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Original article

Protective Effects of Ginger Extract on NAFLD-Associated Male Infertility in Rats: Hormonal Regulation and Semen Quality

Abdalla Elgenaidi[1], Alnagy Ali[2], Hanan Miftah*[3], Fatema Alsaeid[4]

[1]*Department of Biomedical Science, Academy of Graduate Studies, Misurata, Libya*
[2]*General Nursing Department, Faculty of Nursing, Omar El-Mokhtar University, El-Beyda, Libya*
[3]*Zoology Department, Faculty of Science, Omar El-Mokhtar University, El-Beyda, Libya*
[4]*Department of Medical Genetics, Faculty of Health Sciences, Misurata University, Misurata, Libya*
Corresponding authors. hanan.sailh@omu.edu.ly

Abstract
Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent metabolic disorder that extends beyond hepatic pathology to adversely affect male reproductive function through hormonal dysregulation and systemic metabolic disturbances. *Zingiber officinale* (ginger), a medicinal plant rich in bioactive compounds, has been reported to exert beneficial effects on metabolic and reproductive health. The present study investigated the protective role of aqueous ginger extract against NAFLD-induced reproductive dysfunction in male rats. NAFLD was experimentally induced in adult male Wistar rats by feeding a high-fat diet (HFD). Animals were allocated into control, HFD control, and treatment groups receiving either 10% or 20% aqueous ginger extract. Sperm parameters, including concentration, motility, and morphology, were evaluated alongside serum testosterone and luteinizing hormone (LH) levels. Hepatic function was assessed by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. HFD feeding resulted in significant impairments in sperm quality, accompanied by marked reductions in serum testosterone and LH concentrations ($p < 0.001$). Treatment with aqueous ginger extract produced dose-dependent improvements in all reproductive parameters, with the 20% extract restoring sperm quality and hormonal levels to values comparable with controls ($p > 0.05$). In parallel, ginger supplementation significantly reduced serum ALT and AST activities, indicating amelioration of HFD-induced hepatic injury. Aqueous ginger extract effectively attenuates NAFLD-associated male reproductive dysfunction by improving sperm quality, restoring hormonal balance, and protecting hepatic function. These findings highlight ginger's potential as a natural adjunct strategy for managing reproductive and metabolic complications associated with NAFLD and warrant further investigation in clinical settings.
Keywords. Non-alcoholic Fatty Liver Disease, Male Infertility, Zingiber Officinale, Sperm Quality.

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the leading cause of chronic liver disease globally, with its rising incidence occurring in parallel with the worldwide surge in obesity and type 2 diabetes mellitus (1). NAFLD is characterized by excessive lipid deposition in the liver independent of alcohol intake and encompasses a disease continuum from simple steatosis to non-alcoholic steatohepatitis (NASH), which may ultimately progress to cirrhosis or hepatocellular carcinoma. Beyond its well-recognized hepatic and metabolic manifestations, accumulating evidence suggests that NAFLD functions as a systemic disorder with significant implications for male reproductive health (2 -3). Accumulating clinical and experimental evidence indicates that non-alcoholic fatty liver disease in men is commonly accompanied by endocrine disturbances, most notably reduced testosterone levels, thereby increasing susceptibility to infertility. The pathophysiological mechanisms underlying these effects are closely linked to hepatic-derived systemic inflammation, insulin resistance, and oxidative stress (4).

As a critical regulator of metabolic and endocrine homeostasis, the liver plays a pivotal role in sex hormone metabolism; consequently, hepatic dysfunction in non-alcoholic fatty liver disease leads to hormonal imbalance, notably a reduced testosterone-to-estrogen ratio (5). Chronic inflammation and sustained oxidative stress drive the overproduction of reactive oxygen species (ROS), thereby overwhelming cellular antioxidant defenses. Due to the high levels of polyunsaturated fatty acids in sperm membranes and the limited intrinsic antioxidant capacity of testicular tissue, the male reproductive system is particularly vulnerable to oxidative injury. This imbalance triggers lipid peroxidation, DNA fragmentation, and apoptotic pathways, ultimately compromising key sperm parameters, including concentration, motility, and morphology (6-7).

In the absence of established pharmacological interventions specifically targeting the reproductive complications of NAFLD, natural bioactive compounds with antioxidant and anti-inflammatory properties have gained considerable scientific interest. Among these, ginger (Zingiber officinale), a widely used perennial herb, contains phenolic compounds such as gingerols and shogaols that are recognized for their antioxidant, anti-inflammatory, and androgenic effects (8-9). Preclinical evidence indicates that ginger supplementation positively influences male reproductive performance through the elevation of circulating

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testosterone levels, enhancement of sperm quality, and reduction of oxidative stress within the testes. Accordingly, ginger represents a promising therapeutic candidate for counteracting reproductive impairments linked to metabolic conditions, including NAFLD (8).

Accordingly, this study was designed to investigate the protective role of aqueous ginger extract against NAFLD-induced reproductive dysfunction in male rats. The study focuses on evaluating alterations in semen quality, serum sex hormones, and key biochemical markers, while also examining hepatic function indicators. Through this integrated approach, the research aims to elucidate the potential mechanisms by which ginger may mitigate NAFLD-associated reproductive impairments.

Methods

Preparation of Ginger Extracts

Fresh rhizomes of *Zingiber officinale* were obtained from a local market in Misurata, Libya, and authenticated before processing. The rhizomes were thoroughly washed, air-dried at ambient temperature, and mechanically ground into a fine powder. For ethanolic extraction, 200 g of ginger powder was macerated in 400 mL of 95% ethanol for 72 h with intermittent agitation. The extract was filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain a crude extract, which was stored at 4 °C until use. Aqueous ginger extracts were prepared by macerating the powdered rhizomes in distilled water to yield stock solutions of 10% and 20% (w/v), corresponding to approximately 100 mg/mL and 200 mg/mL, respectively. These stock extracts were used to prepare the dosing suspensions administered to rats at the specified treatment doses (300 and 600 mg/kg body weight/day, respectively). The mixtures were filtered through Whatman No. 1 filter paper, and the resulting extracts were stored in amber-colored bottles at 4 °C. To maintain extract stability and biological activity, fresh preparations were made every 2–3 days.

Experimental Animals and Study Design

Thirty-two healthy adult male Wistar albino rats (Rattus norvegicus), aged between 10 and 12 weeks and weighing 102–180 g (mean ± SD: 138.13 ± 18.4 g), were obtained from the University of Derna, Libya. The animals were maintained under standard laboratory conditions at a temperature of 25 ± 4 °C with a 12-hour light/dark cycle and had unrestricted access to food and water. They were allowed to acclimatize for 45 days prior to experimentation. Non-alcoholic fatty liver disease (NAFLD) was induced by feeding the rats a high-fat diet enriched with fat, cholesterol, and fructose for a period of 8–12 weeks, following the protocol described by Vos and Lavine (2013). The induction of disease was confirmed through biochemical indicators of hepatic dysfunction. After successful induction, the rats were randomly divided into five experimental groups, each consisting of eight animals. The first group served as the control and was maintained on a standard diet without treatment. The second group, designated as the positive control, received the high-fat diet without treatment. The third group was administered a low dose of aqueous ginger extract at 300 mg/kg body weight/day, prepared from a 10% w/v stock, in addition to the high-fat diet. The fourth group received a high dose of aqueous ginger extract at 600 mg/kg body weight/day, prepared from a 20% w/v stock, along with the high-fat diet. All treatments were delivered daily by oral gavage throughout the experimental period.

Sample Collection and Analyses

At the end of the experimental period, rats were anesthetized and humanely euthanized in accordance with ethical guidelines. Blood samples were collected by cardiac puncture, allowed to clot at room temperature, and centrifuged at 3000 rpm for 10 min to obtain serum. Serum samples were stored at −20 °C until biochemical and hormonal analyses were performed. Epididymal sperm samples were collected from the cauda epididymis and suspended in pre-warmed physiological saline (37 °C) for semen analysis.

Semen Analysis

Sperm motility was evaluated under a light microscope at 400× magnification and expressed as the percentage of progressively motile, non-progressively motile, and immotile spermatozoa. Sperm concentration ($\times 10^6$/mL) was determined using a Neubauer hemocytometer, while sperm morphology was assessed using eosin–nigrosin–stained smears.

Biochemical and Hormonal Assays

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using standard colorimetric assay kits according to the manufacturers' instructions. Serum testosterone and luteinizing hormone (LH) concentrations were quantified using commercially available ELISA kits following the provided protocols.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Pearson's correlation analysis was applied to assess relationships between biochemical, hormonal, and reproductive

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parameters. Statistical significance was defined at $p < 0.05$. All statistical analyses were conducted using SPSS software (version 26.0).

Results

Impact of Ginger Extract on Sperm Parameters

Feeding rats, a high-fat diet (HFD) resulted in a marked deterioration of all assessed sperm parameters compared with the healthy control group. Administration of aqueous ginger extract, particularly at the 20% concentration, significantly mitigated these adverse effects.

Sperm Motility and Concentration

As summarized in (Table 1), the HFD control group exhibited a pronounced reduction in progressive sperm motility (24.8%) relative to the control group (50.3%). Treatment with 20% aqueous ginger extract significantly improved progressive motility to 38.5%, indicating a partial restoration toward normal values. Similarly, sperm concentration was substantially reduced in the HFD control group (39.0 × 10^6/mL) compared with controls (92.0 × 10^6/mL). Rats receiving 20% ginger extract showed a significant increase in sperm concentration (70.0 × 10^6/mL) relative to the HFD group, although values remained lower than those observed in the control group.

Sperm Morphology: The proportion of morphologically normal spermatozoa was significantly lower in the HFD control group (10.3%) compared with the control group (24.8%). Treatment with 20% ginger extract significantly increased the percentage of normal sperm morphology to 21.0%, reaching values statistically comparable to those of healthy controls.

Table 1. Effects of aqueous ginger extract on sperm motility, concentration, and morphology in HFD-fed rats (mean ± SEM).

Group	Progressive Motility (%)	Sperm Concentration (x10^6/ml)	Normal Morphology (%)
Control	50.3	92.0	24.8
HFD Control	24.8	39.0	10.3
Ginger 10%	28.0	45.0	12.5
Ginger 20%	38.5	70.0	21.0

The dose-dependent effects of aqueous ginger extract on sperm parameters are presented in (Figures 1A–C). Feeding rats, a high-fat diet (HFD) resulted in significant impairments in progressive sperm motility, sperm concentration, and normal sperm morphology compared with the healthy control group. As shown in (Figure 1A), progressive sperm motility was markedly reduced in the HFD group, whereas treatment with 20% aqueous ginger extract significantly improved motility toward control values, while the 10% concentration induced only a modest, non-significant change. Similarly, (Figure 1B) demonstrates a pronounced reduction in sperm concentration following HFD feeding, which was significantly ameliorated by administration of the 20% ginger extract, indicating improved spermatogenic output. Regarding sperm morphology, (Figure 1C) shows that HFD feeding significantly decreased the proportion of morphologically normal spermatozoa, while treatment with 20% ginger extract restored normal morphology and reduced abnormal forms to levels comparable with those of the control group. Collectively, these results indicate a clear dose-dependent protective effect of aqueous ginger extract on sperm quality in HFD-induced NAFLD rats.

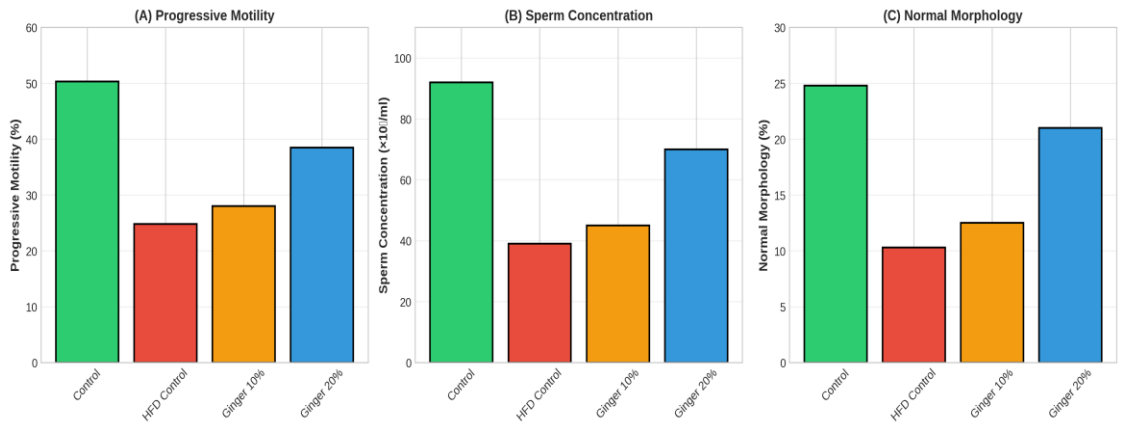

Figure 1. Comprehensive comparison of key sperm parameters across treatment groups. (A) Progressive motility, (B) Sperm concentration, and (C) Normal morphology. The 20% ginger extract shows significant improvement across all parameters compared to the HFD Control group.

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Marked alterations in sperm quality were observed across the experimental groups, with high-fat diet (HFD) feeding resulting in a pronounced deterioration of progressive sperm motility, sperm concentration, and the proportion of morphologically normal sperm compared with healthy controls. These changes were visually reflected by a predominance of lower-intensity color values in the HFD group, indicating reduced parameter levels. Administration of aqueous ginger extract produced a clear concentration-dependent improvement across all evaluated sperm parameters. The Ginger 10% group displayed modest shifts toward intermediate color intensities, corresponding to slight increases in motility, concentration, and normal morphology that remained below control values. In contrast, treatment with 20% aqueous ginger extract resulted in a pronounced transition toward higher-intensity color gradients, closely resembling the pattern observed in the control group. This effect was particularly evident for progressive sperm motility and sperm concentration, indicating a substantial restoration of overall sperm quality. Collectively, the heatmap visualization demonstrates a clear dose-dependent protective effect of aqueous ginger extract against HFD-induced impairments in sperm parameters (Figure 2).

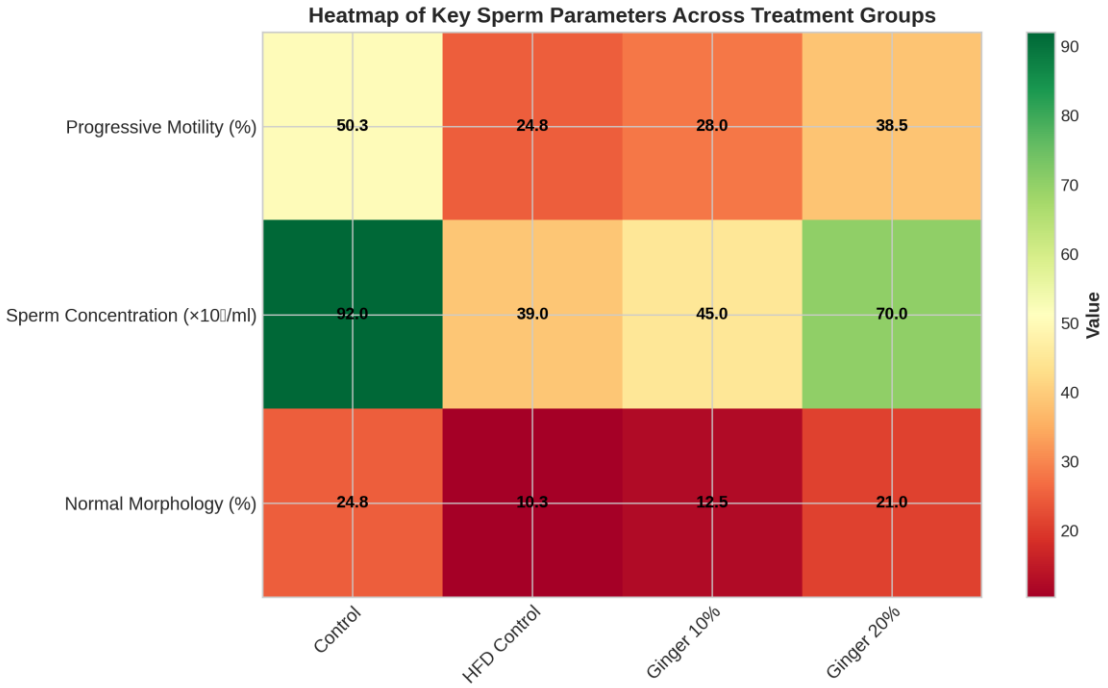

Figure 2. Heatmap showing the distribution of key sperm parameters across treatment groups. The color gradient from red (low values) to green (high values) illustrates the dose-dependent improvement with ginger treatment.

Impact on Hormonal and Biochemical Markers

Serum Luteinizing Hormone and Testosterone Levels

Statistical analysis revealed a highly significant treatment effect on serum luteinizing hormone (LH) concentrations across the experimental groups (one-way ANOVA, $F(3,28) = 282.33$, $p < 0.001$). Rats fed a high-fat diet (HFD) exhibited a marked suppression of LH levels compared with the normal control group. Administration of aqueous ginger extract resulted in a clear dose-dependent restoration of LH concentrations, with the 10% extract producing a partial but significant recovery relative to the HFD group, while treatment with 20% ginger extract fully normalized LH levels to values statistically indistinguishable from those of the control group (Figure 3).

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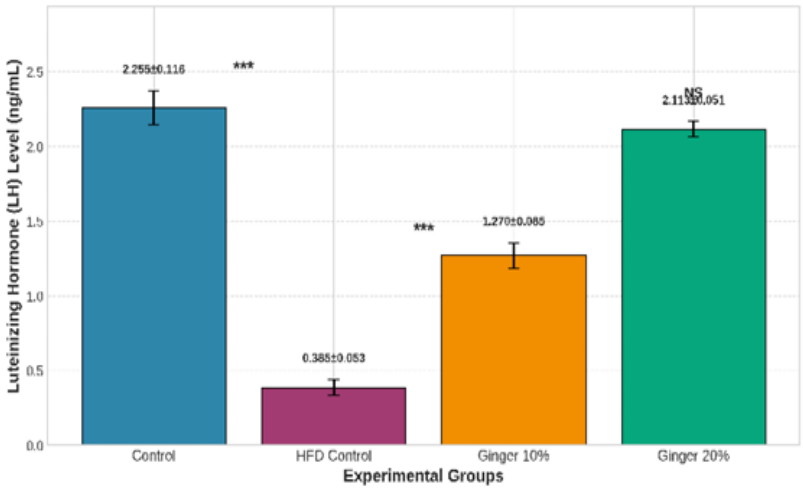

Figure 3. Effect of aqueous ginger extract on serum luteinizing hormone (LH) levels in high-fat diet (HFD)-induced NAFLD rats.

A comparable pattern was observed for serum testosterone levels ($F_{3,12} = 41.88$, $p < 0.001$). HFD feeding caused a pronounced reduction in circulating testosterone compared with controls, whereas supplementation with aqueous ginger extract significantly reversed this decline. Notably, the 20% ginger extract restored testosterone concentrations to near control levels, while the 10% extract induced a moderate yet significant improvement. Collectively, these findings demonstrate a robust, dose-dependent restoration of the hypothalamic pituitary gonadal axis following ginger supplementation (Figure 4).

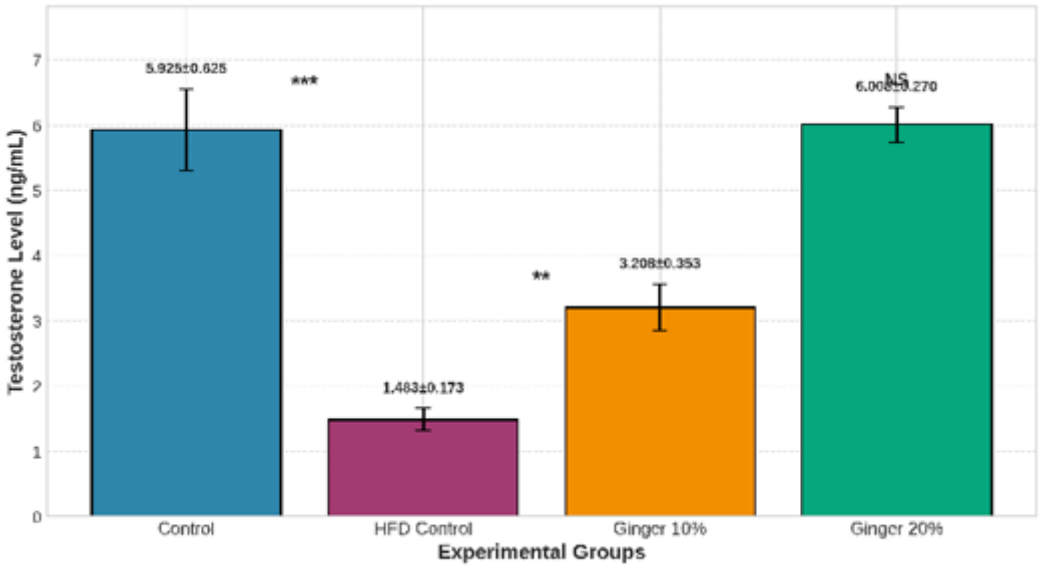

Figure 4. Effect of aqueous ginger extract on serum testosterone levels in high-fat diet (HFD)-induced NAFLD rats.

Effect of Ginger Extract on Serum Aminotransferase Activities

High-fat diet (HFD) feeding markedly elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities compared with the normal control group (Figure 5), confirming the establishment of diet-induced hepatic injury. Administration of ginger extract significantly ameliorated these alterations, although the extent of improvement differed between enzymes. Specifically, the 10% ginger extract group (T2-10%) exhibited the most pronounced reduction in AST levels (174.4 ± 42.4 U/L vs. 580.4 ± 29.0 U/L in the HFD control), corresponding to a 69.95% decrease ($p < 0.001$). The 20% extract group (T3-20%) also reduced AST activity (304.2 ± 116.8 U/L), representing a 47.59% decrease. In contrast, for ALT activity, the positive control group produced the greatest reduction (26.44%), followed by the 20% ginger extract group, which showed a moderate but significant decrease (7.23%). These findings indicate that while ginger extract effectively mitigated HFD-induced hepatic enzyme disturbances, its hepatoprotective effect was more prominent on AST than on ALT, suggesting enzyme-specific sensitivity to treatment. The overall pattern illustrated in Figure 5 highlights the protective potential of ginger extract against diet-induced hepatic dysfunction, aligning with its previously reported antioxidant and metabolic regulatory effects.

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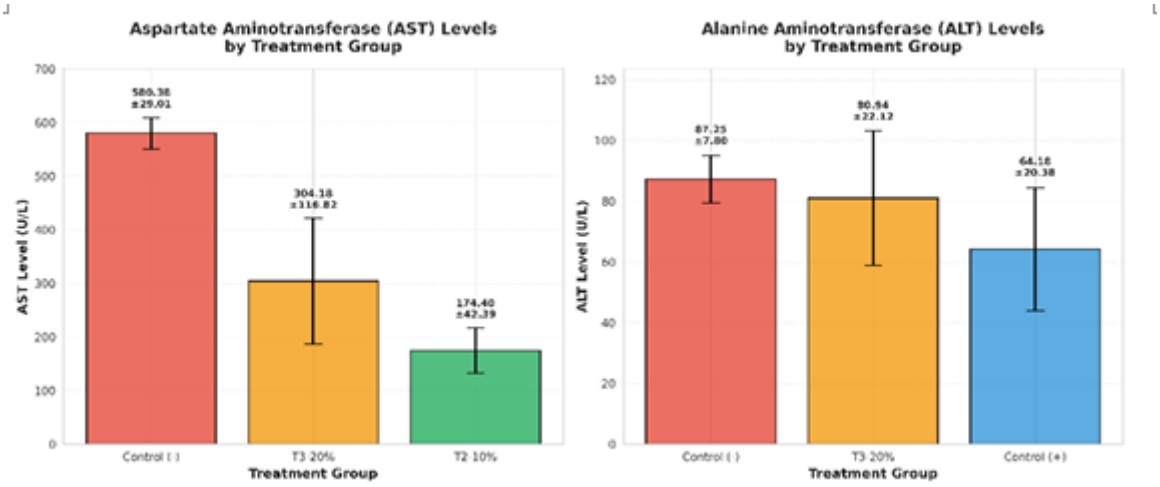

Figure 5. Serum AST and ALT levels in rats fed a high-fat diet (HFD). Control (+) represents HFD-fed rats, T2-10% and T3-20% indicate rats treated with 10% and 20% ginger extract, respectively, while Control (–) represents rats fed a standard diet.

Discussion

The present study provides compelling evidence that aqueous extract of Zingiber officinale exerts significant protective and restorative effects against non-alcoholic fatty liver disease (NAFLD)–associated reproductive dysfunction in male rats. Administration of ginger extract markedly improved sperm concentration, motility, and morphology, accompanied by normalization of serum testosterone and luteinizing hormone (LH) levels. These findings are consistent with previous reports demonstrating the beneficial effects of ginger on male reproductive performance and endocrine balance under pathological conditions (8-9,11). Collectively, the current results extend existing evidence by highlighting ginger's capacity to ameliorate fertility impairment secondary to metabolic liver disease.

The association between NAFLD and male infertility is multifactorial, involving hepatic lipid accumulation, systemic oxidative stress, and disruption of endocrine homeostasis (3-4). Excessive generation of reactive oxygen species (ROS) during hepatic steatosis compromises sperm function by inducing lipid peroxidation of polyunsaturated fatty acid-rich sperm membranes and promoting DNA fragmentation, ultimately impairing sperm motility and viability (7). The observed improvement in sperm quality following ginger treatment may be attributed, at least in part, to the antioxidant properties of its bioactive constituents, including 6-gingerol, 6-shogaol, and zingerone. These compounds have been shown to neutralize ROS and enhance endogenous antioxidant defenses, such as superoxide dismutase, catalase, and glutathione peroxidase, thereby preserving sperm membrane integrity and mitochondrial function (10,12-13).

In addition to its antioxidative actions, ginger supplementation exerted a pronounced effect on hormonal regulation. NAFLD has been strongly linked to hypogonadism and reduced circulating testosterone levels, largely due to impaired hypothalamic–pituitary–gonadal (HPG) axis signaling (1). In the present study, treatment with 20% aqueous ginger extract restored serum testosterone and LH concentrations to near-control levels, indicating recovery of endocrine function. This androgenic effect may be mediated through enhanced pituitary LH secretion and stimulation of Leydig cell steroidogenesis, as well as improved availability of cholesterol substrates required for testosterone biosynthesis, as previously suggested (8). Restoration of hormonal balance likely played a central role in the observed enhancement of spermatogenesis and overall reproductive performance.

Ginger's therapeutic efficacy may also be attributed to its anti-inflammatory properties. Progression of NAFLD is characterized by chronic low-grade inflammation, with elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-6, which adversely affect both hepatic and testicular function (2,14). By suppressing nuclear factor-κB signaling and attenuating inflammatory mediator release, ginger has been shown to alleviate hepatic injury and reduce systemic inflammation (15). These anti-inflammatory effects provide a plausible explanation for the concurrent improvement in liver enzyme profiles and sperm parameters observed in the present study, underscoring the interdependence between hepatic health and male reproductive function.

Despite the strength of these findings, certain limitations should be considered. The study was conducted using an experimental animal model, and extrapolation of the results to human physiology should be approached with caution. Future clinical investigations are required to establish optimal dosing regimens, treatment duration, and long-term safety of ginger supplementation in men with NAFLD-associated infertility. Moreover, further studies aimed at isolating specific bioactive compounds and elucidating their molecular targets would provide deeper insight into the mechanisms underlying ginger's androgenic and hepatoprotective actions.

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Conclusions

In conclusion, the present study demonstrates that aqueous extract of Zingiber officinale exerts a significant protective effect against non-alcoholic fatty liver disease (NAFLD)–associated male reproductive dysfunction in rats. Ginger supplementation effectively improved sperm quality and restored circulating testosterone levels, indicating a recovery of reproductive and endocrine function. These beneficial outcomes are likely attributable to the combined androgenic and hepatoprotective properties of ginger, which contribute to the attenuation of metabolic and hormonal disturbances associated with NAFLD. The concurrent improvement in liver enzyme profiles further underscores the systemic therapeutic potential of ginger in metabolic disorders. Collectively, these findings provide a strong experimental foundation for future clinical studies aimed at validating the efficacy, safety, and optimal dosing of ginger-based interventions in men with NAFLD-related infertility.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed collaboratively. The first draft of the manuscript was written by the authors, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest. Nil

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