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The Ameliorative Effects of Glutathione on Alcohol-Induced Male Testicular Injury using Sprague-Dawley Rats as Experimental Models

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Abstract

*Chronic alcohol consumption is known to induce oxidative stress and lead to significant testicular dysfunction, manifesting as impaired spermatogenesis and altered hormone levels. This study aimed to evaluate whether glutathione, which is a potent antioxidant, could mitigate the detrimental effects of alcohol on testicular tissue and function. Sprague-Dawley rats were divided into four groups: control group, low dose group, medium and high dose receiving varying doses of alcohol following glutathione administration. Serum testosterone levels, seminal parameters, and histopathological evaluations of testicular tissue were conducted to assess the extent of injury and the protective effects of glutathione. The results demonstrate a dose-dependent pattern of disruption. In the testes, levels of MDA, an indicator of oxidative stress, initially decreased in the low-dose group compared to the control group, but increased with medium and high alcohol doses, indicating a shift from decreased to increased oxidative stress. Antioxidant enzyme activity (SOD and CAT) in the testes initially increased in response to lower alcohol doses but decreased at the highest dose. Similar patterns were observed in the epididymis, with MDA levels decreasing at low doses but increasing at medium and high doses, and antioxidant enzyme activity following a similar trend. Also, sperm count and sperm morphology decreased dose-dependently. Hormonal analysis revealed a dose-dependent decrease in FSH, LH, and testosterone levels. These findings indicate that alcohol induces oxidative stress, disrupts antioxidant defense mechanisms, and impairs male reproductive function in a dose-dependent ma*nner.

Keywords: *Glutathione, alcohol-induced injury, testicular injury, Sprague-Dawley rats, oxidative stress, reproductive health.*

INTRODUCTION

Alcohol consumption is a pervasive social behavior associated with various health issues, including reproductive dysfunction. Chronic alcohol exposure is known to induce significant testicular injury characterized by oxidative stress, inflammation, and impaired

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spermatogenesis (Barba *et al.,* 2021). This condition is particularly concerning in males, as it can lead to decreased fertility and various hormonal imbalances (Schmidt *et al.,* 2019). Among the numerous antioxidants evaluated for their protective effects against alcohol-induced toxicity, glutathione (GSH) has emerged as a potent contender. GSH is a tripeptide that plays a crucial role in cellular defense mechanisms by neutralizing reactive oxygen species (ROS) and protecting cells from oxidative damage (Martins and Almeida, 2020). Recent studies involving animal models, particularly Sprague-Dawley rats, have provided significant insights into the ameliorative effects of GSH on testicular injuries induced by alcohol. These studies demonstrate that the administration of GSH can reduce oxidative stress markers, improve histological parameters, and restore testosterone levels (Esmaeili *et al.,* 2023).

While the detrimental effects of alcohol on testicular function are well-documented, and the antioxidant properties of glutathione are established, the present study offers a novel contribution by specifically investigating the ameliorative potential of glutathione against alcohol v-induced testicular injury in Sprague Dawley rats. Unlike previous research that may have broadly examined antioxidant interventions or focused on different models of alcohol exposure or injury, this work uniquely elucidates the protective mechanisms of glutathione within this specific context. By employing a controlled experimental design in Sprague Dawley rats, a widely recognized model for toxicological studies, we aim to provide detailed insights into the ability of glutathione to mitigate the specific histological, biochemical, and potentially hormonal disruptions caused by alcohol exposure in the testes. This investigation promises to offer valuable preclinical evidence for the potential therapeutic application of glutathione in counteracting alcohol-related male reproductive dysfunction.

MATERIALS AND METHODS

Study Area

A total of twenty (20) adult male Sprague-Dawley rats, weighing 146±81g, were obtained from Bowen University's Physiology Department in Nigeria and randomly divided into four groups of 5 rats (A-D). The research was carried out in the Anatomy Department's animal house, they were housed in industrial cages at room temperature and were given one week to get used to their new surroundings. During the experiment, they had unlimited access to water and rat chow *ad libitum*. Ethical approval was obtained from the Ethics and Research committee of Bowen University (BUI/COHES/ANA/25-003)

Collection of Samples

The Alcohol and Glutathione were procured from Fairy health pharmaceuticals, China and were inspected by a chemist. Group A received 0mls of alcohol and 0 mls of Glutathione, B-D received 1.5 mls of 25%, 50% and 75% of Absolute Alcohol and 100mls of Glutathione for a duration of 4 weeks orally. Twenty-four hours after the last dosage of the said administration, the animals were euthanized. The (testes, epididymis and postrate) organs' histoarchitecture were determined by fixing them in Bouin's fluid. The levels of oxidative stress were measured by storing the second testis and epididymis in Sample bottles at a temperature of -20^{o}C. Blood for hormonal function tests was taken from the ocular sinus. A standard protocol was followed to process the Testis and Epididymis tissues for microscopic examination, and 5µm thick paraffin sections were created. Using Olympus and Leica microscopes, photomicrographs were taken at a magnification of 400 and slides were stained with standard hematoxylin and eosin stains (Adebajo *et al.,* 2024).

Measurement of LH, FSH, and Testosterone

The samples were kept at a temperature of -20^{o}Cdegrees Celsius until the analysis day. Using enzyme-linked immunosorbent assay (ELISA) kits (Diagnosis Systems Laboratories, Wester, TX), the serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were measured in male rats across all groups in accordance with the manufacturer's instructions (Adebajo *et al.,* 2024).

Statistical Analysis

Using Graph Pad software version 9.5, the data collected from each group were combined and subjected to statistical analysis using ONE WAY-ANOVA and Post Hoc Tukey's tests. The data results were presented as mean ± SEM (standard error of mean), with a significance threshold of $p<0.05$.

RESULTS

No mortality was observed during the process of the experiment. no gross abnormalities of the limb, tail or head were observed in the adult rats throughout the duration of the study.

Effect of orally administered Alcohol and Glutathione on the organ weight of Testis and Epididymis of male sprague dawle]y rats

There was a decrease in the weight of both testis and epididymis. when treatment groups were compared to control, similar decrease was recorded when medium and high doses were compared to low dose and when high dose was compared to medium dose (Table 2).

TABLE 1: effect of orally administered Alcohol and Glutathione on the organ weight of ovary of male sprague dawley rats

Group	Testis(g)	Epididymis(g)
Control	0.93 ± 0.07	0.67 ± 0.38
Low dose	0.87± 1.12	0.43 ± 0.44
Medium dose	0.56 ± 1.44	0.22± 0.35
High dose	0.34 ± 0.54	0.18 ± 0.10

Values are expressed as Mean ± Standard Error of Mean (SEM). $^{a}p<0.05$ significant compared to control; $^{b}p<0.05$ significant compared with low dose; $^{c}p<0.05$ significant compared with medium dose.

Effect of orally administered Alcohol and Glutathione *on* the hormones of male Sprague Dawley rats

In the values of FSH, LH, and Testosterone a dose-dependent decrease was recorded when treatment groups were compared to control, similar decrease was recorded when medium and high doses were compared to low dose and when high dose was compared to medium dose.

Effect of orally administered Alcohol and Glutathione on the hormones of male sprague dawley rats

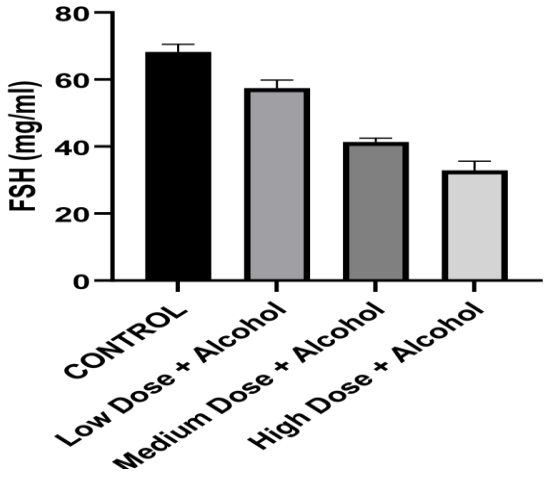


FIG.1.0. FSH

The results of Follicle Stimulating Hormone (FSH) levels demonstrate a dose-dependent decrease, suggesting an inhibition of the hypothalamic-pituitary-gonadal (HPG) axis with increasing alcohol doses. In the Control group (65.86±2.17 mg/mL), FSH levels were at a baseline, reflecting normal gonadal function and hormonal regulation in the testes.
In the Low-dose group (59.84±2.81 mg/mL), FSH levels were slightly reduced compared to the control group. This modest decrease suggests that even low doses of alcohol may interfere with the regulation of FSH. In the Medium-dose group (42.57±1.20 mg/mL), FSH levels decreased further, indicating a more pronounced disruption of the hormonal regulation of spermatogenesis. In the High-dose group (36.00±3.01 mg/mL), FSH levels were significantly lower than in the control and the lower alcohol dose groups, indicating a severe disruption of the hormonal regulation in response to high alcohol doses.

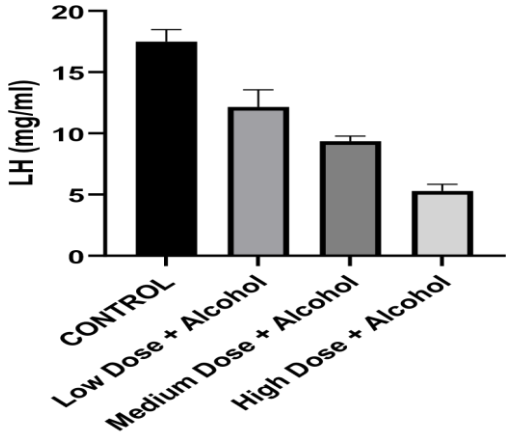


FIG.1.1. LH

The results of Luteinizing Hormone (LH) levels demonstrate a dose-dependent decrease, reflecting a suppression of the hypothalamic-pituitary-gonadal (HPG) axis as alcohol doses increase. In the Control group (16.6±1.12 mg/mL), LH levels were highest, indicating normal hormonal regulation within the testes, which supports healthy reproductive function. In the Low-dose group (12.83±1.55 mg/mL), LH levels decreased compared to the control,

although the decline was relatively modest. In the Medium-dose group (9.37±0.43 mg/mL), LH levels were further reduced, indicating a more substantial disruption of the HPG axis. This decrease suggests that as alcohol exposure increases, it progressively inhibits the release of LH. In the High-dose group (5.51±0.59 mg/mL), LH levels were significantly lower than in all other groups, indicating a severe impairment of the endocrine system.

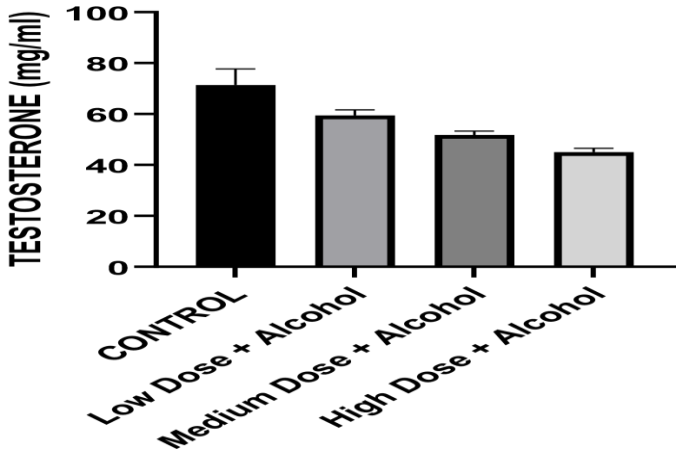


FIG1.2 TESTOSTERONE

The results of testosterone levels demonstrate a dose-dependent decline, suggesting a negative impact of alcohol exposure on male reproductive hormone production. In the Control group (66.01±5.06 mg/mL), testosterone levels were the highest, indicating normal gonadal function and healthy testosterone production under baseline conditions.

In the Low-dose group (60.79±5.56 mg/mL), testosterone levels showed a slight decrease compared to the control, suggesting that low doses of alcohol can lead to a mild suppression of testosterone production. In the Medium-dose group (50.86±2.87 mg/mL), testosterone levels decreased further, indicating a more substantial reduction in hormone production. This decrease suggests that moderate alcohol doses may significantly impair the endocrine system, leading to reduced testosterone synthesis. In the High-dose group (45.20±2.89 mg/mL), testosterone levels were the lowest, showing a significant decline compared to all other groups. This marked reduction indicates that high doses of alcohol severely impair testosterone production.

Effect of orally administered Alcohol and Glutathione on the oxidative stress levels of the Testis and Epididymis in male Sprague Dawley rats

In the values of the MDA, a dose dependent increase was recorded when treatment groups were compared to control and a decrease was seen in the SOD and CAT values when treatment groups were compared to control.

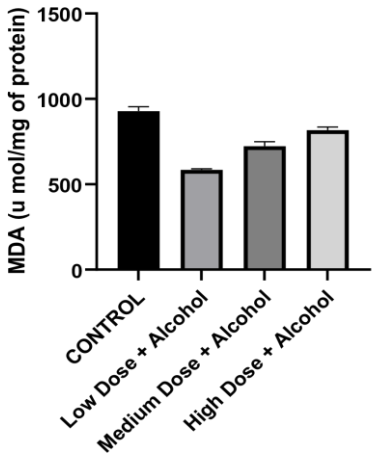


FIG.2.0. MDA TESTES

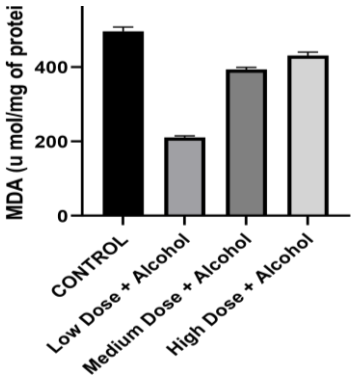


FIG.2.1. MDA EPIDIDYMIS

The results of MDA (Malondialdehyde) levels in the testes demonstrate a dose-dependent variation, indicating a progressive shift from decreased to increased oxidative stress with escalating alcohol doses. In the Control group (936.04±27.29 µmol/mg of protein), MDA levels were the highest, reflecting a baseline level of oxidative stress in the testes under normal conditions, with no intervention. In the Low-dose group (589.67±11.74 µmol/mg of protein), MDA levels showed a significant reduction compared to the control. In the Medium-dose group (752.33±19.35 µmol/mg of protein), MDA levels were higher than in the low-dose group, indicating a shift toward increased oxidative stress. The moderate rise in MDA levels suggests that the alcohol dose at this level begins to surpass any potential protective effects, leading to greater lipid peroxidation and oxidative damage. Finally, the High-dose group (838.25±19.79 µmol/mg of protein) exhibited a slight decrease in MDA levels compared to the medium-dose group, yet the values were still elevated compared to the control.

The results of MDA (Malondialdehyde) levels in the epididymis show a dose-dependent variation, indicating a progressive increase in oxidative stress with higher alcohol doses. In the Control group (483.45±8.22 µmol/mg of protein), MDA levels were the highest, reflecting a baseline level of oxidative stress in the epididymis under normal conditions.

In the Low-dose group (213.54±4.25 µmol/mg of protein), MDA levels were significantly reduced compared to the control. This marked decrease suggests that low alcohol doses may exert a protective effect, potentially by stimulating antioxidant mechanisms or reducing lipid peroxidation in the epididymis. In the Medium-dose group (395.07±7.58 µmol/mg of protein), MDA levels increased relative to the low-dose group, showing that medium doses of alcohol may begin to compromise antioxidant defenses, leading to increased oxidative stress. In the High-dose group (421.56±6.48 µmol/mg of protein), MDA levels further increased, though not dramatically compared to the medium-dose group. The slight increase in MDA levels suggests that at higher doses, oxidative damage continues to rise, although the response plateaus, indicating that the oxidative stress induced by alcohol may reach a certain threshold that does not increase significantly beyond the medium dose.

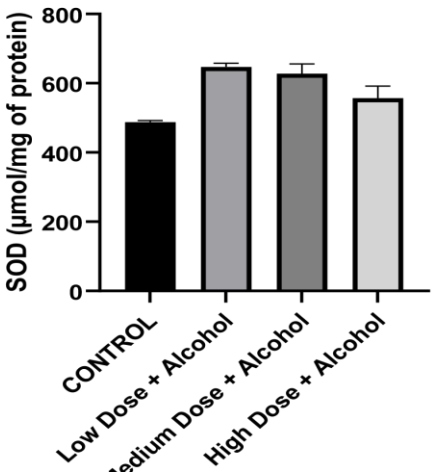


FIG.2.2 SOD TESTES

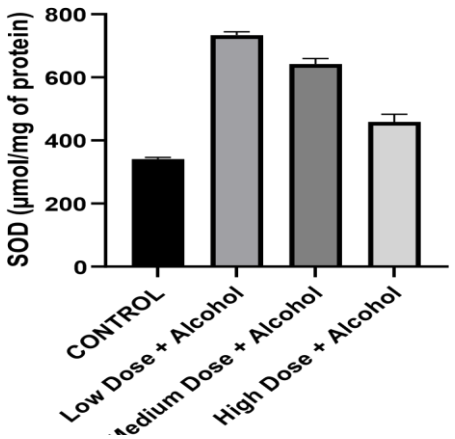


FIG.2.3 SOD EPIDIDYMIS

The results of SOD (Superoxide Dismutase) activity in the testes demonstrate a subtle dose dependent variation, suggesting a progressive increase in antioxidant defense activity at lower alcohol doses, with diminishing effects at higher doses. In the Control group (483.32±4.37 µmol/mg of protein), SOD activity was relatively low, reflecting the baseline antioxidant status in the testes under normal conditions.

In the Low-dose group (637.61±8.29 µmol/mg of protein), SOD activity significantly increased compared to the control. In the Medium-dose group (654.60±19.26 µmol/mg of protein), SOD activity was further elevated, reinforcing the idea that moderate alcohol doses might continue to enhance antioxidant activity in response to oxidative stress. In the Highdose group 597.22±18.37 µmol/mg of protein), SOD activity decreased slightly compared to the medium dose group. While the values were still elevated compared to the control, the reduction in activity suggests that the antioxidant defenses may have reached their threshold or that higher alcohol doses overwhelm the body's ability to produce antioxidant enzymes.

The results of SOD (Superoxide Dismutase) activity in the epididymis show a dose-dependent increase, suggesting a progressive enhancement of antioxidant defense mechanisms in response to alcohol exposure at lower doses, followed by a decline at higher doses. In the Control group (337.12±4.28 µmol/mg of protein), SOD activity was relatively low, reflecting the baseline antioxidant defense capacity in the epididymis under normal conditions.

In the Low-dose group (723.79±7.16 µmol/mg of protein), SOD activity was significantly higher than in the control group. In the Medium-dose group (623.40±7.93 µmol/mg of protein), SOD activity remained elevated compared to the control group but was lower than in the lowdose group. This decrease suggests that although moderate alcohol doses still stimulate an antioxidant response, the protective effect starts to diminish as the dose increases. In the Highdose group (432.69±6.73 µmol/mg of protein), SOD activity was significantly lower than in both the low- and medium-dose groups, indicating that at very high doses, alcohol exposure may overwhelm the antioxidant defenses, leading to a reduction in the body's ability to neutralize oxidative stress.

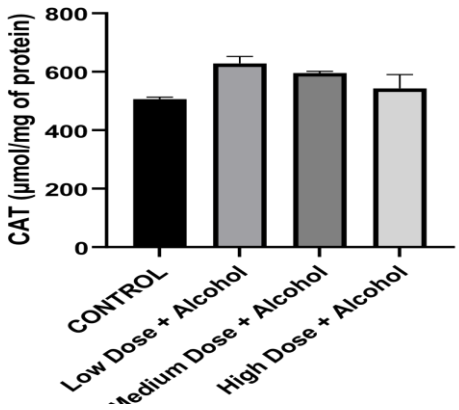


FIG.2.4. CAT TESTES

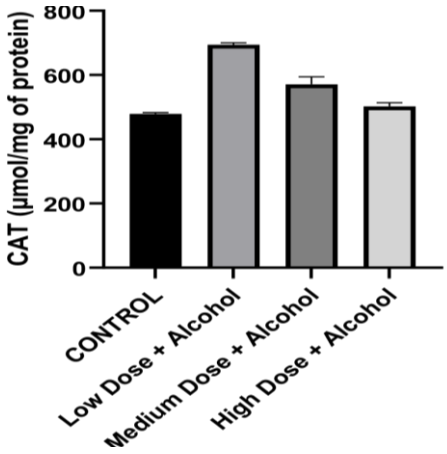


FIG.2.5. CAT EPIDIDYMIS

The results of catalase (CAT) activity in the testes show a slight variation with increasing alcohol doses, suggesting a relatively stable antioxidant response across the different treatment groups. In the Control group (501.33±6.59 µmol/mg of protein), CAT activity was at a baseline level, indicating normal hepatic antioxidant activity in the testes without any intervention.

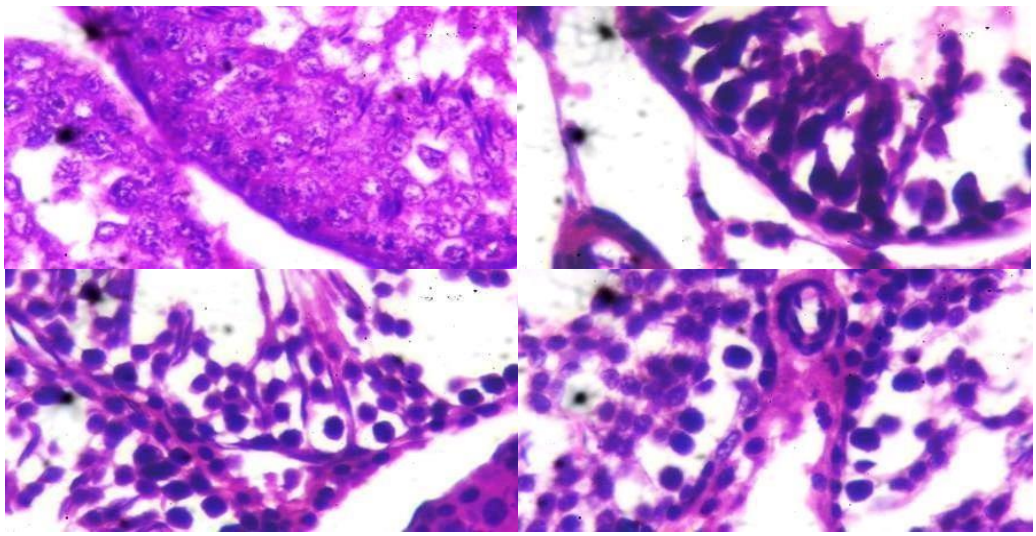
In the Low-dose group (606.33±12.04 µmol/mg of protein), CAT activity showed a significant increase compared to the control. This elevation suggests that low doses of alcohol may trigger an adaptive response by enhancing antioxidant defenses. In the Medium-dose group (601.01±7.59 µmol/mg of protein), CAT activity remained relatively high, but it showed only a slight decrease compared to the low-dose group. In the High-dose group (495.25±16.79 µmol/mg of protein), CAT activity decreased slightly compared to the medium-dose group.

The results of catalase (CAT) activity in the epididymis demonstrate a slight variation across the different alcohol treatment doses, suggesting an adaptive antioxidant response that becomes less pronounced at higher doses. In the Control group (475.5±3.88 µmol/mg of protein), CAT activity was at baseline levels, indicating normal antioxidant activity in the epididymis without any alcohol exposure.

In the Low-dose group (697.21±5.63 µmol/mg of protein), CAT activity increased significantly compared to the control, suggesting that alcohol at low doses stimulates the epididymis to enhance its antioxidant defenses. In the Medium-dose group (589.14±9.73 µmol/mg of protein), CAT activity was slightly reduced compared to the low-dose group, indicating that as the dose of alcohol increases, the effectiveness of the antioxidant response begins to diminish. In the High-dose group (504.43±5.83 µmol/mg of protein), CAT activity was slightly reduced compared to the medium-dose group, suggesting that at very high doses, alcohol may impair the antioxidant response.

Effect of orally administered alcohol and glutathione on the Testicular cytoarchitecture of male sprague dawley rats

Control Low dose



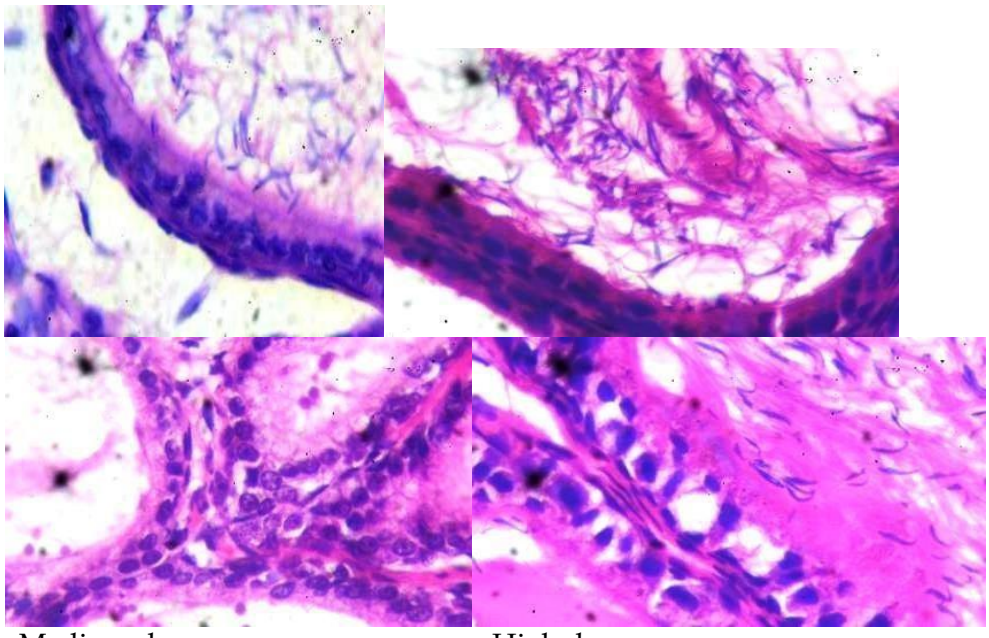
Medium dose High dose

FIG.3.0

Photomicrograph of the normal testicular section stained by Haematoxylin and Eosin showing normal seminiferous tubules lined by , normal and completely developed germinal cells including spermatogonia cells, sertoli and other spermatocytes, their lumen containing spermatozoa (white arrow). The interstitial spaces show normal leydig cells (slender arrow)

Effect of orally administered alcohol and glutathione on the Epididymal cytoarchitecture of male sprague dawley rats

Control. Low dose



Medium dose High dose

FIG.3.1

Photomicrographs of Epididymal sections stained by Haematoxylin and Eosin showing normal epididymal ducts lined by normal epithelial layers with normal smooth muscle layer (blue arrow), the ducts are seen storing spermatozoa within the lumen(white arrow). the interstitial spaces appear normal (slender arrow).

DISCUSSION

Alcohol consumption has been universally recognized as a contributor to oxidative stress, particularly in the male reproductive system. The elevation of malondialdehyde (MDA) levels presented in the medium and high-dose groups in our findings aligns is consistent with findings from recent studies, which reports that alcohol metabolism generates reactive oxygen species (ROS) that lead to lipid peroxidation and cellular damage (Bansal and Bilaspuri, 2019) Also, a contemporary research by Patel et al. (2024), who reported that alcohol metabolization leads to a significant increase in oxidative stress markers, particularly MDA, highlighting a concerning pathway through which alcohol consumption damages male fertility.

Increased MDA levels serve as a biomarker for oxidative stress (Kehrer, 2021), and the results reinforce the notion that higher alcohol concentrations exacerbate oxidative damage in testicular tissues.

The observations of enhanced antioxidant enzyme activities (SOD and CAT) in the low and medium-dose groups suggest an adaptive response aligned with the findings of Kani *et al.* (2020), who noted that lower alcohol exposure can stimulate endogenous antioxidant responses. However, the decline in these activities at higher doses reflects the overwhelming oxidative burden which compromises the antioxidant defense system, mirroring what was reported by Yadav *et al.* (2022). They found that excessive alcohol intake leads to the depletion of antioxidant reserves, rendering tissues more vulnerable to oxidative damage.

The detrimental effects of alcohol on seminal parameters, including decreased sperm count and morphological abnormalities, corroborate prior research that links alcohol exposure to impaired spermatogenesis (Sharma *et al.,* 2021). The effects on sperm quality may arise from direct toxicity to the developing germ cells and supporting Sertoli cells, emphasizing the importance of maintaining a balanced hormonal environment. Alcohol-induced alterations in the hypothalamic-pituitary-gonadal (HPG) axis, reflected in the decreased levels of FSH, LH, and testosterone in the study, align with the work of Siddiqui *et al.* (2023), who similarly highlighted the hormonal disruptions accompanying chronic alcohol consumption and their subsequent impact on male fertility.

Moreover, the observed correlations between alcohol exposure and sperm quality reaffirm findings reported by Gupta et al. (2025), which indicate that increased oxidative stress directly correlates with a decline in sperm parameters, including motility, viability, and morphology. This evidence suggests that oxidative stress may disrupt spermatogenesis and the functionality of Sertoli cells, potentially leading to long-term reproductive challenges.
Glutathione, a potent antioxidant, has been shown to mitigate the adverse effects of oxidative stress in various tissues, including the testes. While the study suggests that glutathione has ameliorative effects, previous research underscores its potential to replenish depleted antioxidant defenses and protect against alcohol-induced testicular injury (Chen *et al.,* 2019).

Recent studies continue to showcase the multifaceted impact of alcohol on male reproductive health.

It is also a key intracellular antioxidant, that has garnered attention as more recent studies suggest its significant potential in counteracting oxidative stress induced by excessive alcohol

consumption. Wang et al. (2024) demonstrated in their investigation that glutathione administration not only restored antioxidant enzyme activity but also significantly improved sperm quality and reduced apoptosis in germ cells affected by alcohol exposure. This aligns with our findings and underscores the utility of glutathione as a therapeutic agent for individuals engaged in chronic alcohol consumption.

It is important to recognize the comprehensive effects that glutathione may have on the hormonal milieu modulated by the hypothalamic-pituitary-gonadal (HPG) axis. Recent evidence from Lee et al. (2025) suggests that glutathione may help stabilize hormonal levels that are often disrupted by chronic alcohol intake, promoting a more conducive environment for spermatogenesis. Their research indicates that glutathione supplementation can effectively restore levels of serum testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), counteracting the dysregulation typically seen with excessive alcohol consumption.

Furthermore, glutathione's role in modulating inflammation adds another layer of complexity to its protective effects. Recent studies indicate that alcohol consumption leads to the activation of inflammatory pathways that can exacerbate testicular damage. Zhao et al. (2024) reported that glutathione can inhibit the production of pro-inflammatory cytokines, thus reducing inflammation and preserving testicular architecture and function. This anti-inflammatory property is particularly crucial given the established relationship between inflammation, oxidative stress, and impaired fertility.

The accumulating body of evidence points to glutathione as a promising adjunct therapy for mitigating alcohol-induced testicular injury. By replenishing antioxidant defenses, restoring hormonal balance, and attenuating inflammatory responses, glutathione holds the potential to safeguard male reproductive health in individuals subjected to chronic alcohol exposure. Future research should focus on identifying the optimal dosing and timing of glutathione administration, as well as exploring its synergistic effects with other antioxidant compounds, to maximize its therapeutic efficacy

CONCLUSION

In conclusion, the study demonstrates that alcohol induces oxidative stress and disrupts antioxidant defense mechanisms and impairs male reproductive function in a dose-dependent manner, leading to impaired sperm parameters and hormonal imbalances. These findings highlight the adverse effects of alcohol on male reproductive health.

There could be further Investigation into Protective Mechanisms as seen in the initial decrease in MDA levels and the increase in SOD and CAT activities at low alcohol doses which suggest the activation of protective mechanisms. The author recommends that caution should be applied in consumption of Alcohol as it has negative impact on the male reproductive health.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this study.

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