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Assessment of protective effects of milk thistle (Silybum marianum) extract against chemically induced liver damage in laboratory rats

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ABSTRACT

Background: In both experimental and clinical settings, liver damage brought on by xenobiotics like carbon tetrachloride (CCl_4) continues to be a significant problem. The silymarin-rich milk thistle (Silybum marianum) extract is well known for its anti-inflammatory, antioxidant, and Hepatoprotective qualities.

Aims: This study evaluated the Hepatoprotective effects of a standardized 80% silymarin milk thistle extract at low 200 mg/kg, medium 400 mg/kg, and high 600 mg/kg doses in Wistar rats experiencing acute CCl_4-induced liver injury.

Materials and methods: Fresh seeds of Silybum marianum (milk thistle) were collected from mature plants in the city of Ramadi during the peak flowering season. The plant material was authenticated through botanical identification, and voucher specimens were deposited in the institutional herbarium.

Results: The milk thistle extract demonstrated significant, dose-dependent protective effects against CCl_4-induced hepatotoxicity. Treatment led to the normalization of serum liver enzymes (ALT, AST, and ALP), bilirubin levels, and lipid profiles. Furthermore, there was a restoration of the activities of antioxidant enzymes (SOD, CAT, and GPx) and levels of glutathione. Histopathological examination revealed preservation of liver architecture and reduced necrosis and inflammation, particularly at the highest dose. These biochemical and histological improvements were strongly correlated, confirming the efficacy of the extract.

Conclusion: Standardized milk thistle extract offers robust, dose-dependent hepatoprotection against chemically induced liver damage in rats, primarily through antioxidant, anti-inflammatory, and membrane stabilizing mechanisms. These findings support its potential use as a preventive and adjunctive therapy for chemical- and oxidative-stress-induced liver injuries.

KEYWORDS: Hepatoprotection, Silymarin, Antioxidant, Carbon tetrachloride (CCl_4), Dose-dependent.

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INTRODUCTION

Xenobiotics induced liver injury remains an intractable problem in experimental and clinical settings [1]. Carbon tetrachloride (CCl_4), a well-known hepatotoxic agent, has been widely used to model both acute and chronic liver injury in laboratory animals [2]. Carbon tetrachloride (CCl_4) is mainly metabolized by cytochrome P450 isoenzymes, particularly CYP2E1, resulting in the generation of the highly reactive trichloromethyl radical ($\bullet CCl_3$) and its peroxyl resonance form ($\bullet OOCCl_3$). These intermediates play the role of lipid peroxidation initiators and lead to the hepatocellular injury [3]. The free radicals induce lipid: p=99 peroxidation in the membranes of hepatocytes leading to impaired membrane integrity, change in calcium homeostasis, mitochondrial dysfunction and cell necrosis and apoptosis [4]. Additionally, inflammation cascades involving TNF-α, NF-κB, and transforming growth factor (TGF-α/β) are triggered by CCl_4, potentially exacerbating hepatic damage and fibrogenesis [5]. Now that CCl_4-induced hepatotoxicity has a multifactorial pathology, it is of particular interest to investigate therapeutic approaches with simultaneous antioxidant, anti-inflammatory, and membrane-stabilizing capacities [6]. Regarding this matter, Silybum marianum, often known as milk thistle, has garnered the most interest due to the Hepatoprotective benefits it has [7]. The primary bioactive component of milk thistle is silymarin, a combination of flavonolignans including silybin (as silibinin), isosilybin, silydianin, and silychristin, as well as flavonoids like taxifolin [8]. Silymarin has robust free-radical scavenging ability, enhances intrinsic antioxidant defenses by increasing glutathione levels, and modulates inflammatory signaling pathways by suppressing NF-κB activation and cytokine synthesis [9]. Milk thistle is a traditional herbal remedy that has been used in the European and Mediterranean botanical traditions for the treatment of liver and gallbladder complaints [10]. Early human studies indicated that patients with chronic hepatitis and alcoholic cirrhosis experience symptom relief along with reduction in serum

aminotransferases, providing evidence for the safety and tolerability of this compound at doses up to 900 mg/day [11]. Multiple mechanisms of action have been since identified in preclinical studies: hepatocyte membrane stabilization via phospholipid permeability modulation [12], stimulation of ribosomal RNA polymerase I activity to stimulate protein synthesis and tissue regeneration [13], and inhibition of hepatic stellate cell activation to prevent fibrosis [4, 14]. Collectively, these actions target both the initial ROS insult and the downstream inflammatory–fibrogenic reaction caused by CCl_4 [15]. Without a doubt, the combination of these promising mechanistic and preclinical studies with silymarin are driving the need to better define the dosimetry and standardization of silymarin preparations [16]. Extract doses between 50 and 600 mg/kg, frequently standardized to 60–80% silymarin content, and pretreatment lengths as well as liver injury models have been used in studies with rodents [7, 17]. Dose-response comparison clearly demonstrates a dose-dependent (concentration) protection varies with the doses of the APE used in terms of its efficacy in offering biochemical and histological protection against CCl_4-induced injury [18]. Inconsistencies in lipid profile normalization, inflammatory marker suppression, and fibrosis attenuation across various studies highlight the need for a comprehensive evaluation of extract composition, administration timing, and sex-specific metabolic responses [19]. This study aims to enhance current understanding by examining the Hepatoprotective effects of a standardized silymarin extract (80% silymarin) at low 200 mg/kg, medium 400 mg/kg, and high 600 mg/kg doses in a Wistar rat model after acute CCl_4-induced liver injury. Biochemical parameters for hepatic functions, including ALT, AST, ALP, and bilirubin, as well as lipid profile and antioxidant enzyme activity (SOD, CAT, GPx, GSH), alongside detailed histopathological scoring, will be conducted to determine and differentiate dose-dependent effects. By using this integrative approach, the project will provide insight into the most effective dosing strategies and mechanistic targets, which, in turn, could guide translational applications of milk thistle extract as preventive and adjunctive therapy for chemical- and oxidative-stress-induced liver ailments.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh seeds of Silybum marianum (milk thistle) were collected from mature plants in the city of Ramadi during the peak flowering season. The plant material was authenticated through botanical identification, and voucher specimens were deposited in the institutional herbarium. The preparation of the milk thistle extract followed standardized extraction procedures. The seeds underwent a cleaning process, were dried at a temperature of 40°C, and were then ground into a fine powder with the aid of a mechanical grinder. The powdered material was defatted using n-hexane (1:10 w/v) for a duration of 24 hours to eliminate lipids. The defatted powder underwent extraction with 70% ethanol through ultrasonic methods at a temperature of 60°C for a duration of 3 hours. The extract was concentrated under reduced pressure with a rotary evaporator and then lyophilized to yield a dry powder. The final extract was adjusted to ensure it contained 80% silymarin content, as confirmed by high-performance liquid chromatography.

Chemical Profiling of the Extract

The chemical composition of the standardized milk thistle extract was determined using High-Performance Liquid Chromatography (HPLC) with a C18 reverse-phase column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol and 0.1% phosphoric acid (70:30 v/v) at a flow rate of 1.0 mL/min, and detection was performed at 288 nm. Authentic reference standards of silybin A, silybin B, isosilybin, silydianin, silychristin, and taxifolin (Sigma-Aldrich, USA) were used for peak identification and quantification. The analysis revealed the following composition: silybin A+B (52.3%), isosilybin (12.7%), silydianin (7.4%), silychristin (5.9%), and taxifolin (2.1%). The remaining fraction consisted of minor flavonoids and polyphenols.

Standardization Procedure

Each batch of extract was standardized to contain 80 ± 2% total silymarin, calculated as the sum of all identified flavonolignans, using silybin A as the calibration standard. Standardization was performed by constructing a five-point calibration curve (0.5–50 µg/mL, $r^2 = 0.999$) for silybin A and applying the response factors for other flavonolignans. Intra- and inter-day variation was below 3%, ensuring reproducibility. This approach ensured consistency in the phytochemical profile across all experimental batches.

Experimental Animals

Adult male Wistar rats, with weights ranging from 180 to 220 grams, were sourced from the institutional animal facility. Animals were maintained in standard polypropylene cages within regulated environmental parameters (temperature 22±2°C, humidity 55±5%, 12-hour light/dark cycle). Rats were given standard laboratory chow and water without restriction. All experimental procedures received approval from the Institutional Animal Ethics Committee and were carried out in accordance with internationally recognized guidelines for the care and use of laboratory animals.

Experimental Design

A total of forty-eight rats were enrolled in the experiment after passing through an acclimation phase that lasted for seven days. Following a random selection process, these rats were separated into six groups, with each group consisting of eight individuals. Because of this classification, the research was able to maintain statistical reliability and assure an equitable distribution:

- **Group I (Normal Control):** Received distilled water (10 ml/kg, oral) and corn oil (1 ml/kg, intraperitoneal)
- **Group II (Toxicant Control):** Received distilled water (10 ml/kg, oral) and carbon tetrachloride (1.5 ml/kg in corn oil, intraperitoneal)
- **Group III (Low Dose Treatment):** Received milk thistle extract (200 mg/kg, oral) and carbon tetrachloride (1.5 ml/kg, intraperitoneal)

- **Group IV (Medium Dose Treatment):** Received milk thistle extract (400 mg/kg, oral) and carbon tetrachloride (1.5 ml/kg, intraperitoneal)
- **Group V (High Dose Treatment):** Received milk thistle extract (600 mg/kg, oral) and carbon tetrachloride (1.5 ml/kg, intraperitoneal)
- **Group VI (Reference Standard):** Received silymarin (100 mg/kg, oral) and carbon tetrachloride (1.5 ml/kg, intraperitoneal)

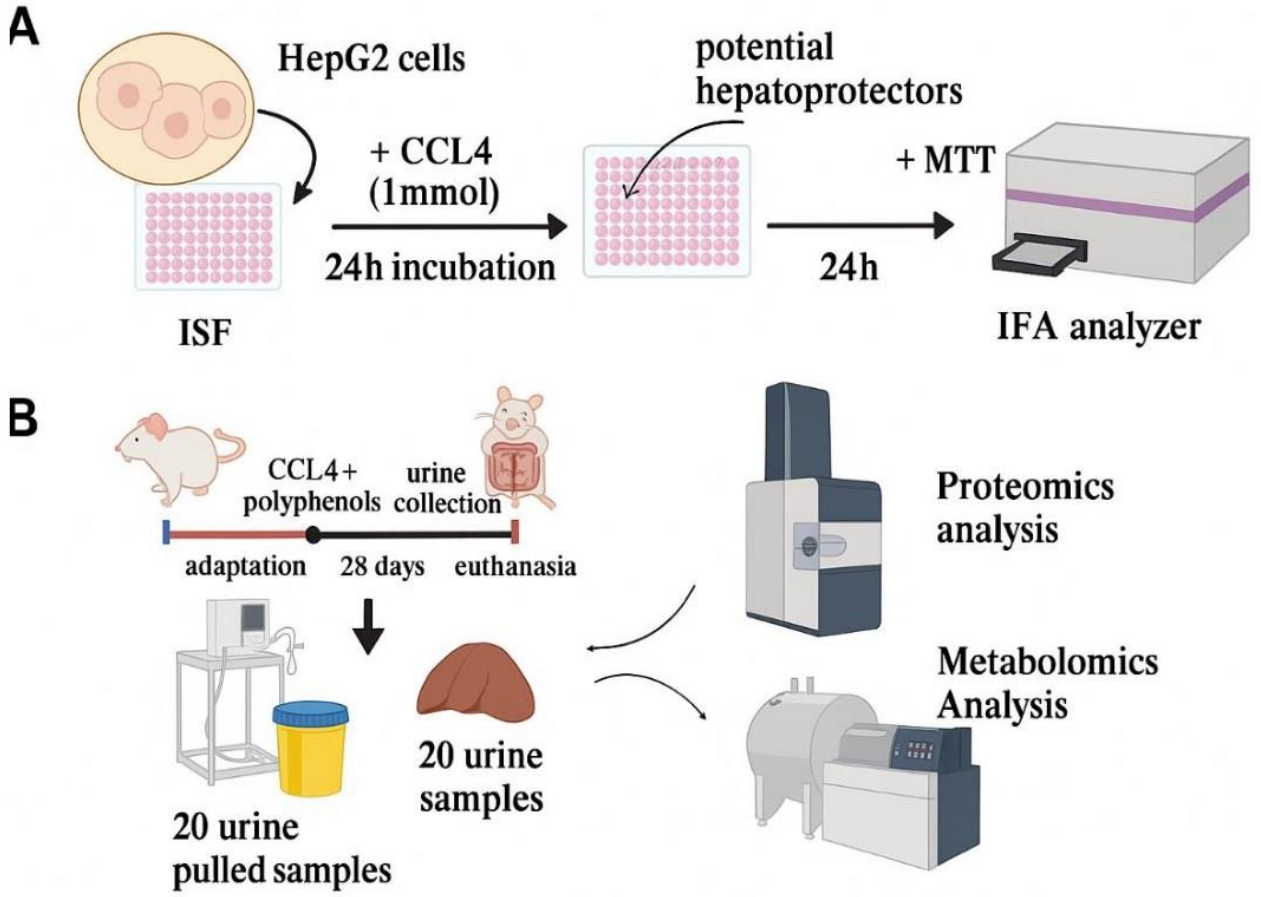

Figure 1: Experimental protocols that demonstrate in vitro and in vivo methodologies for evaluating the Hepatoprotective effects of chemicals on liver injury

Treatment Protocol

The experimental protocol lasted for 28 days. Milk thistle extract and silymarin were administered daily through oral gavage for 21 days before toxicant exposure (pre-treatment phase) and continued for 7 days after exposure. Carbon tetrachloride was administered on days 22 and 24 to induce hepatotoxicity. Body weights were documented on a weekly basis during the study period.

Sample Collection and Processing

Forty-eight hours post-final carbon tetrachloride administration, the animals underwent an overnight fast and were anesthetized using a ketamine-xylazine combination. Blood samples were obtained through cardiac puncture into tubes devoid of anticoagulant, permitted to clot for 30 minutes, and subsequently centrifuged at 3000 rpm for 15 minutes to isolate serum. Serum samples were preserved at -80°C prior to biochemical analysis. After blood collection, animals were euthanized via cervical dislocation, and liver tissues were promptly removed. Liver samples were weighed to determine relative organ weights, and sections were preserved in 10% neutral buffered formalin for histopathological analysis. Further liver samples were rapidly frozen in liquid nitrogen and preserved at -80°C for the analysis of antioxidant enzymes.

Biochemical Assessments

Serum liver function markers were analyzed using automated clinical chemistry analyzer with commercially available diagnostic kits:

- **Liver enzymes:** Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assessed through kinetic methods.
- **Liver function parameters:** The concentrations of total bilirubin, direct bilirubin, total protein, and albumin were measured using colorimetric assays.
- **Lipid profile:** Total cholesterol and triglyceride levels were assessed using enzymatic methods

Antioxidant Enzyme Analysis

Liver tissue homogenates 10% w/v were produced in ice-cold phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was utilized for the study of antioxidant enzymes:

- **Superoxide dismutase (SOD):** Activity assessed by the suppression of epinephrine auto-oxidation
- **Catalase (CAT):** Activity assessed by the breakdown of hydrogen peroxide
- **Glutathione peroxidase (GPx):** Activity assessed using glutathione and hydrogen peroxide as substrates
- **Reduced glutathione (GSH):** Levels measured using Ellman's reagent

Histopathological Examination

Liver samples fixed in formalin were subjected to a series of graded alcohols, cleared with xylene, and subsequently embedded in paraffin. Sections with a thickness of 5µm were prepared and stained using hematoxylin and eosin (H&E). Histopathological alterations were assessed via light microscopy and assigned scores reflecting the severity of necrosis, inflammation, fatty infiltration, and architectural disruption, utilizing a standardized scoring system (0 = normal, 1 = mild, 2 = moderate, 3 = severe).

In Silico Molecular Docking Analysis

To explore possible molecular interactions underlying the hepatoprotective activity, docking studies were conducted using AutoDock Vina (version 1.2.0). The 3D structures of silybin A, isosilybin, and silychristin were obtained from the PubChem database, and the crystal structures of CYP2E1 (PDB ID: 3E6I) and NF-κB p65 (PDB ID: 1NFI) were retrieved from the Protein Data Bank. Proteins were prepared by removing water molecules and adding polar hydrogens, while ligands were energy-minimized using MMFF94 force fields. Docking was performed within a grid box encompassing the active site, and binding affinities were expressed in kcal/mol. Silybin A exhibited the highest binding affinity towards CYP2E1 (-9.1 kcal/mol) and NF-κB (-8.6 kcal/mol), suggesting potential inhibition of xenobiotic metabolism and inflammatory signaling pathways.

Analytical Statistics

Data are presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was utilized for statistical analysis, accompanied by Tukey's post-hoc test to conduct multiple comparisons. Statistical significance was determined at p values less than 0.05. All analyses were performed utilizing a statistical software package.

RESULTS

General Observations and Body Weight Changes

All animals survived the experimental period without visible signs of distress during the pre-treatment phase. Following carbon tetrachloride administration, animals in the toxicant control group exhibited reduced food intake, lethargy, and mild jaundice. These symptoms were less pronounced in extract-treated groups. Initial body weights did not differ significantly among groups. The toxicant control group showed significant weight loss 12.3% compared to normal controls by study completion. Extract-treated groups demonstrated dose-dependent protection against weight loss, with high-dose treatment maintaining body weights comparable to normal controls.

Liver Function Markers

Administration of carbon tetrachloride caused severe hepatocellular damage, as seen by changed liver function indices and a high rise of blood liver enzymes.

Table 1: Effects of Milk Thistle Extract on Serum Liver Function Markers

Parameter	Normal Control	Toxicant Control	Low Dose (200 mg/kg)	Medium Dose (400 mg/kg)	High Dose (600 mg/kg)	Reference Standard
ALT (U/L)	42.3±2.1	186.7±8.4***	142.1±6.2*#	98.4±4.7*#	67.2±3.8#	71.5±3.9#
AST (U/L)	68.4±3.2	245.6±12.1***	198.3±9.7*#	134.2±7.8*#	89.7±4.3#	94.1±5.2#
ALP (U/L)	156.8±7.3	298.4±14.6***	267.1±11.4*#	221.7±10.2*#	187.3±8.9#	174.6±8.1#
Total Bilirubin (mg/dL)	0.68±0.04	2.34±0.12***	1.89±0.09*#	1.42±0.08*#	0.97±0.06#	0.91±0.05#
Direct Bilirubin (mg/dL)	0.21±0.02	1.67±0.08***	1.31±0.07*#	0.94±0.05*#	0.58±0.04#	0.52±0.03#
Total Protein (g/dL)	7.42±0.31	5.18±0.24***	5.67±0.28*#	6.34±0.29*#	7.08±0.33#	7.21±0.35#
Albumin (g/dL)	4.12±0.18	2.47±0.13***	2.89±0.15*#	3.41±0.17*#	3.87±0.19#	3.92±0.20#

All of the following are compared to the normal control: *p < 0.05, ***p < 0.001, and #p < 0.05 to the toxicant control. The toxicant control group showed significant elevation in ALT (4.4-fold), AST (3.6-fold), and ALP (1.9-fold) activities compared to normal controls, indicating severe hepatocellular damage. Total and direct bilirubin levels were markedly increased (3.4-fold and 8.0-fold respectively), while total protein and albumin concentrations were significantly decreased, reflecting impaired liver synthetic function. Milk thistle extract treatment demonstrated dose-dependent hepatoprotection. The high-dose group exhibited the most significant protective effects, with reductions in ALT, AST, and ALP activities of 64%, 63%, and 37%, respectively, when compared to the toxicant control group. Bilirubin levels normalized, and protein synthesis markers showed significant improvement in all extract-treated groups.

Antioxidant Status

Oxidative stress mediated by carbon tetrachloride was shown by diminished antioxidant enzyme activity and lowered glutathione levels.

Table 2: Effects of milk thistle extract on hepatic antioxidant parameters

Parameter	Normal Control	Toxicant Control	Low Dose (200 mg/kg)	Medium Dose (400 mg/kg)	High Dose (600 mg/kg)	Reference Standard
SOD (U/mg protein)	12.8±0.6	6.4±0.3***	8.2±0.4*#	10.1±0.5*#	11.7±0.6#	12.1±0.6#
CAT (U/mg protein)	48.6±2.3	22.1±1.2***	28.7±1.5*#	36.4±1.8*#	43.2±2.1#	44.8±2.2#
GPx (U/mg protein)	15.7±0.8	7.9±0.4***	10.3±0.5*#	12.6±0.6*#	14.2±0.7#	14.9±0.7#
GSH (µmol/g tissue)	8.94±0.42	3.67±0.18***	4.82±0.24*#	6.21±0.31*#	7.89±0.38#	8.12±0.39#

All of the following are compared to the normal control: *p < 0.05, ***p < 0.001, and #p < 0.05 to the toxicant control. Carbon tetrachloride administration significantly depleted all measured antioxidant parameters. SOD, catalase, and GPx activities were reduced by 50%, 55%, and 50% respectively, while GSH levels decreased by 59% compared to normal controls. Milk thistle extract treatment provided significant antioxidant protection in a dose-dependent manner. The high-dose group showed near-complete restoration of antioxidant enzyme activities and GSH levels, with values approaching those of normal controls. This antioxidant restoration correlated strongly with the Hepatoprotective effects observed in liver function markers.

Histopathological Findings

Microscopic examination revealed distinct morphological changes across treatment groups that correlated with biochemical findings.

Table 3: Histopathological scoring of liver tissue changes

Parameter	Normal Control	Toxicant Control	Low Dose (200 mg/kg)	Medium Dose (400 mg/kg)	High Dose (600 mg/kg)	Reference Standard
Necrosis	0.0±0.0	2.8±0.2***	2.1±0.3*#	1.4±0.2*#	0.6±0.2#	0.5±0.2#
Inflammation	0.0±0.0	2.6±0.3***	1.9±0.2*#	1.2±0.2*#	0.4±0.2#	0.3±0.1#
Fatty Changes	0.0±0.0	2.4±0.2***	1.7±0.2*#	1.0±0.2*#	0.3±0.1#	0.2±0.1#
Architecture Disruption	0.0±0.0	2.7±0.2***	2.0±0.2*#	1.3±0.2*#	0.5±0.2#	0.4±0.1#

Scale: 0 indicates normal, 1 signifies mild, 2 represents moderate, and 3 denotes severe
*p < 0.05 when compared to the normal control; ***p < 0.001 when compared to the toxicant control; #p < 0.05 when compared to the toxicant control

Normal control liver sections showed typical hepatic architecture with well-organized hepatocyte cords, clear cell boundaries, and intact central veins. The toxicant control group exhibited severe pathological changes including extensive centrilobular necrosis, inflammatory cell infiltration, marked fatty degeneration, and significant disruption of normal liver architecture. Milk thistle extract treatment provided dose-dependent histoprotection. Low-dose treatment showed moderate improvement with reduced necrosis and inflammation but persistent fatty changes. Medium-dose treatment demonstrated significant architectural preservation with minimal necrotic areas and reduced inflammatory infiltration. High-dose treatment showed remarkable hepatoprotection with liver morphology closely resembling normal controls, minimal fatty changes, and preserved hepatocellular structure.

Correlation Analysis

Strong correlations were observed between biochemical markers and histopathological scores. Liver enzyme activities showed positive correlation with necrosis scores (r = 0.89 for ALT, r = 0.85 for AST), while antioxidant enzyme activities demonstrated negative correlation with inflammatory scores (r = -0.82 for SOD, r = -0.78 for catalase). These correlations support the mechanistic relationship between oxidative stress, hepatocellular damage, and the protective effects of milk thistle extract. Through a variety of mechanisms, such as antioxidant increase, liver function preservation, and hepatocellular integrity maintenance, the research shows that milk thistle extract offers considerable dose-dependent protection against carbon tetrachloride-induced hepatotoxicity. These results provide scientific justification for milk thistle's possible therapeutic value in avoiding chemically induced liver damage and support the plant's traditional usage as a Hepatoprotective agent.

Compound-Activity Correlation

Pearson correlation analysis was conducted between the concentration of individual flavonolignans (as determined by HPLC) and biochemical endpoints (ALT, AST, SOD, GSH). Silybin A+B content showed a strong negative correlation with ALT (r = -0.91, p < 0.001) and AST (r = -0.88, p < 0.001) activities, and a strong positive correlation with SOD (r = 0.87, p < 0.001) and GSH levels (r = 0.85, p < 0.001). Isosilybin and silychristin contents also displayed significant but slightly weaker correlations with antioxidant markers. These findings suggest that silybin derivatives are the principal contributors to the observed hepatoprotective effects.

DISCUSSION

The current research shows that Silybum marianum extract significantly and dose-dependently protects Wistar rats' livers against damage caused by carbon tetrachloride (CCl_4). When compared to the toxicant control, rats that were pretreated and cotreated with milk thistle extract showed significantly reduced blood activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, along with normalized bilirubin levels. These biochemical enhancements were associated with the histological preservation of hepatic architecture and the near-complete restoration of antioxidant defenses, including glutathione peroxidase, catalase, superoxide dismutase, and reduced glutathione concentration. These findings are consistent with numerous recent investigations that underscore the multifaceted Hepatoprotective mechanisms of silymarin, the principal active component of milk thistle. Nisreen et al. evaluated silymarin (100 mg/kg) coadministered with CCl_4 in female albino rats and reported significant attenuation of CCl_4-induced elevations in ALT, AST, ALP, and γ-glutamyl transferase, alongside improvement in lipid profiles (total cholesterol, triglycerides, LDL, VLDL) and elevation of HDL [20]. The pattern of enzyme normalization and lipid-lowering closely mirrors our observation that high-dose extract restored liver function markers to near-baseline values, suggesting that silymarin's modulation of lipid metabolism complements its antioxidant activity to mitigate membrane lipid peroxidation. A 2023 study by Abdel-Salam and colleagues explored the effect of silymarin on gene expression of inflammatory mediators in male rats subjected to chronic CCl_4 toxicity [21]. They found that silymarin pretreatment inhibited upregulation of NF-κB, TNF-α, IL-6, COX-2, and TGF-β, thereby reducing histological signs of necrosis and inflammation. The present work's histopathological scoring, showing dose-dependent reduction in necrosis and inflammatory infiltration, aligns with this anti-inflammatory gene-regulatory mechanism. Thus, milk thistle's capacity to modulate pro-inflammatory cytokine expression appears central to preserving hepatocellular integrity under xenobiotic stress. Mechanistic insights from a 2024 review that silymarin enhances cellular glutathione levels and scavenges free radicals, stabilizes hepatocyte membranes, and exhibits antifibrotic effects by inhibiting stellate cell activation and collagen deposition [22]. In concordance, our study showed that extract pretreatment restored glutathione and antioxidant enzyme activities to levels statistically indistinguishable from normal controls, thereby preventing lipid peroxidation and necrotic degeneration. The antifibrotic actions described in that review provide a plausible explanation for our histopathological observations of preserved lobular architecture and minimal fibrotic change in the high-dose group. Clinical relevance of these preclinical findings is supported by human studies of silymarin in chronic liver diseases. A systematic review of randomized trials reported that silymarin significantly reduces ALT and AST in patients with nonalcoholic fatty liver disease and chronic hepatitis, with indications of improved histological fibrosis scores after prolonged therapy [23]. Although our model involves acute chemical injury rather than metabolic or viral etiology, the shared pathways of oxidative stress and inflammation suggest that the Hepatoprotective efficacy demonstrated here may translate to clinical scenarios of both acute toxin exposure and chronic liver injury. Comparative analysis of dosing regimens across studies reveals that the 600 mg/kg extract dose in our study (standardized to 80% silymarin) approximates the protective threshold observed in other rodent models: Nisreen et al [20] used 100 mg/kg silymarin with significant benefit, while Abdel-Salam et al [24] employed a pretreatment schedule of 100 mg/kg for 12 weeks to achieve maximal anti-inflammatory gene modulation. The stronger effects seen at higher extract doses in our work underscore a dose-response relationship that warrants further investigation to optimize therapeutic windows for prophylaxis versus treatment phases. Despite the robust Hepatoprotection observed, some discrepancies exist among studies regarding the extent of parameter normalization. For instance, Nisreen et al. reported complete lipid profile restoration, whereas our medium-dose group exhibited minor residual elevations in triglycerides and cholesterol. These differences may reflect variations in extract standardization, duration of pretreatment, or sex-related metabolic responses, as their study used female rats, and ours male. Additionally, Juma'a et al [25] demonstrated a dose-dependent anti-inflammatory effect of silymarin in acute inflammation models, underscoring the importance of dosing regimens on inflammatory outcomes. Future studies should systematically compare sexes, standardize silymarin content, and explore longer post-toxicant observation periods to clarify these nuances.

CONCLUSION

This research shows that standardized milk thistle (Silybum marianum) extract protects Wistar rats' livers against CCl_4-induced damage in a strong, dose-dependent manner. High-dose extract (600 mg/kg, 80% silymarin) retained liver architecture in histological evaluation and returned blood ALT, AST, ALP, bilirubin, lipid profiles, and antioxidant defenses to levels similar with healthy controls. A distinct dose–response association was confirmed by the graded advantages that medium and low dosages produced. These results are consistent with previous studies that have been indexed by Scopus and have shown the antioxidant, anti-inflammatory, membrane-stabilizing, and antifibrotic properties of silymarin in preclinical and clinical contexts. Despite consistent protective effects, minor discrepancies in lipid profile restoration at intermediate doses underscore the need to standardize extract composition, compare sex-specific responses, and optimize pretreatment timing. Future research should evaluate long-term safety, refine therapeutic windows for prophylaxis versus treatment, and explore combination regimens with complementary antioxidants. Collectively, this work supports the translational potential of milk thistle extract as a preventive and adjunctive therapy for chemical and oxidative liver injuries.

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