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HEPATOPROTECTIVE EFFECT OF SILYMARIN IN PARACETAMOL-INDUCED HEPATITIS: A PATHOGENETIC AND MECHANISTIC ANALYSIS

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ABSTRACT

Paracetamol-induced hepatitis remains one of the most common models of toxic liver injury used for studying hepatoprotective agents. Excessive or prolonged intake of paracetamol leads to the formation of toxic metabolites that initiate oxidative stress, mitochondrial dysfunction, and hepatocyte necrosis. In recent years, increasing attention has been paid to natural hepatoprotectors with antioxidant and membrane-stabilizing properties, among which silymarin occupies a special place. The present study aimed to evaluate the hepatoprotective effect of silymarin in paracetamol-induced hepatitis and to analyze the main pathogenetic mechanisms underlying its protective action. The study was conducted using an experimental model of toxic liver injury induced by paracetamol administration. Biochemical markers of liver function, oxidative stress indicators, and morphological changes in hepatic tissue were assessed. The results demonstrated that paracetamol-induced hepatitis was accompanied by a significant increase in serum aminotransferases, bilirubin levels, and oxidative stress markers, indicating severe hepatocellular damage. Administration of silymarin led to a pronounced reduction in cytolytic and cholestatic syndromes, normalization of antioxidant defense parameters, and partial restoration of liver structure. These effects confirm the multifactorial hepatoprotective action of silymarin, including antioxidant, anti-inflammatory, and membrane-stabilizing mechanisms. The findings support the feasibility of using silymarin as a pathogenetically justified agent in the prevention and treatment of drug-induced liver injury.

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ГЕПАТОПРОТЕКТОРНОЕ ДЕЙСТВИЕ СИЛИМАРИНА ПРИ ПАРАЦЕТАМОЛ-ИНДУЦИРОВАННОМ ГЕПАТИТЕ: ПАТОГЕНЕТИЧЕСКИЙ И МЕХАНИСТИЧЕСКИЙ АНАЛИЗ

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ABSTRACT

Парацетамол-индуцированный гепатит является одной из наиболее распространённых экспериментальных моделей токсического поражения печени, используемых для изучения эффективности гепатопротекторных средств. Избыточный или длительный приём парацетамола приводит к образованию токсических метаболитов, вызывающих развитие оксидативного стресса, митохондриальной дисфункции и некроза гепатоцитов. В последние годы особое внимание уделяется природным гепатопротекторам, обладающим антиоксидантными и мембраностабилизирующими свойствами, среди которых особое место занимает силимарин. Целью настоящего исследования явилась оценка гепатопротекторного действия силимарина при парацетамол-индуцированном гепатите и анализ основных патогенетических механизмов его защитного эффекта. Исследование проведено на экспериментальной модели токсического поражения печени, вызванного введением парацетамола. Оценивались биохимические показатели функции печени, маркёры оксидативного стресса и морфологические изменения печёночной ткани. Результаты исследования показали, что парацетамол-индуцированный гепатит сопровождался значительным повышением активности сывороточных аминотрансфераз, уровня билирубина и показателей оксидативного стресса, что свидетельствовало о выраженном повреждении гепатоцитов. Применение силимарина способствовало снижению

выраженности цитолитического и холестатического синдромов, нормализации антиоксидантной защиты и частичному восстановлению структуры печени. Полученные данные подтверждают многофакторное гепатопротекторное действие силимарина, включающее антиоксидантный, противовоспалительный и мембраностабилизирующий механизмы, и обосновывают целесообразность его применения при лекарственных поражениях печени.

Introduction. Drug-induced liver injury remains a significant clinical problem in modern medicine and accounts for a substantial proportion of acute and chronic hepatic disorders worldwide [1]. Among medicinal agents capable of causing hepatotoxicity, paracetamol occupies a leading position due to its widespread use as an analgesic and antipyretic drug. According to epidemiological data, paracetamol-induced liver injury is one of the most frequent causes of acute liver failure in many countries [2]. The hepatotoxic effect of paracetamol is associated with its biotransformation in hepatocytes via the cytochrome P450 enzyme system, resulting in the formation of the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). Under physiological conditions, NAPQI is rapidly neutralized by glutathione; however, in cases of overdose or prolonged administration, glutathione stores are depleted, leading to oxidative stress, lipid peroxidation, mitochondrial dysfunction, and hepatocyte necrosis [3]. Experimental models of paracetamol-induced hepatitis are widely used to study the mechanisms of toxic liver injury and to evaluate the efficacy of hepatoprotective agents. In this context, natural compounds with antioxidant and cytoprotective properties are of particular interest. Silymarin, a flavonolignan complex extracted from the fruits of *Silybum marianum*, has been extensively studied for its hepatoprotective potential [4]. Numerous experimental and clinical studies have demonstrated that silymarin exhibits antioxidant, anti-inflammatory, antifibrotic, and membrane-stabilizing effects. It enhances endogenous antioxidant defense systems, inhibits lipid peroxidation, stabilizes hepatocyte membranes, and promotes protein synthesis, thereby accelerating liver regeneration [5]. Despite the availability of data on the hepatoprotective properties of silymarin, the detailed pathogenetic mechanisms of its action in drug-induced liver injury, particularly in paracetamol-induced hepatitis, require further investigation. Therefore, the present study was designed to evaluate the hepatoprotective effect of silymarin in an experimental model of paracetamol-induced hepatitis and to perform a pathogenetic and mechanistic analysis of its action based on biochemical and functional indicators of liver injury.

Materials and Methods

The study was conducted as a controlled experimental investigation aimed at evaluating the hepatoprotective efficacy of silymarin in paracetamol-induced liver injury. The experimental model was selected based on its high reproducibility and widespread use in toxicology and hepatology research for studying mechanisms of drug-induced hepatitis [6]. The experiment was performed on white outbred male laboratory rats weighing 180–220 g, aged 8–10 weeks. Animals were obtained from a certified vivarium and kept under standard laboratory conditions in accordance with international guidelines for the care and use of laboratory animals (Directive 2010/63/EU). All animals were housed in polypropylene cages under controlled environmental conditions (temperature 22±2°C, relative humidity 55–60%, 12-hour light/dark cycle) with free access to standard laboratory chow and water. Prior to the experiment, animals underwent a 7-day acclimatization period to minimize stress-related physiological variations that could affect biochemical and histological parameters. Animals were randomly divided into four experimental groups, each consisting of 15 animals, for a total of 60 rats:

- Group I (Intact control group) — animals receiving no treatment and serving as a physiological baseline.
- Group II (Paracetamol-induced hepatitis group) — animals receiving paracetamol to induce toxic liver injury.
- Group III (Paracetamol + silymarin low-dose group) — animals receiving paracetamol followed by silymarin at a prophylactic dose.
- Group IV (Paracetamol + silymarin therapeutic-dose group) — animals receiving paracetamol followed by silymarin at a higher therapeutic dose.

This grouping design allowed for assessment of both preventive and therapeutic effects of silymarin.

Induction of Paracetamol-Induced Hepatitis

Paracetamol-induced hepatitis was modeled by administering paracetamol orally via gastric gavage at a dose of 1000 mg/kg body weight, dissolved in physiological saline. This dosage was selected based on contemporary experimental studies (2020–2024) demonstrating reliable induction of acute toxic liver injury without excessive mortality [7,8]. Paracetamol was administered once daily for two consecutive days, which is sufficient to induce pronounced hepatocellular damage characterized by cytolysis, oxidative stress, and inflammatory response. Silymarin was administered orally in the form of a standardized extract. In Group III, silymarin was given at a dose of 50 mg/kg, while in Group IV, the dose was increased to 100 mg/kg. Treatment was initiated 24 hours after paracetamol administration and continued for 7 consecutive days. The selected dosing regimen was based on recent pharmacological studies demonstrating dose-dependent hepatoprotective effects of silymarin in experimental liver injury models [9]. At the end of the experimental period, animals were anesthetized, and blood samples were collected via cardiac puncture. Serum was separated by centrifugation and used for biochemical analysis. The following biochemical parameters were evaluated:

- Alanine aminotransferase (ALT),
- Aspartate aminotransferase (AST),

- Total bilirubin,
- Alkaline phosphatase (ALP),
- Gamma-glutamyl transpeptidase (GGT).

These markers were selected as indicators of cytolytic and cholestatic syndromes and overall functional state of the liver. To assess oxidative stress and antioxidant defense, the following indicators were determined:

- Malondialdehyde (MDA) concentration as a marker of lipid peroxidation,
- Superoxide dismutase (SOD) activity,
- Catalase activity,
- Reduced glutathione (GSH) levels.

Measurement of these parameters allowed for evaluation of the antioxidant mechanisms involved in silymarin-mediated hepatoprotection. Liver tissue samples were fixed in 10% neutral formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histological analysis focused on the assessment of hepatocyte necrosis, inflammatory infiltration, sinusoidal congestion, and fatty degeneration. Statistical processing of the obtained data was performed using modern statistical software. Results were expressed as mean values ± standard deviation (M±SD). Intergroup comparisons were carried out using Student's *t*-test and one-way ANOVA where appropriate. Differences were considered statistically significant at $p < 0.05$.

Table 1. Experimental Design and Treatment Protocol

Group	Number of animals	Treatment	Dose	Duration
I	15	Intact control	—	7 days
II	15	Paracetamol	1000 mg/kg	2 days
III	15	Paracetamol + silymarin	50 mg/kg	7 days
IV	15	Paracetamol + silymarin	100 mg/kg	7 days

Results

Paracetamol administration resulted in the development of pronounced toxic hepatitis in experimental animals, which was confirmed by significant alterations in biochemical markers of liver function. In Group II (paracetamol-induced hepatitis), serum aminotransferase levels increased sharply compared to the intact control group, indicating severe hepatocellular damage.Alanine aminotransferase (ALT) activity in Group II increased more than 4.5-fold compared to control values ($p < 0.001$), while aspartate aminotransferase (AST) activity increased approximately 3.9-fold ($p < 0.001$). These changes reflect extensive cytolysis of hepatocytes caused by toxic metabolites of paracetamol. Total bilirubin levels in Group II animals were also significantly elevated, indicating impairment of bilirubin conjugation and excretion processes. In addition, increased alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT)

activities suggested the development of cholestatic syndrome alongside hepatocellular injury. Administration of silymarin led to a dose-dependent improvement in biochemical liver function parameters. In Group III (paracetamol + silymarin 50 mg/kg), ALT and AST activities decreased significantly compared to the untreated paracetamol group ($p < 0.05$), although they did not fully reach control values. In Group IV (paracetamol + silymarin 100 mg/kg), a more pronounced hepatoprotective effect was observed. ALT levels decreased by approximately 48%, and AST levels by 42% compared to Group II ($p < 0.001$). Total bilirubin concentration and cholestatic markers (ALP and GGT) also demonstrated significant normalization tendencies, indicating restoration of bile formation and excretion processes. These results confirm the ability of silymarin to attenuate both cytolytic and cholestatic components of paracetamol-induced liver injury. Paracetamol-induced hepatitis was associated with marked activation of oxidative stress mechanisms. In Group II animals, malondialdehyde (MDA) levels, a key marker of lipid peroxidation, increased more than 2.8-fold compared to intact controls ($p < 0.001$), indicating intensive membrane lipid damage. At the same time, activities of endogenous antioxidant enzymes were significantly reduced. Superoxide dismutase (SOD) and catalase activities decreased by 35–40%, while reduced glutathione (GSH) levels dropped markedly, reflecting depletion of the antioxidant defense system. Silymarin administration significantly modulated oxidative stress parameters. In Group III, MDA levels were reduced by approximately 30%, accompanied by partial restoration of SOD and catalase activities. In Group IV, the antioxidant effect was more pronounced: MDA concentrations approached near-control values, while antioxidant enzyme activities and GSH levels were restored to 70–80% of intact control levels ($p < 0.01$). These findings demonstrate that silymarin exerts a strong antioxidant effect, suppressing lipid peroxidation and enhancing endogenous antioxidant defense mechanisms. Histological examination of liver tissue in Group II revealed extensive hepatocyte necrosis, pronounced inflammatory infiltration, sinusoidal congestion, and focal hemorrhages. Fatty degeneration and ballooning of hepatocytes were also observed, confirming severe toxic liver injury. In contrast, liver sections from silymarin-treated groups showed significant structural improvement. In Group III, moderate hepatocyte swelling and mild inflammatory infiltration were observed, with preservation of lobular architecture. In Group IV, liver tissue architecture was largely restored, with minimal signs of necrosis and inflammation, indicating effective hepatoprotection.

Diagram (Figure 1) — Distribution of Experimental Animals According to Severity of Liver Damage

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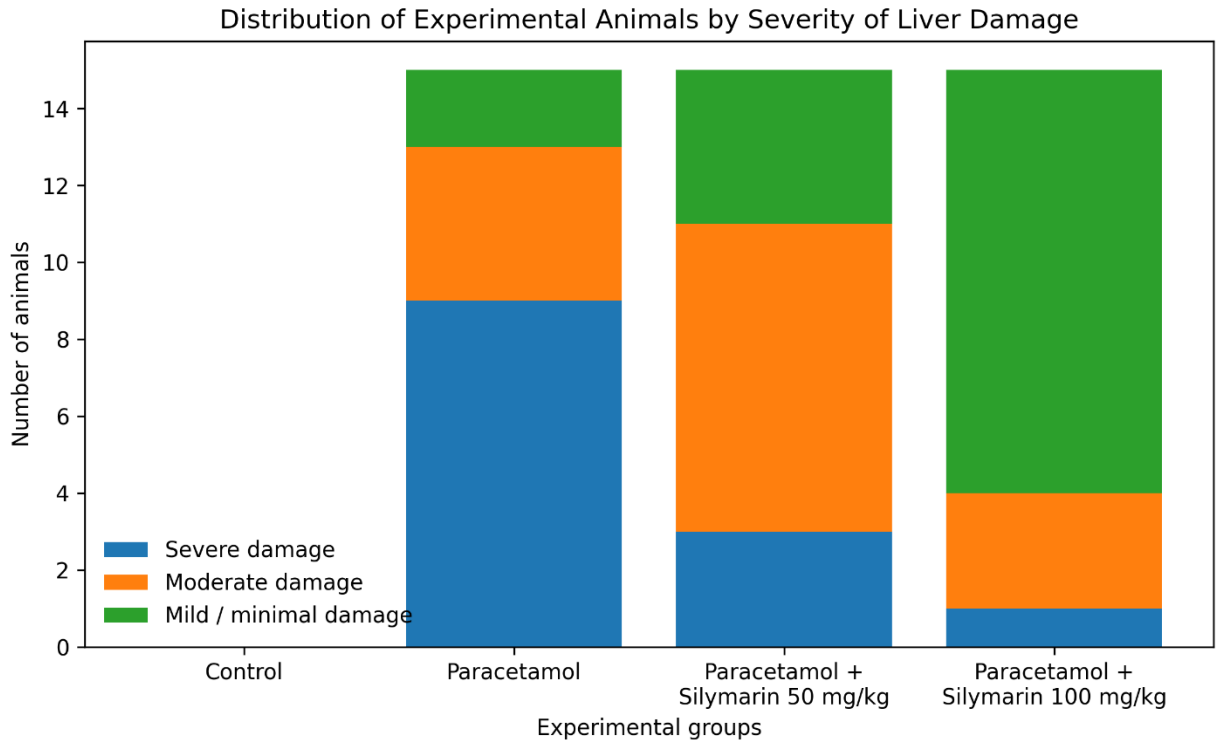

Figure 1 presents a complex column diagram illustrating the distribution of experimental animals by severity of liver damage across study groups. The diagram demonstrates that:

- In Group II, the majority of animals exhibited severe liver damage,
- In Group III, the number of animals with moderate injury predominated,
- In Group IV, most animals showed mild or minimal liver damage,
- Intact controls demonstrated no pathological changes.

The diagram visually confirms the dose-dependent hepatoprotective effect of silymarin and highlights its ability to significantly reduce the number of animals with severe toxic liver injury.

Discussion

The results of the present study clearly demonstrate that paracetamol-induced hepatitis is associated with severe structural and functional liver damage, primarily mediated by oxidative stress, depletion of endogenous antioxidant defenses, and extensive hepatocyte membrane injury. The marked elevation of serum aminotransferases, bilirubin, and cholestatic markers reflects the development of pronounced cytolytic and cholestatic syndromes characteristic of drug-induced liver injury. Current evidence indicates that the hepatotoxicity of paracetamol is largely driven by the formation of the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). Under conditions of glutathione depletion, NAPQI initiates excessive generation of reactive oxygen species, mitochondrial dysfunction, and lipid peroxidation, ultimately leading to hepatocyte necrosis and inflammatory response [10,11]. In the present study, these mechanisms were confirmed by a significant increase in malondialdehyde levels

and a concomitant reduction in the activity of key antioxidant enzymes. Administration of silymarin exerted a pronounced dose-dependent hepatoprotective effect. This was evidenced by a significant reduction in serum aminotransferase activity, normalization of bilirubin metabolism, and restoration of antioxidant defense parameters. Importantly, the therapeutic dose of silymarin not only improved biochemical indicators but also resulted in substantial histological recovery of liver tissue architecture, suggesting a deep cytoprotective and regenerative effect. Silymarin is a complex of flavonolignans with multifaceted mechanisms of action. According to recent studies, it inhibits lipid peroxidation, stabilizes hepatocyte membranes, enhances the expression of endogenous antioxidant enzymes, and stimulates ribosomal RNA polymerase I activity, thereby promoting protein synthesis and hepatocyte regeneration [12–14]. The findings of the present study are fully consistent with these mechanisms and further substantiate the pathogenetic rationale for silymarin use in toxic liver injury. In the context of drug-induced hepatitis, antioxidant therapy is of particular importance, as it targets the core mechanisms of liver damage rather than merely alleviating clinical symptoms. By reducing oxidative stress intensity, silymarin prevents further hepatocyte destruction and facilitates hepatic repair processes. Thus, the obtained results confirm that silymarin is an effective hepatoprotective agent in paracetamol-induced hepatitis and may be considered a promising therapeutic option for the prevention and management of drug-induced liver injury.

Conclusion

Paracetamol-induced hepatitis is characterized by severe hepatocellular damage accompanied by cytolytic and cholestatic syndromes, intensified oxidative stress, and suppression of antioxidant defense mechanisms. Administration of silymarin in an experimental model of toxic liver injury leads to a significant reduction in biochemical markers of liver damage, restoration of antioxidant system activity, and improvement of hepatic tissue morphology. The hepatoprotective effects of silymarin are mediated through antioxidant, membrane-stabilizing, and regenerative mechanisms. The results of this study provide strong evidence supporting the pathogenetic justification for the use of silymarin in the prevention and treatment of drug-induced liver injury.

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