Assessment of Hepatoprotective Potential of a Sinapic Acid on Animal Model of Alcohol Induced Hepatotoxicity

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Abstract

Alcoholic liver disease (ALD) is a leading cause of death among patients with chronic liver disorders. Among the underlying factors contributing to its progression are oxidative stress and pyroptosis. Sinapic acid (SA), a naturally occurring phenolic compound found abundantly in grains, oilseeds, fruits, and vegetables, possesses notable anti-inflammatory, antioxidant, and antimicrobial activities. It is also recognized as a bioactive component in several traditional Chinese medicinal formulations. The present study was designed to investigate the liver-protective potential of sinapic acid in a rat model of ethanol-induced hepatotoxicity. Liver injury was experimentally triggered in Wistar albino rats via prolonged alcohol exposure. To determine the efficacy of SA, various biochemical parameters were analyzed, including serum levels of SGOT, SGPT, ALP, total cholesterol, triglycerides, and HDL. Furthermore, oxidative stress markers were assessed in vivo, such as reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD). Liver tissues were also subjected to histopathological evaluation to examine structural integrity and cellular damage. The results demonstrated that sinapic acid significantly alleviated ethanol-induced liver damage, likely by modulating oxidative stress and inflammation. This study provides novel insights into the therapeutic role of sinapic acid and suggests its promise as a potential treatment strategy for alcohol-related liver disorders, possibly through the suppression of oxidative injury and pyroptosis.

1. INTRODUCTION:

The liver plays an indispensable role in maintaining physiological balance through its diverse functions, including detoxification, biosynthesis, secretion, metabolism, and nutrient storage. However, this vital organ is vulnerable to damage from various pathological conditions such as diabetes, viral infections, and prolonged exposure to harmful substances. One of the most concerning forms of liver injury is alcohol-induced liver injury (ALI), which has become increasingly widespread due to chronic and excessive alcohol consumption [1, 2]. In recent years, the prevalence of ALI has escalated significantly, making it a major contributor to liver-related illnesses and deaths worldwide [3]. This condition places an enormous strain on public health systems

and economic resources. The progression of ALI involves a complex interplay of pathological mechanisms, with oxidative stress identified as a key factor. During alcohol metabolism, the body generates excessive reactive oxygen species (ROS), which initiate lipid peroxidation, DNA damage, and suppress the activity of antioxidant enzymes leading to liver cell injury and dysfunction [4-6].

Furthermore, alcohol consumption impairs lipid metabolism, resulting in fat accumulation within liver cells and contributing to the development of hepatic steatosis [7]. The gut–liver axis, a vital communication network connecting the gastrointestinal tract and liver via the portal vein and biliary channels, is also significantly involved in the pathogenesis of ALI [8]. The gut microbiota, an essential component of this axis, plays a crucial role in liver health. Alcohol disrupts the balance of intestinal microbiota, reducing the production of beneficial short-chain fatty acids (SCFAs), which compromises liver function and enhances disease progression [4, 9-11]. Although drugs like disulfiram, naltrexone, acamprosate, and nalmefene are commonly used to treat alcohol dependency and associated liver conditions, their therapeutic use is often limited due to adverse effects and suboptimal long-term outcomes. Consequently, there is a growing need to explore safer, more effective treatment options that can offer sustainable relief and protection against alcohol-related liver damage [12].

Sinapic acid (SA), also referred to as 4-hydroxy-3, 5-dimethoxycinnamic acid, is a naturally occurring phenolic acid widely distributed in plant-based sources such as oil crops, cereals, vegetables, and berries. It also constitutes an active component in several formulations used in traditional Chinese medicine. Extensive studies have highlighted its notable pharmacological properties, including antioxidant, anti-inflammatory, and antimicrobial effects [13–15]. Evidence suggests that SA effectively attenuates oxidative stress in hepatic and colonic tissues of rats fed a high-fat diet, and plays a role in reducing pyroptosis in diabetic atherosclerosis models [16]. Furthermore, findings by Shin et al. indicate that SA offers protection against liver inflammation induced by carbon tetrachloride (CCl_4) and possesses antifibrotic effects in liver damage caused by dimethylnitrosamine (DMN). However, the therapeutic potential of SA in combating alcohol-related liver disease has yet to be thoroughly investigated [17].

Oxidative stress plays a pivotal role in the onset and progression of alcoholic liver disease (ALD), arising from an imbalance between excessive reactive oxygen species (ROS) production and the body's capacity to neutralize them through its antioxidant defenses [18]. The nuclear factor erythroid 2–related factor 2 (Nrf2) is a key transcription factor involved in cellular protection, known to regulate antioxidant and anti-inflammatory pathways by upregulating heme oxygenase-1 (HO-1) and related protective enzymes [19]. Beyond oxidative damage, several types of programmed cell death contribute to the

complex pathogenesis of ALD. Among these, pyroptosis an inflammation-linked form of cell death has emerged as an important player, offering promising new directions for therapeutic intervention [20]. Due to the side effects and limitations associated with many synthetic medications, there is an increasing focus on natural compounds with potential hepatoprotective effects. In this regard, sinapic acid has gained research interest for its demonstrated ability to combat various liver-related conditions, supporting its potential as a safer alternative for liver disease treatment [21, 22].

The primary objective of this study was to evaluate the hepatoprotective effects of sinapic acid. For this purpose, an alcohol-induced liver injury model was developed in Wistar albino rats. Liver function was assessed by analyzing various biochemical parameters, including serum levels of SGOT, SGPT, ALP, total cholesterol, triglycerides, and HDL. In addition, the in vivo antioxidant capacity was evaluated through measurements of reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD). To support the biochemical data, liver tissues were subjected to histopathological examination to observe structural changes and further validate the protective effects of sinapic acid on liver architecture and function.

2. MATERIAL AND METHODS:

2.1. Drugs and Chemicals:

Sinapic acid was procured from P.C. Chem India, Trichloroacetic acid, Thiobarbituric acid, Hydrogen peroxide, Pyrogallol, Ethanol and Sulphosalicylic acid from LOBA Chemie Pvt. Ltd. ALT Kit, AST kit, ALP kit, Cholesterol Kit, Triglyceride Kit, and HDL Kit this all kit porches from Vishal Diagnostics.

2.2. Experimental animals:

Male Wistar albino rats (6–8 weeks old) were obtained from LACSMI BioFarms (Laboratory Animal Centre for Safe Medical Innovations), located at #12, "Rachana Blossom," Jagdishnagar, Aundh, Pune – 411 007, Maharashtra, bearing CCSEA Registration Number: 1277/PO/RcBt/S/09/CCSEA. The animals were housed in the animal facility at MET's BKC Institute of Pharmacy in polypropylene cages lined with rice husk bedding. Standard laboratory conditions were maintained, including a controlled temperature of 25 ± 2 °C, a 12-hour light/dark cycle, and relative humidity of 55 ± 5%. The rats were provided with unrestricted access to standard pellet feed and drinking water. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee of MET's BKC Institute of Pharmacy (Approval No. MET-IOP-IAEC/2023-24/06), and the study was conducted in full compliance with the guidelines laid down by the Committee for Control and Supervision of Experiments on Animals (CCSEA).

2.3. Experimental design:

Animals were divided into 6 groups of six Wistar Albino Rats each. The experimental design given below has been followed for active phytoconstituent selected for the study. Adult Wistar Albino Rats, weighing about

150-200g (n=6) were used as an experimental model.

The experimental design for the selected active phytoconstituent (sinapic acid) is as follows:

Group I (normal control): Received normal food and water.

Group II (disease control): Received 40% alcohol (3 mL/day, orally, twice daily) for 21 days.

Group III (standard): Received silymarin (50 mg/kg, orally) + 40% alcohol (3 mL/day, orally, twice daily) for 21 days.

Groups IV–VI (test groups): Received 40% alcohol (3 mL/day, orally, twice daily) + sinapic acid (40, 80, or 100 mg/kg, orally, once daily) for 21 days

The grouping of animals is illustrated [23].

At the end of the study blood was withdrawn via "retro-orbital plexus puncture" and was subjected for the biochemical estimation viz., AST, ALT, ALP, TC, TG, HDL; whereas hepatic tissue homogenate was utilized for the evaluation of antioxidant biomarkers viz., LPO, SOD, Catalase, and GSH. Inflammatory, pro-inflammatory and histopathological tests were performed.

2.4. Alcohol induced hepatotoxicity:

Thirty-six male Wistar rats were used for the experiment. The animals were allowed to acclimatize for 7 days after procurement. Temperature (22±2°C) and humidity (45-55%) were controlled. The animals were kept in a 12-hour light/dark cycle. The study period lasted for approximately 28 days. The 36 animals were divided into 6 groups of six animals each. All rats, except those in Group I, were sensitized with alcohol (40%) at a dose of 3 ml/day twice a day through the oral route for 21 days.

3. EVALUATION:

3.1. Biochemical Analysis:

At the end of study, blood was collected from retro-orbital plexus. The collected blood was centrifuged at 3000 rpm for 10 min at 4°C to separate serum for estimation of liver parameters such as AST/SGOT, ALT/SGPT, ALP, Total cholesterol, Triglyceride and HDL. All these parameters were measured by the Standard kits procedures using Bioanalyser.

3.2. Preparation of tissue homogenate:

The Liver was isolated and rapidly transferred to ice-cold Phosphate buffer (PH 7.4) after sacrification of the rats. The Liver were subsequently cross chopped and homogenized in a Phosphate buffer (PH 7.4). Centrifugation at 10,000 rpm for 15 minutes (using a Remi C-24 high speed cooling centrifuge) at a temperature of 4°C. The supernatant was utilized to measure reduced glutathione, lipid peroxidation, superoxide dismutase and catalase.

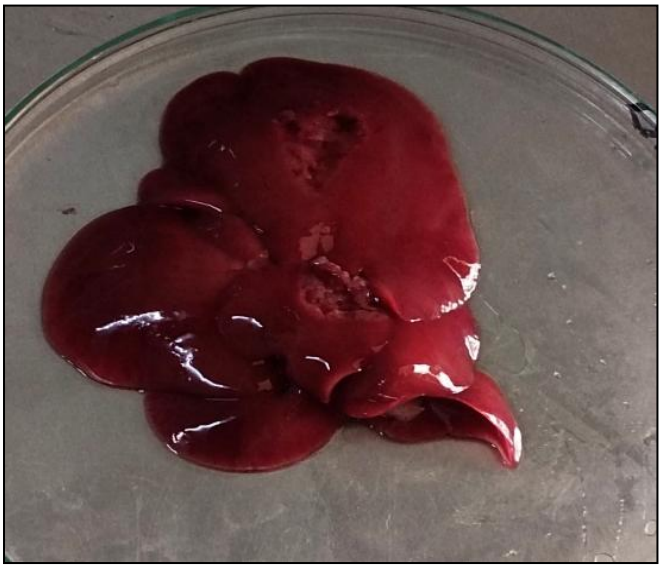

Figure 1: Isolation of Liver

3.3. In vivo antioxidant study:

Estimation of reduced glutathione (RGSH), malondialdehyde (MDA) activity, catalase (CAT) activity, superoxide dismutase (SOD) in homogenized hepatic tissues were examined using commercial kits (Vishal Diagnostics).

3.4. Determination of levels of pro-inflammatory mediators:

Methodology:

3.4.1. RNA Extraction and cDNA Synthesis:

Total RNA was extracted by using TRIZOL method (Sigma, USA) for the gene expression study. The isolated RNA quantification was done by Qubit Fluorometer (Invitrogen, USA). For qRT-PCR analysis, cDNA was synthesized from 2 µg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Invitrogen). Reverse transcription was performed by using thermal cycler (VeritiPro, Applied Biosystems, USA) and PCR conditions are shown in table 1.

Table 1: RNA Extraction and cDNA Synthesis

Settings	Step 1	Step 2	Step 3	Step 4
Temp.	25°C	37°C	85°C	4°C
Time	10 minutes	120 minutes	5 minutes	Hold

3.4.2. Gene Selection:

For this study, selected two target genes includes, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and housekeeping gene, i.e., Glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH). The primers used in the present study are depicted in the Table 2.

Table 2: Table of Gene Selection

Sr. No.	Gene Name	Primer Details	Primer Sequence
Housekeeping gene			
1	GAPDH	Forward	AGTTCAACGGCACAGTCAAG
		Reverse	TACTCAGCACCAGCATCACC
Target Gene			
1	TNF-α	Forward	CTCACACTCAGATCATCTTC
		Reverse	GAGAACCTGGGAGTAGATAAG
2	IL-6	Forward	TAAGCCAACAAGTGGTATTC
		Reverse	AGGTATAGATTCTTCCCCTTG

3.5. Gene Expression Study by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The qRT-PCR was performed using PowerUp™ SYBR™ Green expression assays (Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix, USA) on QuantStudio5 real-time PCR system (Applied Biosystems, USA). In qRT-PCR study, initial UDG activation on 50°C for 2 min.; denaturation was performed at 95 °C for 02 min. This step was followed by the 40 cycles of 95 °C for 15 sec. (denaturation); 57 °C for 60 sec. (annealing and extension) and at 95 °C for 15 sec. 60 °C for 60 sec. 95 °C for 15 sec (dissociation step). The relative amounts of cDNA were normalized to the amount of the endogenous housekeeping control, i.e., GAPDH. Gene expression was given in the fold change. The experiment was performed by delta-delta Ct method (2–ΔCt).

3.6. Histopathological study of liver tissue:

Following standard procedures, the animals were euthanized via cervical dislocation. The liver was carefully excised and immediately fixed in 10% neutral buffered formalin for histopathological examination.

Tissue sections were prepared and stained with hematoxylin and eosin (H&E) to assess structural changes and evaluate the degree of liver fibrosis. All histopathological evaluations were conducted under a light microscope by a board-certified toxicopathologist to ensure accurate interpretation of tissue morphology.

3.7. Statistical analysis:

Data were presented as mean ± standard error of the mean (SEM). Statistical comparisons among groups were carried out using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test to determine significant differences between the control and treatment groups. All statistical analyses were conducted using GraphPad Prism version 8.0. A p-value of less than 0.0001 was considered statistically significant.

4. RESULTS:

4.1. Biochemical Analysis:

4.1.1. Alanine aminotransferase (ALT/SGPT):

The ALT/SGPT level was significantly elevated (P<0.001) in the Disease Control group compared to the Normal Control group. However, treatment with Silymarin (50 mg/kg p.o) and Sinapic acid (40, 80, and 100 mg/kg p.o.) significantly reduced (P<0.001) the ALT/SGPT levels compared to the Disease Control group.

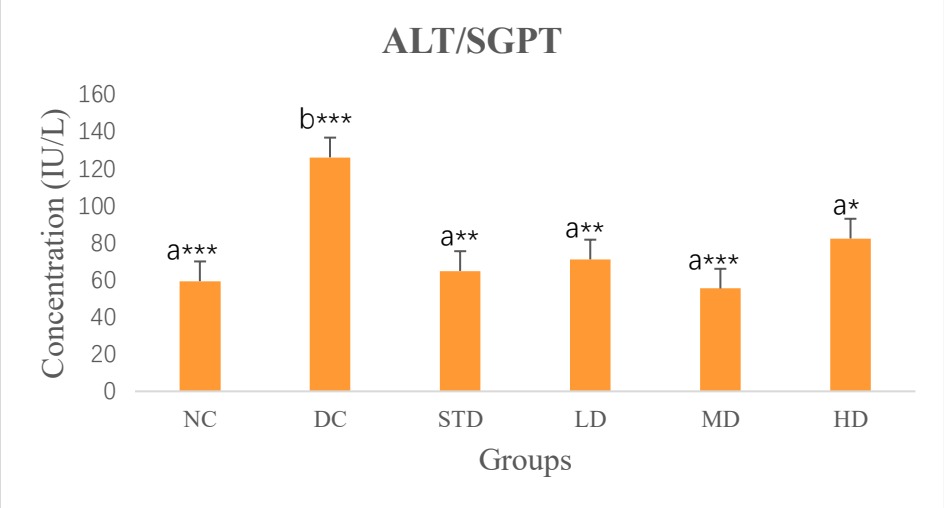

Figure 2: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on ALT/SGPT level (IU/L) in an Alcohol induced hepatotoxicity model in rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data were analyzed using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05.NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.1.2. Aspartate aminotransferase (AST/SGOT):

In Disease Control animals, the AST/SGOT level was significantly increased (P<0.001) compared to Normal Control animals. Treatment with Silymarin (50 mg/kg p.o.) and Sinapic acid (40, 80, and 100 mg/kg p.o.) significantly decreased (P<0.001) the concentration of AST/SGOT compared to the Disease Control group.

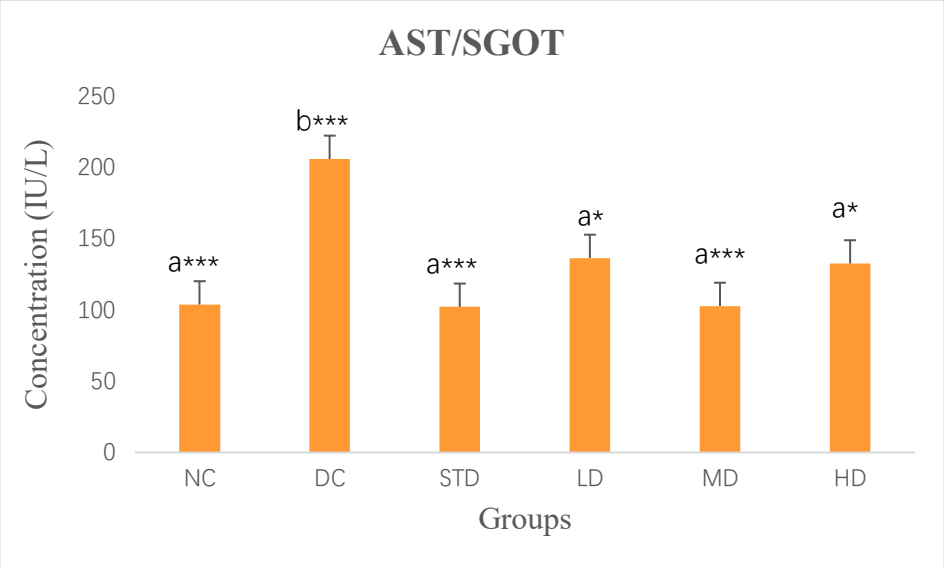

Figure 3: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on level of AST/SGOT (IU/L) in Alcohol induced h

epatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.1.3. Alkaline phosphatase (ALP):

In Disease Control animals, ALP levels were significantly increased (P<0.001) compared to Normal Control animals. Treatment with Silymarin (50 mg/kg p.o.) and Sinapic acid (40, 80, and 100 mg/kg p.o.) significantly decreased (P<0.001) the concentration of ALP compared to the Disease Control group.

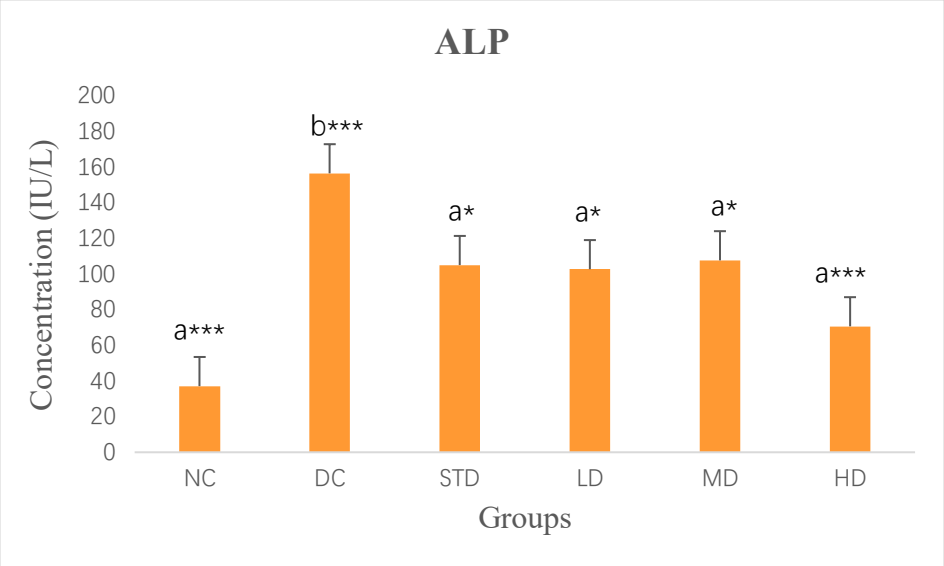

Figure 4: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on level of ALP (IU/L) in Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.1.4. Total cholesterol (TC):

In Disease Control animals, cholesterol levels were significantly increased (P<0.001) compared to Normal Control animals. Treatment with Silymarin (50 mg/kg p.o.) and Sinapic acid (40, 80, and 100 mg/kg p.o.) significantly decreased (P<0.001) the concentration of cholesterol compared to the Disease Control group.

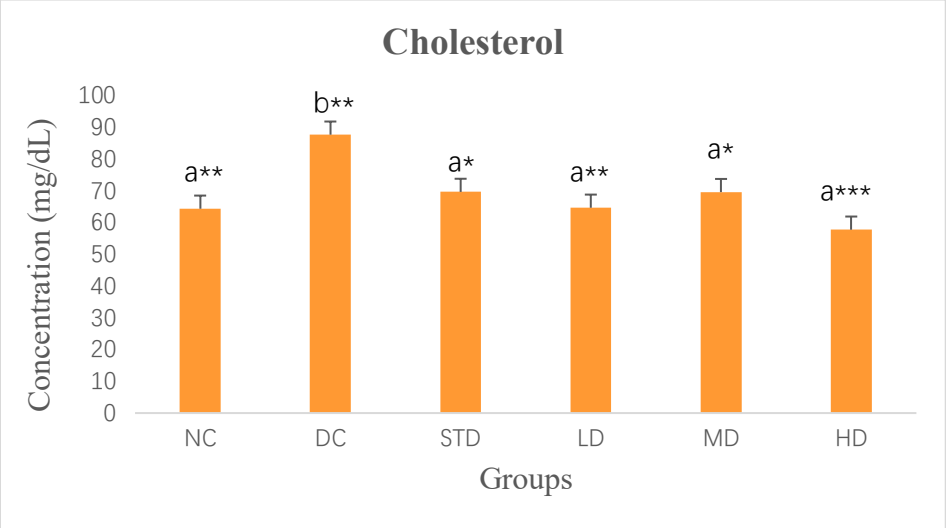

Figure 5: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on level of Cholesterol (mg/dL) in Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.1.5. Triglycerides (TG):

In Disease Control animals, triglyceride levels were significantly increased (P<0.01) compared to Normal Control animals. Treatment with Silymarin (50 mg/kg p.o.) and Sinapic acid (40, 80, and 100 mg/kg p.o.) showed a non-significant decrease in triglyceride levels compared to the Disease Control group; only the medium dose group showed a significant decrease.

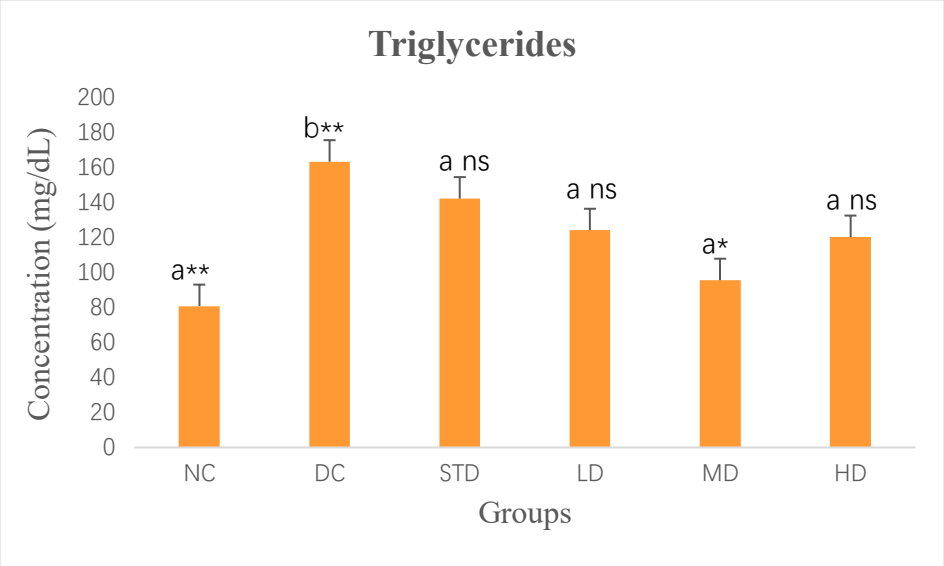

Figure 6: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on level of Triglyceride (mg/dL) in Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose, ns: non-significant.

4.1.6. High-density lipoprotein (HDL):

In Disease Control animals, HDL levels were found to be decreased compared to Normal Control animals. A significant decrease (P<0.01) in HDL concentration was observed in the Disease Control group compared to the Normal Control group. Treatment with Silymarin (50 mg/kg p.o.) and Sinapic acid (40, 80, and 100 mg/kg p.o.) resulted in an increase in HDL concentration, although this increase was not significant when compared to the Disease Control group.

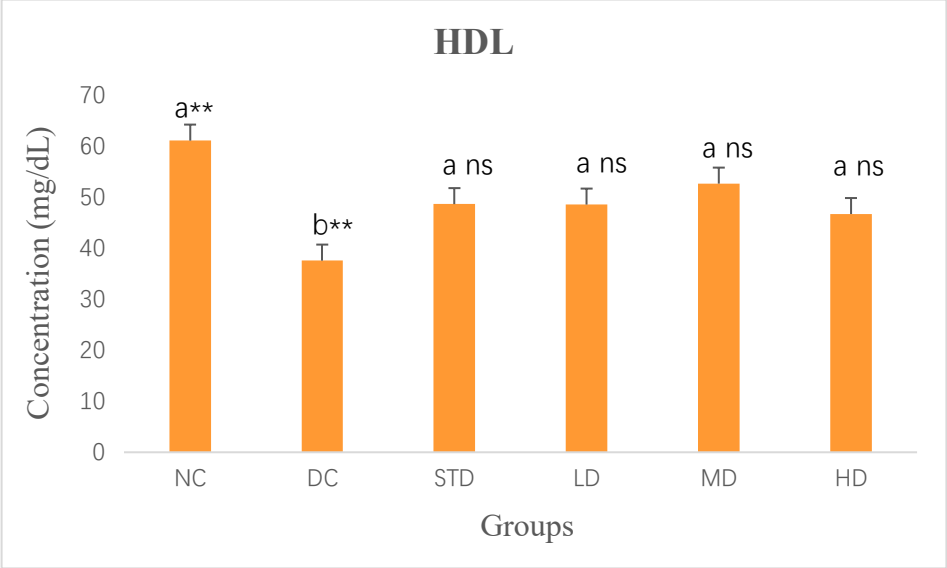

Figure 7: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on level of HDL (mg/dL) in Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose, ns: non-significant.

In the disease control group, rat showed reduction in body weight after the 3rd week in comparison to that of the 1st week. Animals that received Silymarin and Sinapic acid showed increase in body weight after the 3rd week when compared to the 1st week. The effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on body weight (gm) in Alcohol induced hepatotoxicity model of rats in figure 8.

4.2. Body Weight:

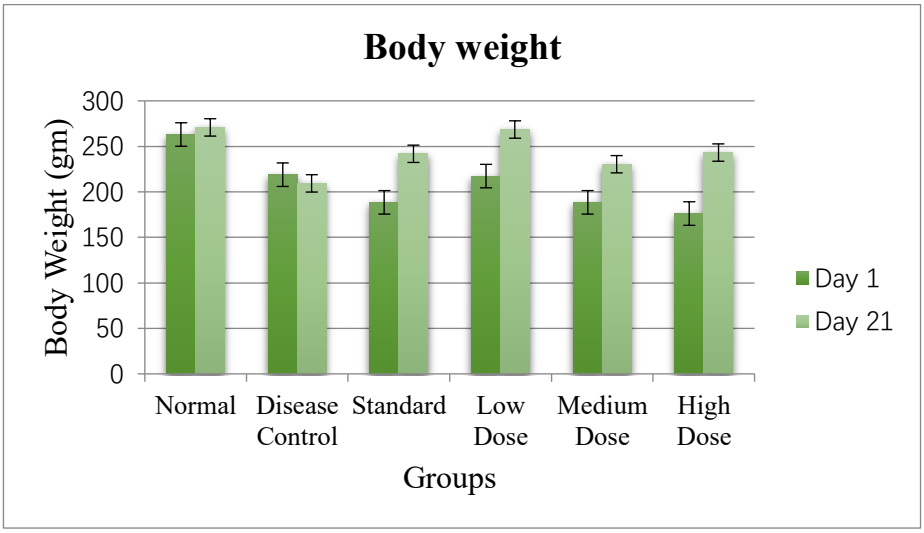

Figure 8: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on body weight (gm) in Alcohol induced hepatotoxicity model of rats.

4.3. In vivo antioxidant study:

4.3.1. Reduced Glutathione (RGSH):

RGSH is primary antioxidant in the cell. In Disease Control animals, RGSH level was found to be decreased as compared to Normal Control animals. Significant ($P<0.01$) decrease in the concentration of RGSH was observed in Disease control group compared with Normal Control group, while treatment with Silymarin (50 mg/Kg p.o), Sinapic acid (40, 80 and 100 mg/kg p.o.) showed significant increase ($P<0.01$) in the concentration of RGSH when compared with Disease Control group.

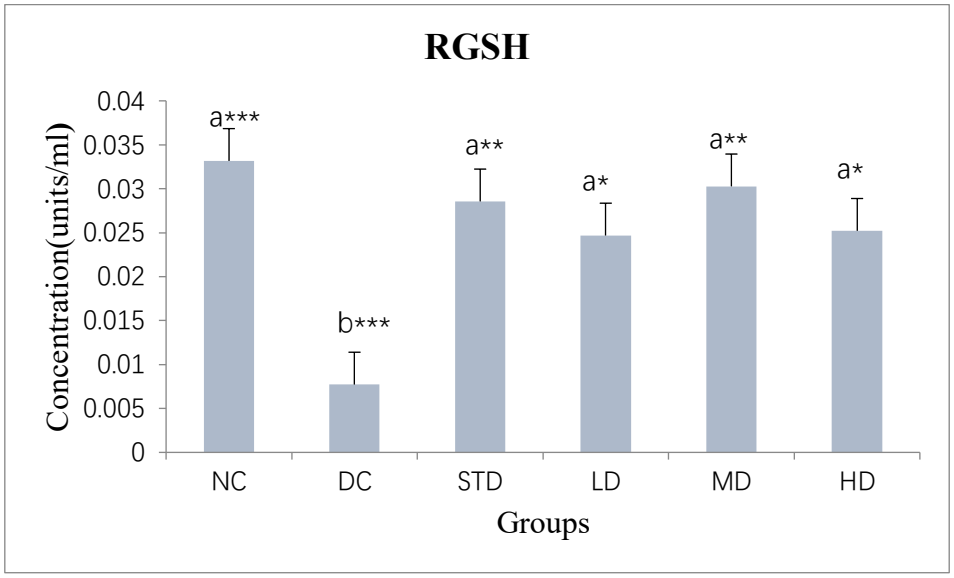

Figure 9: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on level of reduced glutathione in liver tissue homogenate of Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.3.2. Lipid Peroxidation (LPO)/ Malonaldehyde (MDA):

Significant (P<0.001) increase in lipid peroxidation was observed in Disease control group compared with Normal Control group, while treatment with Silymarin (50 mg/Kg p.o), Sinapic acid (40, 80 and 100 mg/kg p.o.) showed significant decrease (P<0.001) in lipid peroxidation when compared with Disease Control group.

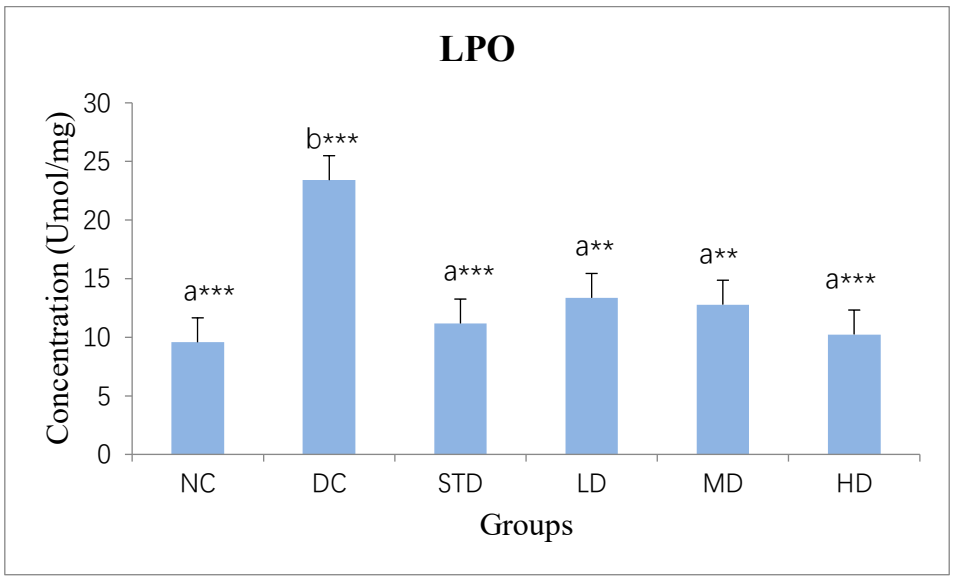

Figure 10: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on lipid Peroxidation in liver tissue homogenate of Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.3.3. Catalase (CAT):

In Disease Control animals, catalase level was found to be decreased as compared to Normal Control animals. Significant (P<0.001) decrease in the concentration of catalase was observed in Disease control group when compared with Normal Control group, while treatment with Silymarin (50 mg/Kg p.o) and Sinapic acid (40, 80 and 100 mg/kg p.o.) showed significant increase (P<0.001) in the concentration of catalase when compared with Disease Control group.

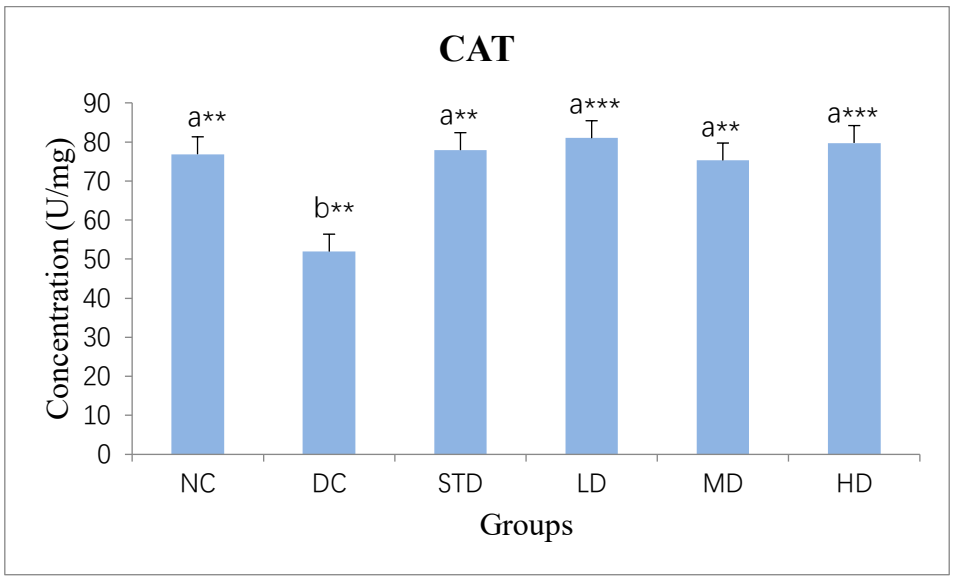

Figure 11: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on catalase in liver tissue homogenate of Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.3.4. Superoxide dismutase (SOD):

In Disease Control animals, superoxide dismutase level was found to be decreased as compared to Normal Control animals. Significant (P<0.001) decrease in the concentration of SOD was observed in Disease control group when compared with Normal Control group, while treatment with Silymarin (50 mg/Kg p.o) and Sinapic acid (40, 80 and 100 mg/kg p.o.) showed increase (not significant) in the concentration of superoxide dismutase when compared with Disease Control group, only medium group showed significant increase in the concentration of superoxide dismutase.

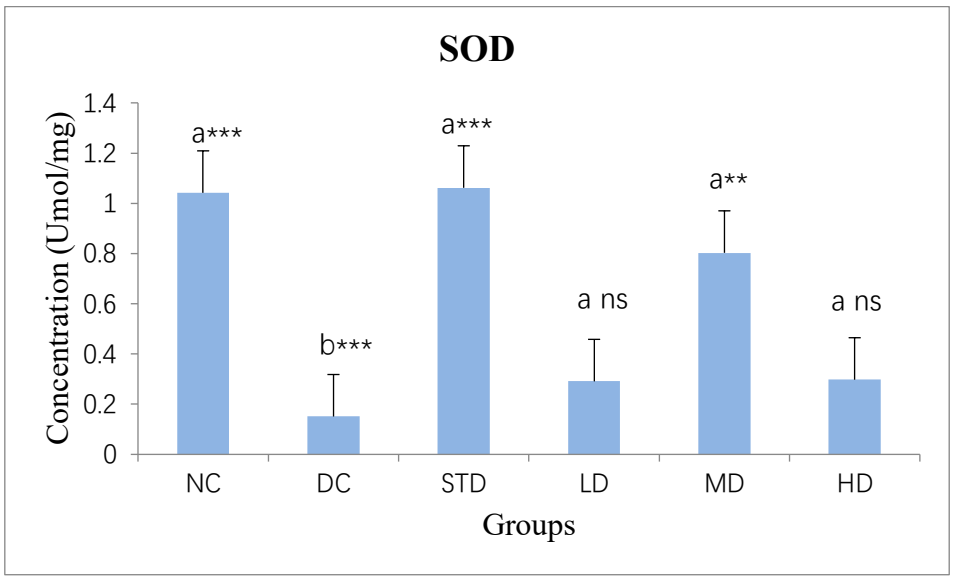

Figure 12: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on superoxide dismutase in liver tissue homogenate of Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose, ns: non-significant.

4.4. Estimation of levels of Pro-inflammatory mediators:

4.4.1. TNF-α:

In Disease Control animals, TNF-α level was found to be increased as compared to Normal Control animals. Significant (P<0.001) increase in the concentration of TNF-α was observed in Disease control group when compared with Normal Control group, while treatment with Silymarin (50 mg/Kg p.o) and Sinapic acid (40, 80 and 100 mg/kg p.o.) showed significant decrease (P<0.001) in the concentration of TNF-α when compared with Disease Control group.

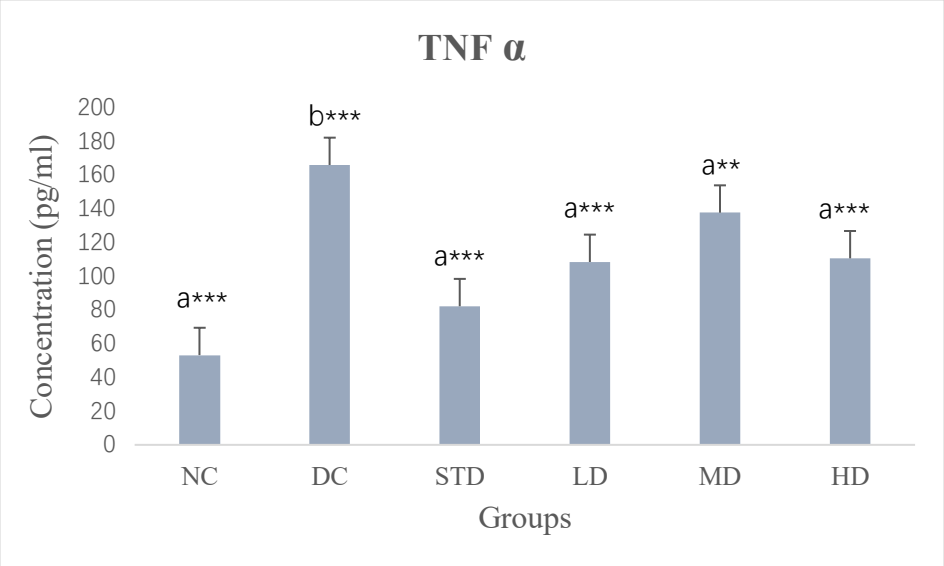

Figure 13: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on TNF α in liver tissue homogenate of Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.4.2. IL-6:

In Disease Control animals, IL-6 level was found to be increased as compared to Normal Control animals. Significant (P<0.001) increase in the concentration of IL-6 was observed in Disease control group when compared with Normal Control group, while treatment with Silymarin (50mg/Kg p.o) and Sinapic acid (40, 80 and 100 mg/kg p.o.) showed significant decrease (P<0.001) in the concentration of IL-6 when compared with Disease Control group.

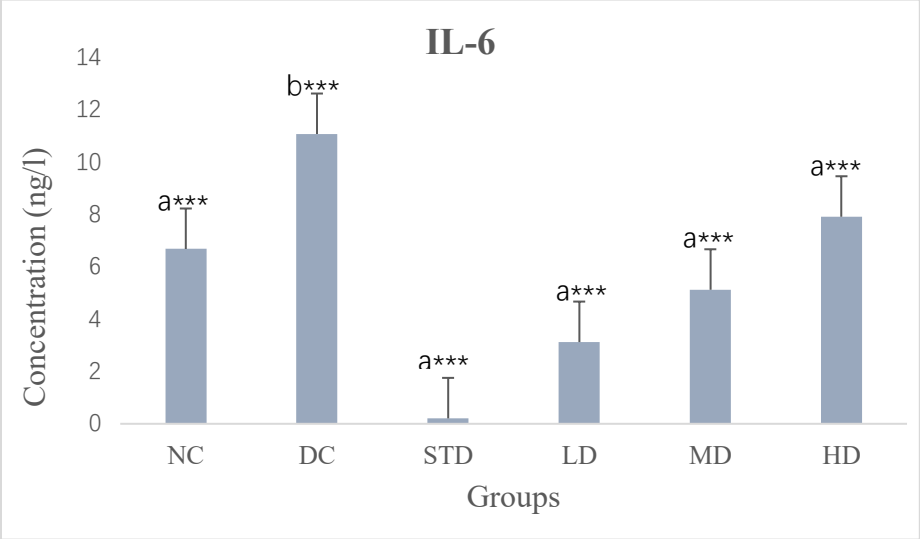

Figure 14: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on IL-6 in liver tissue homogenate of Alcohol induced hepatotoxicity model of rats.

Values are expressed as mean ± S.E.M. (n = 6 in each group). Data was analyzed by using One way ANOVA followed by Tukey's test; a vs Disease Control; b vs Normal Control. ***P<0.001, **P<0.01, *P<0.05. NC: Normal Control, DC: Disease Control, STD: Standard, LD: Low Dose, MD: Medium Dose, HD: High Dose.

4.5. Effect of Sinapic acid on Histology of Liver:

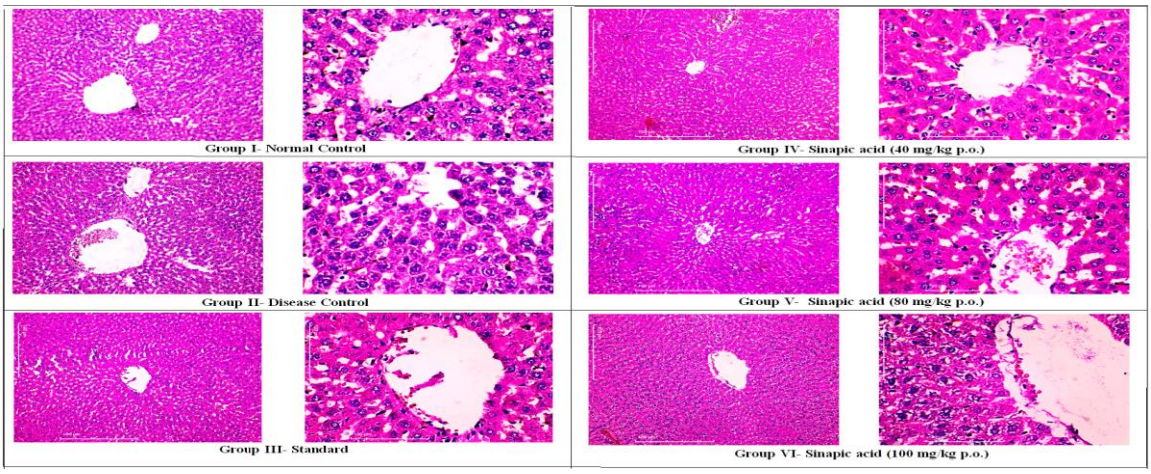

Figure 15: Effect of Sinapic acid (40, 80 and 100 mg/kg p.o.) on Histology of liver of Alcohol induced hepatotoxicity model of rats.

In histopathological studies, it is reported that microscopic examination of liver sample from the Normal Control group showed normal structure and architecture. Normal hepatocytes arranged in cords, obvious sinusoids, and central vein. In Disease Control group, hepatic cells with ballooning degeneration, focal necrotic cell death, and diffuse changes are observed when compared with Normal Control group animals. However, the incidence and severity of these lesions (recorded in Disease Control group) was reduced dose dependently in groups Sinapic acid (40, 80 and 100 mg/kg p.o) when compared with Disease Control group suggestive of reversible nature of the change.

On the basis histopathology findings, it is concluded that animal treated with standard drug, i.e., Silymarin (50mg/Kg, p.o.) and Sinapic acid (80 and 100 mg/kg p.o.) showed ameliorative effect and showed significant protective effect on liver tissue.

Table 3: Histopathology findings

Group	Group No.	Histopathology Observation
Normal Control	I	Normal structure and architecture. Normal hepatocytes arranged in cords, obvious sinusoids, and central vein.
Disease Control	II	Hepatic cells with ballooning degeneration, focal necrotic cell death, and diffuse changes.
Standard (Silymarin 50 mg/kg p.o.)	III	Showing normal sinusoids with structure. Centrilobular hepatocyte. No abnormality detected.
SA (40 mg/kg p.o.)	IV	Dilation of sinusoids with focal hemorrhage. Centrilobular hepatocyte necrosis and initiation of fibrosis. Focal hemorrhagic necrosis. Abnormality detected.
SA (80 mg/kg p.o.)	V	Showing normal sinusoids with structure. Centrilobular hepatocyte. No abnormality detected.
SA (100 mg/kg p.o.)	VI	Showing normal sinusoids with structure. Centrilobular hepatocyte. No abnormality detected.

5. DISCUSSION:

The present study investigated the hepatoprotective potential of sinapic acid in a rat model of alcoholic liver disease (ALD), focusing on its impact on biochemical parameters, liver histopathology, and inflammatory markers. ALD is a chronic and progressive condition triggered by prolonged alcohol intake, typically evolving from fatty liver (steatosis) to alcoholic hepatitis and eventually cirrhosis. Although alcohol use has declined in parts of Europe, a rising trend in countries such as China and the United States has led to an increased global prevalence of ALD.

In this study, rats given alcohol at a dose of 3 mL/day orally exhibited significantly increased liver enzyme levels, indicating hepatic injury. Administration of silymarin (50 mg/kg, p.o.) and sinapic acid at doses of 40, 80, and 100 mg/kg (p.o.) significantly reduced these elevated biomarkers, suggesting a protective effect on liver function. Histopathological evaluation showed normal liver architecture in the Normal Control group, whereas the Disease Control group exhibited marked liver damage. Treatment with sinapic acid, particularly at 80 and 100 mg/kg, and silymarin, significantly improved liver histology, highlighting the potential of sinapic acid to mitigate alcohol-induced hepatic injury.

6. CONCLUSION:

In conclusion, sinapic acid exhibited strong hepatoprotective activity against alcohol-induced liver damage, likely through its ability to counteract oxidative stress and suppress inflammatory responses. The outcomes of this research highlight the potential of sinapic acid as a viable therapeutic agent for the treatment of alcoholic liver disease. Overall, these findings present new insights into the role of sinapic acid in reducing liver toxicity by modulating oxidative stress and pyroptosis, suggesting its promise in developing alternative strategies for managing alcohol-related hepatic conditions.

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