RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v24i2.8

Odigie & Ogeyemhe /*Sokoto Journal of Veterinary Sciences*, **24**(2): 158 - 166.

Aqueous extract of ginger (*Zingiber officinale* Roscoe) rhizome improves testicular histology and reproductive hormones in adult Wistar rats

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Publication History:
Received: 21-02-2026
Revised: 06-04-2026
Accepted: 27-04-2026

Abstract

Ginger is widely used as a dietary spice and traditional remedy, yet evidence on its effects on testicular histology and reproductive hormones remains inconsistent. This study evaluated the effect of graded doses of aqueous ginger rhizome extract on testicular histology and selected reproductive hormones in adult male Wistar rats. Thirty-five adult male Wistar rats weighing 180-200 g were assigned to seven groups of five rats each. The control group received a grower diet and water. Rats in groups B, C and D received aqueous ginger rhizome extract at 100 mg per kg, 200 mg per kg and 400 mg per kg, respectively, for 30 days, while groups E, F and G received the same doses for 60 days. Body and testicular weights were recorded, while serum luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, progesterone, and estradiol were assayed. The testes were examined histologically using haematoxylin and eosin staining. Body and testicular weights did not differ significantly ($p > 0.05$) between control and treated groups at both time points. In the 30-day exposure, luteinizing hormone and follicle-stimulating hormone were significantly ($p < 0.05$) higher at 200 mg/kg and 400 mg/kg, whereas testosterone was highest at 400 mg/kg. After 60 days, follicle-stimulating hormone and estradiol were significantly increased at 400 mg/kg, with a significant ($p < 0.05$) rise in progesterone at the same dose. Histology showed preserved seminiferous tubule architecture across all groups, with luminal accumulation of spermatozoa at 400 mg/kg at both durations. Aqueous ginger rhizome extract preserved testicular histology and produced modest, dose-related endocrine changes without evidence of gonadal toxicity.

Keywords: Aqueous extract, Ginger, Reproductive hormones, Testicular histology, Wistar rats

Introduction

Normal male fertility depends on intact testicular architecture and balanced secretion of reproductive hormones that regulate spermatogenesis (Guyton & Hall, 2015; Lei *et al*., 2025; Maroto *et al.,* 2025). Structural disruption of seminiferous tubules or altered function of Sertoli and Leydig cells may impair

sperm production and compromise reproductive capacity (Feng *et al.,* 2025; Smith & Walker, 2014).

Ginger (*Zingiber officinale* Roscoe) is widely consumed as a dietary spice and used in traditional medicine (Zimazi *et al.,* 2025). Experimental evidence suggests that ginger possesses antioxidant properties that may support testicular integrity and influence reproductive hormone activity (Patel *et al.,* 2025). However, reports on its effects on testicular histology and endocrine function remain inconsistent, largely due to differences in dosage, exposure duration, and extraction methods (Akomolafe & Atoyebi, 2025; Ojukwu & Ibeabuchi, 2025).

There is limited histological evidence describing the effect of aqueous ginger rhizome extract on testicular microarchitecture following repeated oral administration alongside concurrent assessment of reproductive hormone profiles. The present study, therefore investigated the effects of graded doses of aqueous ginger rhizome extract on testicular histology and serum levels of luteinizing hormone (mIU/mL), follicle-stimulating hormone (mIU/mL), prolactin (ng/mL), progesterone (ng/mL), estradiol (pg/mL), and testosterone (ng/mL) in adult male Wistar rats.

Materials and Methods

Experimental animals

A total of 44 adult male Wistar rats weighing between 180 g and 200 g were used for this study. They were obtained from the Animal House, Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed under standard Laboratory conditions with adequate ventilation, a 12-hour light/12-hour dark cycle, ambient temperature maintained at 26 ± 2°C, and relative humidity of approximately 50%. They were fed a standard grower diet and provided clean drinking water *ad libitum*. The rats were allowed to acclimatise for two weeks before the commencement of the experiment. Nine rats were used for acute toxicity (LD_{50}) determination, while 35 rats were used for the main experimental study. All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals.

Ethical consideration

All animal procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals, as outlined in standard ethical frameworks for animal experimentation (Hubrecht & Carter, 2019). Efforts were made to minimize animal suffering and to use the minimum number of animals required to achieve statistical validity. Ethical approval for this study was obtained from the Edo State Ministry of Agriculture and Food Security (Approval No. MAFS/RES/VOL.II/2025/06).

Plant material and authentication

Fresh ginger rhizomes (*Zingiber officinale* Roscoe) were obtained from the Efosa community in Ugbowo, Benin City, N. The plant material was identified and authenticated by qualified botanists. A voucher specimen was prepared and deposited at the Herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria for reference (voucher number: UBHZ404).

Preparation of aqueous ginger rhizome extract

The ginger rhizomes were washed thoroughly with clean water to remove debris and air-dried at room temperature. The dried rhizomes were milled into coarse powder using a Laboratory grinder. Fifty grams of the powdered sample were macerated in 250 mL of distilled water and agitated at 37°C for 24 hours. The mixture was filtered through clean muslin cloth, followed by cotton wool filtration. The filtrate was concentrated using a spray dryer at 45°C to obtain a dry extract. The extract was stored in an airtight dark container at 4°C until use. The extraction procedure was performed under standardised conditions to ensure reproducibility.

Acute toxicity study

The median lethal dose of the aqueous ginger rhizome extract was determined using a modified Lorke method (Lorke, 1983), in line with established acute toxicity testing approaches (OECD, 2019). Three groups of rats comprising 3 rats each (n = 3 per group) were administered single oral doses of 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, and 5000 mg/kg body weight. The animals were observed for seven days for mortality and signs of toxicity, including changes in activity, feeding behaviour, salivation, and physical appearance.

Experimental design and treatment

Thirty-five male rats were randomly assigned to seven groups of five animals each (n=5). A formal sample size calculation was not done before the study. However, the number of animals used is similar to what is commonly used in comparable laboratory studies and was kept low to follow ethical guidelines on reducing animal use. It is important to note that the small sample size may reduce the ability to detect

subtle changes in hormone levels. No positive control group was included in this study, as the experiment was designed to evaluate the dose-dependent effects of aqueous ginger rhizome extract relative to untreated controls. Group A served as the negative control and received distilled water and standard feed (Top Feeds® Growers Mash, Premier Feed Mills Co. Ltd., Nigeria). Groups B, C, and D received aqueous ginger rhizome extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, respectively, for 30 days. Groups E, F, and G received the same doses for 60 days. All treatments were administered orally once daily using a gastric gavage. The doses (100, 200, and 400 mg/kg body weight) were derived from the acute toxicity study, in which no mortality or signs of toxicity were observed up to 5000 mg/kg body weight. These doses represent safe sub-toxic fractions of the highest non-lethal dose and were selected to evaluate dose-dependent biological effects without inducing toxicity.

Sample collection

At the end of each treatment period, the animals were sacrificed by cervical dislocation. Blood samples were collected from the posterior vena cava between 8:00 am and 10:00 am to minimize the effect of circadian variation in hormone levels. The animals were not fasted prior to sample collection. Blood was collected into plain tubes and allowed to clot. Serum was separated by centrifugation and stored at -20°C until hormonal analysis. The testes were carefully excised, trimmed of adherent tissues, weighed, and immediately fixed in Bouin's solution for 24 hours before histological processing.

Hormonal assay

Serum levels of luteinizing hormone, follicle stimulating hormone, and prolactin were measured using immunoenzymometric assay kits (AccuBind ELISA kits, Monobind Inc., Lake Forest, CA, United States). The ELISA kits used are commercially available and validated by the manufacturer for use in rat serum. In addition, assay performance was verified in our laboratory through standard quality control procedures, including the use of controls and replicate measurements, to ensure reliability of the results. Testosterone and progesterone were analyzed with competitive enzyme immunoassay kits (AccuBind ELISA kits, Monobind Inc., Lake Forest, CA, United States), while estradiol was determined using delayed competitive enzyme immunoassay kits (AccuBind ELISA kits, Monobind Inc., Lake Forest, CA, United States). Absorbance readings were taken with a Thermo Scientific Multiskan FC Microplate Photometer (Thermo Fisher Scientific, United States). All procedures followed the manufacturer's instructions precisely.

Physical and gross examination

The animals' behavioural pattern was observed throughout the period of the experiment. The harvested organs (testes) were examined macroscopically during grossing. The gross morphological description of the testes was done taking into cognizance of the color, consistency (hard or soft) and the weight. Then, a representative section of the organs not thicker than 3mm was taken for histomorphological investigation.

Histological processing and examination

The fixed testicular tissues were processed using an automated tissue processor (Leica TP 1020 Automatic Benchtop Tissue Processor, Leica Biosystems, Nussloch, Germany). The tissues were dehydrated through ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax using a paraffin embedding station (Leica EG1150 H Embedding Center, Leica Biosystems, Nussloch, Germany). Sections of 3 µm thickness were obtained using a rotary microtome (Leica RM2235 Rotary Microtome, Leica Biosystems, Nussloch, Germany). The sections were mounted on glass slides, dried on a slide warmer, and stained with haematoxylin and eosin. Stained sections were examined by a histopathologist at the University of Benin Teaching Hospital using a light microscope (Olympus CX23 Light Microscope, Olympus Corporation, Tokyo, Japan), viewed at x40 and x100. Representative photomicrographs were captured using a digital microscope camera (AmScope MU1803 18 MP Camera, United States) for documentation.

Data analysis

Data were expressed as mean ± standard deviation (SD) using the Statistical Package for the Social Sciences (SPSS) software (version 25.0, IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to compare differences among groups, followed by Tukey's post hoc test for multiple comparisons. A probability value of less than 0.05 was considered statistically significant. Separate analyses were conducted for each treatment duration (30 and 60 days). While a two-way ANOVA could assess interaction effects between dose and duration, the present approach was adopted to allow independent evaluation of treatment effects within each time point.

Results

There was no significant difference in body weight among the treated groups compared with the control group after 30 days and 60 days of administration of aqueous ginger rhizome extract (Figure 1A and 1C).

Testicular weights did not differ significantly between the control and treated groups at both time points (Figure 1B and 1D).

After 30 days of treatment, serum luteinizing hormone and follicle-stimulating hormone levels were significantly ($p < 0.05$) higher in rats administered 200 mg/kg and 400 mg/kg of aqueous ginger rhizome extract compared with the control group. Prolactin, progesterone, and estradiol levels did not differ significantly ($p < 0.05$) among groups. Testosterone levels differed across groups, with the highest mean value observed in rats treated with 400 mg/kg ($p < 0.05$); however, this difference was not statistically significant ($p < 0.05$) compared with the control group (Table 1). Though after 60 days of treatment, follicle-stimulating hormone and estradiol levels were significantly ($p < 0.05$) increased in rats administered 400 mg/kg of aqueous ginger rhizome extract compared with the control group ($p < 0.05$). Progesterone levels also showed a significant increase in the 400 mg/kg group. No significant differences were observed in luteinizing hormone, prolactin, or testosterone levels among groups at 60 days (Table 2).

Histological examination of the testes in the control group showed preserved seminiferous tubules with normal arrangement of germ cells at different stages of spermatogenesis and identifiable Sertoli and Leydig cells (Plates I, A). Sections from rats administered 100 mg/kg and 200 mg/kg of aqueous ginger rhizome extract for 30 days and 60 days showed preserved testicular architecture comparable to the control group (Plate I, B1, B2, C1 and C2). In rats administered 400 mg/kg for 30 days and 60 days,

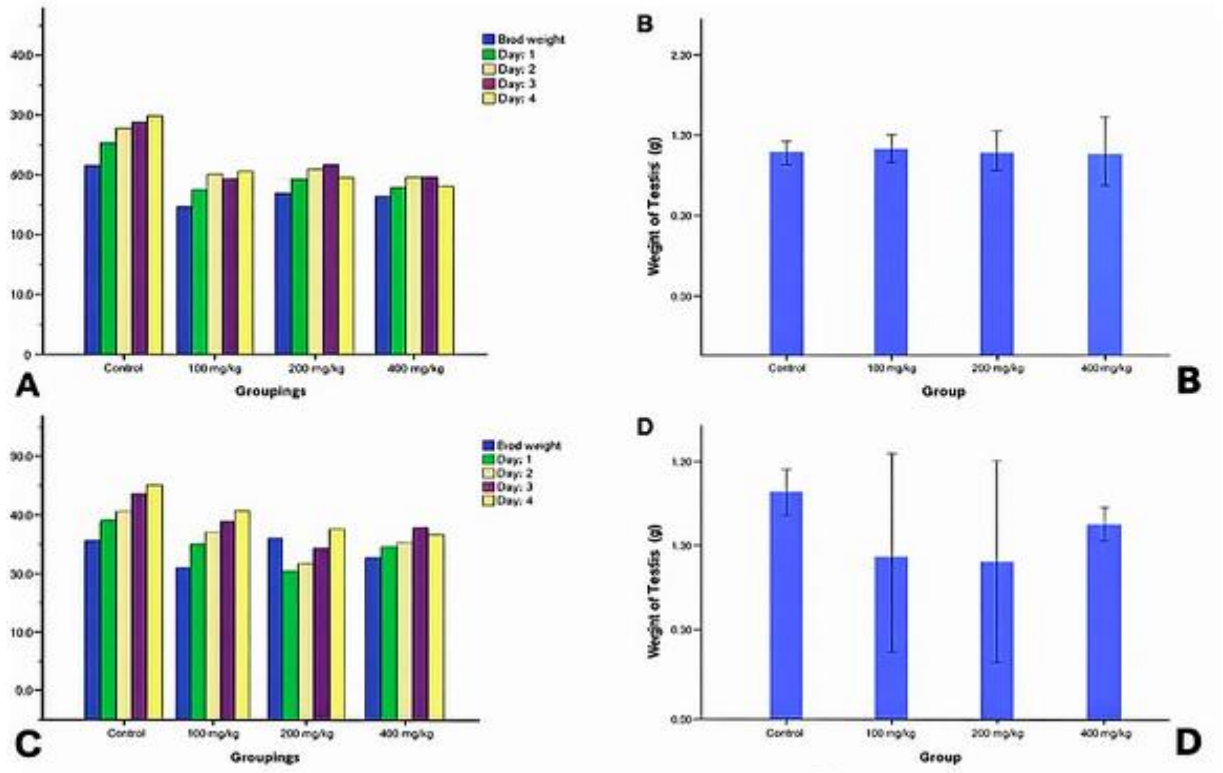

Figure 1: Effects of aqueous ginger rhizome extract on body weight and testicular weight of adult male Wistar rats. (A) Changes in body weight over 30 days in the control and treated groups were observed after administration of 100 mg/kg, 200 mg/kg, and 400 mg/kg of aqueous ginger rhizome extract. (B) Mean testicular weight after 30 days of treatment across the control and treated groups. (C) Changes in body weight over 60 days in the control and treated groups were observed after administration of 100 mg/kg, 200 mg/kg, and 400 mg/kg of aqueous ginger rhizome extract. (D) Mean testicular weight after 60 days of treatment across the control and treated groups. Values are presented as mean ± SD (n = 5)

Table 1: Effect of aqueous ginger rhizome extract on the hormonal profile of adult male Wistar rats after 30 days of administration

Parameter (Unit)	Control	100 mg/kg	200 mg/kg	400 mg/kg	p value
LH (mIU/mL)	0.72 ± 0.18	1.09 ± 0.25	1.85 ± 0.11*	2.40 ± 0.45*	0.002
FSH (mIU/mL)	0.64 ± 0.20	1.10 ± 0.45	1.80 ± 0.45*	2.75 ± 0.34*	0.003
Prolactin (ng/mL)	0.50 ± 0.22	0.75 ± 0.56	0.80 ± 0.22	1.15 ± 0.56	0.260
Progesterone (ng/mL)	0.20 ± 0.22	0.35 ± 0.11	0.25 ± 0.11	0.50 ± 0.22	0.173
Estradiol (pg/mL)	17.50 ± 7.83	20.00 ± 4.47	18.50 ± 3.35	28.50 ± 7.83	0.140
Testosterone (ng/mL)	2.35 ± 0.34	1.80 ± 0.22	2.15 ± 0.34	3.70 ± 0.89	0.016

Values are presented as mean ± standard deviation (SD).
An asterisk (*) symbol indicates a statistically significant difference compared with the control group ($p < 0.05$).

Table 2: Effect of aqueous ginger rhizome extract on the hormonal profile of adult male Wistar rats after 60 days of administration

Parameter (Unit)	Control	100 mg/kg	200 mg/kg	400 mg/kg	p value
LH (mIU/mL)	0.72 ± 0.18	1.55 ± 1.01	1.75 ± 0.56	2.20 ± 0.67	0.098
FSH (mIU/mL)	0.64 ± 0.20	1.35 ± 0.78	1.95 ± 0.34	2.70 ± 0.67*	0.017
Prolactin (ng/mL)	0.50 ± 0.22	0.85 ± 0.56	1.20 ± 0.45	1.40 ± 0.22	0.074
Progesterone (ng/mL)	0.20 ± 0.22	0.15 ± 0.11	0.45 ± 0.11	0.60 ± 0.22	0.043
Estradiol (pg/mL)	17.50 ± 7.83	13.00 ± 4.47	26.50 ± 1.12	31.00 ± 4.47*	0.015
Testosterone (ng/mL)	2.35 ± 0.34	1.95 ± 0.34	2.50 ± 0.22	3.25 ± 0.78	0.049

Values are presented as mean ± standard deviation (SD).
An asterisk (*) symbol indicates a statistically significant difference compared with the control group ($p < 0.05$).

seminiferous tubules showed luminal accumulation of spermatozoa with preserved tubular architecture (Plate I, D1 and D2).

Discussion

In the present study, aqueous ginger rhizome extract did not affect body or testicular weights across the treatment groups, suggesting the absence of overt systemic or gonadal toxicity. The extract produced dose- and duration-related changes in reproductive hormones, with increased luteinizing hormone and follicle-stimulating hormone levels observed at higher doses after 30 days, and increased follicle-stimulating hormone, estradiol, and progesterone levels after 60 days. Histological evaluation showed preserved seminiferous tubule architecture across all groups, with luminal accumulation of spermatozoa observed at the highest dose. These findings suggest that aqueous ginger rhizome extract does not induce testicular damage and may influence the dynamics of reproductive hormone in a dose-dependent manner.

Oxidative stress is a recognized contributor to impaired spermatogenesis and reduced male fertility, largely through damage to germ cells and disruption of testicular function (Wang *et al.,* 2025). Ginger contains bioactive compounds, such as gingerols and shogaols, with established antioxidant properties that may mitigate oxidative injury within the testes (Ayustaningwarno *et al.,* 2024). The absence of changes in body and testicular weights observed in this study further supports the lack of overt systemic or gonadal toxicity, as organ weight stability is commonly used as a general indicator of tissue safety in toxicological assessments (Al-Aamiri, 2025).

Endocrine responses to ginger exposure appeared to vary with dose and duration. At 30 days, gonadotropins were elevated at higher doses, whereas testosterone levels showed a slight increase without a meaningful difference from the control group. However, after 60 days of treatment, significant increases in follicle stimulating hormone, estradiol, and progesterone were observed at the highest dose. These findings suggest that prolonged exposure may enhance endocrine responsiveness, possibly through cumulative effects of bioactive compounds or modulation of hypothalamic-pituitary-gonadal axis activity over time.

The observed increase in estradiol and progesterone levels at higher doses, particularly after prolonged exposure, may reflect enhanced peripheral conversion of testosterone to estradiol via aromatase activity. Elevated estradiol in males is known to exert both negative and positive feedback effects on the

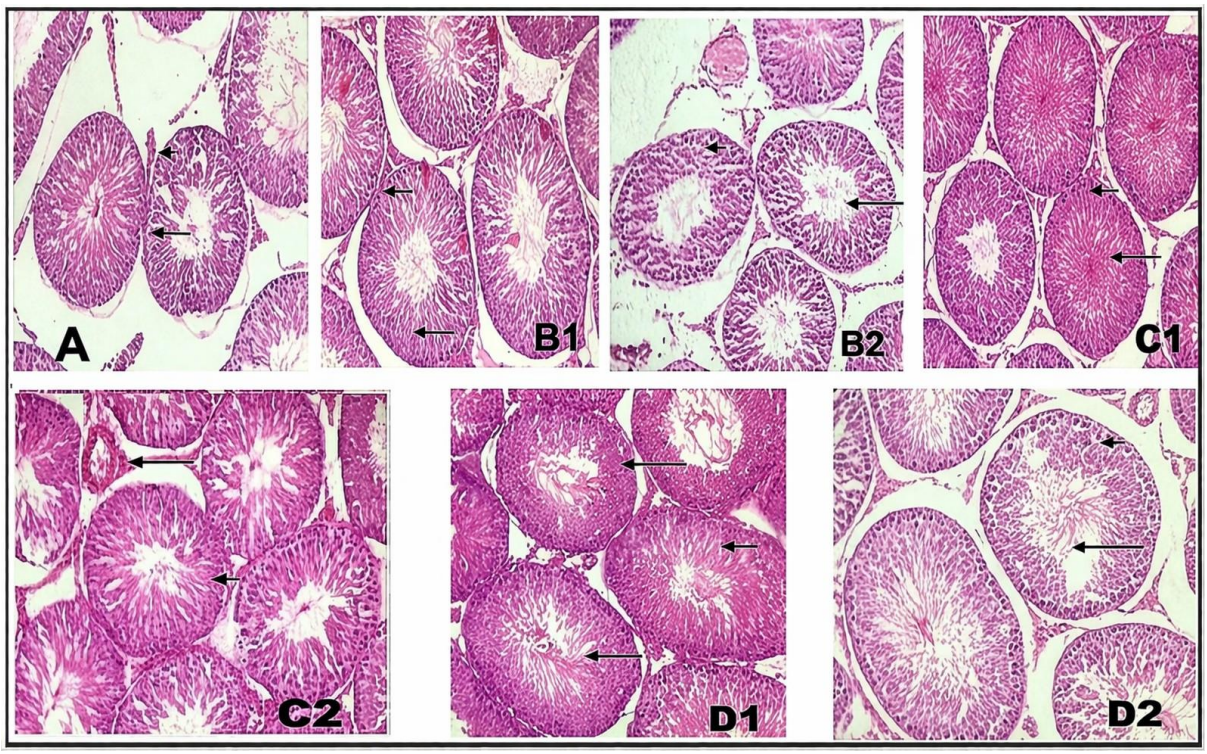

Plate I: Representative photomicrographs of testicular sections from control and treated adult male Wistar rats. (A) The control group showed seminiferous tubules with preserved circular outline, normal arrangement of germ cells at different stages of spermatogenesis, seminiferous epithelium with identifiable Sertoli cell region (short arrows), and interstitial areas containing Leydig cells (long arrows). (B1) Rats administered 100 mg/kg of aqueous ginger rhizome extract for 1 month showed seminiferous tubules with preserved architecture and normal spermatogenic series (arrows indicate seminiferous epithelium and luminal region). (B2) Rats administered 100 mg/kg of aqueous ginger rhizome extract for 2 months showed intact seminiferous tubules with prominent seminiferous epithelium (short arrows) and interstitial areas containing Leydig cells (long arrows). (C1) Rats administered 200 mg/kg of aqueous ginger rhizome extract for 1 month showed preserved seminiferous tubules and normal germinal epithelium (arrows). (C2) Rats administered 200 mg/kg of aqueous ginger rhizome extract for 2 months showed seminiferous tubules at different stages of maturation with identifiable seminiferous epithelium (short arrows), interstitial regions with Leydig cells, and interstitial vessels (long arrows). (D1) Rats administered 400 mg/kg of aqueous ginger rhizome extract for 1 month showed seminiferous tubules with luminal accumulation of spermatozoa (long arrows) and preserved seminiferous epithelium (short arrows). (D2) Rats administered 400 mg/kg of aqueous ginger rhizome extract for 2 months showed increased luminal spermatozoa (long arrows) with intact seminiferous tubules and identifiable seminiferous epithelium and interstitial Leydig cell regions (short arrows). Haematoxylin and eosin stain, magnification ×400

hypothalamic-pituitary-gonadal axis, potentially influencing gonadotropin secretion and overall endocrine balance (Moenter *et al.,* 2020). In addition, increased estradiol levels may alter the testosterone-estradiol ratio, which plays a critical role in male reproductive physiology and has been implicated in hormonal dysregulation under certain conditions (Punjani *et al.,* 2021). Similarly, the rise in progesterone levels may indicate modulation of steroidogenic pathways, as progesterone serves as a precursor in androgen and estrogen biosynthesis. While these hormonal changes may reflect adaptive endocrine responses to ginger exposure, they should not be interpreted as inherently beneficial, as alterations in sex hormone balance can have complex and context-dependent effects on male reproductive function (Ma *et al.,* 2025).

This contrasts with reports of increased testosterone following lower dose ginger powder administration without changes in luteinizing hormone or follicle-stimulating hormone (Abdelfattah *et al.,* 2023). Differences in extract preparation, dosing form, and treatment duration likely account for these discrepancies. Moreover, ginger has been reported to

reduce testosterone and luteinizing hormone levels in models of heavy metal-induced reproductive toxicity, indicating that endocrine responses may vary with underlying physiological or pathological states (Akomolafe & Atoyebi, 2025; Famurewa *et al.,* 2025). The selective increase in luteinizing hormone reported at lower doses in other studies (Ojukwu & Ibeabuchi, 2025), further supports a dose-dependent and context-specific hormonal effect of ginger. The mechanisms underlying these variable endocrine responses remain unclear and warrant targeted mechanistic studies (Verma *et al.,* 2025). While the present findings suggest both dose- and time-related hormonal effects, the analysis used in the present study limits the ability to assess effect of the interaction between dose and duration. Therefore, these observations should be interpreted with caution, and future studies using factorial designs are recommended to better elucidate these relationships.

Histological evaluation demonstrated preservation of seminiferous tubule architecture and organized spermatogenic series across all treated groups, suggesting that aqueous ginger rhizome extract did not produce observable histotoxic changes under the conditions of this study. The presence of prominent interstitial Leydig cells in rats treated for 60 days at lower and intermediate doses suggests maintained or possibly enhanced steroidogenic activity, which is consistent with the known androgen-modulating potential of ginger constituents (Gholami-Ahangaran *et al.,* 2021). Notably, luminal accumulation of spermatozoa observed at the highest dose over both durations is in line with previous reports of improved spermatogenic indices following ginger administration in animal models (Aziz *et al.,* 2025; Lei *et al.,* 2025; Nemati *et al.,* 2023). While these histological findings may suggest a supportive effect on spermatogenesis, they remain descriptive, and the absence of direct semen analysis in this study limits inference regarding functional fertility outcomes.

This study is limited by the lack of quantitative sperm parameters, histomorphometric analysis, oxidative stress biomarkers, and phytochemical profiling of the extract, which would have helped clarify underlying mechanisms and dose-response relationships. In addition, the use of separate one-way ANOVA limits the ability to assess interaction effects between dose and duration. Future work should incorporate semen quality indices, quantitative histomorphometry, testicular oxidative stress markers, and standardized phytochemical characterization to define active constituents and therapeutic windows. Long-term exposure studies and evaluations in disease models of reproductive dysfunction would further establish translational relevance. The findings in this study demonstrates that aqueous ginger rhizome extract preserves testicular histology and produces modest, dose-related endocrine changes without evidence of toxicity. These results support continued investigation of ginger as a dietary adjunct in male reproductive health, with careful attention to dose, duration, and extract standardization.

In conclusion, aqueous ginger rhizome extract preserved testicular histology and produced modest, dose-related changes in reproductive hormones in adult male Wistar rats, with luminal accumulation of spermatozoa observed at the highest dose. These findings suggest potential effects on testicular structure and endocrine function without evidence of overt gonadal toxicity. However, the histological observations are descriptive, and in the absence of quantitative histomorphometric and semen analysis data, conclusions regarding functional fertility outcomes cannot be made and should be interpreted with caution. Further studies incorporating semen analysis, oxidative stress markers, and phytochemical standardization are recommended to clarify mechanisms and define optimal dosing.

Acknowledgements

None

Funding

No funding was received.

Conflict of Interest

The authors declare that there is no conflict of interest.

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