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Experimental Evaluation of Hepatoprotective Activity of Silymarin Against Paracetamol-Induced Hepatotoxicity in Wistar Rats Using Serum Biochemical Assays (ALT, AST, ALP), Oxidative Stress Markers (MDA, SOD, Catalase), and Histopathological Analysis

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Abstract

The liver is a vital organ responsible for numerous physiological functions, including metabolism, detoxification, protein synthesis, and maintenance of biochemical homeostasis. Drug-induced liver injury remains a significant clinical concern worldwide, with paracetamol overdose being one of the most common causes of acute hepatic failure. The present study was undertaken to evaluate the hepatoprotective activity of silymarin against paracetamol-induced hepatotoxicity in Wistar rats using biochemical, antioxidant, and histopathological parameters. Thirty healthy Wistar rats were randomly divided into five groups comprising normal control, toxic control, low-dose silymarin-treated, high-dose silymarin-treated, and standard control groups. Hepatotoxicity was induced by oral administration of paracetamol (2 g/kg body weight), while silymarin was administered at doses of 100 mg/kg and 200 mg/kg body weight for seven consecutive days.

Serum biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were estimated to assess liver function. Oxidative stress was evaluated by measuring malondialdehyde (MDA), superoxide dismutase (SOD), and catalase levels in liver tissue homogenates. Histopathological examination of liver sections stained with hematoxylin and eosin was performed to assess structural alterations.

Paracetamol administration resulted in significant elevation of serum ALT, AST, ALP, and MDA levels, accompanied by marked reductions in SOD and catalase activities, indicating severe hepatic damage and oxidative stress. Histopathological analysis revealed extensive hepatocellular degeneration, necrosis, inflammatory infiltration, and disruption of normal hepatic architecture in the toxic control group. Treatment with silymarin significantly attenuated these alterations in a dose-dependent manner. Silymarin-treated groups exhibited substantial reductions in serum liver enzyme levels and lipid peroxidation, along with restoration of antioxidant enzyme activities. Histological findings demonstrated marked improvement in hepatic architecture, reduced necrosis, and decreased inflammatory changes compared with the toxic control group. The high-dose silymarin group showed near-normal biochemical and histological characteristics.

The findings of this study demonstrate that silymarin possesses significant hepatoprotective activity against paracetamol-induced liver injury, primarily through its antioxidant, membrane-stabilizing, and tissue-regenerative properties. These results support the therapeutic potential of silymarin as an effective natural hepatoprotective agent for the management of drug-induced liver disorders.

1. INTRODUCTION

The liver is the largest internal organ and one of the most metabolically active organs in the human body, performing a wide range of physiological functions essential for survival. It plays a central role in the metabolism of carbohydrates, proteins, and lipids, while also participating in the synthesis of plasma proteins, bile production, storage of glycogen, vitamins, and minerals, and regulation of endocrine functions. Furthermore, the liver serves as the primary detoxification organ, responsible for the biotransformation and elimination of endogenous metabolites, xenobiotics, environmental toxins, and pharmaceutical agents. Due to its strategic anatomical location and continuous exposure to blood-borne substances from the gastrointestinal tract, the liver is highly susceptible to toxic insults and pathological conditions. Maintenance of liver health is therefore crucial for preserving metabolic homeostasis and overall physiological well-being (Trefts et al., 2017; Hall & Hall, 2021).

Hepatotoxicity refers to liver injury resulting from exposure to chemical substances, drugs, herbal products, environmental pollutants, or infectious agents. Drug-induced liver injury (DILI) represents one of the leading causes of acute liver failure worldwide and poses significant challenges in clinical practice and drug development. The mechanisms underlying hepatotoxicity are complex and involve oxidative stress, mitochondrial dysfunction, disruption of cellular calcium homeostasis, immune-mediated responses, inflammation, and apoptosis. Excessive generation of reactive oxygen species (ROS) and depletion of endogenous antioxidant defenses can initiate lipid peroxidation, protein oxidation, and DNA damage, ultimately leading to hepatocellular degeneration and necrosis. Clinically, hepatotoxicity is characterized by elevated serum liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), which serve as important indicators of hepatic injury and dysfunction (Navarro & Senior, 2006; Ramachandran & Jaeschke, 2018).

Among various hepatotoxic agents, paracetamol (acetaminophen) is one of the most extensively used experimental models for investigating liver injury and evaluating hepatoprotective compounds. Paracetamol is a widely prescribed analgesic and antipyretic drug that is generally considered safe at therapeutic doses. However, overdose or prolonged administration can result in severe hepatotoxicity and acute liver failure. Under normal conditions, paracetamol is metabolized primarily through glucuronidation and sulfation pathways in the liver. A small fraction undergoes cytochrome P450-mediated oxidation, particularly by CYP2E1, generating the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). At therapeutic doses, NAPQI is rapidly detoxified through conjugation with glutathione. During overdose conditions, glutathione stores become depleted, leading to the accumulation of NAPQI, which covalently binds to cellular proteins and induces oxidative stress, mitochondrial dysfunction, inflammatory responses, and hepatocellular necrosis. Consequently, paracetamol-induced hepatotoxicity has become a well-established and reproducible experimental model for assessing the efficacy of hepatoprotective agents in laboratory animals (James et al., 2003; Ramachandran & Jaeschke, 2018).

Natural products have gained increasing attention as potential therapeutic agents for the prevention and management of liver diseases. Among these, silymarin has emerged as one of the most extensively investigated hepatoprotective phytoconstituents. Silymarin is a flavonolignan complex isolated from the seeds and fruits of *Silybum marianum* (Milk Thistle), a medicinal plant belonging to the family Asteraceae. The major bioactive constituents of silymarin include silybin (silibinin), isosilybin, silychristin, and silydianin, with silibinin being considered the most pharmacologically active component. Numerous experimental and clinical studies have demonstrated that silymarin possesses antioxidant, anti-inflammatory, antifibrotic, immunomodulatory, and membrane-stabilizing

properties. Its hepatoprotective activity is primarily attributed to its ability to scavenge free radicals, inhibit lipid peroxidation, enhance endogenous antioxidant enzyme activities, preserve cellular glutathione levels, and stabilize hepatocyte membranes against toxic insults (Abenavoli et al., 2018; Federico et al., 2017).

Several investigations have reported the beneficial effects of silymarin against chemically induced liver injury, including paracetamol-mediated hepatotoxicity. Experimental studies have shown that administration of silymarin significantly reduces serum liver enzyme levels, decreases malondialdehyde (MDA) concentrations, enhances antioxidant enzymes such as superoxide dismutase (SOD) and catalase, and improves histopathological architecture of hepatic tissues. These protective effects suggest that silymarin may effectively counteract oxidative stress-mediated liver damage and facilitate hepatic regeneration. Therefore, silymarin continues to be widely used as a standard hepatoprotective agent in preclinical studies evaluating novel therapeutic interventions for liver disorders (Federico et al., 2017; Surai, 2015).

Considering the growing incidence of drug-induced liver injury and the need for effective hepatoprotective therapies, the present study was designed to evaluate the hepatoprotective potential of silymarin against paracetamol-induced hepatotoxicity in Wistar rats. The study aims to investigate the effects of silymarin on serum biochemical markers of liver function, including ALT, AST, and ALP, as well as oxidative stress parameters such as MDA, SOD, and catalase. In addition, histopathological examination of liver tissues will be performed to assess the extent of hepatic protection and tissue recovery following treatment. The findings of this study may contribute to a better understanding of the protective mechanisms of silymarin and support its therapeutic application in the management of liver disorders.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and Reagents

Silymarin was procured from a certified pharmaceutical supplier and used as the hepatoprotective agent in the present study. Paracetamol was obtained in analytical grade purity and utilized for the induction of experimental hepatotoxicity. Commercial diagnostic kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were purchased from a reputed biochemical reagent manufacturer. All chemicals and reagents used in the study were of analytical grade.

The reagents employed for the assessment of oxidative stress biomarkers included thiobarbituric acid (TBA), trichloroacetic acid (TCA), and standard malondialdehyde (MDA) reagents for lipid peroxidation analysis. Superoxide dismutase (SOD) activity was estimated using nitroblue tetrazolium (NBT)-based assay reagents, while catalase activity was determined using hydrogen peroxide substrate and phosphate buffer solutions. Distilled water and other routine laboratory chemicals were used throughout the experimental procedures.

2.1.2 Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 150 and 200 g and aged approximately 8–10 weeks were selected for the study. The animals were procured from a CPCSEA-registered animal breeding facility and acclimatized to laboratory conditions for one week before initiation of the experiment.

The animals were housed in polypropylene cages with sterile paddy husk bedding under standard laboratory conditions maintained at a temperature of 22 ± 2°C, relative humidity of 55 ± 10%, and a 12-hour light/dark cycle. Rats were provided with a standard pellet diet and water ad libitum throughout the experimental period. Proper hygienic conditions were maintained, and animal health was monitored regularly.

2.2 Ethical Approval

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the respective institution before commencement of the study. All procedures involving laboratory animals were conducted in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Necessary measures were taken to minimize animal suffering and reduce the number of animals used in the experiment.

2.3 Experimental Design

A total of thirty healthy Wistar rats were randomly divided into five groups consisting of six animals each (n = 6).

Group I: Normal Control

Animals received the vehicle (0.5% carboxymethyl cellulose) orally for seven consecutive days and served as the normal control group.

Group II: Toxic Control

Animals received the vehicle orally for seven days followed by administration of paracetamol to induce hepatotoxicity and served as the toxic control group.

Group III: Silymarin Low-Dose Treatment

Animals received silymarin at a dose of 100 mg/kg body weight orally for seven consecutive days along with paracetamol-induced hepatotoxicity.

Group IV: Silymarin High-Dose Treatment

Animals received silymarin at a dose of 200 mg/kg body weight orally for seven consecutive days along with paracetamol-induced hepatotoxicity.

Group V: Standard Control

Animals received the standard hepatoprotective treatment with silymarin at 100 mg/kg body weight orally and served as the reference standard group.

2.4 Induction of Hepatotoxicity

Experimental hepatotoxicity was induced by administration of paracetamol at a dose of 2 g/kg body weight orally. The selected dose was administered on the seventh day following pretreatment with silymarin. Paracetamol was suspended in distilled water immediately before administration to ensure uniform dosing.

The induction protocol was designed to produce acute hepatic injury characterized by oxidative stress, elevated liver enzyme levels, and histopathological alterations. Animals were observed for signs of toxicity throughout the study period.

2.5 Drug Administration Protocol

Silymarin was suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally using an intragastric feeding needle. The treatment was continued once daily for seven consecutive days. Low-dose and high-dose treatment groups received 100 mg/kg and 200 mg/kg body weight of silymarin, respectively.

Paracetamol was administered one hour after the final dose of silymarin on the seventh day. The treatment schedule was designed to evaluate the preventive hepatoprotective effect of silymarin against paracetamol-induced liver injury.

2.6 Collection of Blood Samples

Twenty-four hours after paracetamol administration, animals were fasted overnight and anesthetized using ketamine-xylazine anesthesia. Blood samples were collected by retro-orbital puncture or cardiac puncture under aseptic conditions.

The collected blood samples were transferred into sterile centrifuge tubes and allowed to clot at room temperature. Samples were centrifuged at 3000 rpm for 15 minutes to separate the serum. The obtained serum was carefully collected and stored at −20°C until biochemical analysis.

Following blood collection, animals were euthanized humanely, and liver tissues were excised immediately for antioxidant and histopathological investigations.

2.7 Estimation of Serum Biochemical Parameters

Serum biochemical markers were estimated using commercially available diagnostic kits according to the manufacturer's instructions.

2.7.1 Alanine Aminotransferase (ALT)

Serum ALT activity was determined using a kinetic enzymatic method. The assay was based on the conversion of alanine to pyruvate in the presence of ALT. Increased serum ALT levels were considered indicative of hepatocellular damage and membrane leakage.

2.7.2 Aspartate Aminotransferase (AST)

Serum AST activity was measured by an enzymatic colorimetric method. The enzyme catalyzes the transfer of amino groups from aspartate to α-ketoglutarate, producing oxaloacetate and glutamate. Elevated AST levels reflected liver tissue injury and cellular necrosis.

2.7.3 Alkaline Phosphatase (ALP)

Serum ALP activity was estimated using a colorimetric assay based on hydrolysis of p-nitrophenyl phosphate. Increased ALP activity indicated hepatobiliary dysfunction and impaired liver function.

2.8 Estimation of Oxidative Stress Markers

Liver tissues were homogenized in ice-cold phosphate buffer and centrifuged to obtain the post-mitochondrial supernatant for antioxidant enzyme analysis.

2.8.1 Malondialdehyde (MDA)

Lipid peroxidation was assessed by measuring malondialdehyde levels using the thiobarbituric acid reactive substances (TBARS) method. The formation of a pink chromogen resulting from the reaction between MDA and thiobarbituric acid was measured spectrophotometrically at 532 nm. Results were expressed as nmol MDA/mg protein.

2.8.2 Superoxide Dismutase (SOD)

Superoxide dismutase activity was determined based on its ability to inhibit the reduction of nitroblue tetrazolium by superoxide radicals. Absorbance was measured spectrophotometrically, and enzyme activity was expressed as units per milligram protein.

2.8.3 Catalase Activity

Catalase activity was estimated by measuring the decomposition rate of hydrogen peroxide. The decrease in absorbance due to hydrogen peroxide breakdown was monitored spectrophotometrically at 240 nm. Enzyme activity was expressed as units per milligram protein.

2.9 Histopathological Examination

Immediately after sacrifice, liver tissues were carefully excised, washed with normal saline to remove blood, and fixed in 10% neutral buffered formalin for 24–48 hours.

The fixed tissues were processed through graded alcohol series for dehydration, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned into 4–5 µm thick slices using a rotary microtome.

The tissue sections were mounted on glass slides and stained with hematoxylin and eosin (H&E). Histological examination was performed under a light microscope to assess hepatic architecture, cellular degeneration, necrosis, inflammatory infiltration, sinusoidal dilatation, and regeneration of hepatocytes. Representative photomicrographs were

captured for documentation and comparative analysis.

2.10 Statistical Analysis

All experimental data were expressed as Mean ± Standard Error of Mean (SEM) for six animals in each group. Statistical analysis was performed using GraphPad Prism software.

Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A probability value of $p < 0.05$ was considered statistically significant. The results were presented in the form of tables and graphs to facilitate interpretation of the hepatoprotective effects of silymarin against paracetamol-induced liver injury.

3. RESULTS

3.1 Effect of Silymarin on Serum Biochemical Parameters

The effect of silymarin on serum liver function biomarkers, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), is presented in Table 1. Administration of paracetamol in the toxic control group (Group II) resulted in a significant elevation ($p < 0.001$) of serum ALT, AST, and ALP levels compared with the normal control group (Group I), indicating severe hepatic injury and leakage of intracellular enzymes into the bloodstream.

Pretreatment with silymarin significantly attenuated the elevation of serum liver enzymes. The low-dose silymarin treatment group (Group III) exhibited a moderate reduction in ALT, AST, and ALP levels compared with the toxic control group ($p < 0.05$). The high-dose silymarin treatment group (Group IV) demonstrated a more pronounced reduction in enzyme levels ($p < 0.001$), indicating superior hepatoprotective activity. The standard control group (Group V) also showed significant restoration of liver function biomarkers toward normal values.

The observed reduction in serum enzyme levels suggests that silymarin effectively protected hepatocytes from paracetamol-induced damage and preserved the structural integrity of hepatic cell membranes.

Table 1. Effect of Silymarin on Serum Biochemical Parameters in Paracetamol-Induced Hepatotoxicity

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Group I (Normal Control)	34.28 ± 2.11	78.42 ± 3.26	112.65 ± 5.47
Group II (Toxic Control)	125.84 ± 5.62***	218.53 ± 7.85***	289.74 ± 10.32***
Group III (Silymarin 100 mg/kg)	82.47 ± 4.19#	156.72 ± 6.43#	201.56 ± 8.41#
Group IV (Silymarin 200 mg/kg)	48.63 ± 2.87###	96.48 ± 4.12###	132.84 ± 6.27###
Group V (Standard Control)	42.19 ± 2.54###	88.65 ± 3.91###	124.37 ± 5.92###

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

***$p < 0.001$ compared with Normal Control.

#$p < 0.05$, ###$p < 0.001$ compared with Toxic Control.

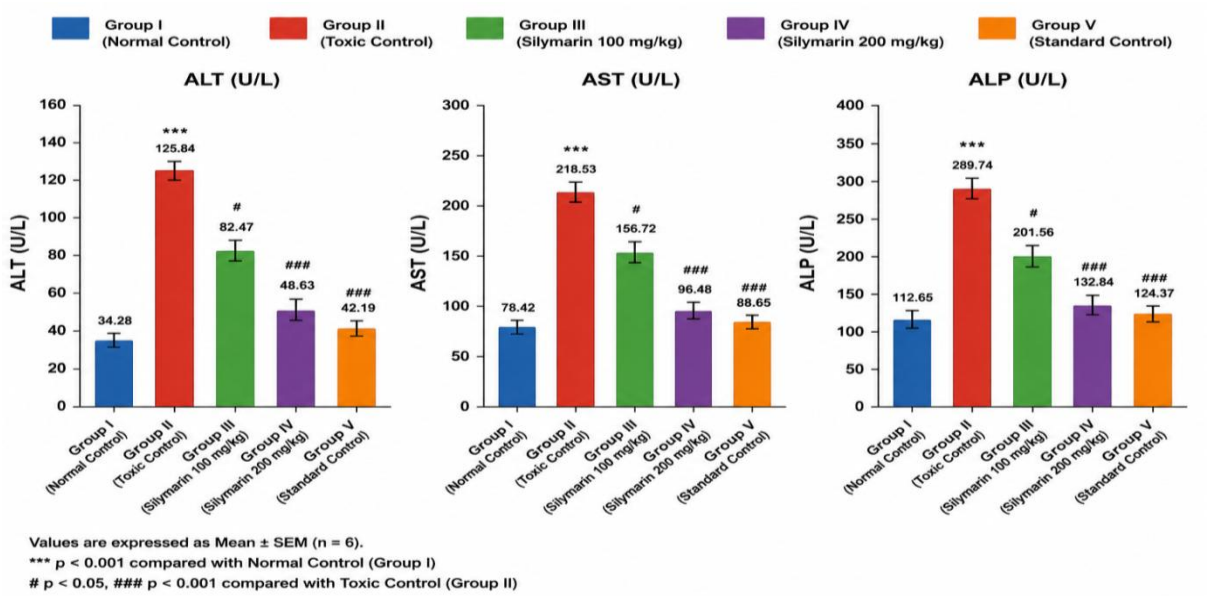

Figure 1. Comparative Graphical Representation of Serum ALT, AST, and ALP Levels

Figure 1 illustrates the comparative changes in serum liver enzyme levels among different experimental groups. The toxic control group exhibited the highest enzyme levels, whereas silymarin-treated groups showed a dose-dependent reduction. The high-dose silymarin group demonstrated values approaching those observed in the normal control group, confirming substantial hepatoprotection.

3.2 Effect of Silymarin on Oxidative Stress Markers

The effects of silymarin on oxidative stress parameters are presented in Table 2. Administration of paracetamol significantly increased hepatic malondialdehyde (MDA) levels while simultaneously reducing the activities of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase in the toxic control group compared with normal animals.

Treatment with silymarin significantly ameliorated oxidative stress by decreasing MDA levels and enhancing antioxidant enzyme activities. The high-dose treatment group demonstrated greater antioxidant protection than the low-dose treatment group. The results indicate that silymarin effectively counteracted lipid peroxidation and restored antioxidant defense mechanisms disrupted by paracetamol toxicity.

Table 2. Effect of Silymarin on Oxidative Stress Markers

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	Catalase (U/mg protein)
Group I (Normal Control)	2.18 ± 0.16	9.82 ± 0.43	52.64 ± 2.17
Group II (Toxic Control)	8.94 ± 0.37***	3.14 ± 0.22***	21.58 ± 1.64***
Group III (Silymarin 100 mg/kg)	5.76 ± 0.28#	5.92 ± 0.31#	35.74 ± 1.82#
Group IV (Silymarin 200 mg/kg)	3.05 ± 0.21###	8.42 ± 0.39###	47.86 ± 2.03###
Group V (Standard Control)	2.64 ± 0.18###	8.96 ± 0.41###	50.35 ± 2.11###

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

***p < 0.001 compared with Normal Control.

#p < 0.05, ###p < 0.001 compared with Toxic Control.

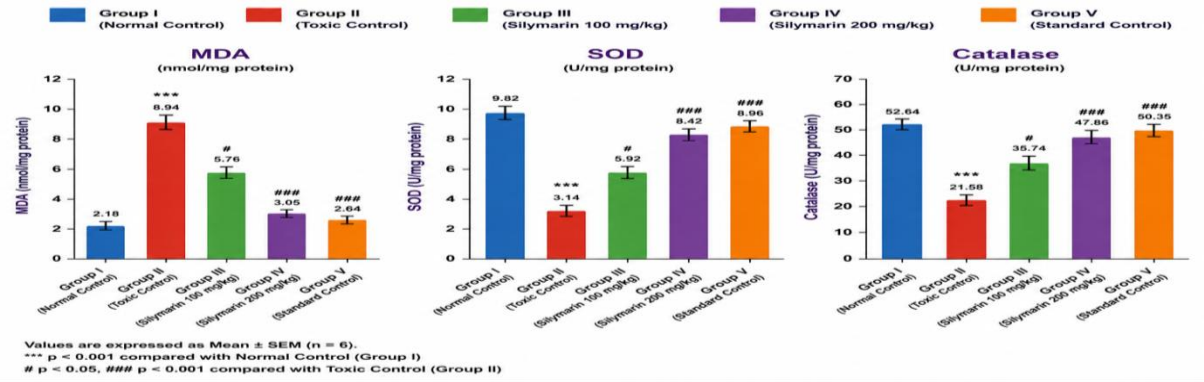

Figure 2. Graphical Comparison of Oxidative Stress Markers

The graphical representation demonstrates a marked increase in MDA levels and significant depletion of antioxidant enzymes following paracetamol administration. Silymarin treatment effectively reduced lipid peroxidation and restored antioxidant status in a dose-dependent manner, with the high-dose group exhibiting near-normal values.

3.3 Histopathological Findings

Histopathological examination of liver tissues further confirmed the biochemical and antioxidant findings. Representative photomicrographs of liver sections stained with hematoxylin and eosin (H&E) are presented in Figure 3.

Group I: Normal Control

Liver sections from the normal control group exhibited normal hepatic architecture characterized by well-arranged hepatocytes, intact central veins, normal hepatic cords, and absence of inflammatory infiltration or cellular degeneration. Hepatocytes appeared polygonal with distinct nuclei and preserved cytoplasmic morphology.

Group II: Toxic Control

The toxic control group displayed severe hepatic damage characterized by extensive hepatocellular degeneration, centrilobular necrosis, sinusoidal congestion, inflammatory cell infiltration, vacuolar degeneration, and disruption of normal hepatic architecture. These pathological changes confirmed successful induction of paracetamol-induced hepatotoxicity.

Group III: Silymarin Low-Dose Treatment

Liver sections from the low-dose silymarin-treated group showed moderate protection against hepatic injury. Reduced necrotic areas, mild inflammatory infiltration, partial restoration of hepatic cords, and improved cellular morphology were observed compared with the toxic control group.

Group IV: Silymarin High-Dose Treatment

The high-dose silymarin-treated group demonstrated marked restoration of normal hepatic architecture. Hepatocytes appeared nearly normal with minimal cellular degeneration and inflammatory infiltration. The central vein and hepatic sinusoids exhibited normal morphology, indicating substantial hepatoprotection.

Group V: Standard Control

The standard control group showed near-normal hepatic architecture with well-preserved hepatocytes, intact hepatic cords, and minimal histopathological abnormalities. The liver tissue closely resembled that of the normal control group.

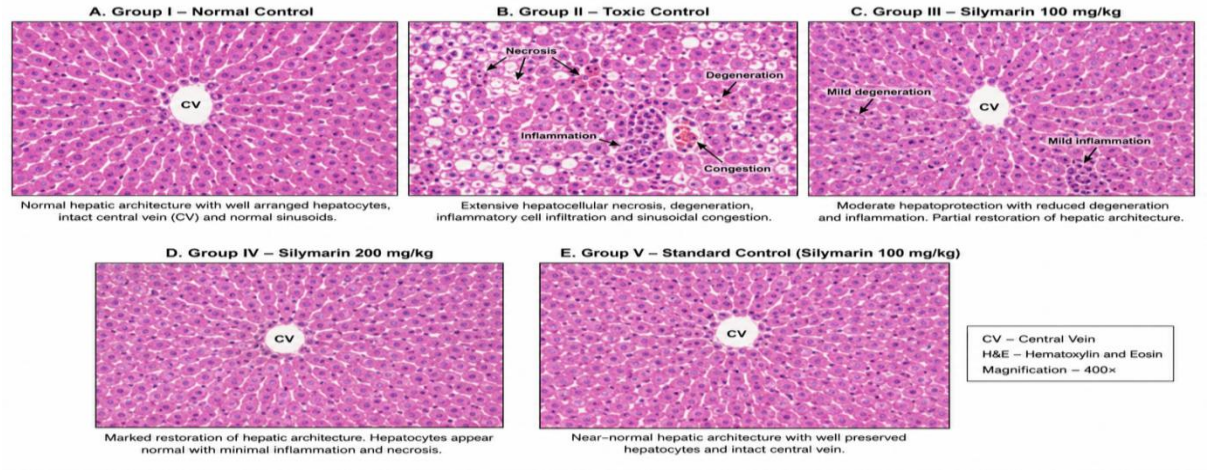

Figure 3. Photomicrographs of Liver Sections (H&E Staining, 400×)

3.4 Statistical Comparison Among Experimental Groups

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed significant differences among experimental groups. The toxic control group exhibited significantly elevated serum liver enzymes and MDA levels along with significantly reduced SOD and catalase activities compared with the normal control group ($p < 0.001$).

Treatment with silymarin produced a statistically significant improvement in all evaluated biochemical and antioxidant parameters. The hepatoprotective effect was found to be dose-dependent, with the high-dose silymarin group demonstrating greater protection than the low-dose group. Histopathological observations corroborated the biochemical findings, showing progressive restoration of liver architecture with increasing doses of silymarin.

Overall, the results indicate that silymarin effectively protects against paracetamol-induced hepatotoxicity by reducing liver enzyme leakage, suppressing oxidative stress, enhancing endogenous antioxidant defenses, and preserving normal hepatic histology.

4. DISCUSSION

Paracetamol-induced hepatotoxicity is one of the most widely accepted experimental models for investigating liver injury and evaluating the efficacy of hepatoprotective agents. The present study successfully established acute hepatic damage in Wistar rats through administration of a toxic dose of paracetamol, as evidenced by significant alterations in serum biochemical parameters, oxidative stress markers, and histopathological architecture. The findings demonstrated that pretreatment with silymarin provided substantial protection against paracetamol-induced liver injury in a dose-dependent manner.

The hepatotoxic effects of paracetamol are primarily attributed to its biotransformation into the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) through the cytochrome P450 enzyme system, particularly CYP2E1. Under normal physiological conditions, NAPQI is rapidly detoxified by conjugation with reduced glutathione (GSH). However, excessive paracetamol administration results in depletion of hepatic glutathione reserves, leading to accumulation of NAPQI within hepatocytes. The reactive metabolite subsequently binds to cellular proteins and mitochondrial components, initiating oxidative stress, mitochondrial dysfunction, inflammatory responses, and cellular necrosis (James et al., 2003; Ramachandran & Jaeschke, 2018). In the present study, the toxic control group exhibited marked elevations in serum ALT, AST, and ALP levels, indicating significant hepatocellular membrane damage and leakage of intracellular enzymes into the circulation. These findings confirm successful induction of liver injury and are consistent with the characteristic biochemical manifestations of

paracetamol toxicity reported in previous studies (Jaeschke et al., 2012).

Assessment of serum liver function biomarkers revealed that silymarin treatment significantly reduced ALT, AST, and ALP levels compared with the toxic control group. ALT and AST are sensitive indicators of hepatocellular injury, whereas ALP is commonly associated with hepatobiliary dysfunction and cholestatic damage. The substantial reduction in these enzyme levels observed in silymarin-treated groups suggests preservation of hepatocyte membrane integrity and protection against cellular leakage. Furthermore, the high-dose silymarin group demonstrated a more pronounced reduction in enzyme levels than the low-dose group, indicating a dose-dependent hepatoprotective response. These observations support the hypothesis that silymarin effectively prevents hepatic damage and promotes restoration of normal liver function following toxic insult.

Oxidative stress plays a pivotal role in the pathogenesis of paracetamol-induced hepatotoxicity. Excessive production of reactive oxygen species and depletion of endogenous antioxidant systems contribute significantly to lipid peroxidation, protein oxidation, and DNA damage. In the present investigation, paracetamol administration caused a significant increase in hepatic malondialdehyde (MDA) levels, accompanied by marked reductions in superoxide dismutase (SOD) and catalase activities. Elevated MDA levels indicate enhanced lipid peroxidation and membrane damage, whereas reduced SOD and catalase activities reflect impairment of the endogenous antioxidant defense system. Treatment with silymarin significantly decreased MDA concentrations and restored antioxidant enzyme activities toward normal values. These findings indicate that silymarin effectively attenuated oxidative stress and protected hepatic tissues from free radical-mediated injury.

The antioxidant effect of silymarin observed in the present study may be attributed to its flavonolignan constituents, particularly silibinin, which possess potent free radical scavenging properties. Silymarin has been reported to inhibit lipid peroxidation, enhance glutathione synthesis, and improve the activity of endogenous antioxidant enzymes, thereby reducing oxidative damage within hepatocytes (Surai, 2015). The restoration of SOD and catalase activities observed in the treated groups further supports the role of silymarin in strengthening the cellular antioxidant defense system and maintaining redox homeostasis.

Histopathological examination provided additional evidence supporting the hepatoprotective activity of silymarin. Liver sections obtained from the normal control group displayed well-preserved hepatic architecture characterized by intact hepatocytes, normal central veins, and organized hepatic cords. In contrast, liver tissues from the toxic control group exhibited extensive hepatocellular degeneration, centrilobular necrosis, inflammatory cell infiltration, sinusoidal congestion, and disruption of normal hepatic architecture. These pathological alterations are characteristic features of paracetamol-induced liver injury and correlate strongly with the observed biochemical abnormalities.

Treatment with silymarin resulted in remarkable histological improvement. The low-dose treatment group exhibited moderate restoration of hepatic architecture with reduced necrosis and inflammation, while the high-dose group demonstrated near-normal liver morphology with minimal cellular degeneration and inflammatory infiltration. The histopathological findings closely paralleled the biochemical and antioxidant results, thereby confirming the protective effect of silymarin against paracetamol-induced hepatic damage. The preservation of hepatic structure observed in treated animals indicates effective prevention of cellular injury and enhanced tissue recovery.

The hepatoprotective activity of silymarin can be explained through multiple complementary mechanisms. First, its potent antioxidant activity reduces oxidative stress by scavenging reactive oxygen species and inhibiting lipid peroxidation. Second, silymarin stabilizes hepatocyte membranes, thereby preventing leakage of intracellular enzymes into the bloodstream. Third, it enhances intracellular glutathione levels and improves the activity of

endogenous antioxidant enzymes such as SOD and catalase. Fourth, silymarin has been shown to modulate inflammatory pathways by suppressing pro-inflammatory cytokines and reducing inflammatory cell infiltration. Additionally, silymarin promotes ribosomal RNA synthesis and protein production, thereby facilitating regeneration and repair of damaged hepatocytes (Abenavoli et al., 2018; Federico et al., 2017). These multifactorial actions collectively contribute to its hepatoprotective efficacy.

The findings of the present study are in agreement with numerous previous investigations demonstrating the protective effects of silymarin against experimentally induced liver injury. Several researchers have reported significant reductions in serum transaminase levels, restoration of antioxidant enzyme activities, and improvement in histopathological features following silymarin administration in various hepatotoxicity models. Similar to the present findings, previous studies have shown that silymarin effectively attenuates paracetamol-induced oxidative stress and preserves hepatic architecture (Surai, 2015; Abenavoli et al., 2018). Although slight variations in dosage regimens, experimental duration, and animal species have been reported across studies, the overall protective trend remains consistent. The dose-dependent improvements observed in the present investigation further validate the therapeutic potential of silymarin as a hepatoprotective agent.

Overall, the results obtained from biochemical assays, antioxidant evaluations, and histopathological examinations collectively demonstrate that silymarin exerts significant hepatoprotective effects against paracetamol-induced liver injury. The observed protection appears to be mediated primarily through antioxidant mechanisms, stabilization of hepatocyte membranes, attenuation of inflammatory responses, and promotion of hepatic regeneration. These findings support the continued use of silymarin as a standard hepatoprotective agent and highlight its potential therapeutic value in the management of drug-induced liver disorders.

5. CONCLUSION

5.1 Summary of Findings

The present study successfully evaluated the hepatoprotective potential of silymarin against paracetamol-induced hepatotoxicity in Wistar rats using serum biochemical assays, oxidative stress markers, and histopathological examination. Administration of paracetamol produced significant hepatic injury, as evidenced by marked elevations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels, along with increased lipid peroxidation and depletion of endogenous antioxidant enzymes. Histopathological analysis further confirmed severe hepatocellular degeneration, necrosis, inflammatory infiltration, and disruption of normal liver architecture in the toxic control group.

Pretreatment with silymarin significantly attenuated paracetamol-induced liver damage in a dose-dependent manner. Silymarin treatment effectively reduced serum liver enzyme levels, indicating preservation of hepatocellular membrane integrity and restoration of liver function. In addition, silymarin significantly decreased malondialdehyde (MDA) levels while enhancing the activities of superoxide dismutase (SOD) and catalase, thereby improving the antioxidant defense system and reducing oxidative stress. Histopathological examination demonstrated considerable restoration of normal hepatic architecture with reduced necrosis, inflammation, and cellular degeneration in silymarin-treated groups. Collectively, the biochemical, antioxidant, and histological findings confirmed that silymarin provided substantial protection against paracetamol-induced hepatotoxicity.

5.2 Significance of the Study

The findings of the present investigation provide strong experimental evidence supporting the hepatoprotective efficacy of silymarin against drug-induced liver injury. The study highlights the importance of natural phytoconstituents as potential therapeutic agents for the prevention and management of hepatic disorders. The observed reduction in liver enzyme levels and oxidative stress

biomarkers, coupled with improvement in histopathological features, demonstrates that silymarin can effectively preserve liver function and structural integrity under toxic conditions.

Furthermore, the results emphasize the critical role of oxidative stress in the pathogenesis of paracetamol-induced hepatotoxicity and reinforce the significance of antioxidant-based therapeutic strategies in liver protection. By enhancing endogenous antioxidant defenses and limiting free radical-mediated cellular damage, silymarin offers a multifaceted approach to hepatoprotection. These findings contribute to the growing body of evidence supporting the use of silymarin as a standard hepatoprotective agent in experimental and clinical settings.

5.3 Future Perspectives

Although the present study demonstrated significant hepatoprotective effects of silymarin, further investigations are warranted to elucidate its precise molecular mechanisms of action. Future studies should focus on molecular and genomic approaches to identify the signaling pathways, gene expression profiles, and regulatory mechanisms involved in silymarin-mediated hepatoprotection. Evaluation of inflammatory mediators, apoptotic markers, mitochondrial function, and oxidative stress-related genes may provide deeper insights into its therapeutic actions.

Additionally, long-term studies using chronic hepatotoxicity and liver fibrosis models are necessary to determine the sustained efficacy and safety of silymarin under prolonged pathological conditions. Such investigations may help establish its role in the prevention and treatment of chronic liver diseases, including fibrosis, cirrhosis, and non-alcoholic fatty liver disease.

Future research should also explore advanced drug delivery systems and novel pharmaceutical formulations to improve the bioavailability and therapeutic effectiveness of silymarin. Nanoparticle-based delivery systems, liposomal formulations, and targeted hepatic delivery approaches may enhance its clinical utility. Moreover, well-designed clinical trials involving larger patient populations are required to validate the translational potential of silymarin and facilitate its integration into evidence-based therapeutic protocols for liver disorders.

In conclusion, the present study demonstrates that silymarin possesses significant hepatoprotective activity against paracetamol-induced liver injury through its antioxidant, membrane-stabilizing, and tissue-regenerative properties. These findings support its potential application as a safe and effective natural hepatoprotective agent and provide a foundation for future preclinical and clinical investigations.

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