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Original Article

Modulatory effects of aqueous rhizome extract of *Zingiber officinale* on oxidative stress, inflammation, and steroidogenesis in alcohol-induced testicular damage in Wistar rats

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ABSTRACT

Background and aim: Male reproductive health is increasingly compromised by environmental and lifestyle factors, with chronic alcohol consumption being a major contributor to testicular dysfunction. Alcohol-induced oxidative stress, inflammation, and disruption of steroidogenesis play key roles in reproductive toxicity. *Zingiber officinale* (ginger) has been widely reported for its antioxidant, anti-inflammatory, and steroidogenic properties. This study investigated the modulatory effects of aqueous rhizome extract of ginger on oxidative stress markers, inflammatory cytokines, and steroidogenic enzymes in alcohol-induced testicular damage in Wistar rats.

Methodology: Thirty male Wistar rats, weighing 100-160 g, were randomly assigned into six groups (n=5/group). Group A received sterile water, while Group B was administered 40% alcohol (3.50 g/kg body weight) for 14 days. Group C received only ginger extract (750 mg/kg body weight) for 14 days. Groups D, E, and F were treated with alcohol for 14 days, followed by low (250 mg/kg body weight), medium (500 mg/kg body weight), and high (750 mg/kg body weight) doses of ginger for another 14 days. Biochemical assays assessed antioxidant enzyme activities (SOD, CAT, MDA), inflammatory cytokines (TNF-α, IL-6, IL-10, IL-4), and steroidogenic enzyme levels (3β-HSD, 17β-HSD).

Results: Alcohol exposure significantly increased oxidative stress markers and pro-inflammatory cytokines while reducing antioxidant enzyme activities and steroidogenic enzyme levels. Ginger administration led to a dose-dependent restoration of antioxidant defenses, reduction in pro-inflammatory cytokines, and improvement in steroidogenic enzyme activities. Notably, lower doses of ginger were more effective in restoring normal testicular function compared to higher doses.

Conclusion: Aqueous rhizome extract of *Zingiber officinale* demonstrates potential as a therapeutic agent in mitigating alcohol-induced reproductive toxicity through its antioxidant, anti-inflammatory, and steroidogenic properties. These findings suggest its possible application in male fertility preservation.

Keywords:
Zingiber officinale; alcohol-induced testicular damage; oxidative stress; inflammation; steroidogenesis; reproductive toxicity.

INTRODUCTION

Male reproductive health is a growing concern due to the increasing prevalence of environmental and lifestyle-related factors that compromise fertility (De Jonge *et al*., 2024). Among these, chronic alcohol consumption has been widely reported to cause reproductive toxicity, particularly in the testes, leading to impaired spermatogenesis, hormonal imbalances, and oxidative stress (Nguyen-Thanh *et al*., 2023; Nyandra *et al*., 2022). Alcohol-induced testicular damage is associated with excessive production of reactive oxygen species (ROS), which overwhelm the antioxidant defense system, resulting in oxidative stress (Silva *et al*., 2022). This oxidative imbalance leads to lipid peroxidation, DNA damage, and protein oxidation, all of which contribute to testicular dysfunction and reduced sperm quality (Neufeld *et al*., 2020). Additionally, chronic alcohol intake has been implicated in the dysregulation of inflammatory pathways (Zhang *et al*., 2022), further exacerbating testicular injury and impairing normal steroidogenesis (Azimzadeh & Jelodar, 2019) crucial in male fertility.

One of the key mechanisms by which alcohol induces testicular damage is through oxidative stress (Villaverde *et al*., 2019), characterized by

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reduced levels of essential antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), while increasing malondialdehyde (MDA), a marker of lipid peroxidation (Onwuka *et al*., 2023). The excessive accumulation of ROS leads to the activation of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), contributing to testicular inflammation (Li *et al*., 2021) and cellular apoptosis (El-Khadragy *et al*., 2021). Equally, anti-inflammatory cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10) play ameliorating roles in counteracting inflammation (Al-Qahtani *et al*., 2024), but their levels are often altered in induced testicular damage (Udefa *et al*., 2020).

Another major consequence of chronic alcohol exposure is its negative impact on steroidogenesis (Li *et al*., 2021), the biochemical process responsible for testosterone production (Ilacqua *et al*., 2018). This disruption is primarily mediated through the inhibition of key steroidogenic enzymes such as 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), which are essential for the synthesis of testosterone from cholesterol precursors (Hou *et al*., 2024). Reduced testosterone levels directly impair spermatogenesis, leading to decreased sperm production, motility, and viability, all of which are critical factors for male fertility (Odetayo *et al*., 2024).

Zingiber officinale, commonly known as ginger, has gained considerable attention due to its potent antioxidant, anti-inflammatory, and steroidogenic properties (Li *et al*., 2021). Ginger contains bioactive compounds such as gingerols, shogaols, and paradols, which have been reported to mitigate oxidative stress and inflammation in various biological systems (Shaukat *et al*., 2023). Studies have shown that ginger supplementation can enhance the activity of antioxidant enzymes, reduce lipid peroxidation, and modulate inflammatory responses (Morvaridzadeh *et al*., 2020). Additionally, ginger has been demonstrated to improve steroidogenesis by enhancing the expression and activity of key steroidogenic enzymes, thereby promoting testosterone production and improving reproductive function (Khwanes *et al*., 2022).

In different protective studies, ginger has been shown to overcome the reproductive toxicity of cyclophosphamide (Mohammadi *et al*., 2014), gentamicin (Zahedi *et al*., 2012; Zahedi & Khaki, 2014) and sodium arsenite (Seif *et al*., 2021) by modulating the effects of ginger on oxidative stress, inflammation, and steroidogenesis to increase sperm counts, viability, motility, and hormones, and improve testicular architecture. However, a study on the curative effect remains unclear, hence, this study.

MATERIALS AND METHODS

Ethical Approval

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.

Sample 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, with the voucher number EB/06006. 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 extract was administered at doses of 250 mg/kg, 500 mg/kg, and 750 mg/kg body weight based on previous studies. Otunola & Afolayan (2017) report that 5000 mg/kg is not toxic to Wistar rats.

Alcohol Preparation

Absolute ethanol (99.7–100% v/v) was purchased from NAFCO Scientific Supplies Limited, Surulere, Lagos, Nigeria (Product Number 28304 7k) and used as the toxicant in this study. A 40% ethanol solution was prepared by diluting absolute ethanol with distilled water. Specifically, 40 mL of absolute ethanol was mixed with 60 mL of distilled water to obtain 100 mL of 40% (v/v) ethanol solution. According to our previous study, Esomchi *et al*. (2025) reported no mortality in rats when induced with 40% alcohol.

Experimental Protocol

Thirty (30) male Wistar rats, weighing 100-160g were randomly assigned into six (6) experimental groups (n = 5 per group). The animals were obtained from the AE-FUNAI animal house and housed in well-ventilated cages under controlled room temperature. The rats were allowed to acclimatize for two weeks and were provided with a standard pellet diet and water *ad libitum*. Group A (Control group) received only distilled water throughout the experiment. Group B (Positive control) was administered 3.50 g/kg body weight per day of 40% ethanol for 14 days. Group C (Ginger control) received 750 mg/kg body weight of ginger extract for 14 days without alcohol induction. Groups D, E, and F (Treatment Groups) were given 40% ethanol for 14 days to induce testicular damage, followed by ginger extract at doses of 250 mg/kg, 500 mg/kg, and 750 mg/kg body weight, respectively, for another 14 days.

Animal Sacrifice and Sample Collection

At the end of the 28-day experimental period, the animals were weighed and sacrificed by cervical dislocation. Blood samples were collected via cardiac puncture, centrifuged at 3000 rpm for 15 minutes, and stored at -80°C for later biochemical assays. The left testis was excised, manually homogenized, and centrifuged at 3000 rpm for 10 minutes in a cold phosphate buffer solution (0.1 M, pH 7.4). The supernatant obtained was used for the analysis

of inflammatory cytokines, steroidogenic enzymes, oxidative stress markers, and antioxidant enzyme activities.

Biochemical Assay

Testicular antioxidant enzyme activities, including Superoxide dismutase (SOD) and catalase (CAT), and malondialdehyde (MDA)levels, were assessed using commercial rat ELISA kits. Caspase-3 and Caspase-9 levels were measured using the CasPASE Assay Kit (G-Bioscience). Pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) and anti-inflammatory cytokines (interleukin-10 (IL-10) and interleukin-4 (IL-4)) were analyzed using Platinum ELISA kits (eBioscience, Waltham, MA, USA) following the manufacturer's protocol. Steroidogenic enzyme activities (3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD)) were assessed using Novex Rat ELISA Kit (Invitrogen, Camarillo, CA, USA).

Data Analysis

Data were analyzed using GraphPad Prism (version 8). Results were presented as Mean ± SD. One-way analysis of variance (ANOVA) was performed, followed by Tukey's *post hoc* test to compare group means. A p-value < 0.05 was considered statistically significant.

RESULTS

Effects of *Z. officinale* on alcohol-induced changes in inflammatory cytokines

The evaluation of testicular cytokines (Figure 1) showed a significant increase in TNF-α and IL-6 levels in the alcohol-treated group compared to the control group ($p < 0.05$). The ginger-only group had TNF-α and IL-6 levels comparable to the control ($p > 0.05$), confirming no inflammatory effect of ginger. Ginger supplementation significantly reduced TNF-α and IL-6 levels in a dose-dependent manner, with the low-dose ginger + alcohol-treated group showing the most reduction ($p < 0.05$ vs. alcohol-treated group). Conversely, IL-10 and IL-4 levels were significantly lower in the alcohol-treated group compared to the control group ($p < 0.05$). The ginger-only group had IL-10 and IL-4 levels similar to the control ($p > 0.05$). Ginger administration significantly increased IL-10 and IL-4 levels ($p < 0.05$), with the medium and lowdose ginger-treated groups restoring IL-10 and IL-4 levels closest to control values respectively.

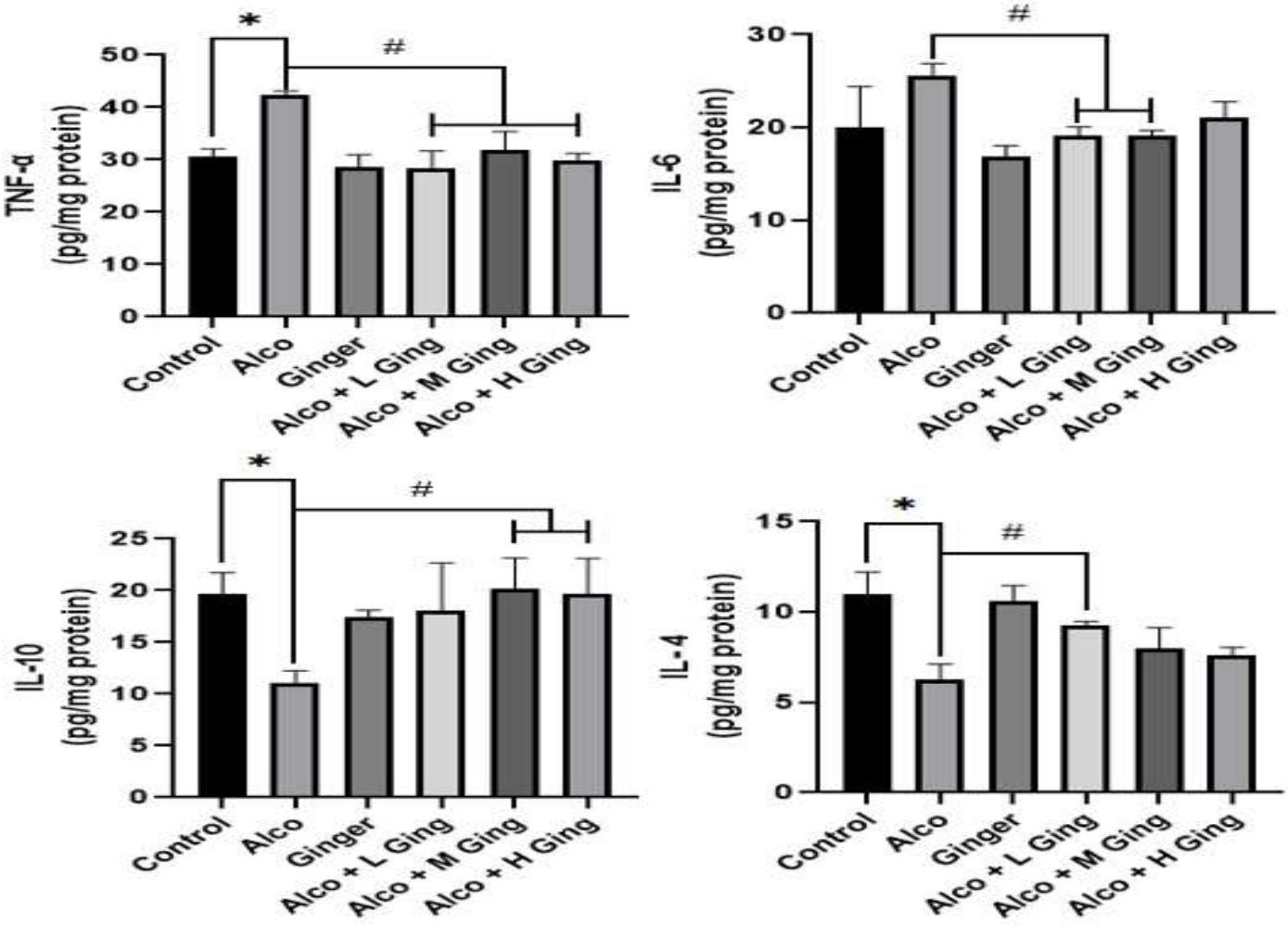

Figure 1: Effects of *Z. officinale* and alcohol on inflammatory cytokines

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

Effects of Ginger on alcohol-induced oxidative stress markers (SOD, CAT, and MDA)

SOD and CAT activities were significantly lower in the alcohol-treated group compared to the control ($p < 0.05$). Ginger supplementation increased both enzymes, with the low-dose ginger + alcohol-treated group showing the highest recovery ($p < 0.05$ vs. alcohol-treated group). MDA levels were significantly elevated in the alcohol-treated group compared to the control. Ginger supplementation reduced MDA, with the low-dose ginger + alcohol-treated group showing the most significant reduction ($p < 0.05$) as presented in Figure 2 below.

Effects of *Z. officinale* on alcohol-induced alterations in testicular steroidogenesis

For 3β-HSD, alcohol-treated group showed a significant reduction compared to Group A ($p < 0.05$). Ginger-only Group (18.30 U/mg) maintained normal levels while ginger-treated groups (D and E) revealed a significant difference when compared to alcohol-treated group ($p < 0.05$). 17β-HSD levels in alcohol-treated group (9.43 U/mg, $p < 0.05$) were significantly lower than Group A. Ginger-treated groups (D, E, and F) showed recovery. Only the low-dose ginger + alcohol-treated group showed significant recovery ($p < 0.05$) as presented in Figure 3.

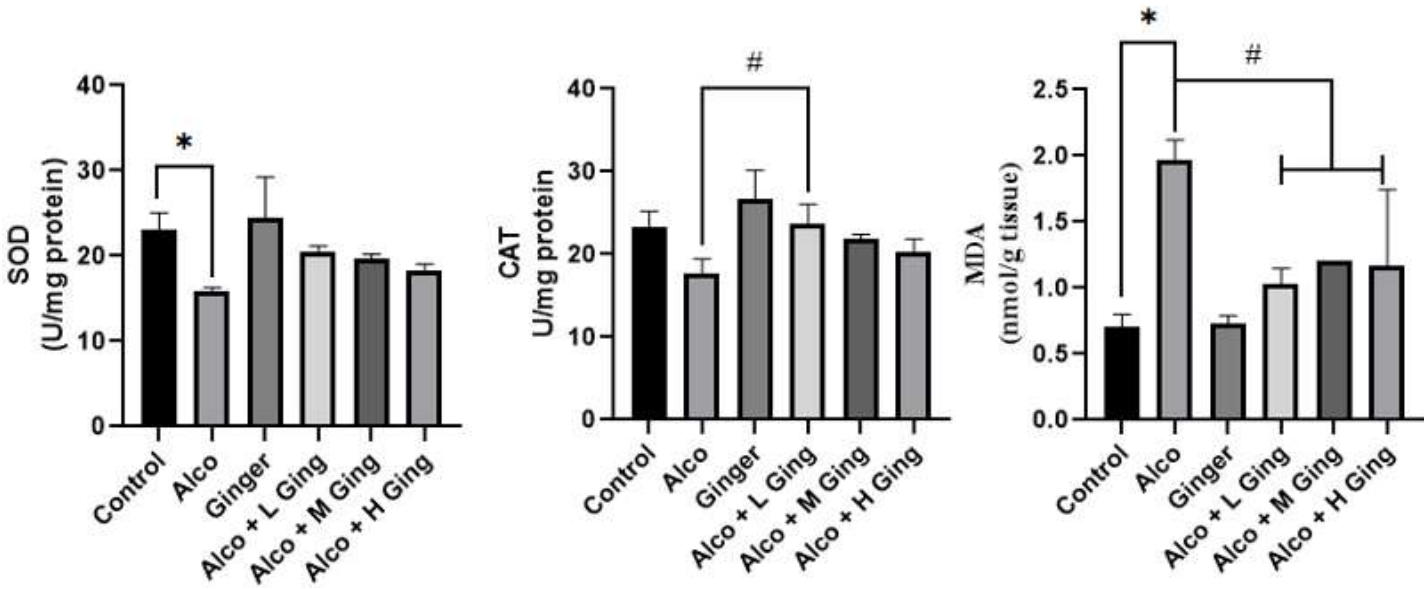

Figure 2: Ginger and Alcohol Effects on Antioxidant and Oxidative Markers
Values represent Mean ± SD. * represent significance difference when compared to A; # represents significance difference when compared to B.

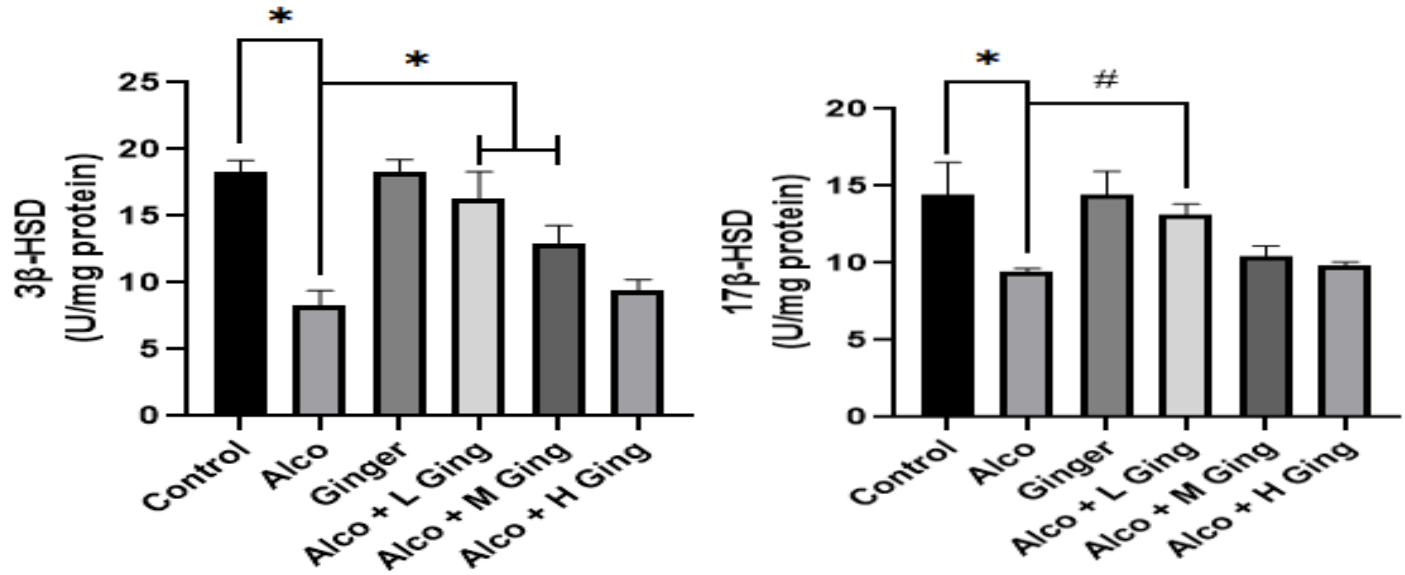

Figure 3: Effects of *Z. officinale* and Alcohol on Testicular Steroidogenesis
Values represent Mean ± SD. * represent significance difference when compared to A; # represents significance difference when compared to B.

DISCUSSION

Infertility is currently on the rise and is a significant global health issue (Kyrgiafini & Mamuris, 2023). Lifestyle factors are reported to possess significant roles in causing male infertility on a global scale (Balawender & Orkisz, 2020) with alcohol consumption among them (Leisegang & Dutta, 2021). Consumption of alcohol has been considered one of the most prevalent diets in different societies since time immemorial (Duca *et al*., 2019), where they perform various functions (Nyandra *et al*., 2022). The mechanism of alcohol-induced infertility is through the increase of oxidative stress (Silva *et al*., 2022; Esomchi *et al*., 2025), a negative impact on steroidogenesis and activation of pro-inflammatory cytokines (Li *et al*., 2021) and deactivation of anti-inflammatory cytokines (Tharmalingam *et al*., 2024). This study evaluated the modulatory effects of ginger on oxidative stress, inflammation, and steroidogenesis in alcohol-induced testicular damage.

This study demonstrated that ginger treatment significantly reduced the levels of TNF-α and IL-6 in a dose-dependent manner. Studies have shown that alcohol can elevate TNF-α and IL-6 levels in the testes, which contributes to oxidative stress and further aggravates tissue damage (Dong *et al*., 2016; Zhang *et al*., 2022). Specifically, TNF-α is known to come from spermatids, spermatocytes, and macrophages, playing a crucial role in testicular health (Han *et al*., 2019) while IL-6 plays a critical role in protecting sperm cells and regulating Sertoli cell and germ cell functions (Loveland *et al*., 2017). Our data supports these findings, where the alcohol-only group showed significantly higher TNF-α and IL-6 levels compared to the control. This suggests that alcohol induces a pro-inflammatory environment in the testicular tissue. The observed reductions in TNF-α and IL-6 following ginger treatment corroborate previous studies by Li *et al*. (2021), Zhang *et al*. (2016) and Banihani (2018), where ginger's anti-inflammatory effects led to lower levels, indicating its protective effect against alcohol-induced inflammation. Turning to the anti-inflammatory cytokines, our study found a significant increase in IL-10 and IL-4 levels in all ginger-treated groups compared to the alcohol-only group where they were found to be lowest. IL-10 and IL-4 are known for their ameliorating roles against inflammation by inhibiting pro-inflammatory cytokines (Leclercq *et al*., 2012). This finding aligns with studies by Zhang *et al*. (2013), García-Marchena *et al*. (2020) and Mao *et al*. (2019), who observed that ginger's active compounds could regulate anti-inflammatory pathways. Ginger and its active compounds, such as 6-gingerol and 6-shogaol, have been shown to inhibit the NF-κB signalling pathway, a key mediator of inflammation, which likely contributes to the observed reduction in TNF-α and IL-6 and increase in IL-10 and IL-4 (Zhang *et al*., 2016; Mao *et al*., 2019).

Ethanol abuse induces oxidative stress through mitochondrial damage, homocysteine and acetaldehyde production, disruption in essential elements' homeostasis and increased microsomal proliferation (Akbari *et al*., 2017, 2020) thereby creating an imbalance between oxidants and antioxidants. This imbalance results in oxidative damage to cellular structures, including lipids, proteins, and DNA (Bhattacharya *et al*., 2020). The present study supports these findings, as alcohol administration led to a significant decrease in the activities of key antioxidant enzymes, such as SOD and CAT, and an increase in MDA, a marker of lipid peroxidation. These alterations are consistent with previous studies showing alcohol's role in diminishing antioxidant defenses while enhancing oxidative stress (Akbari *et al*., 2020; Li *et al*., 2021). As oxidative stress persists, it impairs Leydig cell function, which can lead to reduced testosterone production and spermatogenesis, ultimately contributing to male infertility (Cinthya Riris *et al*., 2021). However, most plants are antioxidant in nature, mitigating the increase of oxidative stress (Ovie et al., 2023; Igwe et al., 2024). This is consistent with previous studies that have reported the ability of ginger to modulate oxidative stress and enhance antioxidant enzyme activity in various tissues (Akinyemi *et al*., 2013; Mazani *et al*., 2020).

Steroidogenesis, which encompasses the synthesis of hormones such as TT, LH and FSH is essential for the regulation of male reproductive health and spermatogenesis (Ilacqua *et al*., 2018). Disruption in the production or regulation of these hormones has been well-documented as a contributing factor in both animal and human studies to conditions that compromise reproductive health, such as infertility (Santillo *et al*., 2020). Alcohol exposure significantly reduced the activity of these enzymes, as seen in previous research that has documented alcohol's damaging impact on steroidogenic pathways. Studies by Jana *et al*. (2010) and Muthusami & Chinnaswamy (2005) demonstrate that alcohol disrupts steroidogenesis by downregulating critical enzymes, leading to diminished hormone synthesis in the testes. Alcohol-induced oxidative stress, coupled with an inflammatory response, interferes with essential regulatory proteins and enzymes which are pivotal for transporting cholesterol into mitochondria, the initial step in steroid hormone production (Li *et al*., 2021). Ginger administration showed a curative and dose-dependent restorative effect on enzyme activity, counteracting the enzyme suppression caused by alcohol. Consistent with the findings of Aktan *et al*. (2006) and Khwanes *et al*. (2022), ginger's bioactive compounds likely exert antioxidant and anti-inflammatory effects, which help restore enzyme levels and support testicular function. Li *et al*. (2021) further corroborate this observation, noting that ginger treatment increased 17β-HSD levels, promoting testosterone production and mitigating oxidative damage in the testes. The dose-dependent recovery observed in ginger-treated groups does not align with previous studies by Mohammadi *et al*. (2014) and Akbari *et al*. (2017), who found that ginger's efficacy in restoring steroidogenesis increases with dosage, whereas ours was better at lower doses, suggesting that a high dose of ginger could be inflammatory.

Conclusion: The findings of this study demonstrate that *Zingiber officinale* exhibits significant modulatory effects on oxidative stress, inflammation, and steroidogenesis in alcohol-induced testicular damage, with a dose-dependent trend. These results suggest that ginger supplementation may serve as a potential therapeutic intervention for mitigating alcohol-induced reproductive toxicity in males.

Competing interests: None declared

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