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RESEARCH ARTICLE

Evaluation of the Hepatoprotective Activity of Silymarin Against Acetaminophen-Induced Oxidative Hepatic Injury in Wistar Rats

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Abstract: The liver serves as the central hub for the metabolic transformation and detoxification of diverse xenobiotics, rendering it inherently susceptible to chemical-induced pathologies. Acetaminophen (paracetamol) represents a widely utilized analgesic and antipyretic agent that, while safe at therapeutic concentrations, precipitates acute hepatotoxicity when administered at supratherapeutic levels. This injury originates from the metabolic conversion of acetaminophen into N-acetyl-p-benzoquinone imine (NAPQI), a reactive intermediate that depletes hepatic glutathione reservoirs, triggers oxidative stress, and induces hepatocellular necrosis. While N-acetylcysteine remains the primary clinical intervention, its efficacy is often limited by a narrow therapeutic window and potential adverse reactions. Consequently, the search for phytotherapeutic alternatives has identified silymarin, a flavonolignan complex derived from Silybum marianum, as a potent hepatoprotective candidate. Experimental evaluation in male Wistar rats shows that silymarin significantly mitigates hepatic damage induced by acute acetaminophen exposure. Administration of silymarin at doses of 100 mg/kg and 200 mg/kg results in a marked reduction of serum biomarkers, including alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, while simultaneously restoring endogeneous antioxidant levels. Histopathological analysis corroborates these biochemical findings, revealing a preservation of hepatic architecture and a reduction in centrilobular necrosis. These observations indicate that silymarin exerts its protective effects through robust free-radical scavenging, membrane stabilization, and the stimulation of hepatocyte regeneration. The findings provide substantial evidence for the integration of silymarin as a natural therapeutic strategy for managing drug-induced liver injury.

Keywords: Hepatoprotection; Silymarin; Acetaminophen-Induced Toxicity; Oxidative Stress; Liver Regeneration.

1. Introduction

The liver is an indispensable organ responsible for maintaining metabolic homeostasis, regulating nutrient processing, and orchestrating the biotransformation of endogenous and exogenous compounds. Due to its unique anatomical position and high blood flow, it serves as the primary site for the detoxification of drugs and environmental toxins [1]. This metabolic activity is largely driven by the cytochrome P450 enzyme system, which facilitates Phase I and Phase II reactions to render hydrophobic molecules more water-soluble for excretion [2]. However, the very processes intended to detoxify chemicals can sometimes lead to the generation of reactive intermediates, placing the liver at significant risk for toxicological insult.

Drug-induced liver injury (DILI) remains a major challenge in clinical medicine and a leading cause of acute liver failure in developed nations [3]. DILI can be classified as either predictable (dose-dependent) or idiosyncratic (dose-independent). The mechanisms underlying such damage typically involve mitochondrial dysfunction, the induction of oxidative stress, and the activation of innate immune responses that exacerbate hepatocellular damage [4]. Among the various pharmaceutical agents associated with DILI, acetaminophen is the most frequently cited due to its widespread availability and the predictable nature of its toxicity at high doses.

Acetaminophen is metabolized primarily via glucuronidation and sulfation pathways. However, a small fraction undergoes oxidation by CYP2E1 to produce N-acetyl-p-benzoquinone imine (NAPQI). Under normal conditions, NAPQI is rapidly neutralized by conjugation with reduced glutathione (GSH) [5]. In the event of an overdose, the excess production of NAPQI overwhelms the hepatic GSH supply, leading to the covalent binding of the toxic metabolite to mitochondrial proteins [6]. This binding triggers a cascade of events, including the overproduction of reactive oxygen species (ROS), lipid peroxidation of cellular membranes, and eventually, the induction of the mitochondrial permeability transition pore, which culminates in centrilobular necrosis [7].

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N-acetylcysteine (NAC) is the gold standard for treating acetaminophen overdose, functioning primarily by providing the necessary cysteine for GSH replenishment and acting as a direct antioxidant [8]. Despite its efficacy, NAC treatment must be initiated shortly after ingestion to be fully effective, and its use is sometimes complicated by anaphylactoid reactions or gastrointestinal distress [9]. There is, therefore, a scientific imperative to identify safer, naturally derived compounds that can provide robust hepatoprotection and support liver repair through diverse mechanisms.

Silymarin, a mixture of flavonolignans including silybin, silychristin, and silydianin, has been utilized in traditional medicine for centuries to treat various hepatic ailments [10]. Extracted from the seeds of *Silybum marianum* (milk thistle), silymarin is recognized for its multifaceted pharmacological profile, encompassing antioxidant, anti-inflammatory, and anti-fibrotic properties [11]. It acts as a membrane stabilizer by altering the outer structure of hepatocyte membranes, thereby preventing the entry of toxins. Silymarin improves protein synthesis by stimulating RNA polymerase I, which facilitates the regeneration of damaged hepatic tissue [12].

Figure 1. Leaves and Flowers of Silymarin

Paracetamol (N-acetyl-p-aminophenol) is a p-aminophenol derivative characterized by its analgesic and antipyretic properties. It is chemically identified by the molecular formula $C_8H_9NO_2$ and consists of a benzene ring core substituted by a hydroxyl group and an amide group in the para-position.

The primary therapeutic action of paracetamol is attributed to the inhibition of cyclooxygenase enzymes (COX-1 and COX-2) within the central nervous system, which leads to reduced prostaglandin synthesis in the hypothalamus [13]. Unlike non-steroidal anti-inflammatory drugs (NSAIDs), it possesses negligible peripheral anti-inflammatory activity. Clinically, it is the first-line treatment for mild-to-moderate pain and febrile conditions, with a maximum recommended adult dose of 4 grams per day [14].

At therapeutic doses, over 90% of paracetamol is metabolized into non-toxic glucuronide and sulfate conjugates. The remaining portion is processed by the CYP450 system (specifically CYP2E1, CYP1A2, and CYP3A4) into NAPQI [15]. In cases of acute toxicity (typically >150 mg/kg in humans), the depletion of GSH allows NAPQI to induce irreversible cellular damage. This predictability makes paracetamol the most widely used experimental model for evaluating the efficacy of potential hepatoprotective agents in rodents.

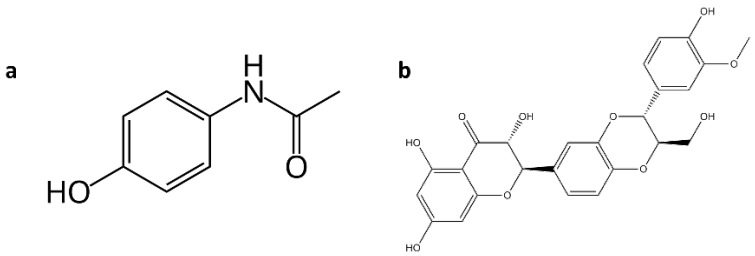

Figure 2. Chemical Structure of a. Paracetamol b. Silymarin

Silymarin is the standardized extract of milk thistle, with silybin (silibinin) representing the most bioactive component (approximately 50–70% of the complex). The primary mechanism of silymarin involves the neutralization of free radicals and the inhibition of lipid peroxidation. It serves as a direct scavenger of superoxide, hydroxyl, and peroxyl radicals [16]. Additionally, silymarin influences the expression of endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, thereby strengthening the cellular defense against oxidative insult [17].

Silymarin stabilizes the hepatocellular plasma membrane by interacting with lipid components, which prevents the penetration of various toxins into the cell interior [18]. It also promotes the synthesis of ribosomal RNA, leading to increased protein production. This stimulatory effect on the metabolic machinery of the hepatocyte is crucial for the accelerated repair and regeneration of liver tissue following injury [19].

2. Materials and Methods

2.1. Experimental Animals

The study utilized healthy adult male Wistar albino rats, weighing between 150 and 250 grams. The animals were sourced from a registered breeding facility and acclimatized to the laboratory environment for a period of seven days prior to the commencement of the experiment. They were maintained under standardized environmental conditions, including a controlled temperature range of 20–26°C, relative humidity of 30–70%, and a regulated 12-hour light/dark cycle. The rats were provided with a standard pellet diet and water *ad libitum*.

All experimental protocols were executed in strict accordance with the guidelines prescribed by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The study design was carried out according to the principles of the 3Rs (Replacement, Reduction, and Refinement) to ensure minimal animal distress. The experimental procedures received formal approval (IAEC/KGRL/2025/126) from the Institutional Animal Ethics Committee (IAEC), ensuring that the use of animals was scientifically justified and ethically sound.

2.2. Chemicals and Test Formulations

Acetaminophen (paracetamol) was utilized as the hepatotoxic inducing agent, administered at a concentration determined to consistently produce centrilobular necrosis. Silymarin was obtained as a standardized milk thistle extract. For oral administration, silymarin and paracetamol were prepared as homogeneous suspensions using 0.5–1% carboxymethyl cellulose (CMC) as the vehicle. All other chemicals and diagnostic kits used for biochemical estimations were of analytical grade.

2.3. Experimental Design

The animals were randomly assigned to five experimental groups, with each group comprising six individuals (n = 6). The grouping and dosing schedule were structured as follows:

2.3.1. Group I: Normal Control

Animals in this group received the vehicle (1% CMC, 1 ml/kg, p.o.) daily for five consecutive days. This group served as the baseline for normal physiological and biochemical parameters.

2.3.2. Group II: Disease Control (Hepatotoxic Group)

To induce acute hepatic injury, animals were administered a single supratherapeutic dose of paracetamol (2 g/kg, p.o.) on the fifth day of the study. This group was used to assess the maximum extent of drug-induced damage without intervention.

Table 1. Experimental Grouping and Dosing Schedule

Group	Nomenclature	Treatment Protocol	Dose & Route	Objective
I	Normal Control	1% CMC (Vehicle)	1 ml/kg, p.o.	Establish baseline parameters
II	Disease Control	Paracetamol (PCM)	2 g/kg, p.o. (Day 5)	Induce acute hepatotoxicity
III	Silymarin Low Dose	Silymarin + PCM	100 mg/kg + 2 g/kg, p.o.	Evaluate low-dose protection
IV	Silymarin High Dose	Silymarin + PCM	200 mg/kg + 2 g/kg, p.o.	Evaluate high-dose protection
V	Post-Treatment	PCM then Silymarin	2 g/kg (D1-4) then Silymarin (D5-7)	Evaluate curative potential

2.3.3. Group III: Silymarin Low Dose (Pre-treatment)

Rats were pre-treated with silymarin at a dose of 100 mg/kg (p.o.) once daily for four days. On the fifth day, paracetamol (2 g/kg, p.o.) was administered to evaluate the protective efficacy of a lower silymarin concentration.

2.3.4. Group IV: Silymarin High Dose (Pre-treatment)

This group received a higher dose of silymarin (200 mg/kg, p.o.) daily for four days. On the fifth day, the animals were challenged with paracetamol (2 g/kg, p.o.) to determine dose-dependent hepatoprotective effects.

2.3.5. Group V: Silymarin Post-treatment (Curative Model)

To evaluate the regenerative potential of the formulation, animals were first subjected to hepatic injury via paracetamol administration for four days. From the fifth day onwards, silymarin (200 mg/kg, p.o.) was administered for three subsequent days to assess its ability to reverse established damage.

2.4. Sample Collection and Processing

Following the final treatment session, the animals were subjected to an overnight fast. Blood samples were collected via the retro-orbital plexus under light anesthesia. The blood was allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes to separate the serum. The resulting serum was stored at -20°C for subsequent biochemical analysis. Immediately following blood collection, the animals were humanely euthanized, and the livers were excised, rinsed in ice-cold saline, and subjected to macroscopic and histopathological evaluation.

2.5. Biochemical Parameters Estimation

The serum was analyzed for liver function biomarkers, including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) using standardized enzymatic kits. Additionally, total bilirubin, direct bilirubin, total protein, and albumin levels were quantified. These parameters provided a quantitative measure of hepatocellular integrity and secretory function.

2.6. Histopathological Examination

Excised liver tissues were fixed in 10% neutral buffered formalin. The tissues were then dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. Thin sections (5 µm) were cut using a microtome and stained with Hematoxylin and Eosin (H&E). The slides were examined under a light microscope to assess structural alterations, including necrosis, inflammatory infiltration, and vacuolation.

2.7. Statistical Analysis

Data were expressed as Mean ± Standard Error of the Mean (SEM). Statistical significance was determined using one-way Analysis of Variance (ANOVA), followed by a suitable post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of Silymarin on Serum Biochemical Markers

The administration of a hepatotoxic dose of paracetamol (Group II) resulted in a profound elevation of serum enzymes compared to the normal control group (Group I). Specifically, ALT and AST levels increased by approximately 2.5-fold, while ALP and bilirubin levels showed significant surges, indicating severe leakage of these enzymes from damaged hepatocytes into the systemic circulation.

Table 2. Effect of Silymarin on Serum Liver Enzymes and Bilirubin Levels

Parameter	Normal Control (I)	Disease Control (II)	Silymarin Low Dose (III)	Silymarin High Dose (IV)
AST (SGOT) (U/L)	48.0 ± 2.2	115.0 ± 5.2*	76.0 ± 3.5#	58.0 ± 2.9##
ALT (SGPT) (U/L)	42.0 ± 2.0	110.0 ± 5.0*	70.0 ± 3.2#	52.0 ± 2.6##
ALP (U/L)	130.0 ± 5.8	340.0 ± 11.0*	240.0 ± 8.2#	170.0 ± 6.8##
Total Bilirubin (mg/dL)	0.70 ± 0.04	2.60 ± 0.11*	1.40 ± 0.07#	0.90 ± 0.05##
Direct Bilirubin (mg/dL)	0.17 ± 0.02	1.30 ± 0.06*	0.65 ± 0.03#	0.28 ± 0.02##

Values are expressed as Mean ± SEM (n=6). () $p < 0.05$ vs. Normal Control; (#) $p < 0.05$ vs. Disease Control; (##) $p < 0.01$ vs. Disease Control*

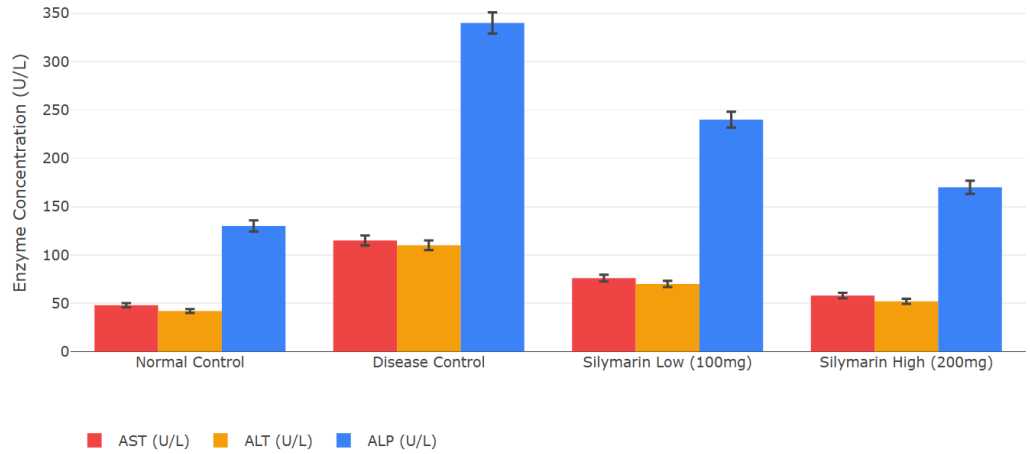

Figure 3. Comparison of Liver Function Biomarkers (AST, ALT, ALP)

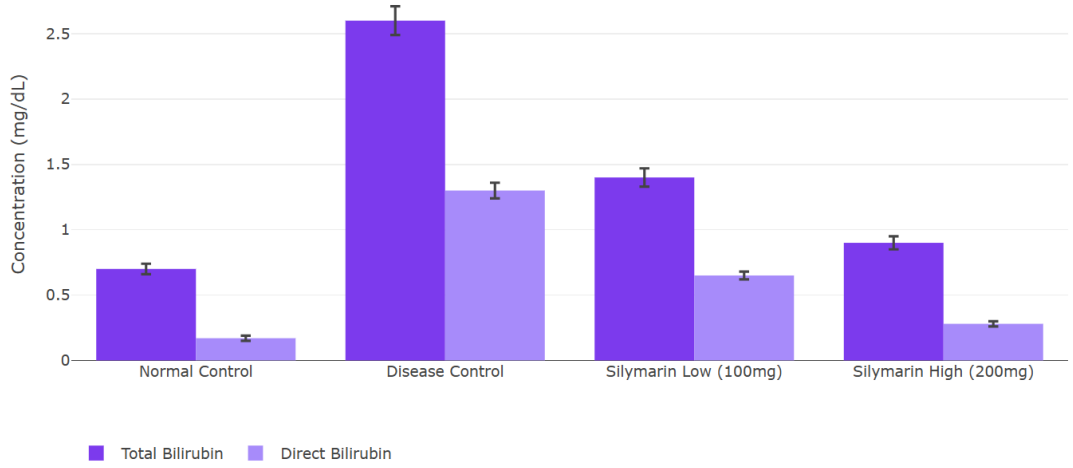

Figure 4. Effect of Silymarin on Serum Bilirubin

Pre-treatment with silymarin (Groups III and IV) significantly attenuated these elevations in a dose-dependent manner. The high-dose group (200 mg/kg) exhibited the most substantial protection, with enzyme levels closely approaching the values observed in the normal control group. Post-treatment (Group V) also showed a restorative effect on biochemical markers, although the degree of normalization was less pronounced than that observed in the pre-treatment high-dose group.

Table 3. Effect of Silymarin on Serum Protein Profile and A/G Ratio

Parameter	Normal Control (I)	Disease Control (II)	Silymarin Low Dose (III)	Silymarin High Dose (IV)
Total Protein (g/dL)	7.3 ± 0.3	5.9 ± 0.2*	6.8 ± 0.3#	7.2 ± 0.2##
Albumin (g/dL)	4.3 ± 0.2	3.2 ± 0.1*	3.8 ± 0.1#	4.1 ± 0.2##
Globulin (g/dL)	3.0 ± 0.2	4.1 ± 0.3*	3.0 ± 0.2#	3.1 ± 0.2##
A/G Ratio	1.43 ± 0.07	0.78 ± 0.04*	1.27 ± 0.06#	1.32 ± 0.06##

*Values are expressed as Mean ± SEM (n=6). () $p < 0.05$ vs. Normal Control; (#) $p < 0.05$ vs. Disease Control; (##) $p < 0.01$ vs. Disease Control.**

3.2. Macroscopic Evaluation of Liver Tissue

Macroscopic examination of the livers from Group I revealed a healthy, reddish-brown color with a smooth, glistening surface and firm consistency. In contrast, livers from Group II (Disease Control) appeared enlarged and pale, with visible mottling and a granular surface texture indicative of extensive necrosis and congestion.

Livers from the silymarin-treated groups showed a remarkable recovery in physical appearance. The high-dose pre-treatment group (Group IV) displayed a morphology nearly indistinguishable from the normal control, whereas the low-dose and post-treatment groups showed moderate improvements in coloration and surface texture.

Table 4. Observed Liver (Macroscopical)Morphology

Group	Coloration	Surface Texture	Lobular Architecture	Overall Status
Normal Control	Reddish-brown	Smooth/Glistening	Well-defined	Normal
Disease Control	Pale/Darkened	Irregular/Granular	Distorted	Severe Injury
Silymarin Low	Near-normal	Slightly Irregular	Improved	Moderate Protection
Silymarin High	Reddish-brown	Smooth	Preserved	High Protection

3.3. Histopathological Observations

The histological analysis of liver sections from the Normal Control group showed intact lobular architecture with hepatocytes arranged in radiating cords around the central vein. In the Paracetamol-treated group, severe pathological changes were observed, characterized by extensive centrilobular necrosis, ballooning degeneration of hepatocytes, and significant infiltration of inflammatory cells.

Table 5. Semiquantitative Scoring of Histopathological Alterations

Histological Feature	Group I	Group II	Group III	Group IV
Centrilobular Necrosis	-	+++	++	-
Ballooning Degeneration	-	+++	++	+
Inflammatory Infiltration	-	+++	++	+
Sinusoidal Congestion	-	++	+	-
Hepatic Architecture	Intact	Distorted	Partial Recovery	Normalized

Scoring: (-) Absence of lesion; (+) Mild; (++) Moderate; (+++) Severe/Widespread.

The silymarin-treated groups (100 and 200 mg/kg) exhibited a dose-dependent reduction in these pathological markers. In the 200 mg/kg pre-treatment group, the hepatic architecture was remarkably preserved, showing only minimal inflammatory changes and no evidence of significant necrosis. The post-treatment group also showed signs of active regeneration, with a reduction in the area of necrotic tissue compared to the disease control.

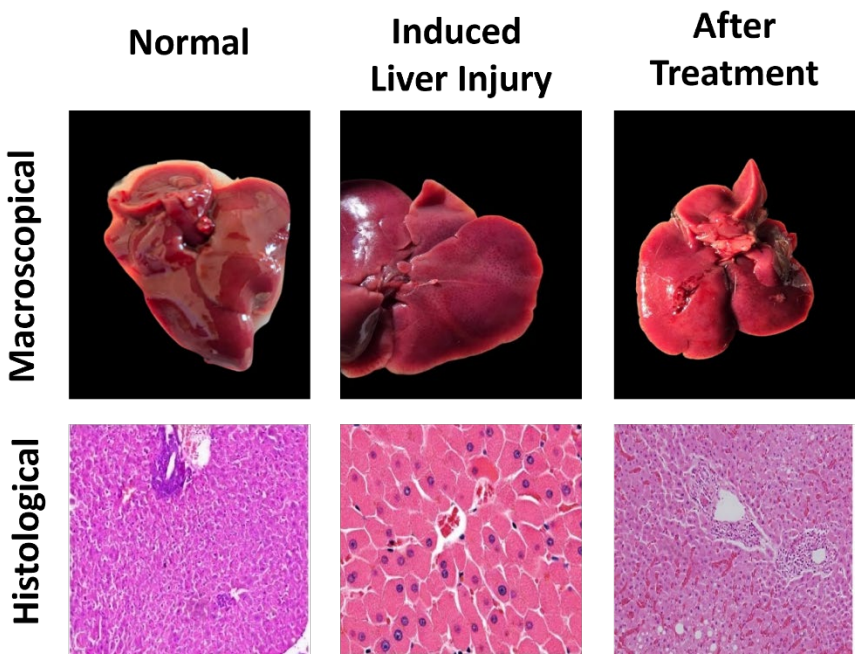

Figure 5. Macroscopical Histopathological Sections (H & E staining) of Liver Tissue

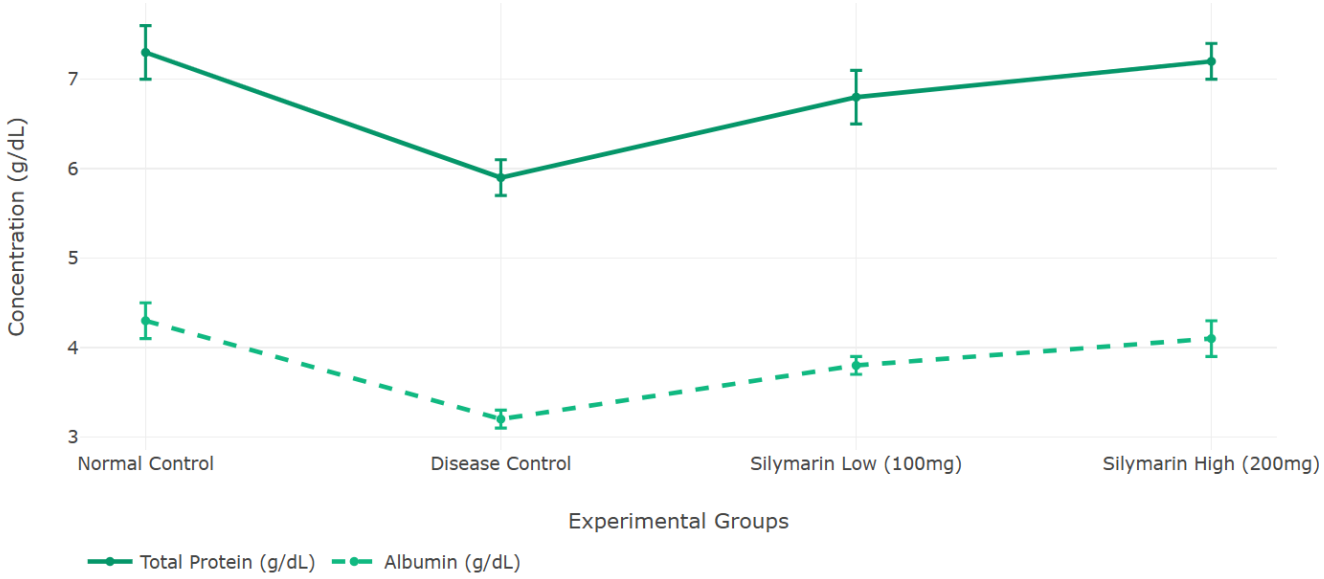

Figure 6. Effect of Silymarin on Serum Total Protein and Albumin Levels

4. Discussion

The induction of hepatic injury by paracetamol is a well-established experimental model that mimics the clinical progression of acute liver failure. The pathogenesis is primarily driven by the metabolic activation of paracetamol into the highly reactive electrophile NAPQI [20]. The results of this study confirm that a supratherapeutic dose of paracetamol leads to the depletion of hepatic glutathione, which subsequently allows NAPQI to trigger widespread oxidative stress and lipid peroxidation [21]. The significant elevation of serum AST, ALT, and ALP in the disease control group serves as a clear indicator of loss of membrane integrity and cellular leakage, which is corroborated by the observed centrilobular necrosis in histopathological sections.

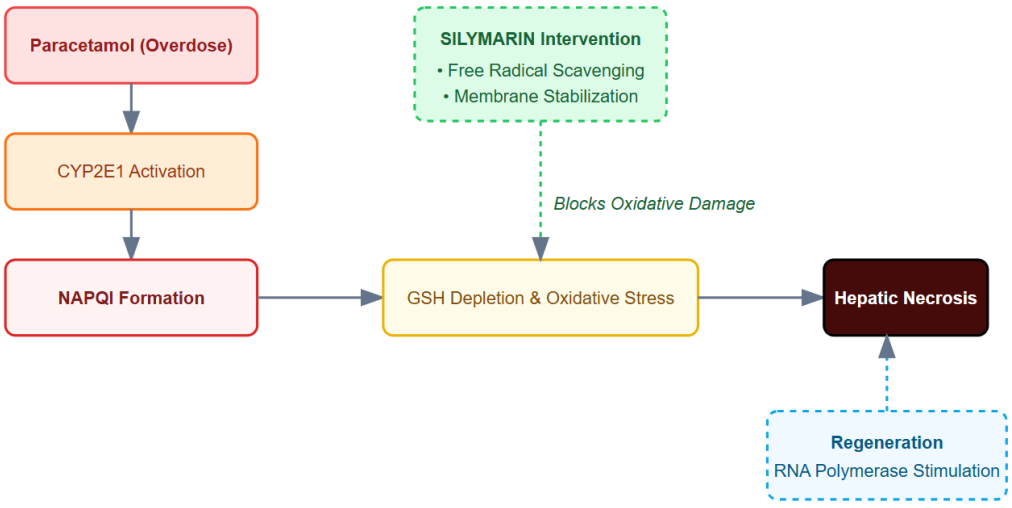

Figure 7. Mechanism of Paracetamol-Induced Hepatotoxicity and Silymarin Intervention

The hepatoprotective activity of Silymarin is likely mediated through its ability to act as a potent antioxidant and free-radical scavenger. Silymarin prevents the initial phase of hepatocellular damage by neutralizing reactive intermediates and inhibiting lipid peroxidation [22]. The dose-dependent reduction in serum enzymes observed in Groups III and IV suggests that silymarin successfully maintains the structural integrity of the hepatocyte plasma membrane, thereby preventing the leakage of intracellular enzymes. This membrane-stabilizing effect is a hallmark of flavonolignans, which alter the lipid composition of the membrane to hinder the entry of toxic metabolites [23].

The restorative effects seen in the post-treatment group (Group V) highlight silymarin's role in promoting liver regeneration. Silymarin accelerates the replacement of damaged hepatocytes with healthy tissue by stimulating RNA polymerase I and increasing ribosomal protein synthesis [24]. While pre-treatment provided superior protection, the curative potential observed in the post-treatment model underscores the therapeutic versatility of silymarin in managing ongoing hepatic insult. The preservation of total protein and albumin levels in the silymarin-treated groups further supports its role in maintaining the synthetic capacity of the liver, which is often compromised during severe toxication [25].

5. Conclusion

The present study shows that silymarin possesses significant hepatoprotective and regenerative properties against acetaminophen-induced liver injury in Wistar rats. The administration of silymarin, particularly as a pre-treatment at higher dosages, effectively mitigates biochemical markers of hepatic damage and preserves the structural integrity of the liver architecture. These effects are attributed to the synergistic action of free-radical scavenging, membrane stabilization, and the enhancement of protein synthesis. While clinical management of drug-induced liver injury remains complex, these findings reinforce the potential of silymarin as a robust natural therapeutic agent capable of preventing and reversing oxidative hepatic damage. Molecular studies are required to elucidate the specific signaling pathways through which silymarin modulates the cellular response to acute metabolic stress.

Compliance with ethical standards

Acknowledgements

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Conflict of interest statement

The authors declare that they have no potential conflicts of interest or competing interests with respect to the research, authorship, and/or publication of this manuscript. No financial support or products from any third-party institutions influenced the outcome of this study.

Statement of ethical approval

The experimental protocol was reviewed and approved (IAEC/KGRL/2025/126) by the Institutional Animal Ethics Committee (IAEC) of the institute and was conducted in strict accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), formerly known as CPCSEA, Government of India. All procedures were performed under appropriate ethical oversight to ensure animal welfare and the application of the 3Rs principles.

Statement of informed consent

The present research work does not contain any studies performed on human subjects by any of the authors. Therefore, the statement of informed consent is not applicable to this study.

References

[1] Kaplowitz N. Drug-induced liver injury. Clin Infect Dis. 2004;38(Supplement_2):S44-S48.

[2] Grant DM. Detoxification pathways in the liver. J Inherit Metab Dis. 1991;14(4):421-430.

[3] Ostapowicz G, Fontana RJ, Schiødt FV, Larson A, Davern TJ, Han SH, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 2002;137(12):947-954.

[4] Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. Toxicol Sci. 2002;65(2):166-176.

[5] Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. Handb Exp Pharmacol. 2010;(196):369-405.

[6] Jaeschke H, Bajt ML. Intracellular signaling mechanisms of acetaminophen-induced liver cell death. Toxicol Sci. 2006;89(1):31-41.

[7] McGill MR, Williams CD, Xie Y, Ramachandran A, Jaeschke H. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. Toxicol Appl Pharmacol. 2012;264(3):387-394.

[8] Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). N Engl J Med. 1988;319(24):1557-1562.

[9] Yarema M, Chopra P, Sivilotti ML, Johnson D, Nettel-Aguirre A, Victorino C, et al. Anaphylactoid reactions to intravenous N-acetylcysteine: a prospective case-control study. Postgrad Med J. 2018;94(1110):210-215.

[10] Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs. 2001;61(14):2035-2063.

[11] Gazák R, Walterová D, Kren V. Silybin and silymarin—new and emerging applications in medicine. Curr Med Chem. 2007;14(3):315-338.

[12] Sonnenbichler J, Zetl I. Stimulating influence of a flavonolignan derivative on proliferation, RNA synthesis and protein synthesis in liver cells. In: Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties. Alan R. Liss, Inc.; 1988. p. 363-374.

[13] Graham GG, Scott KF. Mechanism of action of paracetamol. Am J Ther. 2005;12(1):46-55.

[14] Prescott LF. Paracetamol: past, present, and future. Am J Ther. 2000;7(2):143-148.

[15] Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. Proc Natl Acad Sci U S A. 1984;81(5):1327-1331.

[16] Braidy N, Poljak A, Marjo C, Rutlidge H, Rich A, Jugder BE, et al. Identification of 7-hydroxysilybin in the seeds of Silybum marianum. Molecules. 2017;22(12):2066.

[17] Valenzuela A, Garrido A. Biochemical bases of the pharmacological action of the flavonoid silymarin and of its main constituent, silybin. Biol Res. 1994;27(2):105-112.

[18] Ramasamy K, Agarwal R. Multitargeted therapy of cancer by silymarin. Cancer Lett. 2008;269(2):352-362.

[19] Wellington K, Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. BioDrugs. 2001;15(7):465-489.

[20] Nelson SD. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Semin Liver Dis. 1990;10(04):267-278.

[21] Knight TR, Ho YS, Farhood A, Jaeschke H. Peroxynitrite is a critical mediator of acetaminophen hepatotoxicity in murine central veins: evidence from glutathione peroxidase-1-deficient mice. Chem Biol Interact. 2002;139(2):141-158.

[22] Fehér J, Lengyel G. Silymarin in the prevention and treatment of liver diseases and primary liver cancer. Curr Pharm Biotechnol. 2012;13(1):210-217.

[23] Polyak SJ, Ferenci P, Pawlotsky JM. Silymarin and HCV infection. Antivir Ther. 2013;18(2):141-147.

[24] Machicao F, Sonnenbichler J. Mechanism of the stimulation of RNA synthesis in rat liver nuclei by silybin. Hoppe Seylers Z Physiol Chem. 1977;358(2):141-147.

[25] Muriel P, Mourelle M. Prevention by silymarin of membrane alterations in acute CCl4 liver damage. J Appl Toxicol. 1990;10(4):275-279.