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Hepatoprotective Potential of *Moringa oleifera* Extract Against Alcohol–Induced Oxidative Stress and Liver Damage in Wistar Albino Rats

M. Venkata Chandrudu[1], Anand Konkala[2], V. Madan Mohan Rao[3], R. Chandra Mouli[4], B. Jameela Beebi[5], and D. Veera Nagendra Kumar*[6]

[1]*Department of Zoology, School of Life Sciences, Rayalaseema University, Kurnool, Andhra Pradesh, India*
[2]*Department of Zoology, Government City College (Autonomous), Hyderabad, Telangana, India*
[3]*Department of Botany, Government Degree College, Chintalapudi, Andhra Pradesh, India*
[4]*Department of Zoology, SVA Government College (A), Srikalahasti, Andhra Pradesh, India*
[5]*Department of Zoology, SKP Government Degree College, Guntakal, Andhra Pradesh, India*
[6]*Department of Zoology, Government College for Men (A), Kadapa, Andhra Pradesh, India*

ABSTRACT

This study was conducted and to assess hepatoprotective potential of Moringa oleifera leaf extract on Alcohol-induced oxidative stress and to assess the liver damage in Wistar albino rats. Thirty rats were divided into five groups: normal as control, alcohol control, silymarin (100 mg/kg) and M. oleifera extract treated groups (200 and 400 mg/kg). Chronic ethanol administration had significantly decreased bodys weight gain (11.9 ± 1.8 g) and increased the relative liver weight (5.92 ± 0.18%) in comparison with the normal control group. Ethanol also increased serum ALT (112.4 ± 4.5 U/L), AST (218.7 ± 6.8 U/L), ALP (286.4 ± 8.2 U/L), the bilirubin (2.14 ± 0.09 mg/dL), and decreased total protein (4.26 ± 0.15 g/dL) and albumin (2.42 ± 0.10 g/dL). Moreover, elevated MDA levels (5.94 ± 0.18 nmol/mg protein) and reduced SOD, CAT and GSH levels indicated oxidative stress induced by ethanol. M. oleifera treatment, especially at 400 mg/kg, significantly restored biochemical and antioxidant parameters to near normal values and ameliorated liver histoarchitecture. The current results suggest that Moringa oleiferahas significant hepatoprotective and antioxidant activities against Alcohol-induced hepatic injury.

Keywords: *Moringa oleifera, Hepatoprotection, Ethanol-induced hepatotoxicity, Oxidative stress, Antioxidant enzymes, Liver function biomarkers, Wistar albino rats.*

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Corresponding Author: *D. Veera Nagendra Kumar*

E-mail Address: *veeranagendrakumar@gmail.com*

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1. INTRODUCTION

Liver is one of the vital organ for metabolism, essential for detoxifying the body, synthesizing proteins, managing carbohydrates, regulating lipids, and maintaining physiological balance. Due to its pivotal role in processing foreign substances, the liver is particularly vulnerable to harm from environmental toxins, medications, alcohol, and infectious agents. Chronic alcohol consumption, in particular, is a leading cause of liver diseases globally, significantly impacting morbidity and mortality rates. Alcohol-related liver damage includes a range of pathological conditions, from the steatosis and the hepatitis to the fibrosis, cirrhosis, and hepatocellular carcinoma. The cytochrome known as P450 2E1 (CYP2E1) and alcohol dehydrogenase pathways in the liver are the main sites of ethanol metabolism. Oxidative stress, lipid peroxidation, mitochondrial malfunction, inflammation, and hepatocellular damage result from the overproduction of reactive oxygen species (ROS) throughout this process.

Proteins, lipids, and nucleic acids are harmed by oxidative stress, which is caused by an imbalance between the creation of free radicals and the antioxidant defense system. Alcohol-induced hepatic injury is frequently linked to elevated to the serum levels of bilirubin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALAP), which are significant indicators of liver dysfunction.

Liver illnesses now significant global health concern, contributing significantly to morbidity, death, and medical costs. A large percentage of liver-related deaths are caused by alcohol-associated liver disease (ALD), the most common causes of chronic liver injury globally [1-2]. Hepatic steatosis, alcoholic hepatitis, fibrosis, cirrhosis, and hepatocellular cancer have all become more common as a result of rising rates of excessive alcohol consumption and shifting lifestyle choices. Effective pharmacological treatments for alcohol-induced liver damage are still scarce despite advancements in diagnostic and therapeutic techniques, underscoring the need for safer and

more potent hepatoprotective drugs. As prospective substitutes for manufactured hepatoprotective medications, medicinal plants have garnered significant interest. Different bioactive substances found in natural goods have the ability to simultaneously target several disease pathways. In experimental models of liver injury, some important phytochemicals, such as flavonoids, phenolic acids, alkaloids, terpenoids, and the glycosides, have shown notable hepatoprotective effect [3-4]. The scavenging free radicals, regulating antioxidant enzymes, inhibiting inflammatory mediators, and stabilizing cellular membranes, these substances provide protection. Because of its remarkable nutritional and pharmacological qualities, *Moringa oleifera* Lam. holds a prominent position among the therapeutic plants studied for hepatoprotective action. Medicinal plants are known from the ages for its novel actions known as traditional or herbal medicine and famous across the Asia, Africa, and the South America continents.

The hepatoprotective activity of *M. oleifera* against chemically induced liver injury has been shown in multiple experimental investigations. Its capacity to inhibit lipid peroxidation, boost antioxidant enzyme activity, stabilize hepatocyte membranes, and regulate inflammatory pathways has all been linked to its beneficial properties. According to a recent systematic analysis, *M. oleifera leaf* extract supports its therapeutic use in liver illnesses by considerably improving liver histology and reducing hepatic damage in experimental animals [5,6]. Further research into the hepatoprotective effectiveness of *M. oleifera* is necessary given the rising incidence of alcohol-related liver disorders and the growing interest in plant-based therapies. In order to assess the preventive effects of *Moringa oleifera* leaf extract against ethanol induces the oxidative stress and liver damages, the current study was conducted. In order to clarify the possible processes behind its hepatoprotective action, the study sought to evaluate liver function biomarkers, antioxidant status, and histological changes.

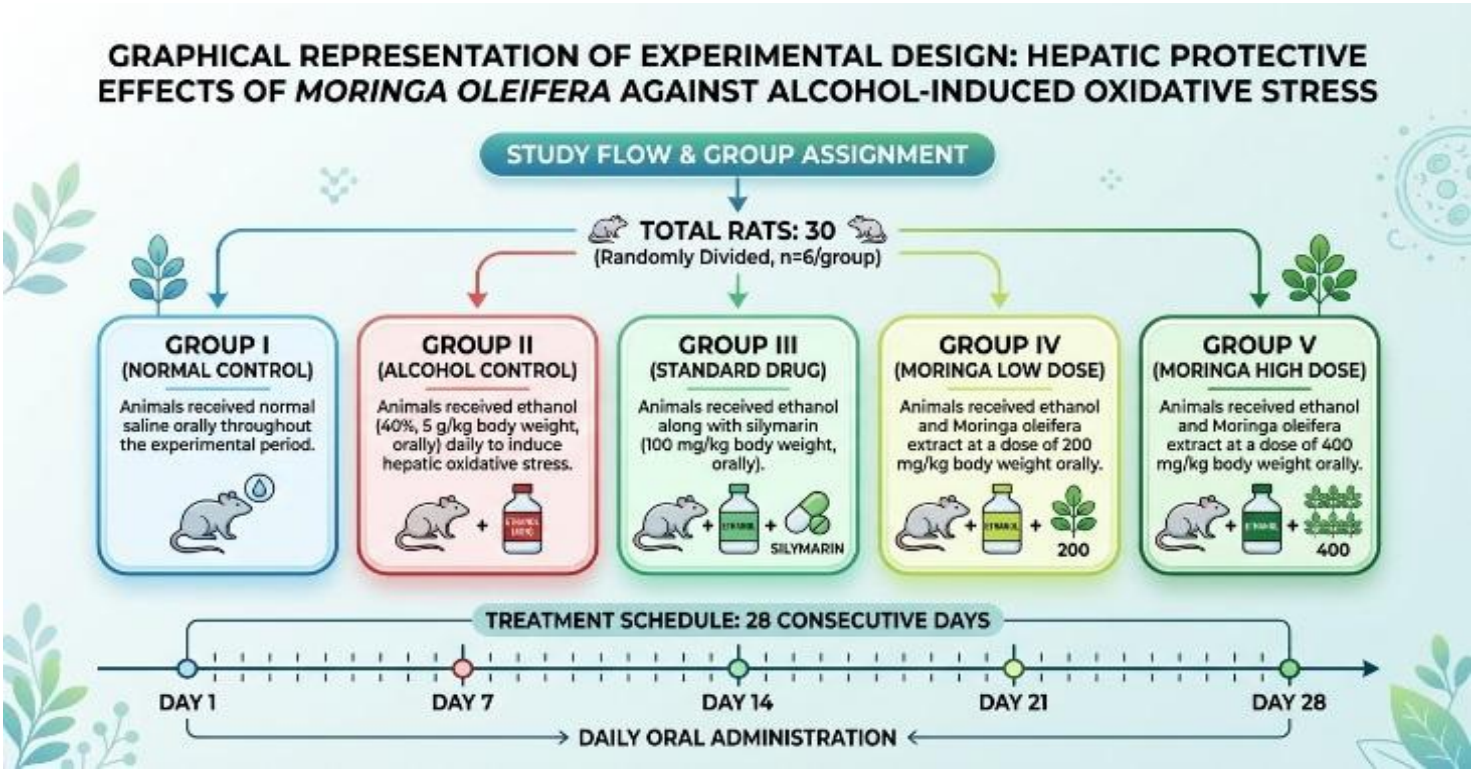

Fig.1: Graphical Representation

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

Fresh *Moringa oleifera* leaves gathered from mature, robust plants that were growing nearby. A certified taxonomist from the Department of Botany verified the authenticity of the plant sample. After properly cleaning with distilled with water, to get rid of any dust or debris that might have stuck to them, they were slightly dried for 10-14 days and maintained the room temperature before being ground into a fine powder viz of mechanical grinder.

2.2. Preparation of Plant Extract

In a Soxhlet system, the powdered leaf material was extracted for 48 hours using 70% ethanol. Rotating vacuum evaporator used and the extracted material kept under low pressure after it passed through the filtered through the Whatman No. 1 filter paper. And to create a semisolid bulk, the concentrated extract was further dried in a desiccator.

After calculating the extract's per centage yield, the dried extract was kept at 4 °C for further experiment.

2.3. Experimental Animals

A certified animal breeding facility provided healthy adult male Wistar albino rats weigh of 180 and 220 g. And then kept in polypropylene cages under typical laboratory settings, which included a 12-hour light/dark cycle, a temperature of 22 ± 2°C, and a relative humidity of 55–65%. Rats had unlimited access to drinking water and a normal pellet diet. Before the experiment began, the animals were given a week to get used to the lab environment. Experimental Duration: 28 consecutive days

2.4. Ethical Approval

Institutional Animal Ethics Committee [IAEC] with the number of [Regd. No. IAEC/VI/04/RIPER/2024] approved for experimental techniques in accordance with the Committee for maintain and control and the supervision of experiments on animals' recommendations.

Table 1: Experimental Design and Treatment Schedule

Group	Experimental Group	Treatment Administered	Dose and Route
I	Normal Control	Normal saline	Oral administration throughout the experimental period
II	Alcohol Control	Ethanol	40% ethanol, 5 g/kg body weight, orally, daily
III	Standard Drug	Ethanol + Silymarin	Ethanol (40%, 5 g/kg, orally) + Silymarin (100 mg/kg body weight, orally)
IV	*Moringa oleifera* Low Dose	Ethanol + *Moringa oleifera* Extract	Ethanol (40%, 5 g/kg, orally) + *Moringa oleifera* extract (200 mg/kg body weight, orally)
V	*Moringa oleifera* High Dose	Ethanol + *Moringa oleifera* Extract	Ethanol (40%, 5 g/kg, orally) + *Moringa oleifera* extract (400 mg/kg body weight, orally)

2.5. Sample Collection

Rats were fasted overnight and given light ether anesthesia at the conclusion of the treatment period. Heart puncture was used to obtain blood samples, which were then left to clot. Centrifugation at 3000 rpm kept for the 15 minutes was used to extract the serum, which was then kept at -20°C for biochemical analysis. The liver was removed right away, cleaned with ice-cold saline, blotted dry, weighed, and prepared for histological and oxidative analyses.

2.6. Assessment of Liver Function Biomarkers

Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALAP), total bilirubin, total protein, and the albumin were among the hepatic marker enzymes whose serum levels were measured using commercially available diagnostic kits in accordance with the manufacturer's instructions.

2.7. Determination of Oxidative Stress Markers

Malondialdehyde levels were determined according to the method of [7]. Superoxide dismutase activity in tissue homogenates was assayed following the method of [8] using a Hitachi U-2000 spectrophotometer. Absorbance was recorded at 480 nm for 4 min. SOD activity was defined as the amount of enzyme required to inhibit 50% of epinephrine oxidation and was expressed as U/mg protein. Catalase activity was measured at room temperature by the method of [9]. Reduced glutathione concentrations in liver homogenates were determined as described by [10]. All enzyme activities were normalized to protein content. Total protein was estimated by the method of Lowry et al. using bovine serum albumin as the standard.

2.8. Histopathological Examination

For 48 hours, small pieces of liver tissue were preserved at the 10% neutral buffered formalin. Tissues cleaned with the help of xylene, embedded of paraffin wax, dehydrated using a series of graded alcohols. A rotary microtome was used to prepare 5 µm thick sections, which were then stained with hematoxylin and eosin (H and E). Light-microscope used to analyze the histological alterations, which were then captured on camera for record.

2.9. Statistical Analysis

Mean ± standard error of mean was used to express experimental results. Statistical analysis performed and one-way analysis of variance [ANOVA] followed of Tukey's multiple comparison test. At $p < 0.05$, the differences deemed statistically significant.

3. Results

3.1. Body weight and the liver weight changes

Impact of *Moringa oleifera* extract on body weight and relative liver weight in rats given ethanol is displayed in Table 2. The normal control group, alcohol administration considerably decreased body weight gain and raised relative liver weight. The alcohol control group had the largest relative liver weight (5.92 ± 0.18%) and the lowest body weight gain (11.9 ± 1.8 g). Silymarin treatment considerably decreased liver enlargement and improved body weight growth. In a similar vein, compared to the alcohol control group, *Moringa oleifera* extract at doses of 200 and 400 mg/kg considerably enhanced body weight gain and lowered relative liver weight. Compared to the lower dose, the higher dose (400 mg/kg) shown more improvement. These results imply that *Moringa oleifera* provides dose-dependent protection against ethanol-induced changes in body and liver weight.

Table 2: Effect of Moringa oleifera Extract on Body Weight and Relative Liver Weight in Alcohol-Induced Hepatotoxicity in Wistar Albino Rats

Group	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)	Relative Liver Weight (%)
Normal Control	185.3 ± 3.2	228.6 ± 4.1	43.3 ± 2.1	3.48 ± 0.12
Alcohol Control	186.5 ± 2.8	198.4 ± 3.6***	11.9 ± 1.8***	5.92 ± 0.18***
Silymarin (100 mg/kg)	184.8 ± 3.1	223.7 ± 3.9###	38.9 ± 2.0###	3.71 ± 0.11###
M. oleifera (200 mg/kg)	186.1 ± 3.0	214.2 ± 3.8##	28.1 ± 1.9##	4.38 ± 0.14##
M. oleifera (400 mg/kg)	185.7 ± 2.9	221.5 ± 4.0###	35.8 ± 2.2###	3.86 ± 0.13###

Values are expressed as Mean ± SEM (n = 6).

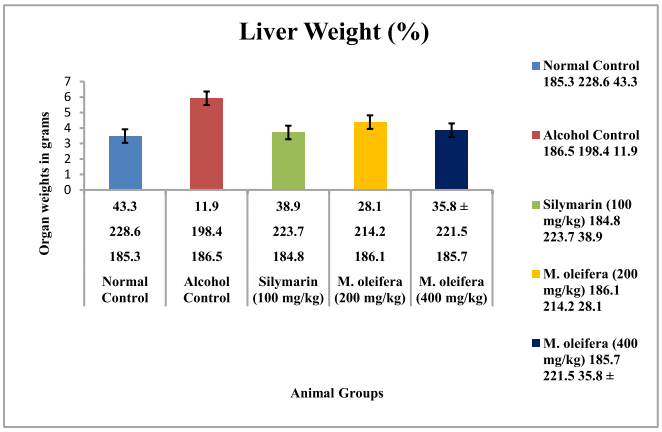

Fig 2. Organ weights %

3.2. Biochemical parameters

Moringa oleifera extract on serum liver function indicators in ethanol-induced hepatotoxicity is shown in Table 3. When compared to the normal control group, alcohol delivery dramatically reduced total protein and albumin levels while increasing ALAT, ASAT, ALAP, and total bilirubin levels. These changes show that ethanol consumption has seriously damaged the liver. Silymarin treatment significantly brought all liver function markers back to normal. Similarly, while raising total protein and albumin levels, *Moringa oleifera* extract at 200 and 400 mg/kg dramatically decreased the high levels of ALAT, ASAT, ALAP, and bilirubin. At the higher dose (400 mg/kg), the hepatoprotective effect was more noticeable, and the findings were similar to those of the conventional medication silymarin. These results show that Moringa oleifera extract can shield the liver from biochemical changes brought on by ethanol.

Table 3: Effect of Moringa oleifera Extract on Liver Function Biomarkers in Alcohol-Induced Hepatotoxicity

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
Normal Control	36.8 ± 2.1	82.5 ± 3.4	118.6 ± 4.2	0.68 ± 0.04	7.24 ± 0.18	4.18 ± 0.12
Alcohol Control	112.4 ± 4.5***	218.7 ± 6.8***	286.4 ± 8.2***	2.14 ± 0.09***	4.26 ± 0.15***	2.42 ± 0.10***
Silymarin (100 mg/kg)	44.7 ± 2.4###	96.8 ± 4.1###	132.5 ± 5.6###	0.82 ± 0.05###	6.95 ± 0.16###	4.02 ± 0.11###
M. oleifera (200 mg/kg)	67.5 ± 3.1##	138.2 ± 5.3##	188.6 ± 6.2##	1.32 ± 0.07##	5.94 ± 0.17##	3.42 ± 0.12##
M. oleifera (400 mg/kg)	51.8 ± 2.8###	108.4 ± 4.5###	148.7 ± 5.4###	0.96 ± 0.06###	6.72 ± 0.15###	3.88 ± 0.10###

Values are expressed as Mean ± SEM (n = 6).

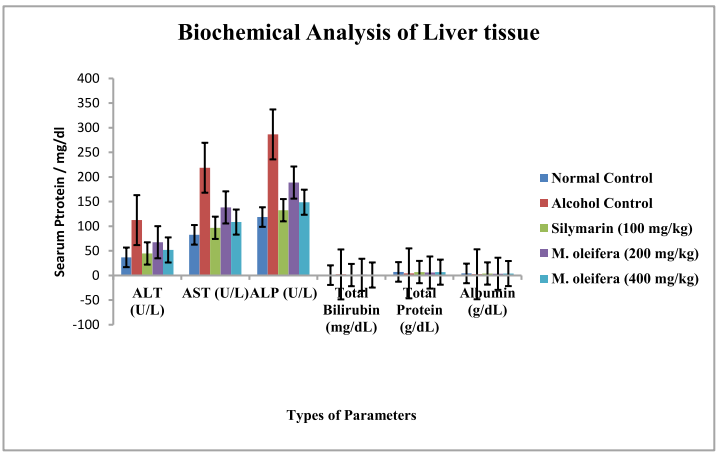

Fig 3: Analysis of Liver Tissue through Biochemical Parameters

3.3. Antioxidant enzyme levels

The impact of Moringa oleifera extract on hepatic oxidative stress indicators in ethanol-induced hepatotoxicity is shown in Table 4. The normal control group, ethanol administration dramatically raised MDA levels and greatly reduced the antioxidant enzymes SOD, CAT, and GSH, indicating increased oxidative stress. Lipid peroxidation was considerably decreased, and antioxidant enzyme activity was restored after silymarin treatment. Similarly, compared to the alcohol control group, *Moringa oleifera* extract at 200 and 400 mg/kg considerably reduced MDA levels while raising SOD, CAT, and GSH levels. The larger dose (400 mg/kg) yielded values that were comparable to those seen in the group treated with silymarin and demonstrated increased antioxidant activity. These findings imply that by strengthening the body's natural antioxidant defense mechanism, *Moringa oleifera* successfully reduces oxidative stress brought on by ethanol.

Table 4: Effect of Moringa oleifera Extract on Hepatic Oxidative Stress Parameters in Alcohol-Induced Hepatotoxicity

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)
Normal Control	1.86 ± 0.08	10.42 ± 0.34	62.5 ± 2.4	8.82 ± 0.31
Alcohol Control	5.94 ± 0.18***	4.18 ± 0.21***	28.4 ± 1.8***	3.26 ± 0.15***
Silymarin (100 mg/kg)	2.14 ± 0.10###	9.76 ± 0.28###	58.8 ± 2.1###	8.21 ± 0.26###
M. oleifera (200 mg/kg)	3.68 ± 0.14##	7.12 ± 0.25##	46.3 ± 1.9##	6.34 ± 0.22##
M. oleifera (400 mg/kg)	2.48 ± 0.11###	8.96 ± 0.30###	55.4 ± 2.2###	7.92 ± 0.24###

Values are expressed as Mean ± SEM (n = 6).

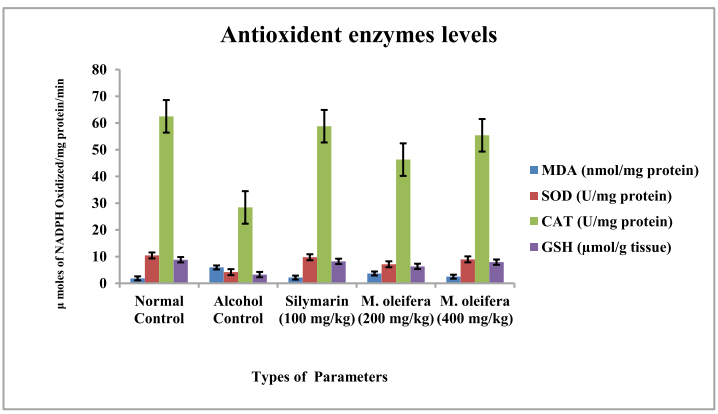

Fig 4: Antioxidant enzymes

3.4. Histopathological Analysis of Liver Tissue

The control group's hepatic architecture, which is characterized by well-organized hepatocytes, intact central veins, and normal sinusoidal spaces, was found by histopathological analysis of H&E-stained liver sections. The alcohol control group, on the other hand, showed signs of ethanol-induced liver damage, including fatty degeneration, hepatocellular necrosis, inflammatory cell infiltration, and sinusoidal dilatation. After receiving 100 mg/kg of silymarin, the liver's histology significantly improved, exhibiting almost normal hepatic architecture with just some congestion. Rats given 200 mg/kg of Moringa oleifera extract showed a moderate degree of recovery, with mild inflammatory cell infiltration and fewer lipid alterations. The high-dose Moringa oleifera group (400 mg/kg) showed minor pathological changes, reduced cellular degeneration, and a significant restoration of normal hepatic architecture with well-preserved hepatocytes. These histological results validate *Moringa oleifera's* hepato protective efficacy against ethanol-induced liver injury and support the biochemical findings.

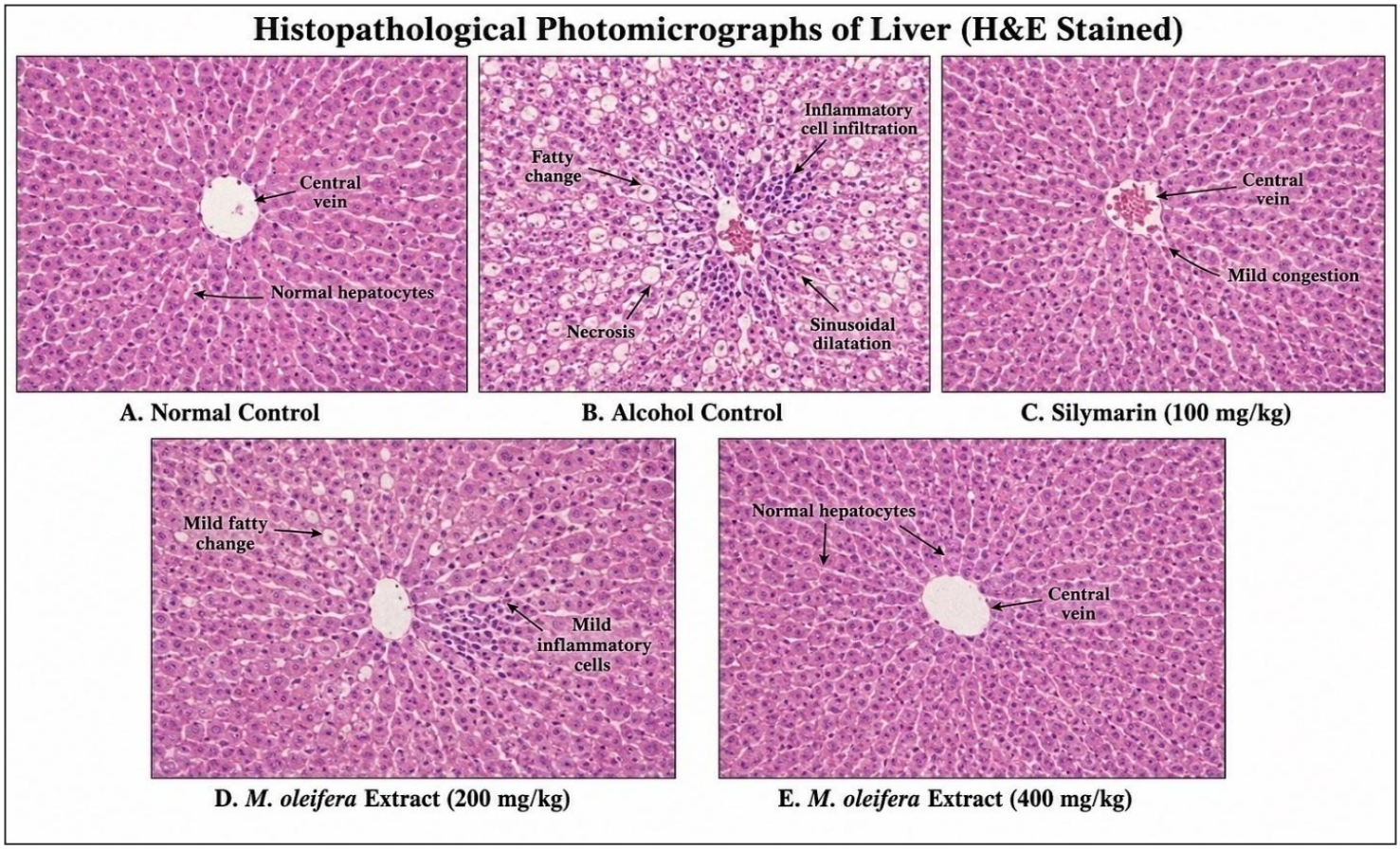

Fig 5: Liver Sections with proofing of Hispatological evidences

4. DISCUSSION

This examination the hepatoprotective effectiveness of *Moringa oleifera* leaf extract against the oxidative stress and liver damage caused by Alcohol in Wistar albino rats. Significant changes in body weight, liver weight, liver function biomarkers, antioxidant status, and hepatic histoarchitecture were observed after long-term Alcohol administration. These alterations were successfully reversed by treatment with *M. oleifera* extract, indicating its strong hepatoprotective and antioxidant qualities. The substantial decrease in body weight gain and rise in relative liver weight after Alcohol delivery was one of the study's main conclusions. Alcohol-induced weight loss can be linked to tissue deterioration, oxidative stress, metabolic disorders, and poor food utilization. The alcohol control group's increased relative liver weight suggests hepatomegaly brought on by inflammation, cellular edema, and lipid infiltration. When *M. oleifera* extract was administered, body weight growth was considerably improved, and liver enlargement was decreased, indicating protection against metabolic disorders brought on by Alcohol. [11] observed similar findings, noting that supplementing experimental animals subjected to oxidative stress with M. oleifera improved physiological and metabolic markers.

Serum liver function biomarkers are well-known measures of cellular damage and hepatic integrity. Alcohol administration significantly raised the levels of ALT, AST, ALP, and total bilirubin in the current study while lowering the concentrations of albumin and total protein. Increased transaminase activity is indicative of intracellular enzyme leaking into the bloodstream as a result of hepatic membrane injury. While decreased protein and albumin concentrations point to poor liver synthesis activity, elevated bilirubin levels indicate impaired hepatic excretory function.

Hepatocyte membrane stabilization and liver function preservation were demonstrated by the considerable restoration of these parameters toward normal values following treatment with extracts of *M. oleifera*. These results align with the findings of [12], who emphasized *M. oleifera's* hepato protective capability against a variety of experimental models of liver injury.

One important aspect of the pathophysiology of alcohol-induced liver damage is oxidative stress. Excessive reactive oxygen species produced by CYP2E1's metabolism of Alcohol cause lipid peroxidation and cellular damage. Rats given Alcohol showed noticeably higher MDA levels in the current investigation, which suggests greater lipid peroxidation. Significant drops in SOD, CAT, and GSH levels were noted concurrently, indicating the depletion of endogenous antioxidant defenses. These results are consistent with other investigations that identified oxidative stress as a primary mechanism of hepatotoxicity caused by Alcohol [13, 14, 15]

These results align with the findings of Camilleri and Blundell (2024), who emphasized M. *oleifera's* hepatoprotective capability against a variety of experimental models of liver injury. One important aspect of the pathophysiology of alcohol-induced liver damage is oxidative stress. Excessive reactive oxygen species produced by CYP2E1's metabolism of Alcohol cause lipid peroxidation and cellular damage. Rats given Alcohol showed noticeably higher MDA levels in the current investigation, which suggests greater lipid peroxidation. Significant drops in SOD, CAT, and GSH levels were noted concurrently, indicating the depletion of endogenous antioxidant defenses. These results are consistent with other investigations that identified oxidative stress as a primary mechanism of hepatotoxicity caused by Alcohol [13]).

The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway may be activated by *M. oleifera's* anti oxidant action, according to recent molecular research. Nrf2 activation increases cellular tolerance to oxidative stress by promoting the transcription of cytoprotective proteins and antioxidant enzymes. It has been demonstrated that M. oleifera suppresses inflammatory responses mediated by nuclear factor-kappa B (NF-κB), which lowers the production of pro-inflammatory cytokines like TNF-α and IL-6 [16]. The hepatoprotective benefits seen in this study may be a result of both systems working together.

The biochemical results were further corroborated by histopathological analysis. Hepatocellular degeneration, fatty changes, inflammatory infiltration, sinusoidal dilatation, and necrotic lesions were among the many pathological changes found in liver sections from animals treated with alcohol. These findings have been reported in earlier research on alcoholic liver disease and are typical characteristics of Alcohol-induced hepatic injury [17,18]. Hepatic architecture was significantly improved, cellular degeneration was decreased and normal hepatocyte organization was restored after treatment with M. oleifera extract. The liver morphology of the high-dose treatment group was almost normal and similar to that of the silymarin-treated group. [19] showed similar histological changes and concluded that M. oleifera greatly reduces liver damage in a variety of experimental models. [20] reported that ethanol extracts of Bacopa were able to increase SOD, CAT and GPx activities in renal tissue of the alcohol treated rat.

The current study's hepatoprotective activity seems to be dosage-dependent, with the 400 mg/kg dose yielding better results than the 200 mg/kg dose. This result implies that increased phytoconstituent concentrations offer improved defense against oxidative stress and liver damage. The therapeutic potential of M. oleifera as a natural substitute for treating alcohol-induced liver problems was demonstrated by the high-dose extract's efficacy, which was comparable to that of silymarin, a well-known hepatoprotective drug, the current study's findings are consistent with recent research showing M. oleifera's positive effects on liver function. The extract's hepatoprotective efficacy is confirmed by its capacity to improve histopathological abnormalities, lower lipid peroxidation, restore antioxidant enzyme activities, and regulate liver function indicators. Synergistic antioxidant, anti-inflammatory, and membrane-stabilizing processes are probably responsible for these protective benefits.

5. Conclusion

The use of *Moringa oleifera* as a potential hepatoprotective drug against ethanol-induced oxidative stress and liver injury is thus supported by the current findings, which offer strong experimental evidence. To clarify its exact mechanisms of action and therapeutic usefulness in human liver illnesses, more research using molecular biomarkers, gene expression analysis, and clinical studies is necessary.

Abbreviations: ALT—Alanine Aminotransferase; AST—Aspartate Aminotransferase; ALP—Alkaline Phosphatase; MDA—Malondialdehyde; SOD—Superoxide Dismutase; CAT—Catalase; GSH—Reduced Glutathione.

Conflict of interest: None

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