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Mitigating Alcohol-Induced Liver Enzyme Alterations in Wistar Rats Through Forced Swimming Exercise

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Abstract

This study investigates the impact of forced swimming exercise on alcohol-induced alterations in hepatic enzyme biomarkers in Wistar rats (mean weight 150-200g). Twenty Wistar rats were randomly divided into four groups of five rats each: a normal control group, an exercise-only group, an alcohol-only group, and an exercise plus alcohol group. The control group received 0.9% normal saline 5 days a week for 12 weeks. The exercise group underwent forced swimming for 5 minutes a day, 5 days a week, for 12 weeks. The alcohol group received 20% ethanol orally at a dose of 2.0g/kg body weight. The combined exercise and alcohol group followed the same protocols as the exercise and alcohol groups. At the end of the 12-week period, all animals were euthanized, and blood samples were collected for analysis of hepatic enzyme biomarkers, including ALT, AST, and GGT, using an automated serum biochemistry analyzer. Results showed significant elevation of hepatic enzyme biomarkers in the alcohol-only group, while the normal control, exercise-only, and exercise plus alcohol groups exhibited decreased enzyme levels. These findings indicate that exercise mitigates alcohol-induced liver enzyme alterations in adult male Wistar rats. The study suggests that exercise training may be effective in alleviating liver damage from chronic alcohol consumption and could potentially prevent liver failure and hepatocellular carcinoma.

Keywords: Alcohol, Exercise, Liver enzymes

INTRODUCTION

Alcohol has been a part of human culture for millennia, recognized as the world's oldest and most widely consumed alcoholic beverage, and ranks as the third most popular drink after water and tea. Approximately two billion people worldwide consume alcoholic beverages. The term "alcohol" originates from the Latin word "bibere," meaning "to drink" (Ngongang et al., 2019). Alcohol consumption is a major global public health issue, with Alcoholic Liver Disease (ALD) being the second leading cause of death globally and the most common in industrialized countries (Kilian et al., 2020).

In the United States, an estimated 75% of the population consumes alcohol, with 7.4% meeting the criteria for alcohol abuse. Annually, 100,000 deaths are attributed to alcohol-related disorders, with 20% resulting from liver cirrhosis. The prevalence is higher in men and among non-black populations, although black individuals have a higher incidence of liver cirrhosis (Ogamba et al, 2021). In Europe, alcohol consumption is responsible for 6.5% of all deaths, with recent estimates indicating that one in seven deaths in men and one in thirteen deaths in women aged 15-64 years is due to alcohol consumption. Alcohol Use Disorders (AUD) are the leading cause of liver cirrhosis and are a major cause of death among adults (Burnette et al., 2022).

In Asian countries like India, alcohol is becoming the most common cause of chronic liver disease (Albillos et al., 2022). In Africa, liver cirrhosis results in 53,000 to 103,000 deaths per year. Nigeria reports a high incidence of chronic liver disease, with varying prevalence across different geopolitical areas (Ofori et al., 2022). Studies indicate a high prevalence of liver cirrhosis in Jos, North Central Nigeria, particularly among younger age groups, with many cases progressing to hepatocellular carcinoma (Enenche et al., 2024).

Ethanol is primarily metabolized in the liver through oxidative enzymatic processes involving alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), leading to acetaldehyde and acetate formation. This metabolism generates reactive oxygen species (ROS), resulting in oxidative stress and damage to liver cells, microtubules, and mitochondria (Yan et al., 2020). Chronic alcohol use induces the microsomal ethanol-oxidizing system (MEOS), particularly the cytochrome P450 enzyme (CYP2E1), which produces excessive ROS, recruiting immune cells and pro-inflammatory cytokines that cause inflammation, apoptosis, and fibrosis of hepatocytes (Parola & Pinzani, 2019).

Regular physical exercise is widely recognized for its numerous health benefits, including improved cardiovascular health, enhanced muscle strength, and better overall metabolic function(Galam et al, 2023). Importantly, exercise has also been shown to exert protective effects against oxidative stress(Bloomer, 2008) making it a potential therapeutic strategy for conditions such as Alcoholic Liver Disease (ALD).

Evidence strongly links high alcohol consumption with increased incidence of liver disease (Hammerich & Tacke, 2023). Laboratory investigations of ALD focus on the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), where an AST/ALT ratio greater than 2:1 indicates a deficiency in the enzyme pyridoxine phosphate. Typically, AST and ALT concentrations are less than 500 picograms. ALD encompasses a spectrum from simple steatosis to alcoholic steatohepatitis, progressive fibrosis, and cirrhosis. Although the overall prevalence of ALD remained stable between 2001-2016 (8.1%-8.8%), the proportion of ALD with stage 3 fibrosis and above increased from 2.2% to 6.6%. Consequently, chronic liver disease and cirrhosis rank as the 12th leading cause of death in the United States (Dang et al., 2020).

MATERIALS AND METHODS

Chemicals

The following chemicals were used: 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 (K2HPO4), dihydrogen phosphate (H2PO4), and hydrogen peroxide.

Disposables

Glass slides, cover slips, hand gloves, sample bottles, and cotton wool.

Equipment

JENWAY 6310 spectrophotometer, microtome, thermostatic water bath, thermometer, centrifuge, and hand dryer.

Other Materials

Staining jar, plastic cages with wire mesh cover, orogastric tube, rat feeding bottles, beakers, syringes, staining rag, and towels.

Ethical Clearance

Ethical clearance was obtained from the ethics committee of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. All procedures adhered to ethical guidelines for the use of laboratory animals in research.

Research Animals

Male Wistar rats were sourced from the experimental animal house unit of the University of Jos, Jos Plateau State, Nigeria.

Preservation, Feeding, and Acclimatization

The Wistar rats were humanely handled and housed in plastic cages. They were fed an isocaloric diet and given water ad libitum. Environmental conditions included a relative humidity and temperature of approximately 25℃ and a natural 12-hour light/dark cycle. The animals were acclimatized for one week, and their body weights were monitored to ensure they ranged between 150g and 170g prior to the study.

Research Design

Sample Size Determination

Sample size for one-way ANOVA was determined using the appropriate statistical formula.

Animal Grouping

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

- Exercise Group: Subjected to forced swimming in cylindrical tanks (60 cm diameter, 100 cm height) filled with water (30-45 cm depth) for 30 minutes/day, 5 days/week, for 12 weeks. After swimming, rats were dried with a towel and hand dryer at normal temperature.

- Alcohol Group: Administered 20% ethanol at a dose of 2.0 g/kg body weight/day via an orogastric tube for 12 weeks.

- Exercise and Alcohol Group: Exposed to forced swimming as described for the Exercise Group, followed by ethanol administration as described for the Alcohol Group.

Specimen Collection and Assessment

Blood Sampling

After 12 weeks, all animals were anesthetized with chloroform and euthanized. Blood samples were collected from the jugular vein into plain sample bottles for serum biochemical assays (liver function tests).

Evaluation of Samples

Serum samples were analyzed for liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum glutamate pyruvate transaminase (SGGTP) using an automated serum biochemistry analyzer (Cobas C 111, England). Briefly, 500µl of preserved serum was placed in a 1cm light path cuvette and inserted into the analyzer, standardized at a wavelength of 546 nm and a temperature of 37°C.

Statistical Analysis

Data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used to evaluate significant differences between groups, with a p-value < 0.05 considered significant. Tukey's post-hoc test was applied to evaluate significant differences between groups using SPSS version 21.

RESULTS

Table below: gives the Summary of Enzyme Activities

Table 1: Effect of exercise on alcohol induced changes in transaminases activity

Parameter IU/L	Group-1 (Control)	Group- 2 (Exercise)	Group-3 (Alcohol)	Group- 4 (Exercise+Alcohol).
AST	14.75 ± 0.96^{a}	16.33 ± 0.58^{a}	19.40 ± 1.67^{b}	15.00 ± 1.00^{a}
ALT	8.60 ± 0.89^{a}	$10.50\pm0.71^{a,c}$	12.67 ± 0.58^{b}	11.00 ± 1.00^{c}
GGT	20.40 ± 0.078^{a}	16.55 ± 1.43^{a}	37.38 ± 5.54^{b}	21.89 ± 0.31^{a}

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

Values expressed with different superscript alphabet letter a,b,c are statistically significant ($P<0.05$), N= 5

Aspartate Aminotransferase (AST) Activity

- Group III (Alcohol) showed a significant increase ($P<0.05$) in serum AST activity compared to Group I (Normal Control) and Group II (Exercise).

- The highest AST activity was observed in Group III (Alcohol) (19.40 ± 1.67 IU/L), while the lowest was in Group I (Normal Control) (14.75 ± 0.96 IU/L).

- Group IV (Exercise + Alcohol) had a significantly lower AST activity compared to Group III (Alcohol) ($P<0.05$).

Alanine Aminotransferase (ALT) Activity

- Group III (Alcohol) also showed a significant elevation ($P<0.05$) in serum ALT activity compared to Groups I (Normal Control) and II (Exercise).

- There was a significant decrease in serum ALT activity in Group IV (Exercise + Alcohol) compared to Group III (Alcohol) ($P<0.05$).

Gamma-Glutamyl Transferase (GGT) Activity

- Serum GGT activity was significantly higher ($P<0.05$) in Group III (Alcohol) compared to Groups I (Normal Control) and II (Exercise).

- The highest GGT activity was in Group III (Alcohol) (37.38 ± 5.54 IU/L), and the lowest was in Group II (Exercise) (16.55 ± 1.43 IU/L).

DISCUSSION

Chronic alcohol consumption is widely recognized for its detrimental effects on liver function. The present study demonstrates that chronic alcohol intake significantly elevates liver enzyme activities (ALT, AST, and GGT), which are key biomarkers for hepatocyte injury. These findings align with previous research, such as Yang et al. (2019), which indicated that ethanol induces hepatotoxicity, leading to increased levels of these enzymes. Specifically, rats in the alcohol-administered group (Group III) exhibited significant increases in ALT, AST, and GGT activities by 67%, 76%, and 55%, respectively, compared to the normal control group.

This elevation in liver enzymes is consistent with the metabolic effects of chronic alcohol consumption, as reported by Nguyen et al. (2018). The metabolic processing of alcohol, particularly through cytochrome P450 2E1, increases serum transaminase levels. Similarly, Saka (2020) reported elevated AST and ALT levels in rabbits administered ethanol, attributing the rise to ethanol's ability to disrupt biomembranes, increasing their fluidity and causing enzyme release (Jiang et al., 2020). The liver, being the primary organ for detoxification, generates potentially harmful by-products, including free radicals, which contribute to hepatocyte damage and subsequent elevation of ALT, AST, and GGT levels (Kumar et al., 2024).

ALT is primarily a liver-specific enzyme involved in converting food into energy. Elevated serum ALT levels typically indicate liver cell injury or irritation, likely induced by chronic alcohol consumption (Bhagriya, 2022). AST, while primarily found in the liver, is also present in the heart and skeletal muscles, with increased levels observed in conditions such as myocardial infarction and muscle trauma (Lv et al., 2022). GGT, present in the liver, kidney, pancreas, heart, and brain, when elevated, indicates hepatic dysfunction (Ofori et al., 2022).

The study also found that exercise significantly reduced the elevated levels of these liver enzymes in the alcohol-exposed rats. This suggests a hepatoprotective effect of exercise, supporting findings by Jowhari et al. (2022) that exercise reduces AST, ALT, and GGT in non-alcoholic fatty liver disease. The beneficial effects of exercise may be attributed to the increased force production and energy demand during muscle contraction, which activates metabolic pathways responsible for ATP generation and impacts liver transaminase activity. Silva et al. (2022) also noted that endurance training positively affects energy metabolism and liver enzymes, with transcriptional responses to exercise playing a crucial role in controlling liver transaminase and oxidative stress biomarkers.

This research highlights the potential benefits of exercise in mitigating liver damage caused by chronic alcohol consumption. Although our understanding of the molecular mechanisms underlying the protective effects of exercise on liver enzymes is still developing, this study provides a foundation for further research. As our knowledge expands, there will be more opportunities to utilize exercise as a therapeutic strategy to combat the adverse effects of chronic alcohol consumption, which poses significant public health challenges.

CONCLUSION

The findings of this study reveal that chronic alcohol consumption elevates liver enzyme activities, indicative of hepatic injury. However, regular exercise significantly mitigates these effects, suggesting a protective role against alcohol-induced liver damage. Further research into the molecular mechanisms involved may enhance our ability to leverage exercise in preventing and treating liver diseases associated with chronic alcohol consumption.

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