modulation of lipid profiles and liver health, our findings indicate that similar considerations must be given to how herbal extracts influence reproductive health, including organ weights, histopathological changes and hormone levels.<sup>24</sup> Study on the antifertility activity of *Michelia champaca* aligns with our study in demonstrating the capacity of herbal extracts to influence reproductive health and fertility. Both studies highlight that herbal extracts can alter reproductive organ structure, decrease organ weights and modulate hormonal levels in a manner that affects fertility. The dose-dependent nature of these effects, as shown in our study, underscores the need for careful consideration of dosing to either utilize these properties for antifertility purposes or avoid unintended disruption of reproductive function.

The histological analysis reveals that the elevated dosage (250 mg) causes significant ovarian follicular degeneration, alterations in stromal density and tissue disorganization, suggesting potential disturbances in cellular and tissue homeostasis. The effects may be influenced by oxidative stress, a well-established outcome of certain bioactive components in herbal preparations. Oxidative stress can hinder folliculogenesis by elevating Reactive Oxygen Species (ROS), resulting in granulosa cell death and follicular atresia. Subsequent research may investigate the significance of oxidative indicators, such as malondialdehyde or glutathione concentrations, to validate this theory.

The dose-dependent decrease in ovarian weight corresponds with histological findings of follicular degeneration and stromal changes, indicating disruption of normal ovarian function. A probable reason is the disturbance of endocrine signaling pathways, especially those governing the Hypothalamic-Pituitary-Gonadal (HPG) axis. The observed fluctuations in FSH and estrogen levels at varying doses corroborate this concept. The 100 mg dosage marginally inhibits FSH and estrogen levels, but the 250 mg dosage significantly increases them, demonstrating a biphasic action. These hormonal oscillations may arise from the extract's interaction with steroidogenic enzymes, such as aromatase or  $3\beta$ -HSD, which are essential for estrogen production. Investigating enzyme expression and activity might elucidate the manner in which the herbal extract influences steroidogenesis.

The histological results of altered stromal density and fibrotic areas indicate the activation of inflammatory or fibrotic pathways, perhaps via cytokine upregulation or extracellular matrix remodeling. These processes may further compromise ovarian and uterine function, highlighting the necessity to assess inflammatory markers such as IL-1 $\beta$  or TNF- $\alpha$ . Moreover, apoptotic mechanisms seem to be crucial, as indicated by granulosa cell condensation and pyknotic characteristics. The herbal extract may stimulate pro-apoptotic factors (e.g., Bax,

caspases) or suppress anti-apoptotic proteins (e.g., Bcl-2), leading to follicular degeneration. Molecular experiments aimed at these apoptotic markers might yield more conclusive information.

## CONCLUSION

our investigation revealed that the herbal extract displayed dose-dependent effects on the reproductive health of female rats. Elevated dosages resulted in substantial modifications to ovarian architecture, reproductive organ mass and hormonal concentrations, indicating possible toxicity and effects on fertility. These findings correspond with earlier research investigating the impacts of several herbal extracts, emphasizing the significance of dosage in ascertaining the therapeutic advantages relative to undesirable consequences. The findings highlight the necessity for careful implementation and additional research of herbal extracts to guarantee their safe application in reproductive health.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**TCM:** Traditional Chinese Medicine; **ROS:** Reactive Oxygen Species; **ZTN:** *Ziziphus spina-christi Trigonella foenum-graecum Nigella sativa*; **PCOS:** Poly Cystic Ovarian Syndrome; **ELISA:** Enzyme-Linked Immunosorbent Assay; **FSH:** Follicle-Stimulating Hormone; **LH:** Luteinizing Hormone; **HPG:** Hypothalamic-Pituitary-Gonadal.

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