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Swimming Away the Damage: Exercise Combats Alcohol-Induced Liver Stress in Wistar Rats

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Abstract

This study investigated the impact of forced swimming exercise on alcohol-induced oxidative stress biomarkers and liver histo-architecture in Wistar rats (mean weight 150 to 200g). The rats were randomly assigned to four groups of five: normal control, exercise only, alcohol only, and exercise plus alcohol. The control group received 0.9% saline for 5 days a week over 12 weeks. The exercise group underwent forced swimming for 5 minutes daily, 5 days a week, for 12 weeks. The alcohol group was given 20% ethanol orally at a dose of 2.0 g/kg body weight. The exercise plus alcohol group received both treatments as described for the exercise and alcohol groups. At the end of the exposure period, all animals were euthanized, and blood and liver tissue samples were collected for analysis of oxidative stress biomarkers (SOD, CAT, GPx, and MDA) and liver histo-architecture. The ethanol-exposed group showed significantly elevated oxidative stress markers, whereas the normal, exercise, and exercise plus alcohol groups exhibited decreased levels. Marked hepatocellular necrosis and perivascular inflammation were observed in the ethanol group, along with moderate central vein congestion. In contrast, the normal and exercise groups displayed normal hepatocellular architecture with no inflammatory cells and clear central veins. The exercise plus alcohol group showed largely normal liver architecture, with very mild necrotic cells, no inflammatory cells, and a clearly visible central vein, indicating that exercise

mitigated alcohol-induced liver changes in adult male Wistar rats. These findings suggest that exercise training can alleviate oxidative stress and prevent liver architecture damage following chronic alcohol consumption, potentially helping to prevent liver failure and hepatocellular carcinoma.

Keywords: Alcohol-induced liver damage, Oxidative stress biomarkers, Forced swimming exercise, Wistar rats, Hepatocellular architecture

INTRODUCTION

Alcohol has been an integral part of human culture for thousands of years, consistently ranking among the world's most popular beverages, surpassed only by water and tea. Today, nearly two billion people worldwide consume various forms of alcohol, including beer, wine, spirits, and traditional beverages such as palm wine, burukutu, and ogogoro (Ngongang et al., 2019; Aniemena et al., 2021). The word "beer" itself is derived from the Latin term "bibere," meaning "to drink." Beer production involves the steeping of cereal grains in water, followed by fermentation with yeast (Evans et al., 2022).

However, alcohol consumption poses significant public health challenges worldwide. Alcoholic Liver Disease (ALD) is the second leading cause of death globally and the most common in industrialized nations (Kilian et al., 2020). In the United States, 75% of the population consumes alcohol, with 7.4% meeting the criteria for alcohol abuse. Each year, alcohol-related disorders lead to 100,000 deaths, with 20% resulting from liver cirrhosis, predominantly affecting men and non-black populations (Ogamba et al., 2021). Similarly, in Europe, alcohol consumption is responsible for 6.5% of all deaths, with one in seven men and one in thirteen women aged 15-64 dying from alcohol-related causes (Burnette et al., 2022). In Asia, particularly India, alcohol is becoming a leading cause of chronic liver disease (Albillos et al., 2022).

In Africa, liver cirrhosis accounts for between 53,000 and 103,000 deaths annually. In Nigeria, chronic liver disease is prevalent, with significant variations across different regions (Ofori et al., 2022). A study conducted in Jos, North Central Nigeria, highlighted a high prevalence of liver cirrhosis, particularly among younger individuals, many of whom had progressed to hepatocellular carcinoma (Enenche et al., 2024).

Alcohol is primarily metabolized in the liver by enzymes such as alcohol dehydrogenase (ADH), which converts ethanol to acetaldehyde. Acetaldehyde is then transformed into acetate by aldehyde dehydrogenase (ALDH). This metabolic process generates reactive oxygen species (ROS), leading to oxidative stress and hepatocellular damage (Yan et al., 2020). Chronic alcohol consumption further exacerbates oxidative stress by inducing the microsomal ethanol-oxidizing system (MEOS), particularly the cytochrome P450 enzyme (CYP2E1). This system produces excessive ROS, triggering inflammation, apoptosis, and fibrosis in hepatocytes (Parola & Pinzani, 2019). The oxidative stress induced by alcohol impairs the body's endogenous antioxidant defenses, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), contributing to the progression of ALD (Fernández-Rodríguez et al., 2022).

Laboratory investigations of ALD often involve the assessment of liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), where an AST/ALT ratio greater than 2:1 is typically observed. These enzymes, usually present at levels below 500 IU/L, provide important diagnostic markers for liver damage (Acharya et al., 2021). Treatment strategies for ALD include alcohol cessation and nutritional support for early-stage disease, while advanced cases may require liver transplantation. Emerging treatments involve the use of bone marrow and hematopoietic stem cells (Zajkowska et al., 2021). Pharmacological interventions, such as silymarin, S-adenosyl methionine, infliximab, and etanercept, have also been explored, although corticosteroids remain indicated for severe liver inflammation (Tornai et al., 2020).

Exercise has numerous health benefits, influencing almost every organ and tissue. It enhances immune function, reduces the risk of chronic diseases like cardiovascular disease and diabetes, and improves mental health by reducing anxiety and depression (Anjum et al., 2022). On a cellular level, exercise activates pathways that regulate metabolism, gene expression, and protein synthesis (Galam et al., 2023). Specifically, exercise has been shown to increase the activity of antioxidant enzymes such as SOD, CAT, and GPx, while reducing oxidative stress markers like malondialdehyde (MDA) (Chaudhary et al., 2023; Rodríguez et al., 2021). Furthermore, exercise has a protective effect on liver histopathology, mitigating alcohol-induced damage and improving liver enzyme levels in patients with nonalcoholic fatty liver disease (NAFLD) (Keymasi et al., 2020). Regular exercise also enhances cognitive health, promoting learning, memory, and resilience to stress (Holden et al., 2020). Due to its comprehensive health benefits, exercise is often

referred to as a "miracle drug" (Baena-Morales et al., 2023). National and international health organizations have recognized these benefits, issuing guidelines to encourage regular physical activity (Xia et al., 2023).

This study aims to explore the potential of exercise as a therapeutic intervention for ALD, offering insights into developing effective strategies to combat the adverse effects of chronic alcohol consumption.

MATERIALS AND METHODS

Materials

- **Chemicals**: Formaldehyde, Xylene, Ethanol, Hematoxylin, Eosin, Paraffin wax, 2-Nitrobenzoic acid, 2-Nitro-5-thiobenzoic acid, 2-Thiobarbituric acid, 30% Trichloroacetic acid, Phosphate buffer (pH 7.0), Dipotassium Hydrogen Phosphate (K_2HPO_4), Dihydrogen Phosphate (H_2PO_4), Hydrogen peroxide.

- **Disposables**: Glass slides, cover slips, hand gloves, sample bottles, cotton wool.

- **Equipment**: JENWAY 6310 Spectrophotometer, Microtome, Thermostatic water bath, Thermometer, Centrifuge, Hand dryer.

- **Other Materials**: Staining jar, plastic cages with wire mesh cover, orogastric tube, rat feeding bottles, beaker, syringe, staining rag, towel.

Ethical Clearance

Ethical clearance was obtained from the ethics committee of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. The ethical guidelines governing the use of laboratory animals for research purposes were strictly adhered to.

Research Animals

Male Wistar rats bred in the Experimental Animal House Unit of the University of Jos, Plateau State, Nigeria, were used for this research.

Preservation, Feeding, and Acclimatization

The Wistar rats were humanely handled and kept in plastic cages. They were fed based on an isocaloric feed formula previously used by the unit, with water provided ad-libitum. The environment was maintained at a relative humidity and temperature of about 25℃, with a natural photoperiod of 12-hour light and 12-hour dark cycles. The animals were allowed a week for acclimatization. The rats' body weights were monitored to ensure a range of 150g – 170g before the commencement of the research.

Research Design

Sample Size

Sample size determination for one-way ANOVA was adopted and n was determined as N=5

Animal Grouping

- Control Group: Rats received 0.9% normal saline daily for 12 weeks.

- Exercise Group: Rats underwent forced swim exercise in a cylindrical tank (60 cm diameter, 100 cm height, 30–45 cm water depth) for 30 minutes/day, 5 days/week for 12 weeks (Ashcroft et al., 2020).

- Alcohol Group: Rats received 20% ethanol at a dose of 2.0 g/kg body weight/day via an orogastric tube for 12 weeks (Husain & Somani, 1997).

- Exercise and Alcohol Group: Rats were exposed to the same forced swim exercise and ethanol regimen as described above.

Specimen Collection and Assessment

Blood Sampling

Blood samples were collected from the jugular vein into plain sample bottles for serum biochemical assays (e.g., oxidative stress biomarkers).

Dissection and Harvesting of Vital Organs

After blood sample collection, the rats were euthanized, and their livers were harvested. Part of the liver was homogenized in phosphate-buffered saline for tissue homogenate, and the other part was immersed in 10% buffered formalin for histological processing.

Evaluation of Samples

Oxidative Stress Biomarkers (Serum and Liver Homogenate)

Lipid Peroxidation (LPO)

Lipid peroxidation was assessed by measuring Malondialdehyde (MDA) levels, following a method described by Lewden (1996):

Catalase Activity

Catalase activity was determined using a the method described by Claiborne (1985):

Superoxide Dismutase (SOD) Activity

SOD activity was measured according to the method described by Beyer (1987):

Histological Analysis

Liver tissue processing followed the method described by Akunna et al. (2008):

Statistical Analysis

Data were expressed as mean ± SEM. One-way ANOVA was used to evaluate significant differences between groups ($p < 0.05$). Tukey post-hoc tests were used for group comparisons using SPSS version 21.

RESULTS

The results are summarized in the following tables1 and 2 for biochemical parameters while plate I-IV shows photo-micrograph of the liver histology:

The study observed significant differences in hepatic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) activities across different groups.

Table 1 : Effect of exercise on alcohol induced changes in serum oxidative stress bio-markers.

Parameters (nmol/mg prtin) (IU/mg prtin)	Group- 1	Group-2	Group- 3	Group- 4
SOD	23.99±1.09[a]	25.64±1.23[b]	34.43±3.30[c]	27.26±0.90[a]
CAT	11.57±0.01[a]	10.82±1.08[a]	21.75±0.88[b]	12.38±0.88[a]
GPx	1.52±0.08[a]	1.76±0.36[a]	3.32±0.44[b]	2.25±0.69[a]
MDA	1.02±0.54[a]	1.30±0.31[a]	4.48±0.67[b]	1.79±0.65[a]

Data analyzed by one way ANOVA followed by turkey post hoc test.

Value expressed with different superscript alphabet letter a,b,c and d are statistically significant (P<0.05)

KEY: Prtin= protein

- SOD Activity: Serum SOD activity significantly increased (P<0.05) in Group III (23.7 ± 1.12 IU/L) compared to Groups I, II, and IV. The lowest activity was observed in Group I (14.05 ± 1.53 IU/L).

- CAT Activity: Serum CAT activity was significantly higher (P<0.05) in Group III (15.72 ± 0.74 IU/L) compared to Groups I, II, and IV, with Group II showing the lowest activity (11.87 ± 0.89 IU/L).

- GPx Activit: Serum GPx activity significantly increased (P<0.05) in Group III compared to Groups I and II, with a significant decrease (P<0.05) observed in Group IV compared to Group III.

- MDA Concentration: Serum MDA concentration was significantly elevated (P<0.05) in Group III compared to Groups I and II, but significantly decreased (P<0.05) in Group IV compared to Group III.

Table 2 : Effect of exercise on alcohol induced changes in oxidative stress bio-markers Liver tissue (homogenate).

Parameter (nmol/mg prtin) (IU/mg prtin)	Group- 1	Group-2	Group- 3	Group- 4
SOD	14.05±1.53[a]	17.23±0.45[b]	23.78±1.12[c]	22.36±0.68[d]
CAT	11.94±0.14[a]	11.87±0.89[a]	15.72±0.74[b]	13.41±0.83[a]
GPx	1.60±0.29[a]	1.69±0.07[a]	2.91±0.13[b]	2.04±0.67[a]
MDA	1.76±0.23[a]	1.93±0.07[a]	4.26±0.43[b]	2.24±0.55[a]

- SOD Activity: There was a significant increase ($P<0.05$) in hepatic SOD activity in Group III compared to Groups I and II. However, Group IV showed a significant decrease ($P<0.05$) in SOD activity compared to Group III.

- CAT Activity: The hepatic CAT activity was significantly increased ($P<0.05$) in Group III compared to Groups I, II, and IV, with the highest activity recorded in Group III (21.75 ± 0.88 IU/L) and the lowest in Group II (10.82 ± 1.08 IU/L).

- GPx Activity: The hepatic GPx activity significantly increased ($P<0.05$) in Group III compared to Groups I and II. Although there was no significant difference ($P>0.05$) between Groups III and IV, Group IV showed a 67% decrease in GPx activity compared to Group III.

- MDA Concentration: The hepatic MDA concentration was significantly higher ($P<0.05$) in Group III compared to Groups I and II. Group IV exhibited a significant decrease ($P<0.05$) in MDA concentration compared to Group III.

Effect of Exercise on Alcohol-Induced Changes in Hepatocellular Architecture

- Group I: Photomicrographs showed no apparent pathological lesions, with normal hepatocellular architecture and clearly visible central veins (Plate I).

- Group II: Similar to Group I, no histopathological lesions were observed (Plate II).

- Group III: Photomicrographs revealed marked hepatocellular necrosis with perivascular inflammation and moderate congestion of the central vein (Plate III).

- Group IV: Mild necrosis was observed, with no evidence of inflammation (Plate IV).

Control Group

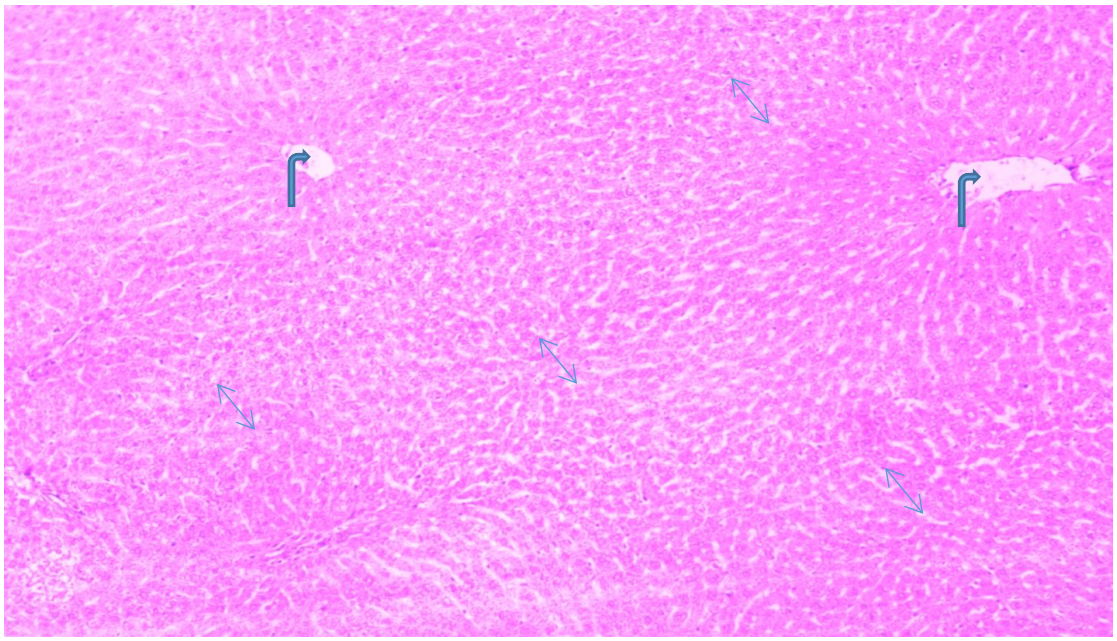

Figure 1

Plate I (Group I): Photomicrograph of the liver showing normal hepatocellular architecture (normal hepatocytes with normal central veins). H & E stain, Mag. X 100.

Key

- Normal central vein
- Normal hepatocellular architecture

Exercise Group

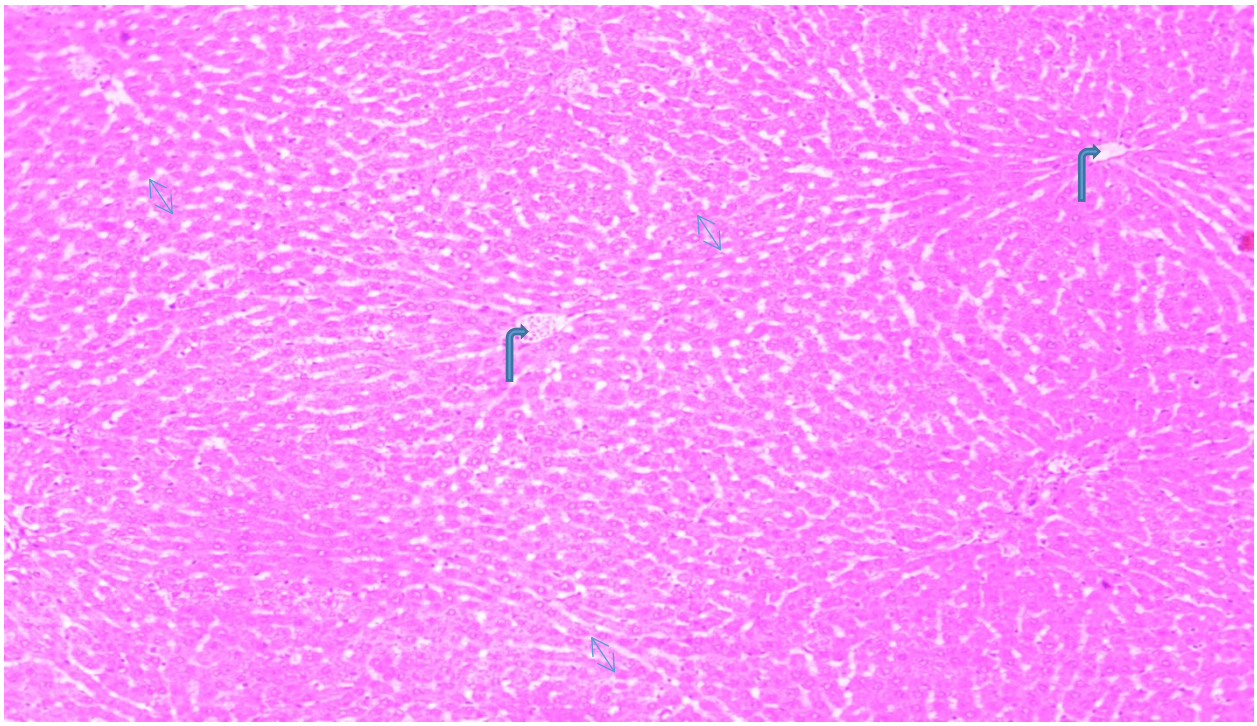

Figure 2

Plate II (Group II): Photomicrograph of the liver showing normal hepatocellular architecture (normal hepatocytes with normal central veins). H & E stain, Mag. X 100.

Key

- Normal hepatocellular architecture

- Normal central vein

Alcohol Group

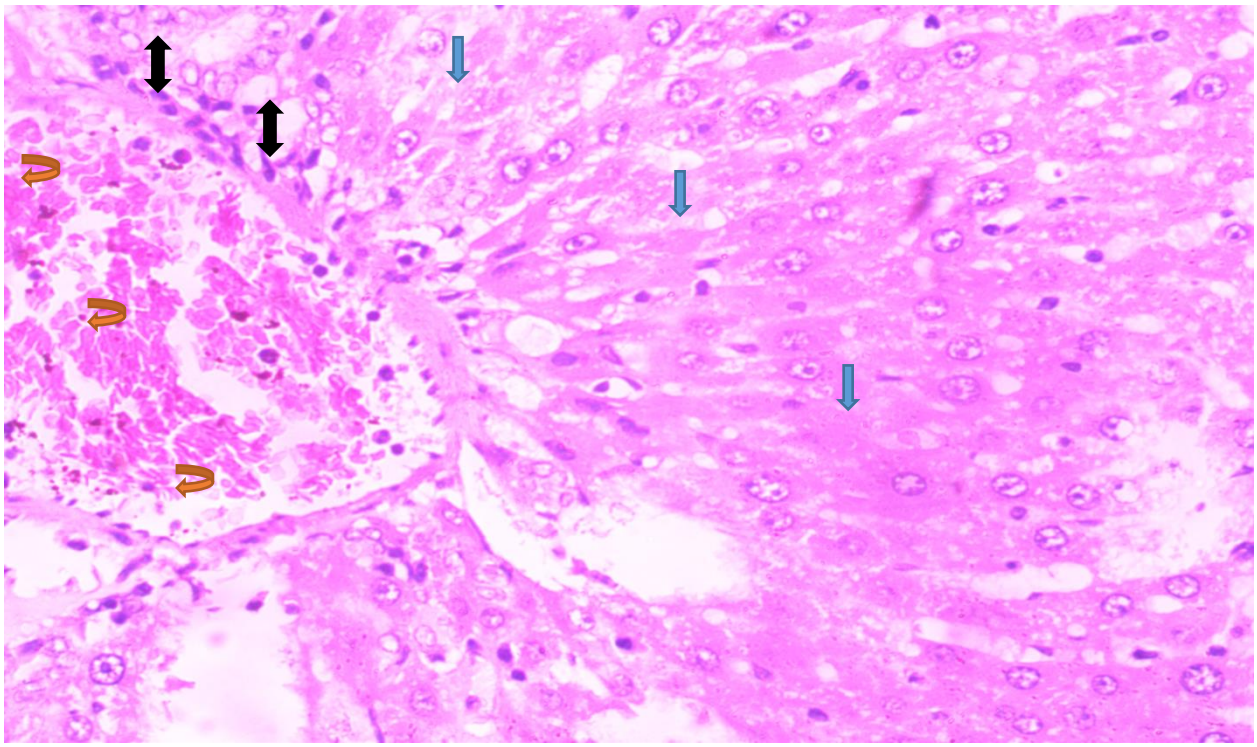

Figure 3

Plate III (Group III): Photomicrograph of the liver showing marked hepatocellular necrosis with mild perivascular inflammation and moderately congested central vein. H & E stain, Mag. X 400.

key

- Necrosis

- Congestion

- Inflammation

Execise and Alcohol Group

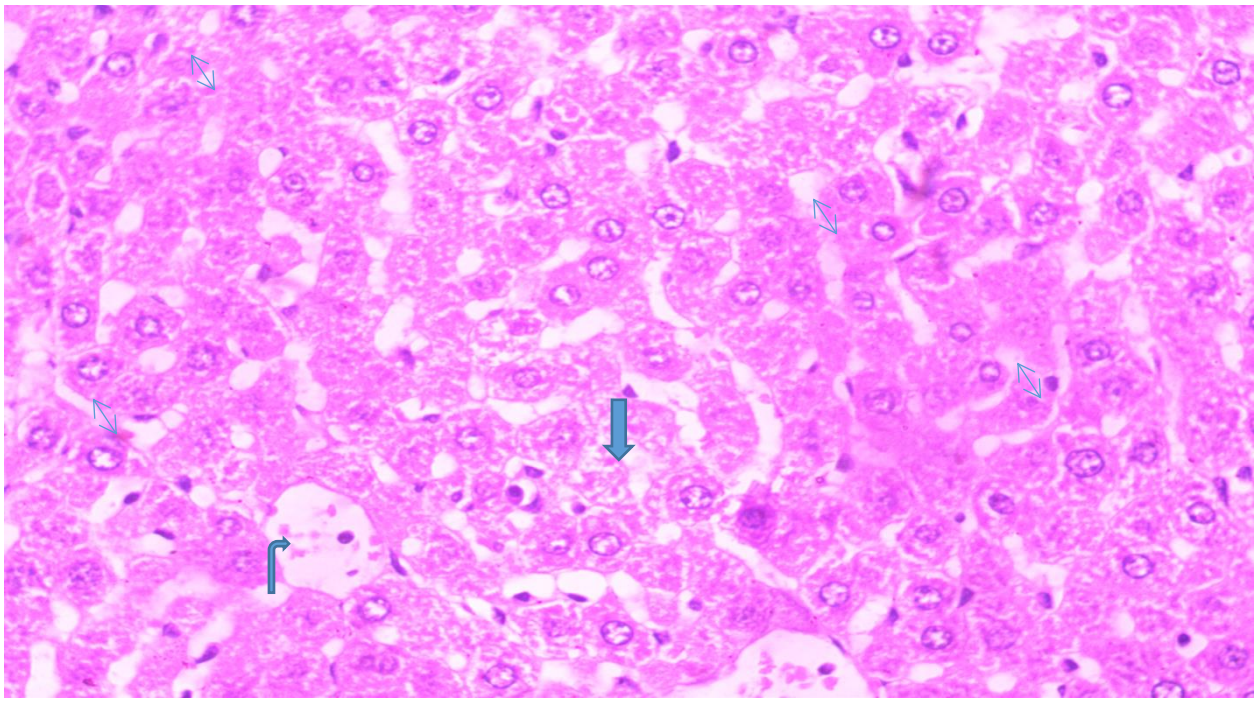

Figure 4

Plate IV (Group IV): Photomicrograph of the liver showing normal hepatocellular architecture with mild necrosis and absence of inflammatory cells. H & E stain, Mag. X 400.

key

- Necrosis

- Normal central vein

- Normal hepatocellular architecture

DISCUSSION

Chronic alcohol consumption is widely recognized as a major cause of hepatic dysfunction. Malondialdehyde (MDA), a key product of polyunsaturated fatty acid peroxidation, serves as a crucial marker of lipid peroxidation induced by reactive oxygen species (ROS). In this study, the elevated serum and hepatic homogenate MDA concentrations observed in alcohol-exposed rats align with findings by Ibrahim et al. (2022), who reported that ROS

activates c-Jun N-terminal kinase (JNK), leading to the expression of the activator protein 1 (AP-1) transcription factor. This sequence contributes to cellular hyper-regeneration and lipid peroxidation, resulting in increased MDA production. Similarly, Shakya et al. (2020) demonstrated that ethanol-mediated CYP2E1 induction leads to heightened ROS levels, lipid peroxidation, and the accumulation of MDA and 4-hydroxynonenal (4HNE).

In this study, forced exercise effectively mitigated the elevated MDA concentrations induced by chronic alcohol consumption. This effect may be attributed to the physiological adaptations triggered by exercise, such as enhanced skeletal muscle blood flow, increased oxygen consumption, and improved fatty acid mobilization and transport to tissues. Simioni et al. (2018) also noted that moderate exercise enhances antioxidant defenses and reduces liver lipid peroxidation, supporting the protective role of exercise observed in this study.

Chronic alcohol consumption was found to increase the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in both serum and hepatic tissues. This increase may be due to the liver's metabolic response to alcohol, which generates ROS and threatens hepatocellular architecture, as evidenced in the alcohol-exposed rats. These findings are consistent with Tan et al. (2020), who reported that alcohol metabolism-induced oxidative stress leads to direct hepatocyte damage through mitochondrial dysfunction and endoplasmic reticulum (ER) stress, activating intrinsic cell death pathways. Additionally, oxidative stress associated with alcohol metabolism has been linked to hepatic carcinogenesis in alcohol-exposed rodents due to DNA oxidation.

The increased activities of SOD, CAT, and GPx observed in this study suggest a compensatory mechanism by the liver to counteract the elevated ROS levels. Specifically, SOD catalyzes the dismutation of superoxide anion (O_2•) into hydrogen peroxide (H2O2), which is then detoxified by CAT or GPx into water and oxygen. Barbosa et al. (2020) noted that this increased activity in antioxidant enzymes may be a response to eliminate excess O_2•. The findings are further supported by Bitanihirwe (2021), who reported that initial high levels of oxidative stress activate antioxidant defenses to scavenge free radicals and prevent harmful chain reactions.

In contrast, a significant decrease in the activities of serum SOD, CAT, and GPx was observed in the forced exercise group. This result may be attributed to increased oxygen consumption during exercise and the breakdown of oxygen at the tissue level during

aerobic respiration. Tian et al. (2023) suggested that regular exercise up-regulates SOD gene expression, enhancing SOD activity in red blood cells while reducing NADPH oxidase-mediated ROS production and oxidative stress. However, antioxidant enzyme activities increase post-exercise to counteract ROS production, indicating that SOD is sensitive to the overproduction of superoxide and hydrogen peroxide (Lu et al., 2021). Exercise also reduces insulin resistance, which is associated with decreased inflammation and oxidative stress (Whillier, 2020).

Histopathological analysis revealed well-preserved liver architecture with mild necrotic changes and a normal central vein in the forced exercise group. This observation is consistent with findings by Adedapo et al. (2020), who reported no abnormal features in the liver histology of exercised rabbits. The protective effects of exercise on liver histoarchitecture highlight its broader health benefits, which extend to influencing metabolism and function in other tissues.

This study underscores the beneficial effects of exercise in counteracting molecular changes induced by chronic alcohol consumption. Although our understanding of the molecular mechanisms underlying exercise-induced liver protection is still developing, this research provides a foundation for future studies. As our knowledge of liver enzyme responses and oxidative stress biomarkers continues to expand, there will be increased opportunities to develop therapeutic strategies to mitigate the adverse effects of chronic alcohol consumption.

CONCLUSION

This study demonstrates that chronic alcohol consumption significantly disrupts hepatic function by increasing oxidative stress, as evidenced by elevated levels of malondialdehyde (MDA) and the heightened activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These changes underscore the detrimental impact of alcohol on liver health, promoting oxidative damage and impairing cellular architecture.

Importantly, the study also highlights the protective role of exercise in mitigating alcohol-induced hepatic damage. Forced exercise was shown to effectively reduce MDA levels and moderate the activities of antioxidant enzymes, suggesting a restoration of the oxidative

balance. Additionally, exercise preserved liver histoarchitecture, reducing necrosis and inflammation, which are commonly associated with chronic alcohol exposure.

The findings suggest that regular exercise could be a valuable intervention to counteract the oxidative stress and hepatic damage induced by chronic alcohol consumption. While further research is needed to fully elucidate the molecular mechanisms involved, this study provides promising evidence that exercise can serve as a preventive measure to protect against alcohol-related liver damage.

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