© IIJP 2025 | Volume 1, Issue 3, pp. 464-478 | ISSN: 2636-4484

INVESTIGATION ON THE IMPACT OF ALCOHOL ON THE KIDNEY AND LIVER OF ADULT MALE SPRAGUE-DAWLEY RATS USING GLUTATHIONE AS AN ANTIOXIDANT

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ARTICLE INFO

Article No.: 056

Accepted Date: 30/08/2025

Published Date: 20/09/2025

Type: Research

ABSTRACT

The impact of alcohol consumption on the liver and kidney functions remains a critical area of investigation, particularly concerning oxidative stress-induced damage. This study examines the effects of chronic alcohol exposure on the liver and kidney tissues of adult male Sprague-Dawley rats and evaluates the potential protective role of glutathione, a key antioxidant. Rats were divided into control, alcohol-exposed, and alcohol-exposed with glutathione supplementation groups. Chronic alcohol administration was used to induce oxidative stress, and glutathione was administered to assess its protective effects. Biochemical assays were conducted to measure markers of liver and kidney injury, including serum transaminases, creatinine, and blood urea nitrogen (BUN). Additionally, histopathological examination of liver and kidney tissues was performed to assess structural damage. The results demonstrated significant increase ($p<0.05$) in liver and kidney injury markers in alcohol-exposed rats, accompanied by histopathological alterations such as hepatocellular necrosis and renal tubular degeneration. However, glutathione supplementation attenuated these changes, reducing oxidative stress and mitigating organ damage. These findings highlight the detrimental effects of alcohol on kidney and liver function and suggest that glutathione may serve as a potential therapeutic agent for protecting against alcohol-induced organ toxicity.

Keywords: Glutathione, Hepatocellular Necrosis, Renal Tubular Degeneration, Oxidative Stress, Sprague-Dawley Rats.

Introduction

The effects of long-term alcohol usage on the liver and kidney are the main topic of this investigation, both of which are essential for detoxification and waste removal, making them particularly vulnerable to alcohol-related damage (Nevzorova et al., 2020). Chronic alcohol intake induces oxidative stress, leading to liver and kidney dysfunction (Tan et al., 2020). In the liver, this manifests as alcoholic liver disease (ALD), which encompasses a spectrum of hepatic injuries, ranging from fatty liver to cirrhosis, with mitochondrial damage playing a key role (Nassir and Ibdah 2014). In the kidneys, alcohol leads to nephrotoxicity, causing structural damage and significant renal dysfunction, including reduced creatinine clearance and structural alterations in renal tissues (Barnett and Cummings 2018).Electron microscopy reveals damage to renal epithelial cells, particularly in the distal tubules and Henle's loops resembling tubular necrosis observed with other nephrotoxic agents (Zhang et al., 2020).The study highlights glutathione (GSH), a tripeptide composed of glutamine, cysteine and glycine and it is an antioxidant that helps protect cells from oxidative damage, as a potential protective agent against alcohol-induced organ damage (Labarrere and Kassab 2022). Previous research suggests that GSH supplementation could reduce oxidative stress and mitigate liver injury (Honda, 2017). This study aims to further investigate the protective effects of GSH in Sprague-Dawley rats whose liver anatomy is closely related to humans to better understand its potential in treating alcohol-induced liver and kidney damage.

Materials and Methods

Animal management and care

A total of twenty adult male Sprague-Dawley rats in the study, ranging in weight from 80-120g.The rats were kept at Bowen University's Department of Anatomy Animal House under normal conditions after being acclimated for two weeks. They were housed in clean, well-ventilated plastic cages, fed water and pellets, and maintained in a clean environment. Every day, wood shavings were used as bedding to provide hygienic conditions.

Drug Dilution

The treatment groups received 1.5ml of 25%, 50% and 75% Alcohol.

Experimental Design

Twenty adult male Sprague-Dawley rats were used as experimental models and they were divided into four groups, Group (A-D). Each of the group consisted of 5 selected rats, where the control group had rats with less weights.

Group A functioned as the control group, and the rats were administered 1ml of distilled water over a duration of 4 weeks.

Group B rats received a low dose of 1.5ml of 25% absolute alcohol and 100mg/kg of glutathione concurrently orally alone over a duration of 4 weeks.

Group C rats were administered a medium dose of 1.5ml of 50% absolute alcohol and 100mg/kg of glutathione concurrently orally alone for 4 weeks.

Group D rats were administered a high dose of 1.5ml of 75% absolute alcohol and 100mg/kg of glutathione concurrently orally alone over a duration of 4 weeks.

After 4 weeks of administration, the rats were euthanized and the kidneys and liver were harvested for histology and oxidative stress markers. The ocular sinuses provided blood for the analysis.

Blood sampling and function test: The ocular sinus provided blood and it was centrifuged. The kidneys and liver were frozen before homogenization for the oxidative stress markers.

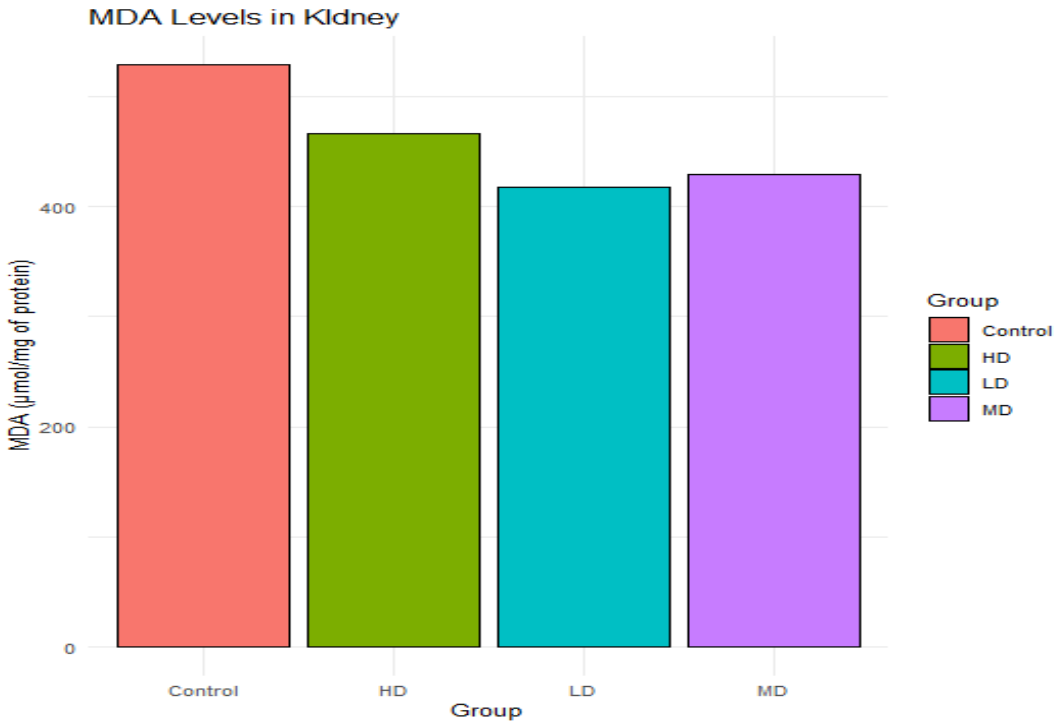
Histological Procedures: The kidneys and liver were harvested, preserved and processed for histological examination following a standard methodology. 5mm thick paraffin sections were made for microscopic investigation.

Statistical Analysis: Graph Pad software version 9.5 was used; each group's data was aggregated and statistically analyzed using ONE-WAY ANOVA. The data results were presented as mean± SEM (Standard Error of Mean), with a significant threshold of $p<0.05$.

Results

Effects of orally administered alcohol and glutathione on the kidney oxidative stress markers in male Sprague-dawley rats

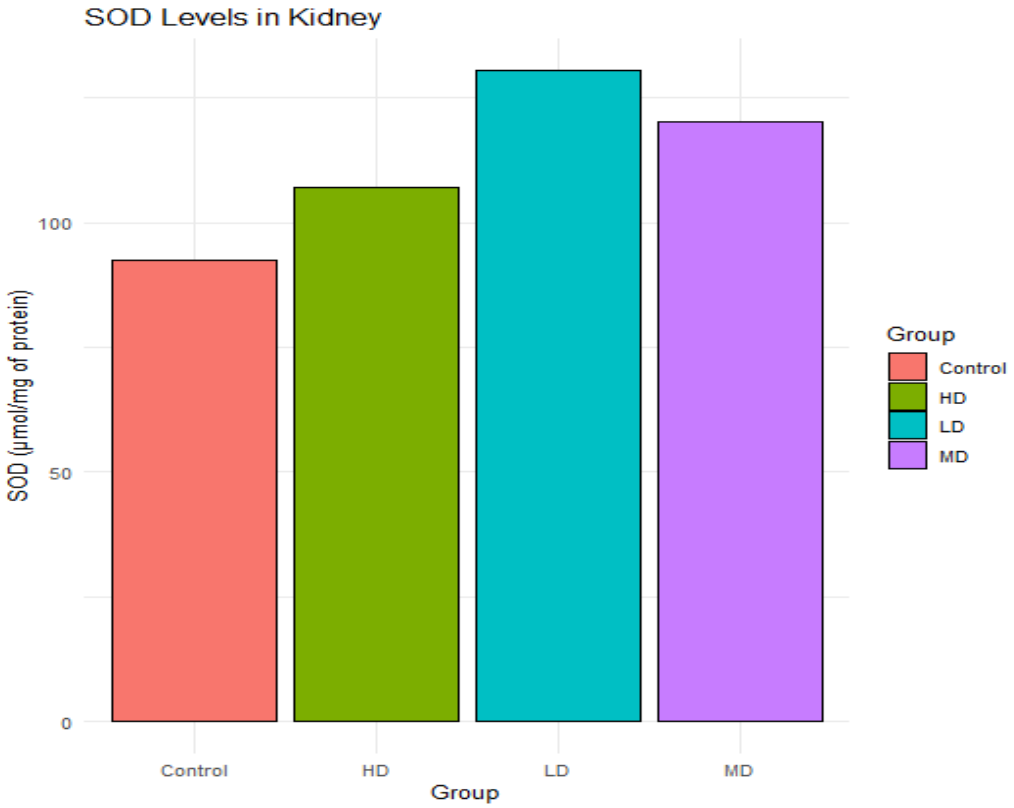
Malondialdehyde (MDA) Levels in Kidney



There is a statistically significant difference in MDA levels among the Control, LD, MD, and HD groups ($p < 0.0001$). The high F-value ($F = 5202.76$) indicates that the between-group variance is substantially greater than the within-group variance, which suggests that the treatment at different dosage levels has a strong effect on MDA concentrations.
Specifically, the Control group exhibited the highest MDA level (528.42 µmol/mg), followed by HD (466.73 µmol/mg), MD (428.69 µmol/mg), and LD (417.81 µmol/mg). This pattern indicates a dose-dependent reduction in MDA levels at lower doses (LD and MD), with a slight elevation at the highest dose (HD), potentially suggesting a non-linear antioxidant response or compensatory mechanism at higher concentrations.

This result supports the hypothesis that the test substance influences lipid peroxidation, as reflected in MDA levels, and highlights the potential antioxidant role of the treatment.

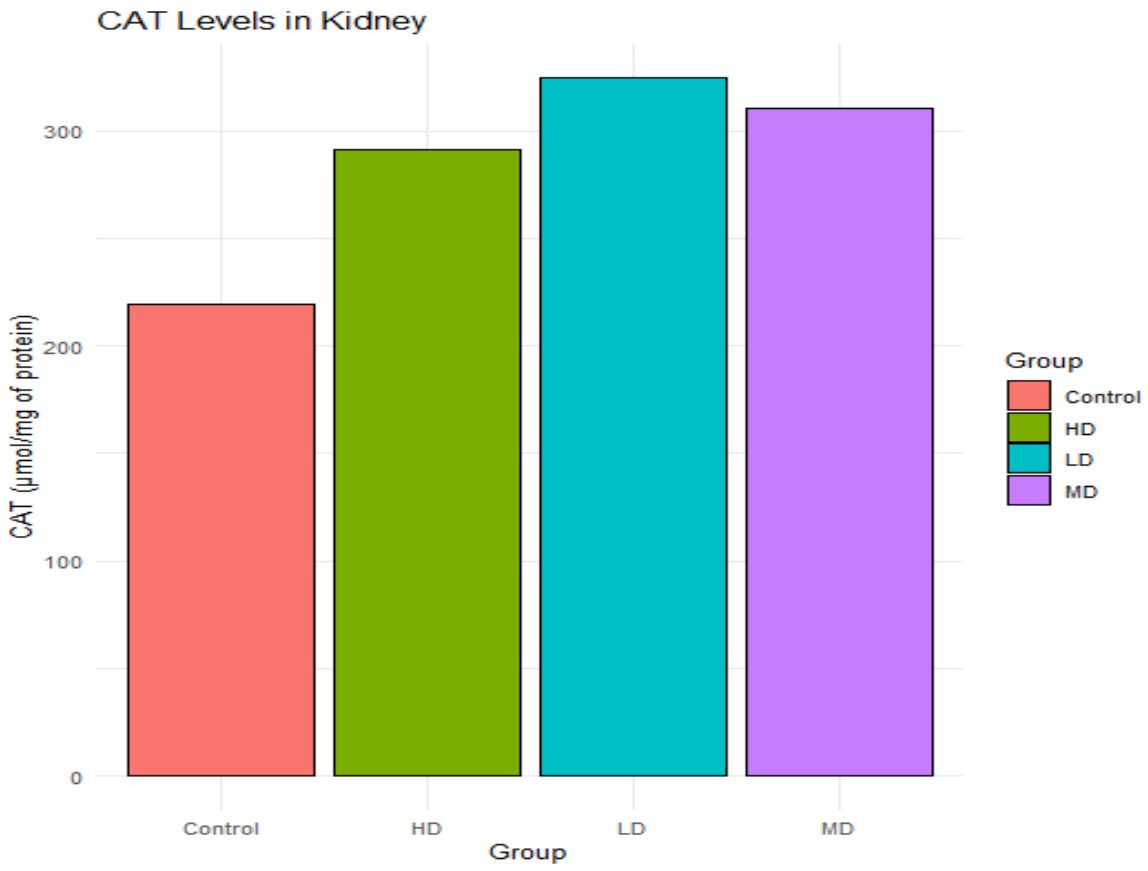
Superoxide Dismutase (SOD) Levels in Kidney



There is a statistically significant difference in Superoxide Dismutase (SOD) levels among the Control, LD, MD, and HD groups ($p < 0.0001$, $F = 179.58$). The high F-value indicates that the variation between the groups is substantially greater than the variation within groups, suggesting that the different dosage levels had a strong influence on SOD activity.
The LD group had the highest SOD level (130.396 IU/mg), followed by the MD group (120.096 IU/mg) and HD group (107.076 IU/mg), while the Control group recorded the lowest value (92.37 IU/mg). This trend implies that SOD activity increases following treatment, particularly at the low

dose, but gradually declines with higher dosages, potentially due to enzyme inhibition, feedback mechanisms, or oxidative stress overload at elevated doses. This result indicates that the treatment significantly enhances antioxidant enzyme activity at moderate exposure levels but may induce enzyme fatigue at higher concentrations.

Catalase (CAT) Levels in Kidney

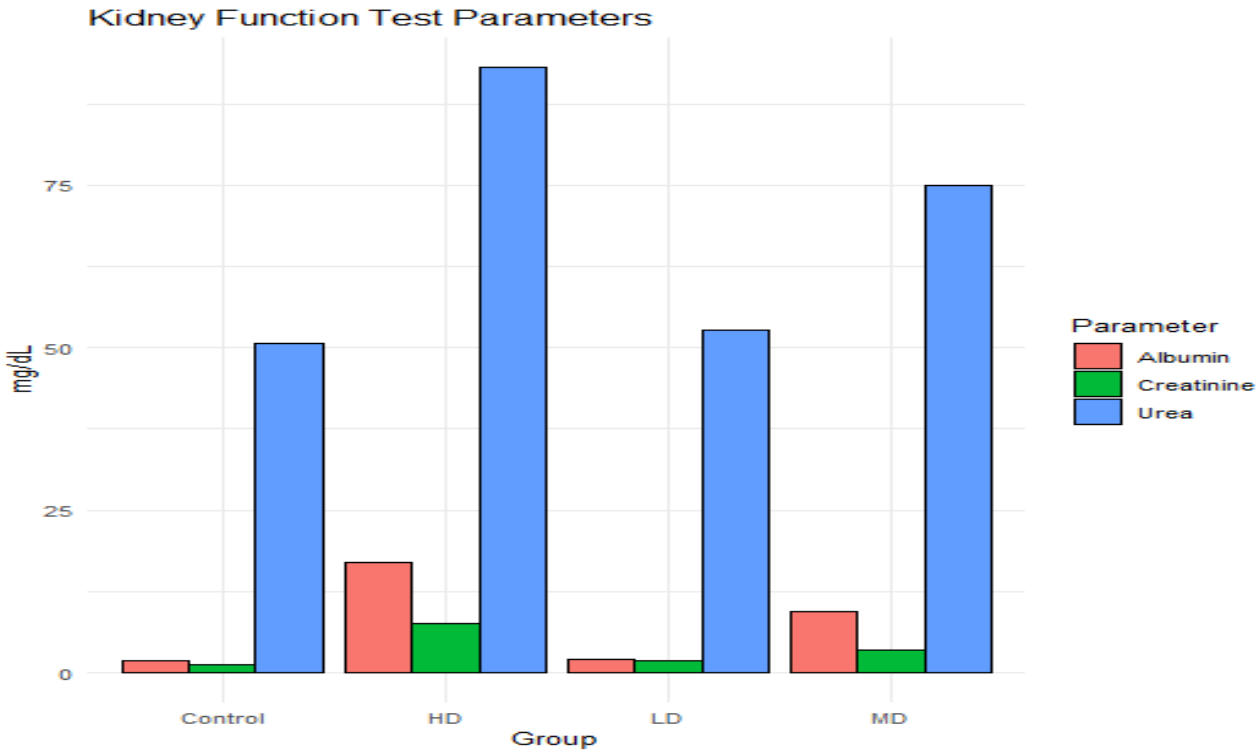


There is a statistically significant difference in CAT (Catalase) levels among the Control, LD, MD, and HD groups ($p < 0.0001$, $F = 1073.24$). The high F-value signifies that the differences between group means are much larger than the variability within groups, confirming that the treatment dosages had a pronounced impact on CAT activity.

The LD group recorded the highest catalase activity (324.49 IU/mg), followed by the MD group (310.20 IU/mg) and then the HD group (291.47 IU/mg), while the Control group showed the lowest level (219.51 IU/mg). This pattern indicates that the treatment enhanced catalase activity, particularly at low and medium doses. However, there's a gradual decline in CAT activity as the dose increases beyond the medium level, which might suggest oxidative saturation or feedback

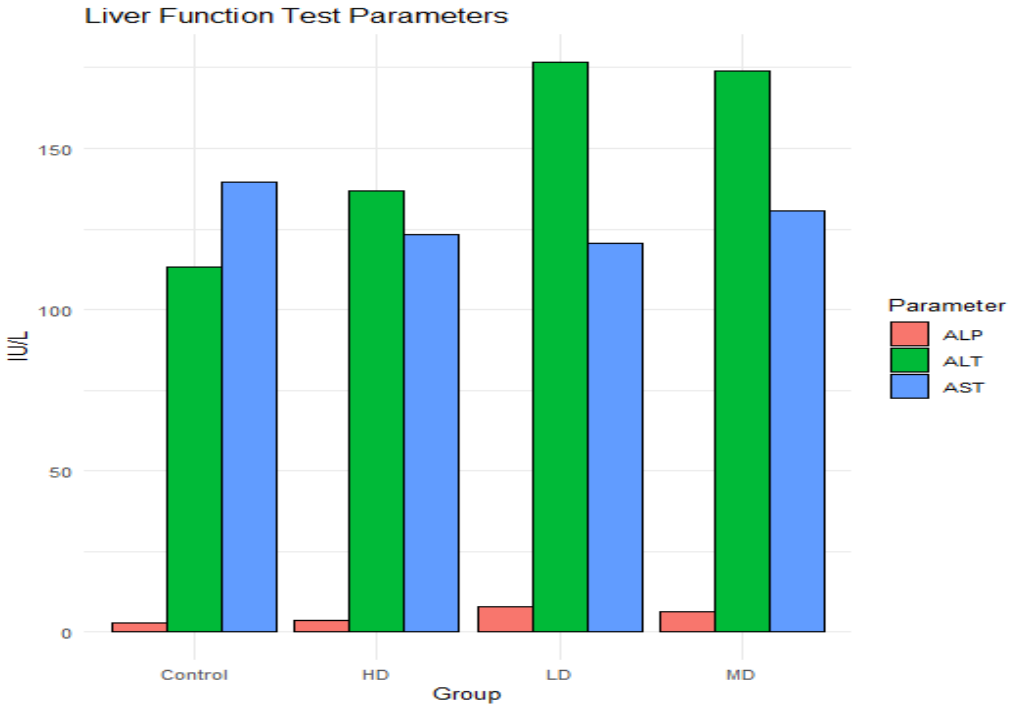
inhibition at higher dosages. These findings demonstrate that the treatment significantly boosts antioxidant defense by increasing catalase activity, especially at lower doses.

Effects of orally administered alcohol and glutathione on the kidney function parameters.



Creatinine, Urea, and Albumin levels were significantly different across the groups ($p < 0.0001$, $F = 20054.76$ for Creatinine, 10033.95 for Urea, and 19160.88 for Albumin). The HD group had the highest creatinine and urea levels, reflecting severe kidney dysfunction ($p < 0.0001$). The sharp increase in albumin levels in MD and HD suggests potential nephrotic changes associated with high-dose exposure.

Effects of administration of alcohol and glutathione on the liver function parameters.

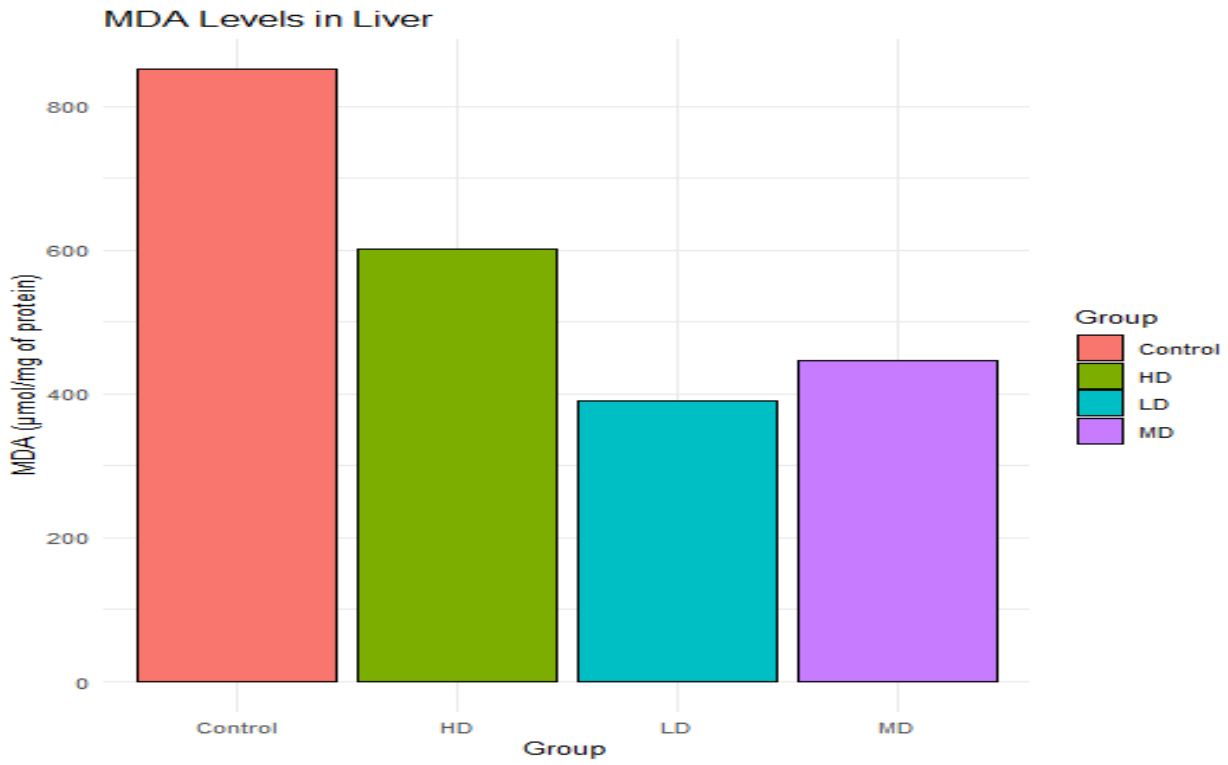


AST, ALP, and ALT levels were significantly different across the groups ($p < 0.0001$, $F = 2351.34$ for AST, 825.64 for ALP, and 7864.71 for ALT). The LD and MD groups had significantly elevated ALT levels, indicating liver stress ($p < 0.0001$). The HD group exhibited a reduction in ALT levels compared to MD, suggesting a possible adaptation to liver stress.

Effects of the oral administration of alcohol and glutathione on liver biochemical stress markers

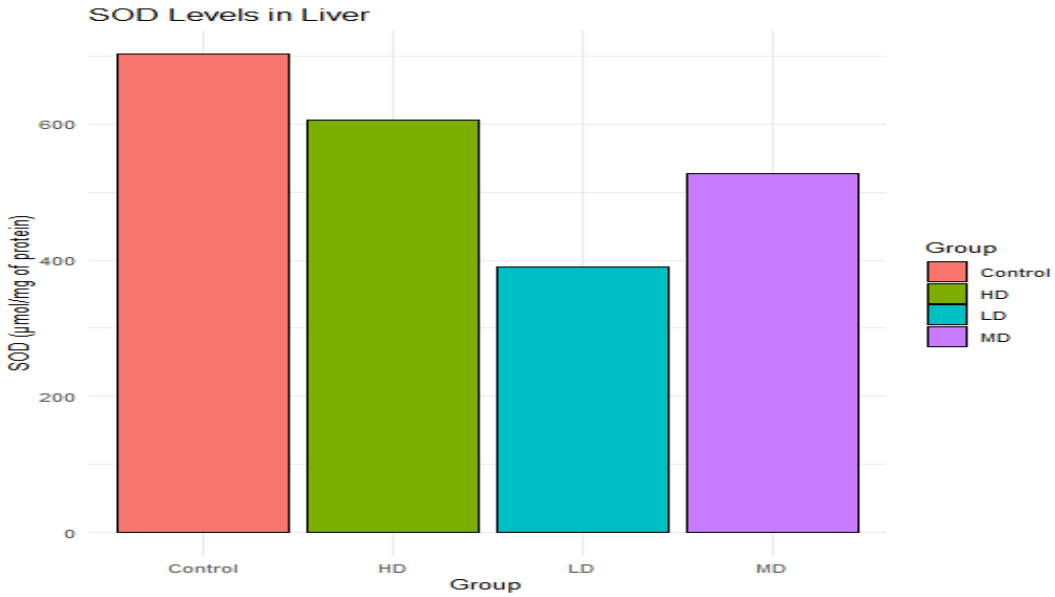
Malondialdehyde (MDA) Levels in Liver

The MDA (µmol/mg of protein) levels varied across different groups:



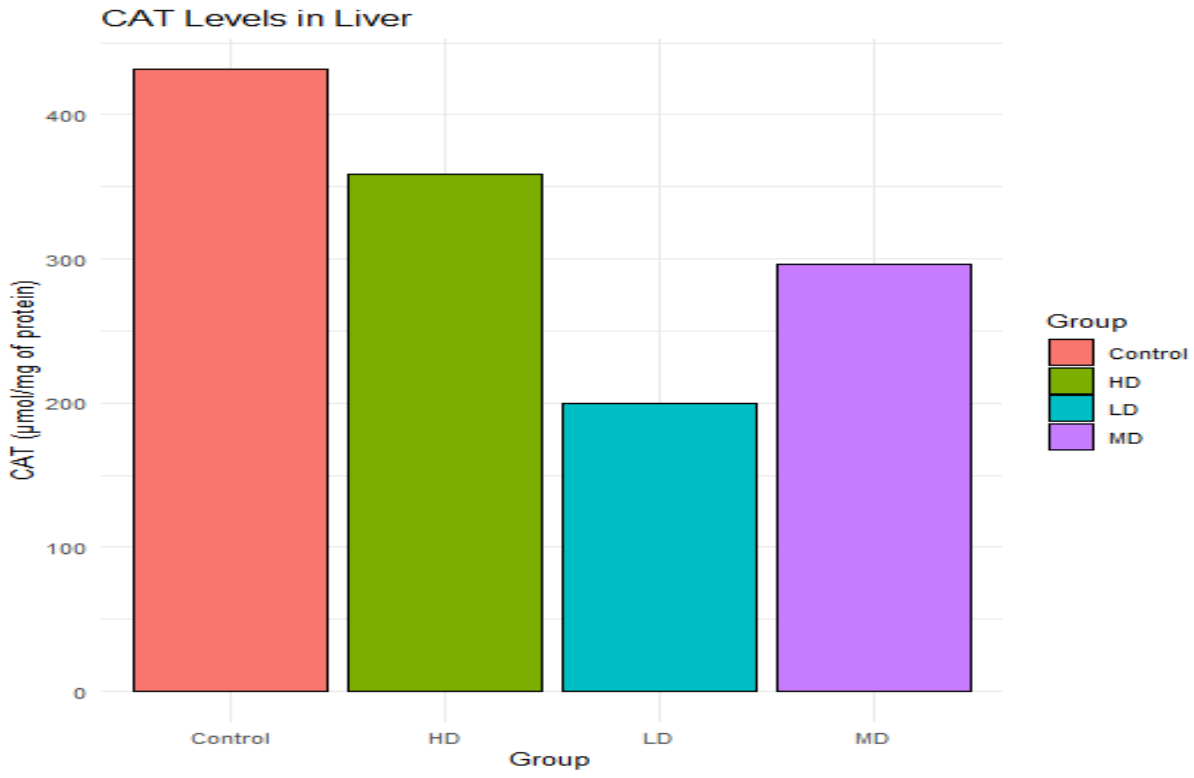
MDA levels were significantly lower in LD and MD groups when compared to the control ($p < 0.0001$, $F = 304.32$). The HD group exhibited a significant increase in MDA levels compared to LD and MD ($p < 0.0020$, $t = 9.22$).

Superoxide Dismutase (SOD) Levels in Liver



SOD levels were significantly lower in LD groups compared to the control ($p < 0.0001$, $F = 16778.55$). The HD group showed a higher SOD level than the MD group ($p < 0.0008$, $t = 7.54$)

.**Catalase (CAT) Levels in Liver**



CAT levels were significantly lower in LD and MD groups when compared to the control ($p < 0.0001$, $F = 22864.72$). The HD group had slightly increased CAT levels compared to MD but remained lower than the control ($p < 0.002$, $t = 6.87$)

Effects of orally administered alcohol and glutathione on the histology of the Liver

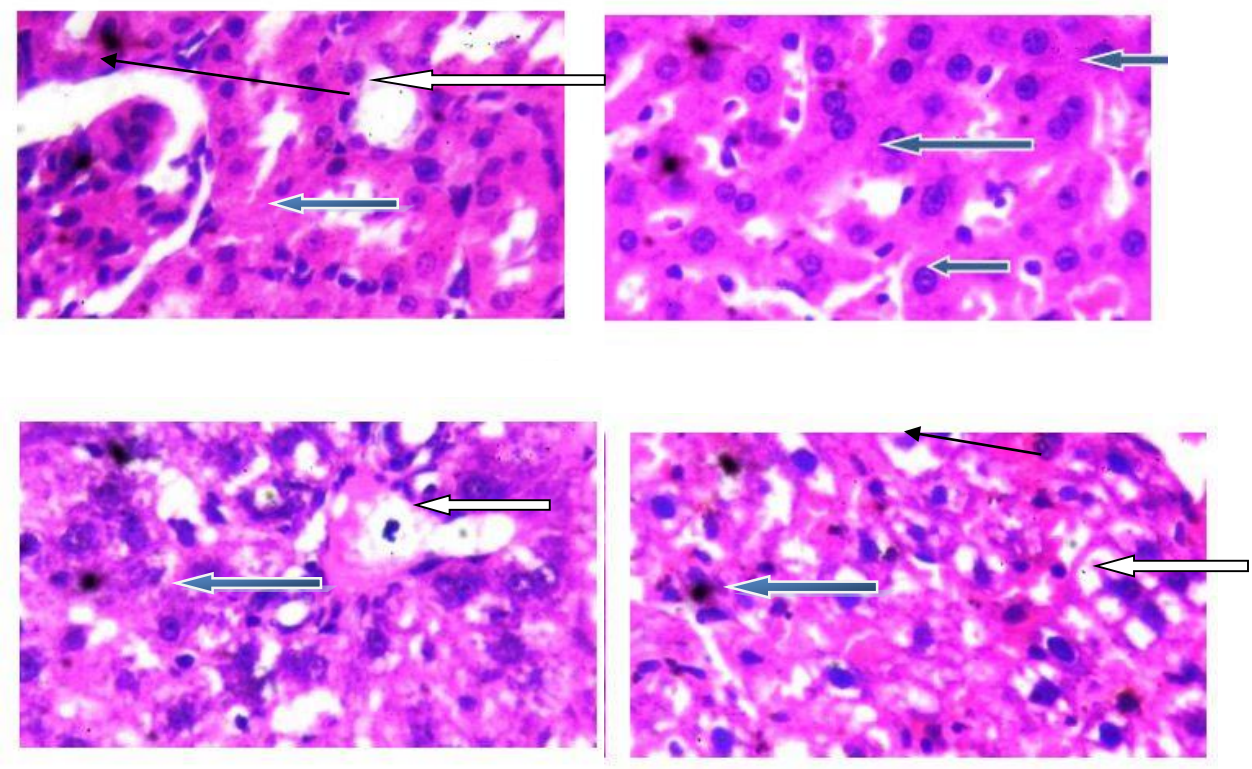


Plate A (Control) Plate B (Low dose) Plate C (Medium dose) Plate D (High dose)

Plate A: Photomicrograph of a liver section stained by Haematoxylin & Eosin showing normal liver parenchyma, the hepatocytes show normal morphology (blue arrow) and the venules appear normal (white arrow). The sinusoids appear normal (slender arrow)

Plate B: Photomicrograph of a liver section stained by Haematoxylin & Eosin showing normal liver parenchyma, the hepatocytes show normal morphology (blue arrow) and the venules appear normal (blue arrow). The sinusoids appear slightly dilated with attendance of red cells (blue arrow)

Plate C: Photomicrograph of a liver section stained by Haematoxylin & Eosin showing liver parenchyma, the hepatocytes show moderate to severe cytoplasmic vacuolation (blue arrow) and the venules appear normal (white arrow). The sinusoids appear mildly infiltrated by inflammatory cells.

Plate D: Photomicrograph of a liver section stained by Haematoxylin & Eosin showing liver parenchyma, the hepatocytes show moderate to severe hepatic steatosis (blue arrow) and the

venules appear normal (white arrow). The liver tissue shows extended area of fat degeneration (green arrow). The sinusoids appear normal (slender arrow)

Effects of orally administered alcohol and glutathione on the kidney

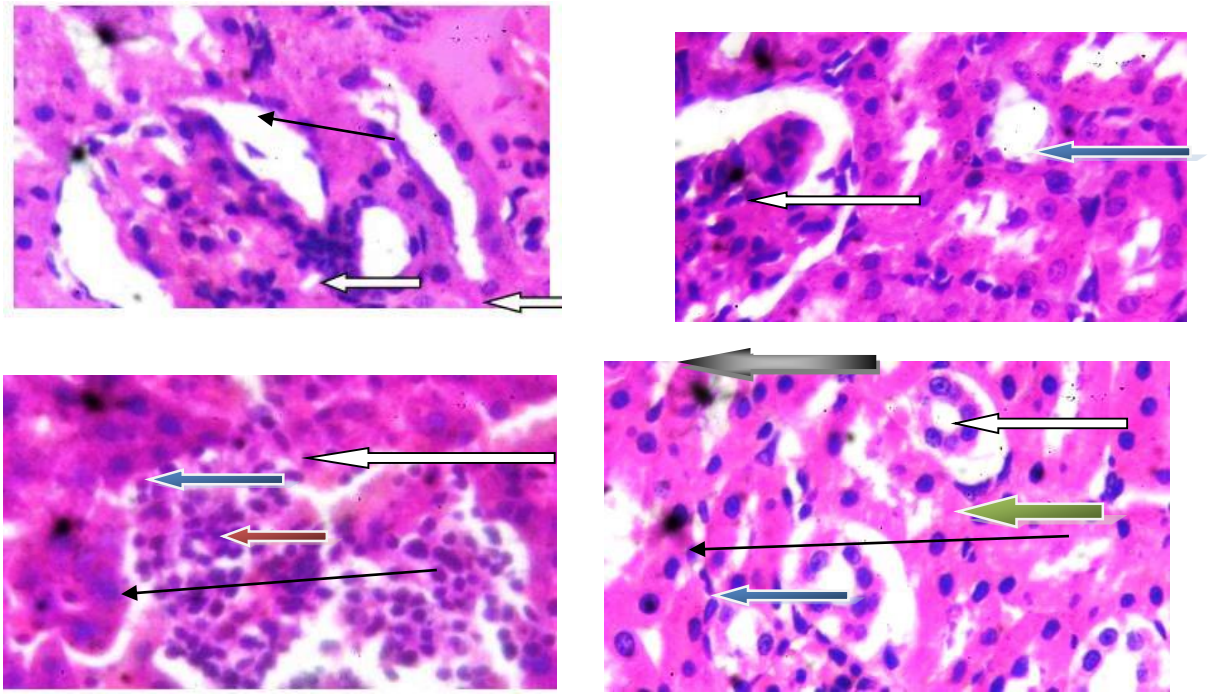


Plate A (Control) Plate B (Low dose) Plate C (Medium dose) Plate D (High dose)

Plate A: Photomicrographs of kidney sections stained by Haematoxylin and Eosin showing the renal cortex with normal glomeruli showing normal mesangial cells and capsular spaces (white arrow), there are normal renal tubules lined by normal cuboidal epithelial cells seen (blue arrow), the interstitial spaces appear normal (slender arrow)

Plate B: Photomicrographs of kidney sections stained by Haematoxylin and Eosin showing the renal cortex with normal glomeruli showing normal mesangial cells and capsular spaces (white arrow), there are normal renal tubules lined by normal cuboidal epithelial cells seen (blue arrow), the interstitial spaces show focus of infiltrates.

Plate C: Photomicrographs of kidney sections stained by Haematoxylin and Eosin showing the renal cortex with normal glomeruli showing normal mesangial cells and capsular spaces (white arrow), there are normal renal tubules lined by normal cuboidal epithelial cells (blue arrow) and few tubules show eosinophilic cast within their lumen (red arrow), the interstitial spaces show moderate aggregate of inflammatory cells (slender arrow)

Plate D: Photomicrographs of kidney sections stained by Haematoxylin and Eosin showing the renal cortex with normal glomeruli showing (white arrow) glomeruli with hyperplasia and vacuolation (black arrow) seen, there are normal renal tubules lined by normal cuboidal epithelial

cells seen (blue arrow) and few tubules with epithelial desquamation (green arrow), the interstitial spaces appear mildly infiltrated by inflammatory (slender arrow)

Discussion

The long-term use of alcohol has been extensively studied for its detrimental effects on the liver and kidney function, primarily due to oxidative stress and the depletion of antioxidant defenses (Roseline *et al.,* 2024). Glutathione plays a crucial role in detoxifying harmful metabolites in the liver. Alcohol can impair the synthesis and recycling of GSH, leading to a state of GSH depletion in the liver. Alcohol also affects the kidneys, leading to damage to renal cells (Bergasa,2021). Chronic alcohol use can cause inflammation and fibrosis in the kidneys. GSH helps prevent these pathological changes by modulating the inflammatory pathways, it also protects renal tissue by preventing oxidative damage (Ratliff *et al.,* 2016). In the liver function test, Alanine Aminotransferase is elevated among the groups and it indicates hepatocellular damage, Increased AST reflects cell damage and may indicate a more extensive or systemic tissue injury from alcohol. ALP may indicate cholestasis or bile duct obstruction (Maindonald and Jugdoyal 2020). Creatinine, an essential test that measured how well the kidneys filtered waste from the blood. It is closely related with renal function. In rats treated with alcohol, creatinine levels increased due to synergistic toxic effects (Costa-Valle *et al; 2018*). Glutathione may help in restoring kidney filtration. Urea is a significant metabolic product that contains nitrogen, urea levels increased which points to renal dysfunction, most likely brought on by inflammatory reactions and oxidative stress (Alani, 2021).

The sharp increase in albumin levels suggests potential nephrotic changes associated with high-dose exposure. Protein leakage, a defining feature of nephrotic-like changes in rats, can also be the cause of the sharp rise in albumin levels in rats treated with alcohol and glutathione afterwards. This study reveals that the administration of alcohol and glutathione significantly increased Malondialdehyde (MDA) levels in the liver. MDA is a product of lipid peroxidation, a key indicator of oxidative stress damage to cell membranes (Rilwanu, 2021). Alcohol consumption can deplete glutathione levels by impairing the liver antioxidant defense. The MDA levels in the kidney indicates a dose reduction in the medium dose and low dose with a slight increase in the high dose, which means the test has a great effect on lipid peroxidation (Ogbanya *et al.,* 2024). The significant reduction in hepatic superoxide dismutase levels observed in the low dose suggests that the administered dose was insufficient to counteract alcohol-induced oxidative stress. The high dose showed a higher level than the medium dose because the medium dose provided partial protection against alcohol-induced oxidative stress while then higher dose effectively replenished hepatic GSH levels (Contreras-Zentalla *et al.,* 2022). The level of SOD activity was increased in the low dose group of the kidney; the result indicates that the treatment enhanced antioxidant enzyme activity but may induce stress at higher concentrations. Catalase, an essential enzyme that protects cell from oxidative damage, increased following the alcohol and glutathione treatment in

the high dose group in the liver and in the low dose of the kidney because the findings demonstrate that the treatment boosts antioxidant defense (Anwar *et al*., 2024).

Conclusion

Chronic alcohol intake significantly impairs liver and kidney function by triggering oxidative stress and weakening the body's antioxidant defenses. Studies in adult male Sprague-Dawley rats have shown that long-term ethanol exposure lowers glutathione levels, diminishes glutathione peroxidase activity and elevates oxidative stress markers such as protein carbonyls and lipid peroxidation. These biochemical alterations not only contribute to liver dysfunction but may also compromise kidney health. Since glutathione plays a central role in counteracting oxidative stress, antioxidant supplementation emerges as a potential strategy to protect against alcohol-induced toxicity. Gaining a deeper insight into these mechanisms could help in the developing effective therapies to reduce alcohol-related oxidative damage and safeguard overall organ function.

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Source of Funding

All of the participating authors provided funding for this study.

Conflict of Interest

The authors affirm that they have no competing interest regarding the publication of this study.

Ethical Approval

The approval of this study was obtained from the Ethics and Research Committee of Bowen University (BUI/COHES/ANA/25-003)

Acknowledgement

The authors are grateful to College of Health Sciences of Bowen University for providing the facilities needed to carry out this study.