



Aqueous *Zingiber officinale* rhizome extract impacts ovarian histology and reproductive hormones in adult female Wistar rats

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Abstract

Zingiber officinale (ginger) is widely used in traditional medicine; however, evidence regarding its dose- and duration-dependent effects on ovarian structure and reproductive hormones is limited. This study investigated the effects of graded oral doses of aqueous *Z. officinale* rhizome extract on ovarian histomorphology and some reproductive hormones in adult female Wistar rats. Thirty-five rats weighing 180-200 g (approximately 8-10 weeks old) were randomized into seven groups (n=5). Control animals received distilled water. Groups B-D received 100, 200, and 400 mg/kg daily for 30 days by oral gavage; groups E-G received the same doses for 60 days. Body and ovarian weights were recorded, and serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, progesterone, estradiol, and testosterone were measured by ELISA. Ovaries were processed for histology and examined after haematoxylin and eosin staining. No mortality or overt toxicity occurred during the acute study, and body and ovarian weights did not differ across groups. After 30 days, LH, FSH, and prolactin increased at 200 and 400 mg/kg, while progesterone and estradiol increased only at 400 mg/kg; testosterone showed no significant change. After 60 days, LH, FSH, and prolactin increased at 200 mg/kg, whereas prolactin decreased and testosterone increased markedly at 400 mg/kg. Histology revealed preserved ovarian architecture at 100 and 200 mg/kg after both 30 and 60 days, and at 400 mg/kg after 30 days. Prolonged exposure at 400 mg/kg was associated with expanded corpora lutea and increased developing follicles, consistent with enhanced follicular activity. Aqueous *Z. officinale* induced dose- and time-dependent endocrine changes with histological features suggesting increased folliculogenesis at prolonged high dose. These findings indicate dose-dependent ovarian and hormonal alterations, particularly at prolonged high dose, which may have reproductive implications. Further studies are needed to evaluate reproductive safety and mechanisms following long-term high-dose exposure.

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Introduction

Female fertility depends on normal ovarian structure and balanced secretion of reproductive hormones that regulate follicular development, ovulation, and luteal function (Holesh *et al.*, 2025). Alterations in ovarian histoarchitecture or disturbances in luteinizing hormone, follicle stimulating hormone, prolactin, progesterone, estradiol, and androgens may impair folliculogenesis and reduce reproductive capacity (Bendarska-Czerwińska *et al.*, 2023).

In recent years, there has been growing interest in the use of medicinal plants for the prevention and management of various disease conditions, including reproductive disorders, due to their bioactive constituents and relatively favorable safety profiles (Ayustaningwarno *et al.*, 2024; Patel *et al.*, 2025). Among these, *Zingiber officinale* Roscoe (ginger) is widely used both as a culinary spice and in traditional medicine. The rhizome contains bioactive compounds such as gingerols, shogaols, paradols, and zingerone (Patel *et al.*, 2025), which possess antioxidant and anti-inflammatory activities that may influence reproductive tissues through modulation of oxidative stress and cellular responses (Patel *et al.*, 2025; Zimazi *et al.*, 2025).

Experimental studies have reported reproductive effects of ginger; however, findings vary depending on dose, duration of exposure, and method of extraction. For instance, some studies report improved fertility parameters and protective effects on reproductive tissues, whereas others indicate alterations in reproductive hormone levels and potential endocrine disruption at higher doses or prolonged exposure (Akomolafe & Atoyebi, 2025; Ayustaningwarno *et al.*, 2024; Ojukwu & Ibeabuchi, 2025).

The ovary is a dynamic organ that undergoes continuous remodeling during follicular growth and corpus luteum formation (Wang & Yang, 2025). Angiogenic and endocrine interactions play critical roles in these processes, and their disruption may contribute to ovulatory dysfunction and infertility (Jankowska-Ziemak *et al.*, 2025; Zhu *et al.*, 2025). Despite existing reports on the reproductive effects of ginger, information regarding its influence on ovarian microstructure alongside concurrent endocrine changes following repeated oral exposure remains limited.

Therefore, the purpose of this study was to investigate the effects of graded oral doses of aqueous *Z. officinale* rhizome extract administered for 30 and 60 days on ovarian histomorphology and selected reproductive hormones in adult female

Wistar rats. The study was designed to provide experimental evidence on ovarian structural responses and associated endocrine changes under subacute exposure conditions.

Materials and Methods

Experimental animals

Thirty-five adult female Wistar rats weighing 180 to 200 g (between 8-10 weeks old) were obtained from the Animal House Facility, Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were housed in standard laboratory cages under controlled environmental conditions with normal light and dark cycles and adequate ventilation. Rats were fed standard grower mash (Top Feeds®, Premier Feed Mills Co. Ltd., Nigeria) twice daily, which provides balanced nutrients required for maintenance of normal growth, metabolic activity, and reproductive function, and provided clean drinking water *ad libitum*. A two-week acclimatization period was allowed before treatment commenced. Note that the estrous cycle stage of the animals was not monitored prior to or during treatment.

Ethical consideration

Ethical approval for this study was obtained from the Edo State Ministry of Agriculture and Food Security (Approval No. MAFS/RES/VOL.II/2025/07). All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals as outlined by the National Research Council Guide for the Care and Use of Laboratory Animals (Hubrecht & Carter, 2019).

Plant material and authentication

Fresh rhizomes of *Zingiber officinale* Roscoe were obtained from the Efosa community in Ugbowo axis, Benin City, Nigeria (Latitude 6.3976° N, Longitude 5.6090° E). The plant material was identified and authenticated by a taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A voucher specimen (UBHz404) was prepared and deposited in the departmental herbarium for reference.

Preparation of aqueous ginger rhizome extract

The rhizomes were washed thoroughly and cut into small pieces. The pieces were air-dried at room temperature for two weeks and milled into coarse powder using a laboratory grinder (Retsch SM 100, Retsch GmbH, Haan, Germany). Fifty grams of the

powdered sample were macerated in 250 mL of distilled water and agitated on an orbital shaker (GFL 3015, GFL GmbH, Burgwedel, Germany) at 37°C for 24 hours. The temperature of 37°C was maintained using a temperature-controlled orbital shaker incubator, which allowed simultaneous agitation and regulation of extraction temperature throughout the 24-hour maceration period. The mixture was filtered through muslin cloth and cotton wool. The filtrate was dried using a laboratory spray dryer (Büchi Mini Spray Dryer B-290, Büchi Labortechnik AG, Flawil, Switzerland) at 45°C to obtain a dry extract. The dried extract was stored in airtight amber bottles at 4°C until use.

Acute toxicity study

Acute oral toxicity of the aqueous extract was evaluated using the Lorke method (Lorke, 1983). Rats were divided into five groups of two animals per group. Single oral doses of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight were administered to the respective groups. Animals were observed continuously for the first 24 hours and thereafter daily for seven days for mortality and signs of toxicity including lethargy, reduced feed intake, salivation, diarrhoea, weakness, and abnormal behaviour. The Lorke's (1983) method was adopted as a preliminary toxicity screening procedure that permits estimation of acute toxicity using a relatively small number of animals.

Experimental design and treatment

The animals were randomly assigned into seven groups of five rats each. Group A served as the control and received distilled water. Groups B, C and D received aqueous ginger extract at doses of 100, 200 and 400 mg per kg body weight, respectively, for 30 days. Groups E, F and G received the same respective doses for 60 days. All administrations were carried out once daily by oral gavage using stainless steel feeding needles.

Sample collection

At the end of the treatment period, rats were anaesthetized using light chloroform inhalation. A midline abdominal incision (laparotomy) was performed, and blood samples were collected by venepuncture of the posterior vena cava using sterile needles and transferred into plain sample bottles. Following blood collection, the animals were euthanized by cervical dislocation. Samples were allowed to clot at room temperature and centrifuged at 3000 rpm for 10 minutes using a bench centrifuge (Eppendorf 5702, Eppendorf AG, Hamburg,

Germany). Serum was stored at -20°C until analysis. Ovaries were then excised, cleared of adherent tissues, weighed using an analytical balance (Mettler Toledo ME204, Mettler Toledo, Greifensee, Switzerland), and fixed in 10 percent neutral buffered formalin.

Physical and gross examination

Animals were monitored daily for behavioural changes and general health status. At necropsy, ovaries were examined for colour, consistency and gross abnormalities. Tissue slices not exceeding 3 mm in thickness were selected for histological processing.

Histological processing and examination

Histological processing followed standard histological procedures as outlined by Suvana *et al.* (2019). Fixed ovarian tissues were processed using an automated tissue processor (Leica TP 1020, Leica Biosystems, Nussloch, Germany). Tissues were dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax using a paraffin embedding station (Leica EG1150 H, Leica Biosystems, Nussloch, Germany). Sections of 3 µm thickness were cut using a rotary microtome (Leica RM2235, Leica Biosystems, Nussloch, Germany) and mounted on clean glass slides. Sections were dried on a slide warmer (Thermo Scientific SlideMate AS, Thermo Fisher Scientific, Waltham, MA, United States) and stained with haematoxylin and eosin. Stained sections were examined using a light microscope (Olympus CX23, Olympus Corporation, Tokyo, Japan). Histological evaluation was performed in a blinded manner by two independents qualified pathologists at the University of Benin Teaching Hospital, Benin City, Nigeria. Assessment focused on ovarian architecture, follicular stages, and corpora lutea. Quantitative follicular counting was not performed. Photomicrographs were captured with a digital microscope camera (AmScope MU1803, AmScope, Irvine, CA, United States).

Hormonal assay

Serum luteinizing hormone, follicle stimulating hormone and prolactin were determined using immunoassay kits (AccuBind ELISA kits, Monobind Inc., Lake Forest, CA, United States). Progesterone and testosterone were measured using competitive enzyme immunoassay kits (AccuBind ELISA kits, Monobind Inc., Lake Forest, CA, United States). Estradiol was measured using delayed competitive enzyme immunoassay kits (AccuBind ELISA kits, Monobind Inc., Lake Forest, CA, United States).

States). Absorbance was read using a microplate photometer (Multiskan FC, Thermo Fisher Scientific, Waltham, MA, United States). All assays were performed according to the manufacturers' protocols.

Statistical analysis

Results were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was carried out using Statistical Package for the Social Sciences software (SPSS version 25.0, IBM Corp., Armonk, NY, United States). One-way analysis of variance (ANOVA) was used to compare group means, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

Acute toxicity

Single oral administration of aqueous *Z. officinale* extract at doses of 1000, 2000, 3000, 4000 and 5000 mg/kg produced no mortality within the seven-day observation period. Transient reduction in feed intake and mild weight loss was observed within the first 24 hours at 5000 mg/kg, with recovery observed by day 2. No overt signs of toxicity were recorded thereafter.

Body and ovarian weights

Body weight changes over 30 days showed comparable trends across control and treated groups, with no consistent dose-related pattern (Figure 1A). Initial body weights: control (200.3 ± 5.8 g), 100 mg/kg group (215.2 ± 6.2 g), 200 mg/kg group (220.1 ± 6.0 g), and 400 mg/kg group (218.4 ± 5.9 g). After 30 days of treatment, body weights were: control (215.2 ± 6.5 g), 100 mg/kg group (225.1 ± 6.8 g), 200 mg/kg group (230.4 ± 7.1 g), and 400 mg/kg group (210.3 ± 6.4 g). After 60 days, body weight progression remained similar among groups, with no evidence of treatment-related weight loss or gain (Figure 1C). Body weights at day 60 were: control (290.4 ± 8.5 g), 100 mg/kg group (200.3 ± 6.9 g), 200 mg/kg group (225.1 ± 7.2 g), and 400 mg/kg group (200.3 ± 6.7 g). Mean ovarian weights did not differ significantly between control and treated groups after 30 days (Figure 1B) or 60 days of administration (Figure 1D). Across both durations, ovarian weights remained within the control range, indicating no dose-related effect on ovarian mass.

Reproductive hormone profile

After 30 days of treatment, luteinizing hormone and follicle-stimulating hormone were significantly higher

at 200 and 400 mg/kg compared with control. Prolactin increased significantly at 200 and 400 mg/kg. Progesterone and estradiol showed significant elevation at 400 mg/kg only. Testosterone did not differ significantly among groups (Table 1). Following 60 days of exposure, luteinizing hormone, follicle stimulating hormone and prolactin were significantly increased at 200 mg/kg relative to control. At 400 mg/kg, prolactin and testosterone showed significant changes, while progesterone and estradiol differed across groups without a consistent dose related trend (Table 2).

Ovarian histology

The control ovaries showed preserved ovarian architecture with prominent corpora lutea and well-organized follicular structures (Plate 1A). Ovaries from rats treated with 100 mg/kg for 30 and 60 days demonstrated intact follicular organization with identifiable corpora lutea and preserved granulosa cell layers (Plate 1B1 and B2). At 200 mg/kg, ovarian sections obtained after 30 and 60 days showed well-formed follicles at different stages of development with preserved stromal organization (Plate 1C1 and C2). At 400 mg/kg, ovarian sections obtained after 30 days showed intact histoarchitecture with visible corpora lutea and developing follicles (Plate 1D1). After 60 days, ovaries showed expanded corpora lutea with prominent primary and secondary follicles, consistent with increased follicular activity (Plate 1D2).

Discussion

The study tested different oral doses of ginger extract in adult female rats for 30 and 60 days. It found no change in body or ovary weight. Some reproductive hormones changed with dose and time. The highest dose for longer use showed signs of increased ovarian follicle activity, linking hormonal changes with ovarian structure. These observations address the study aim of defining ovarian structural responses alongside endocrine changes following subacute exposure to ginger.

The absence of significant changes in body and ovarian weights across doses and durations indicates that the extract did not induce overt systemic toxicity or gross ovarian atrophy. This supports the safety profile of ginger reported in dietary and preclinical studies and aligns with recent evidence that ginger-based interventions can improve reproductive parameters without compromising general health status (Akomolafe & Atoyebi, 2025; Zimazi *et al.*, 2025).

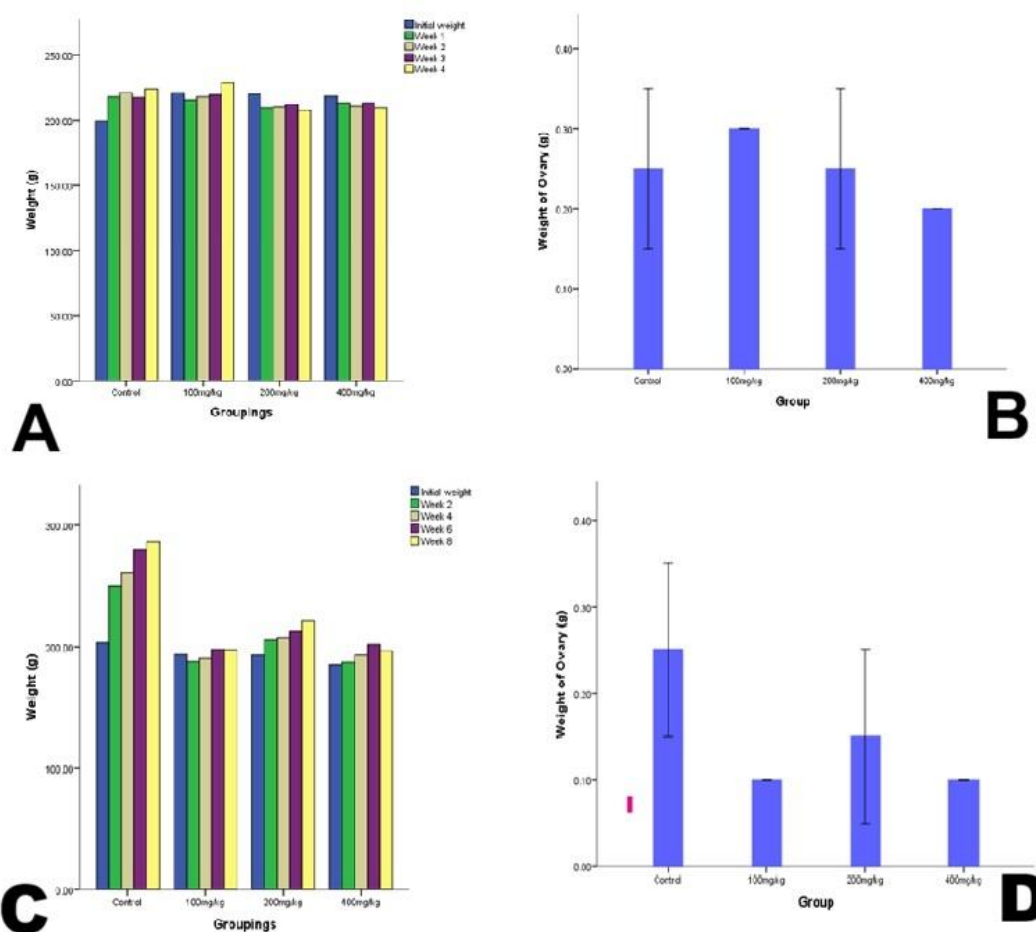


Figure 1. Effects of graded oral doses of aqueous *Z. officinale* extract on body and ovarian weights of adult female Wistar rats. (A) Weekly body weight changes over 30 days across control and treated groups (100, 200, 400 mg/kg), showing no consistent dose-related deviation from baseline. (B) Mean ovarian weights after 30 days of treatment, indicating no significant differences between control and extract-treated groups. (C) Body weight progression over 60 days, with comparable trends across all groups and no evidence of treatment-related weight loss or gain. (D) Ovarian weights following 60 days of exposure, showing values within the control range across doses. Values are presented as mean $\pm$ SD (n=5).

The lack of gross weight changes also reduces the likelihood that the observed histological findings were secondary to nonspecific toxicity.

Endocrine responses varied with dose and duration (Kolnikaj *et al.*, 2024), however, in this study, short-term exposure was associated with dose-dependent increases in luteinizing hormone, follicle-stimulating hormone, and prolactin at 200 and 400 mg/kg, with progesterone and estradiol increasing only at 400 mg/kg, while testosterone remained unchanged. In contrast, prolonged exposure showed a biphasic pattern, with luteinizing hormone, follicle-stimulating hormone, and prolactin increasing at 200 mg/kg but decreasing at 400 mg/kg, whereas testosterone increased markedly at the highest dose. These

findings suggest dose- and time-dependent endocrine alterations following ginger exposure, which may involve hypothalamic-pituitary-ovarian interactions; however, the present data cannot distinguish central axis modulation from direct ovarian steroidogenic effects. Ovulation and follicular maturation are tightly regulated by coordinated changes in luteinizing hormone and follicle-stimulating hormone, with downstream effects on ovarian steroidogenesis (Holesh *et al.*, 2025; Bendarska-Czerwińska *et al.*, 2023). The hormonal shifts observed here are therefore biologically plausible and may reflect adaptive endocrine responses to phytochemical exposure, which is in line with the study by Stiefel & Stintzing (2023).

Table 1: Effects of graded oral doses of aqueous *Z. officinale* extract on reproductive hormone levels in adult female Wistar rats after 30 days of treatment

Parameter (Unit)	Control	100 mg/kg	200 mg/kg	400 mg/kg	p value
LH (mIU/mL)	2.60 ± 0.45	2.87 ± 0.22	4.60 ± 0.89*	6.05 ± 0.34*	0.002
FSH (mIU/mL)	2.35 ± 0.34	3.25 ± 0.56	4.05 ± 0.34*	5.25 ± 0.56*	0.002
Prolactin (ng/mL)	4.05 ± 0.34	5.70 ± 0.67	7.35 ± 1.12*	8.45 ± 1.68*	0.006
Progesterone (ng/mL)	1.65 ± 0.34	1.70 ± 0.45	2.50 ± 0.67	3.05 ± 0.34*	0.023
Estradiol (pg/mL)	48.00 ± 6.71	47.00 ± 8.94	66.50 ± 10.07	73.50 ± 12.30*	0.028
Testosterone (ng/mL)	0.30 ± 0.22	0.25 ± 0.11	0.35 ± 0.11	0.45 ± 0.11	0.310

*Values are presented as mean ± SD (n = 5).

One-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons.

Asterisk (*) indicates a statistically significant difference compared with the control group ($p < 0.05$)

Table 2: Effects of graded oral doses of aqueous *Z. officinale* extract on reproductive hormone levels in adult female Wistar rats after 60 days of treatment

Parameter (Unit)	Control	100 mg/kg	200 mg/kg	400 mg/kg	p value
LH (mIU/mL)	2.60 ± 0.45	3.15 ± 0.11	3.70 ± 0.45*	1.70 ± 0.45	0.005
FSH (mIU/mL)	2.35 ± 0.34	3.10 ± 0.45	3.90 ± 0.22*	1.70 ± 0.22	0.002
Prolactin (ng/mL)	4.05 ± 0.34	4.90 ± 0.22	6.75 ± 1.12*	1.15 ± 0.56*	<0.001
Progesterone (ng/mL)	1.65 ± 0.34	1.60 ± 0.89	2.30 ± 0.45	0.30 ± 0.22	0.018
Estradiol (pg/mL)	48.00 ± 6.71	49.00 ± 22.36	62.50 ± 10.07	21.50 ± 10.07	0.037
Testosterone (ng/mL)	0.30 ± 0.22	0.15 ± 0.11	0.30 ± 0.22	20.20 ± 0.45*	0.001

*Values are presented as mean ± SD (n = 5).

One-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons.

Asterisk (*) indicates a statistically significant difference compared with the control group ($p < 0.05$)

The rise in testosterone at the highest dose after prolonged treatment suggests a shift in steroidogenic balance toward androgen production. Elevated testosterone alongside reduced gonadotropins at 400 mg/kg indicates possible feedback suppression within the hypothalamic-pituitary-ovarian axis. However, the elevation of testosterone at 60 days without a corresponding increase in luteinizing hormone may reflect mechanisms beyond hypothalamic-pituitary-ovarian axis modulation. This pattern suggests possible direct ovarian steroidogenic effects, including increased theca cell androgen synthesis or reduced aromatization of androgens to estradiol. Bioactive constituents of ginger, such as gingerols, shogaols, and zingerone, have been reported to influence steroidogenic pathways through modulation of steroidogenic acute regulatory (StAR) protein expression and CYP19A1 (aromatase) activity, thereby altering androgen-estrogen balance. Experimental evidence indicates that zingerone can modulate circulating steroid hormones and steroidogenic markers, supporting direct effects on steroidogenesis (Jerang *et al.*, 2025). These findings may therefore reflect direct ovarian stimulation or

altered steroid metabolism rather than central hypothalamic-pituitary regulation. In the absence of hypothalamic tissue assessment, ex vivo pituitary stimulation assays, or ovarian culture experiments, the present data cannot distinguish central from peripheral sites of action, and interpretation is therefore limited to systemic endocrine alterations associated with ginger exposure (Nai *et al.*, 2025). Increased androgen levels may influence follicular dynamics and steroid interactions, potentially altering estrogen-progesterone balance. Similar observations have been discussed by Lazarev *et al.* (2026), who reported that elevated testosterone levels modify endocrine interactions and may affect reproductive hormonal regulation.

Histological assessment showed preserved ovarian architecture at lower doses and durations, and prolonged high-dose exposure was associated with expanded corpora lutea and increased representation of developing follicles. These structural features are consistent with enhanced follicular activity and luteal development, processes that depend on adequate angiogenesis and stromal support (Zaniker *et al.*, 2024).

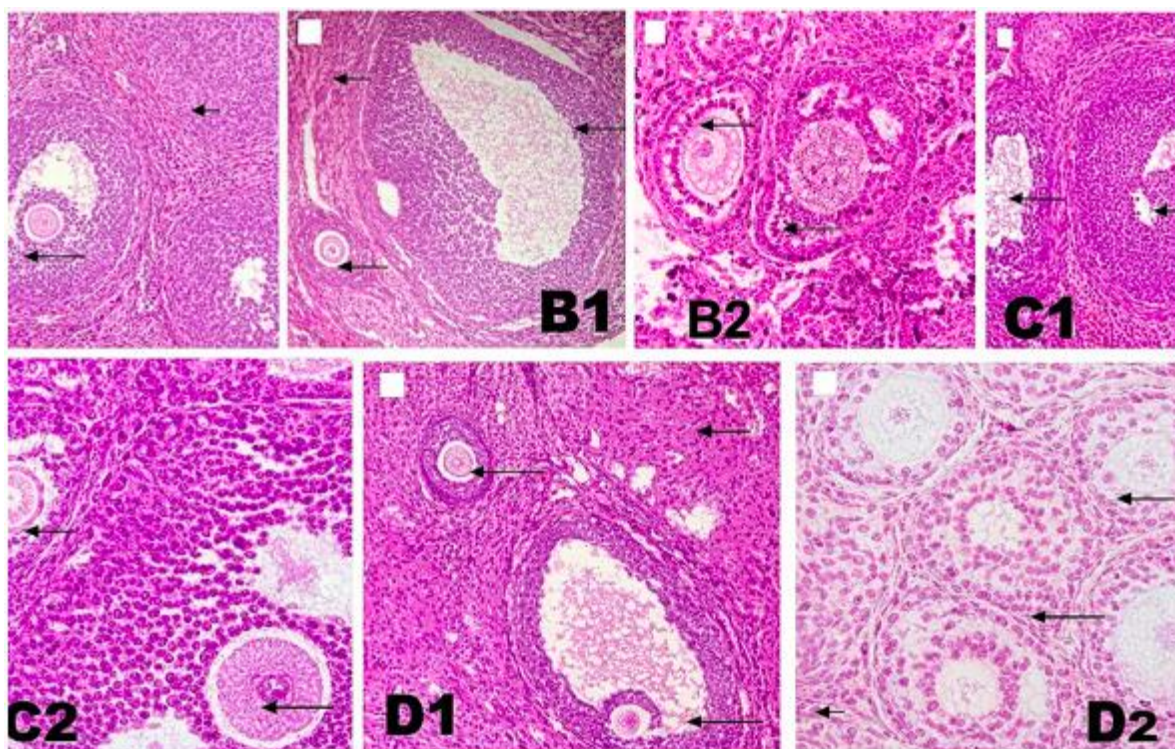


Plate I: Representative photomicrographs of the ovarian sections from control and treated adult female Wistar rats. (A) Control animals showing normal ovarian architecture with multiple corpora lutea, intact germinal epithelium and well-defined granulosa layer surrounding an eccentrically placed oocyte. B1 (100 mg/kg, 1 month): Preserved ovarian structure with identifiable corpus luteum and follicles at different stages of development. B2 (100 mg/kg, 2 months): Well organized follicular units with prominent oocytes and intact granulosa cell layers, indicating maintained follicular maturation. C1 (200 mg/kg, 1 month): Ovarian cortex showing developing follicles with preserved stromal organization and intact follicular layers. C2 (200 mg/kg, 2 months): Increased representation of growing follicles with prominent oocytes and orderly granulosa cell arrangement. D1 (400 mg/kg, 1 month): Intact ovarian histoarchitecture with visible corpus luteum and developing follicles. D2 (400 mg/kg, 2 months): Expanded corpus luteum with prominent primary and secondary follicles, consistent with enhanced folliculogenic activity. Haematoxylin and eosin stain, magnification $\times 400$.

The follicular changes observed in this study may therefore reflect modulation of local ovarian microenvironment rather than tissue injury. Recent work highlights the central role of growth factors and signaling pathways in ovarian angiogenesis and follicular progression (Jankowska Ziemak *et al.*, 2025), as well as the contribution of ovarian mechanics to follicular development (Wang & Yang, 2025).

This aligns with evidence that ginger possesses antioxidant and anti-inflammatory properties that can influence tissue remodeling and vascular responses (Ayustaningwarno *et al.*, 2024; Patel *et al.*, 2025). Conversely, chronic ovarian remodeling and fibrosis are features of ovarian aging and inflammatory states (Zhu *et al.*, 2025). The absence of fibrotic or degenerative features in the present study suggests that the observed changes are more

consistent with functional remodeling than pathology under the exposure conditions used.

Our findings are concordant with prior animal studies showing reproductive modulation by ginger. Dietary ginger has been reported to improve fertility related parameters in metabolic stress models (Akomolafe & Atoyebi, 2025), while aqueous ginger extracts have shown protective effects on male gonadal tissues following ischemic injury (Ojukwu & Ibeabuchi, 2025). Together with the present data, these studies support a context dependent reproductive effect of ginger that varies with dose, duration, and physiological state (Matin *et al.*, 2025). The present work extends this evidence to ovarian histomorphology in females and highlights potential endocrine trade offs at higher doses with prolonged use.

This study is limited to a small group size, and only serum hormones and routine histology were measured. Markers of angiogenesis, oxidative stress, and signaling pathways were not assessed. The oestrous cycle was not controlled, which may affect hormone results. Future studies should control cycle stage, include follicle counts and molecular markers, optimize dose selection to further clarify the reproductive effects of *Z. officinale*. A two-way ANOVA assessing interaction between dose and duration was not performed and may provide additional insight in future studies.

In conclusion, ginger extract caused no obvious toxicity or weight changes in female rats but produced dose- and time-dependent hormone changes and signs of increased follicular activity at high, prolonged doses. This suggests possible benefits at moderate intake but potential hormonal disturbance with long-term high use. Further studies are needed to define safe doses and active compounds.

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No funding was received.

Conflict of Interest

The authors declare that there is no conflict of interest.

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