

Full-Length Research Article

Impact of *Zingiber officinale* on Testicular Morphometry, Sperm Quality, and Hormonal Profiles in Alcohol-Induced Toxicity

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Summary: Alcohol consumption is known to induce reproductive toxicity, leading to adverse effects on testicular morphology, sperm quality, and DNA integrity in males. *Zingiber officinale* (ginger), known for its antioxidant and anti-inflammatory properties, may counteract these effects. This study aimed to investigate the impact of ginger on testicular morphometry, sperm quality, and hormonal profiles in alcohol-induced toxicity. 30 male Wistar rats were divided into six groups (n=5/group). Group A (control) received normal saline. Group B was exposed to 40% alcohol (3.50 g/kg body weight) from Days 15–28. Group C received ginger (750 mg/kg) during the same period. Groups D, E, and F were treated with alcohol for 14 days, followed by low (250 mg/kg), medium (500 mg/kg), and high (750 mg/kg) doses of ginger, respectively, from Days 15–28. The study evaluated changes in body and testicular morphometry, antioxidant enzyme and hormonal changes. Semen analysis included sperm motility, count, and morphology, while sperm chromatin/DNA integrity was assessed using aniline blue and toluidine blue staining. Alcohol reduced body weight gain (16.5 g vs. 38.5 g in Control), testicular volume ($1.11 \pm 0.03 \text{ mm}^3$ vs. $2.16 \pm 0.27 \text{ mm}^3$), GSI, sperm quality, hormonal levels, and GSH, reflecting oxidative damage. Ginger treatment, particularly at 250 mg/kg, restored body weight gain (29.7 g), testicular volume ($1.75 \pm 0.24 \text{ mm}^3$), GSI ($1.25 \pm 0.12\%$), sperm parameters, LH (5.08 mIU/mL), FSH, and GSH levels, and reduced sperm DNA damage. Higher ginger doses (500–750 mg/kg) showed diminished efficacy, suggesting dose optimisation. Ginger's antioxidant properties, likely mediated by gingerols and shogaols, counteract alcohol-induced reproductive toxicity. Ginger improves reproductive health and mitigates alcohol-induced toxicity in a dose-dependent manner. Moderate doses show optimal benefits, while high doses may be detrimental. These findings support ginger's potential as a natural therapeutic agent for reproductive health.

Keywords: Alcohol-induced toxicity, antioxidant, ginger, semen quality, testicular morphometry, *Zingiber officinale*.

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INTRODUCTION

Infertility, known as the inability to conceive after months of regular, unprotected sexual intercourse (at least for 12 months), is a significant health matter (Kyrgiafina and Mamuris, 2023). Globally, infertility affects about 15% of couples of reproductive age (Assidi, 2022). Males account for about 50% of total infertility cases worldwide. The prevalence is said to increase at 0.3% annually, leading to an uneven geographical distribution ranging from 20 to 70% (Sun *et al.*, 2019). Nonetheless, the rates of male infertility are underreported due to different factors such as cultural, societal, religious and patriarchal influences that hinder precise sampling and analysis (Mehra *et al.*, 2018). Male infertility includes any health condition that hinders the chances of conception. It can result from abnormal sperm

function or obstructions preventing ejaculation (Agarwal *et al.*, 2021).

Numerous factors are reported to influence male infertility, including anatomic-pathophysiological factors, environmental factors, the process of ageing and lifestyle factors (Skoraacka *et al.*, 2020). Lifestyle-related factors play a significant role in causing male infertility globally (Balawender and Orkisz, 2020). An example of these lifestyle-related factors is alcohol consumption, which has gained considerable attention due to its global prevalence and modifiable nature (Anwara *et al.*, 2025). Alcohol is considered one of the most prevalent dietary factors that people are exposed to and is very prevalent in many societies, with almost 60% of the global population aged 15 years and over reported to have consumed alcoholic drinks in a single year (Finelli *et al.*, 2021). The consumption of alcoholic beverages has been a part of the socio-cultural

heritage of most populations since ancient times (Duca *et al.*, 2019). Indeed, alcohol has been viewed as an integral part of a meal and, in some cases, as a remedy for infectious diseases or even as a cleaning agent (Neufeld *et al.*, 2020). Alcohol consumption poses a significant threat to male reproductive health, affecting millions worldwide and impacting testicular structure and function through oxidative stress mechanisms (Assidi, 2022). Chronic alcohol exposure leads to histological, hormonal, and biochemical alterations, often culminating in reduced semen quality, including impaired sperm concentration and motility, which are key markers of male infertility (Neufeld *et al.*, 2020).

Since alcohol causes these distortions via the increase of reactive oxygen species (ROS), many therapeutic approaches focus on synthetic or natural antioxidants derived from natural sources to mitigate the damage caused by an elevation in ROS (Ovie *et al.*, 2023). An example of these natural antioxidants is *Zingiber officinale*, commonly known as ginger. The ginger rhizome is utilised for its aromatic smell and strong taste (Mahomoodally *et al.*, 2021). Moreover, ginger is extensively employed in folk medicine for its numerous health benefits in treating various diseases, including chronic conditions such as cancer, diabetes, Alzheimer's, ulcers, cardiovascular disease, as well as depression (Kukula-Koch *et al.*, 2018). The positive impact of ginger on these diseases primarily stems from its antioxidant, antimicrobial, and anti-inflammatory properties (Ballester *et al.*, 2022). In different protective studies, ginger has been shown to overcome the reproductive toxicity of cyclophosphamide (Mohammadi *et al.*, 2014), gentamicin (Zahedi and Khaki, 2014), sodium arsenite (Seif *et al.*, 2021), and ethanol (Li *et al.*, 2021) and increase sperm counts, viability, motility, and hormones and improve testicular architecture. Despite these promising findings, the curative potential of testicular morphometry, sperm quality, and hormonal profiles in alcohol-induced toxicity remains underexplored; hence, this study.

MATERIALS AND METHODS

Study Location: This study was carried out in the animal unit of the Anatomy department at Alex Ekwueme Federal University Ndufu Alike Ikwo (AE-FUNAI), Ebonyi State, Nigeria. Ethical approval was sought from the Research and Ethical Committee of the Faculty of Basic Medical Sciences with the code AE-FUNAI/FBMS/EAHC/24/006.

Samples Collection, Identification and Preparations: Fresh rhizomes of *Zingiber officinale* were purchased at "Ogbe Hausa" in Abakaliki Local Government Area,

Ebonyi State, Nigeria. The samples were identified in the Applied Biology Department at Ebonyi State University, Abakaliki, Nigeria. The rhizomes were washed, dried at room temperature, and mechanically milled into a fine powder. The aqueous extract was prepared by soaking 100 g of powdered rhizome in 1 L of distilled water for 24 hours with intermittent stirring. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was evaporated using a water bath at 40°C to obtain a concentrated extract. The doses of 250 mg/kg, 500 mg/kg, and 750 mg/kg of *Zingiber officinale* extract were classified as low, medium, and high, respectively, based on prior studies that reported no toxicity at doses up to 5000 mg/kg in Wistar rats (Ogunola and Afolayan, 2017) and aligned with previous reproductive studies (Khaki *et al.*, 2008; Morakinyo *et al.*, 2008) to investigate dose-dependent effects.

Animal Care and Treatment: The alcohol (99.7- 100% v/v GPR absolute ethanol) produced by NAFCO Scientific Supplies Limited, Surulere, Lagos, Nigeria, with Product Number 28304 7k, was purchased which was used as the toxicant for this experiment. According to the study by Biney *et al.* (2020) 42% had no mortality in rats.

Thirty (30) male Wistar rats randomly divided into six (6) groups (Groups A-F), each comprising five (5) randomised rats, were obtained from the animal house at AE-FUNAI and were kept at room temperature (20–22°C). The animals were housed in well-ventilated cages with suitable environmental conditions. During their acclimatisation process, which lasted for two weeks at the animal house, the rats were provided with standard feed only. The experimental groups and treatment protocols are shown in Table 1.

Animal Sacrifice and Sample Collection: On the 28th day of the experiment, animals were weighed and sacrificed. Blood samples were collected by heart puncture and centrifuged (3000 rpm for 15 min) to obtain sera, and they were then separated and stored at -80 °C for later hormonal assays. After blood collection, testicular parameters (weight, volume and gonadosomatic/testes index) were performed. The left testis was manually homogenised and centrifuged at 3000 rpm for 10 min in a cold phosphate buffer (pH 7.4, 0.1M). The obtained supernatant was used to evaluate the level of antioxidant enzyme activity.

Testis Morphometry

Testis Weight: The weight of each testis was recorded immediately after extraction using an electronic scale.

Table 1:

Experimental Groups and Treatment Protocols

| Groups | Description | Days | |
|--------|------------------------------|-------------------------|----------------------------|
| | | 1 – 14 | 15 – 28 |
| A | Control | Sterile water | Sterile water |
| B | Alcohol-Only | Sterile water | 40% Alcohol (3.50 g/kg) |
| C | Ginger-Only (High Dose) | Sterile water | Ginger extract (750 mg/kg) |
| D | Alcohol + Low-Dose Ginger | 40% Alcohol (3.50 g/kg) | Ginger extract (250 mg/kg) |
| E | Alcohol + Medium-Dose Ginger | 40% Alcohol (3.50 g/kg) | Ginger extract (500 mg/kg) |
| F | Alcohol + High-Dose Ginger | 40% Alcohol (3.50 g/kg) | Ginger extract (750 mg/kg) |

Testis Volume: A sliding digital Vernier calliper was used to measure the width and length of each testis. The testis volume was then calculated using the spheroid formula: Testis volume = width² × length × 0.523 (mm³)

Gonadosomatic/Testes Index: The final body and testis weights were used to calculate the gonadosomatic index using the formula previously reported by Ukoha *et al.* (2014).

$$\text{Gonadosomatic index} = \frac{\text{Testis weight}}{\text{Body weight}} \times 100(\%)$$

Biochemical Assays

Hormonal Assay: The serum levels of reproductive hormones—luteinizing hormone (LH), follicle-stimulating hormone (FSH), and inhibin B (INB)—were measured using enzyme-linked immunosorbent assay (ELISA) kits. The ELISA kits for LH and FSH were obtained from NIADDK, NIH (USA), while the kit for INB was sourced from Diagnostic Systems Laboratories (DSL-10-84100i; Webster, TX, USA), following the manufacturer's protocols, as reported by Famurewa *et al.* (2023).

Glutathione (GSH) Level: Testicular activities of antioxidant enzyme GSH were analysed in homogenate supernatant at 4°C using commercial rat ELISA kits according to the manufacturer's instructions.

Semen Analysis: Spermatozoa were collected by making a small incision (1 ml) in the caudal epididymis, followed by evaluation of sperm count, motility, and morphology. The sperm count was determined using an improved Neubauer hemocytometer. Epididymal sperm motility was calculated by measuring the number of motile spermatozoa per unit area and was expressed as a percentage of motility. Sperm morphology was analysed using the Wall and Ewas stain, with examination conducted under a microscope as previously detailed (Igwe *et al.*, 2024).

Sperm Chromatin Evaluation: Standard cytochemical methods, incorporating aniline blue (AB) and toluidine blue (TB), were employed to evaluate chromatin condensation and DNA integrity. AB was selectively utilised to stain lysine-rich histones. Air-dried smears obtained from washed semen samples were positioned in 0.2 M phosphate buffer (pH 7.2) containing 3% buffered glutaraldehyde for 30 minutes at room temperature. Subsequently, each smear underwent staining in 4% acetic acid (pH 3.5) with a 5% aqueous solution of AB for 7 minutes. During the light microscopic evaluation, a meticulous count of 200 spermatozoa was conducted across various sections of each slide, utilizing a ×100 eyepiece magnification (Pourmasumi *et al.*, 2019). Sperm heads stained pale blue/colourless and dark blue were considered normal (AB-) and abnormal sperm (AB+), respectively.

On the other hand, TB served as a metachromatic dye, offering insight into nuclear chromatin condensation and the quality and quantity of DNA fragmentation in sperm. Air-dried sperm smears were fixed using a mixture of 96% ethanol and acetone (1:1) for 30 minutes at a temperature of 4°C. Subsequently, the slides underwent a 5-minute incubation in 0.1 N HCl at 4°C, followed by thorough

washing with distilled water three times for 2 minutes each. Finally, staining took place for 10 minutes at room temperature using 0.05% TB in 50% citrate phosphate. In the evaluation process, a minimum of 200 spermatozoa were counted in each sample using light microscopy with a ×100 eyepiece magnification (Pourmasumi *et al.*, 2019). Normal sperm is pale blue, and abnormal sperm is dark blue or violet purple. For each sample, the normal (TB-) and abnormal (TB+) spermatozoa were reported as percentages.

Data Analysis: Data were subjected to analysis of variance using GraphPad Prism version 8 and presented as Means ± SD. Group means of parametric data were compared using a one-way analysis of variance, followed by Turkey's post hoc test. $p < 0.05$ was considered statistically significant.

RESULTS

Body Weight Analysis: The body weight analysis demonstrates weight changes among the groups as presented in Table 2. Group A exhibited a steady increase, resulting in a total weight change of 38.50 g. Group B showed a minimal weight gain of only 16.50 g when compared to Group A, indicating the detrimental effects of alcohol on weight. Group C displayed a weight increase of 20.50 g, whilst Groups D, E, and F were exposed to alcohol and treated with varying doses of ginger. D gained 29.00 g, while Group E had a slight weight loss of 0.75 g, and Group F gained 13.25 g.

Table 2: Body weight analysis among the experimental groups.

| Groups | Initial Weight (g) | Final Weight (g) | Weight change (g) |
|--------|--------------------|------------------|-------------------|
| A | 113.20±7.46 | 152.00±9.06 | 38.50±11.68 |
| B | 131±25.86 | 147.50±22.34 | 16.50±11.39 |
| C | 152.25±15.44 | 172.75±31.82 | 20.50±33.41 |
| D | 131±12.88 | 160.00±19.55 | 29.00±31.35 |
| E | 150±4.47 | 149.75±23.47 | -0.75±24.51 |
| F | 158.40±20.74 | 171.00±1.16 | 13.25±24.73 |

Values represent Mean ± SD

Table 3: Testes Morphometry

| Groups | Testes Weight (g) | Testes volume (mm ³) | Gonadosomatic index (%) |
|--------|-------------------|----------------------------------|-------------------------|
| A | 1.57±0.39 | 2.16±1.76 | 1.04±0.28 |
| B | 1.57±0.32 | 1.10±0.29 | 1.10±0.27 |
| C | 1.81±0.13 | 2.61±1.31 | 1.10±0.31 |
| D | 1.75±0.24 | 1.08±0.28 | 1.20±0.12 |
| E | 1.66±0.28 | 1.18±0.08 | 1.11±0.12 |
| F | 1.65±0.21 | 1.31±0.19 | 0.96±0.13 |

Values represent Mean ± SD.

Testicular Morphometry: The evaluation of testes morphometry (Table 3) revealed no statistically significant differences ($p > 0.05$) in testes weight, testes volume, and gonadosomatic index among the experimental groups. The control group showed a mean testis weight of 1.57 ± 0.39 g, while the highest value was observed in Group C (1.81 ± 0.13 g), but this increase was not significant ($p > 0.05$). Testis volume was also highest in Group C (2.61 ± 1.31 mm³), with the lowest in Group D

($1.08 \pm 0.28 \text{ mm}^3$); however, these differences did not reach statistical significance. The gonadosomatic index ranged from $0.96 \pm 0.13\%$ in Group F to $1.20 \pm 0.12\%$ in Group D, but no significant variation was observed across the groups ($p > 0.05$).

Semen Analysis: As shown in Table 4, the semen analysis revealed significant differences in motility, count and morphology across the groups. Group B exhibited significantly reduced progressive motile sperms when compared to Group A indicating significant impairment. Group C demonstrated notable improvement when compared to A. Groups D, E and F recorded motility levels significantly different from Group B. Regarding sperm

count, Group B had a lower count compared to Group A. Groups C, D, E, and F showed counts higher than Group B. In terms of morphology, the results revealed that Groups A, C, D, and E maintained high percentages of normal sperm (around 95%), while Group B had a slightly lower percentage of 95.33 ± 0.58 . Notably, Group B displayed higher instances of head defects, with pinhead defects reported at 3.33 ± 0.58 ($p < 0.05$) compared to Group A. Additionally, in terms of midpiece defects, Group B had a bent midpiece at 0.33 ± 0.58 , while all other groups reported 0.00 ± 0.00 . Concerning tail defects, Group B exhibited a higher incidence of headless tails at 1.33 ± 0.58 compared to Group A, which had 1.00 ± 0.00 .

Table 4:
Showing the semen analysis after exposure to alcohol and ginger

| Groups | A | B | C | D | E | F | |
|----------------------------|--------------------|------------------|--------------------|------------------|---------------------|----------------------|----------------------|
| Motility (%) | Progressive motile | 71.67 ± 2.89 | $36.67 \pm 2.89^*$ | 75.00 ± 0.00 | $51.67 \pm 2.89^\#$ | $51.67 \pm 10.41^\#$ | $40 \pm 13.23^\#$ |
| | Sluggish motile | 23.33 ± 2.89 | 30.00 ± 5.00 | 20.00 ± 0.00 | 26.67 ± 2.89 | 28.33 ± 2.89 | $36.67 \pm 10.40^\#$ |
| | Non-motile | 5.00 ± 0.00 | $33.33 \pm 2.89^*$ | 5.00 ± 0.00 | 21.67 ± 2.89 | $20.00 \pm 10.00^\#$ | 23.33 ± 2.89 |
| Count ($10^6/\text{ml}$) | | 37.33 ± 6.43 | 20.00 ± 7.00 | 38.33 ± 8.39 | 34.00 ± 5.00 | 27.33 ± 11.02 | 33.00 ± 12.12 |
| Morphology (%) | Normal sperm | 97.33 ± 0.58 | 95.33 ± 0.58 | 95.67 ± 0.58 | 96.67 ± 1.53 | 96.00 ± 1.00 | 95.67 ± 0.58 |
| Head defects | Round Head | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | Pinhead | 1.00 ± 1.00 | $3.33 \pm 0.58^*$ | 1.67 ± 0.58 | 0.67 ± 0.58 | 1.67 ± 1.14 | $1.33 \pm 0.58^\#$ |
| Midpiece defects | Bent midpiece | 0 | 0.33 ± 0.58 | 0 | 1.00 ± 1.00 | 1.33 ± 1.15 | 1.33 ± 0.58 |
| | Coiled midpiece | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Tail defects | Headless tail | 1.00 ± 0.00 | 1.33 ± 0.58 | 1.33 ± 0.58 | 1.33 ± 0.58 | 1.00 ± 1.00 | 0.33 ± 0.58 |
| | Coiled tail | 0.67 ± 0.58 | 1.33 ± 0.58 | 0 | 0.33 ± 0.58 | 0 | 1.33 ± 0.00 |
| | Absence of tail | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | Loop tail | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Values represent Mean \pm SD. * represents a significant difference when compared to A; # represents a significant difference when compared to B.

Table 4:
Effects of alcohol and ginger on sperm chromatin

| Groups | Aniline Blue | | Toluidine Blue | |
|--------|---------------------|--------------------|---------------------|--------------------|
| | AB- | AB+ | TB- | TB+ |
| A | 96.83 ± 1.61 | 3.17 ± 1.60 | 99.33 ± 0.76 | 0.67 ± 0.76 |
| B | 92.17 ± 3.62 | $7.83 \pm 3.62^*$ | $89.00 \pm 1.73^*$ | $12.33 \pm 4.04^*$ |
| C | 98.33 ± 1.04 | 2.00 ± 0.50 | 99.50 ± 0.50 | 0.50 ± 0.50 |
| D | $97.37 \pm 0.78^\#$ | $2.67 \pm 0.76^\#$ | $95.17 \pm 1.89^\#$ | $4.83 \pm 1.89^\#$ |
| E | $97.83 \pm 0.29^\#$ | $2.67 \pm 0.58^\#$ | $97.33 \pm 1.26^\#$ | $2.67 \pm 1.26^\#$ |
| F | $97.33 \pm 0.58^\#$ | $2.17 \pm 0.29^\#$ | $99.00 \pm 0.87^\#$ | $1.00 \pm 0.87^\#$ |

Values represent Mean \pm SD. * represents a significant difference when compared to A; # represents a significant difference when compared to B. KEYS: normal chromatin (AB-); abnormal chromatin (AB+); normal DNA (TB-); abnormal DNA (TB+).

Sperm Chromatin: The evaluation of sperm chromatin integrity (Table 4) showed significant differences ($p < 0.05$) among the experimental groups. Group B had a significantly higher percentage of abnormal chromatin (AB+) and abnormal DNA (TB+) compared to the Control group ($p < 0.05$). Group C exhibited normal chromatin (AB-) and DNA integrity (TB-) levels comparable to the Control group ($p > 0.05$). Ginger supplementation in alcohol-treated groups (D, E and F) significantly reduced the percentage of spermatozoa with abnormal chromatin (AB+) and DNA damage (TB+) compared to Group B ($p < 0.05$).

Hormonal Assay and Antioxidant Enzyme Levels: Serum hormone levels in Table 5 showed significant differences ($p < 0.05$) among the experimental groups. Group B exhibited a significant reduction in FSH and INB levels compared to the Control group ($p < 0.05$), while LH

levels were also lower but not statistically significant ($p > 0.05$). Group C showed LH, FSH, and INB levels comparable to the Control group ($p > 0.05$). In the alcohol-treated groups supplemented with ginger (groups D, E and F), FSH and INB levels were significantly higher than in Group B ($p < 0.05$), approaching values similar to the Control group, while LH levels remained statistically comparable across all groups ($p > 0.05$).

Furthermore, the testicular GSH levels showed significant differences ($p < 0.05$) among the experimental groups. Group B had a significantly lower GSH level ($12.93 \pm 2.41 \text{ mmol/g tissue}$) compared to the Control group ($23.07 \pm 1.76 \text{ mmol/g tissue}$) ($p < 0.05$). Group C showed a GSH level ($25.47 \pm 3.83 \text{ mmol/g tissue}$) comparable to the Control group ($p > 0.05$). Ginger supplementation in alcohol-treated groups (D, E and F) significantly increased GSH levels compared to the alcohol-only group ($p < 0.05$), although these values remained slightly lower than those of the Control group ($p > 0.05$), as shown in Table 5.

DISCUSSION

This study investigated the effects of alcohol-induced toxicity and aqueous *Zingiber officinale* (ginger) extract on testicular morphometry, sperm quality, hormonal profiles, and antioxidant levels in male Wistar rats. Our findings demonstrate that alcohol impairs testicular function, reduces body weight gain, and disrupts hormonal and antioxidant defenses, while ginger treatment, particularly at a low dose (250 mg/kg), mitigates these effects, improving testicular

volume, gonadosomatic index (GSI), sperm parameters, hormonal levels, and glutathione (GSH) activity. These results align with previous studies on alcohol's reproductive toxicity and ginger's ameliorative effects, while contributing novel insights into ginger's role in preserving sperm DNA integrity.

Table 5:

Effects of ginger and alcohol on levels of LH, FSH, INB and GSH

| Group s | LH (mIU/mL) | FSH (mIU/mL) | IHB (pg/mL) | GSH (mmol/g tissue) |
|---------|-------------|--------------|-------------|---------------------|
| A | 3.27±0.40 | 6.57±0.32 | 31.33±1.00 | 23.07±1.76 |
| B | 2.23±0.47 | 2.63±1.51* | 22.03±3.04* | 12.93±2.41* |
| C | 3.00±0.26 | 6.03±0.40 | 33.00±1.35 | 25.47±3.83 |
| D | 2.70±0.10 | 5.07±0.32* | 30.83±2.18* | 21.33±1.53# |
| E | 2.47±1.19 | 4.30±0.36 | 27.40±0.95 | 19.67±0.58# |
| F | 2.23±0.70 | 4.07±0.55 | 24.67±2.53 | 18.53±1.37 |

Values represent Mean ± SD. * represents a significant difference when compared to A; # represents a significant difference when compared to B.

Alcohol exposure was associated with an increase in body weight, which is consistent with the findings of Kołota *et al.* (2019), who observed weight gain in Wistar rats after four weeks of 10% ethanol consumption. However, other studies have reported weight loss or stagnation following prolonged alcohol exposure (Milat *et al.*, 2017), as seen in this study. This reduction in weight gain likely reflects alcohol's effects on appetite, nutrient absorption, and metabolic function. Ginger treatment partially restored body weight gain, suggesting ginger's potential to counteract alcohol's metabolic disruptions, aligning with findings from Mahamoud and Elnour (2013) and Misawa *et al.* (2015), possibly via its antioxidant properties (Esomchi *et al.*, 2025). Testicular morphometry was also affected where alcohol exposure in the alcohol-only group reduced testicular volume and GSI, consistent with Oremosu and Akang (2015), who reported testicular atrophy due to ethanol-induced oxidative stress and seminiferous tubule damage. Testicular weight remained similar between the alcohol-only and control groups, likely due to variations in body weight, as noted by Kołota *et al.* (2019). However, the GSI, which normalises testicular weight to body weight, was a more reliable metric, showing improvements in ginger-treated groups, particularly the alcohol + low-dose ginger group, suggesting ginger's ameliorating effect against alcohol-induced testicular damage (Esomchi *et al.*, 2025). Ginger treatment, especially at 250 mg/kg, increased testicular volume and weight, aligning with Morakinyo *et al.* (2008) and Khaki *et al.* (2008), who attributed ginger's curative effects to its antioxidant properties, likely mediated by gingerols and shogaols.

Sperm morphology and seminal fluid parameters are considered primary morphological and physicochemical diagnostic markers of male infertility (Assidi, 2022). Sperm parameters were significantly impaired in the alcohol-only group, with reduced motility, count, and morphology, corroborating studies that link alcohol to oxidative stress and lipid peroxidation in sperm membranes (Oremosu and

Akang, 2015). Ginger treatment markedly improved these parameters, with Group D showing the highest recovery in motility and count, consistent with Gholami-Ahangaran *et al.* (2021). A novel finding of this study is ginger's curative effect on sperm DNA integrity, assessed via TB and AB staining. Alcohol increased abnormal DNA percentages in the alcohol-only group, reflecting compromised chromatin condensation and DNA damage, as reported by Rahimpour *et al.* (2013) and Bai *et al.* (2020). Ginger treatment, particularly at 250 mg/kg, reduced DNA abnormalities, suggesting a potential role in stabilising sperm chromatin, an area previously underexplored in alcohol toxicity models.

Alcohol significantly reduced LH, FSH and INB levels in the alcohol-only group, disrupting the hypothalamic-pituitary-gonadal (HPG) axis, as noted by Akbari and Jelodar (2013) and Emokpae and Osabuohien (2020). In contrast, some studies report elevated gonadotropins due to compensatory feedback mechanisms (Muthusami and Chinnaswamy, 2005) highlighting variability in alcohol's effects based on dose and duration. Ginger treatment restored LH, FSH, and INB levels, aligning with Morakinyo *et al.* (2008), likely due to ginger's modulation of oxidative stress and androgen synthesis. Similarly, GSH levels, a key antioxidant, were reduced in the alcohol-only group, consistent with Basaki *et al.* (2012), but increased in ginger-treated groups, particularly ginger-only and alcohol + low-dose ginger, supporting Li *et al.* (2021).

The dose-dependent effects of ginger were evident, with the alcohol + low-dose ginger group (250 mg/kg) showing optimal recovery across most parameters, while higher doses (500 and 750 mg/kg) yielded diminishing benefits, possibly due to potential toxicity, as suggested by Khwanes *et al.* (2022). While the current study demonstrates the curative function against alcohol-induced reproductive toxicity, Biney *et al.* (2020) reports that the extent of recovery from alcohol-induced toxicity is influenced by the severity and duration of alcohol exposure.

CONCLUSION

This study demonstrates that alcohol exposure in male Wistar rats impairs body weight gain, testicular morphometry, sperm quality (motility, count, morphology, DNA integrity), hormonal profiles (LH, FSH, INB), and antioxidant defenses (GSH). Aqueous *Zingiber officinale* extract, particularly at a low dose (250 mg/kg), significantly mitigates these effects, restoring body weight gain, testicular volume, GSI, sperm parameters, hormonal levels, and GSH activity. A novel finding is ginger's effect on sperm DNA integrity, reducing alcohol-induced chromatin damage, which warrants further investigation. These results highlight ginger's therapeutic potential in ameliorating alcohol-induced reproductive toxicity, with low-dose treatment offering optimal benefits.

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