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# SYNERGISTIC HEPATOPROTECTIVE EFFECTS OF PHYTOCONSTITUENTS IN COUNTERACTING CCL4-INDUCED LIVER INJURY

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## ABSTRACT

Hepatotoxicity, characterized by liver damage due to various agents, poses a significant health challenge worldwide. The liver, one of the largest and most significant organs in the human body, plays a vital role in metabolism and excretion, contributing to the maintenance of homeostasis. It is involved in nearly every metabolic pathway and is responsible for crucial functions such as detoxification, bile secretion, vitamin storage, and the metabolism of proteins, lipids, and carbohydrates. Consequently, the health of the liver is essential for overall well-being. However, liver disorders pose a significant global health challenge due to their poor prognosis and high mortality rates, stemming from insufficient prevention and treatment options. Common hepatic conditions include autoimmune diseases, viral hepatitis, non-alcoholic fatty liver disease, and alcohol-induced liver disease. Despite ongoing research, the precise causes of many liver diseases remain unclear. This study investigates the combined effects of phytoconstituents from herbal plants, specifically vanillin acid and thymol, in preventing or treating carbon tetrachloride-induced hepatotoxicity. The concept of synergy suggests that blending these compounds may enhance their efficacy beyond that of individual herbs. Additionally, using a combination of herbs could reduce the risk of adverse effects commonly associated with high doses of certain herbal medicines, particularly those with hepatotoxic potential. By allowing for lower dosages of each herb, this approach may mitigate side effects while still achieving therapeutic outcomes. The research further examines levels of antioxidants, electrolyte profiles, biochemical markers, and histological changes to elucidate the potential mechanisms behind their therapeutic effects.

**KEYWORDS:** Hepatotoxicity, Carbon tetrachloride, drug induced hepatotoxicity, milk thistle, vanillic acid, Thymol.

## INTRODUCTION

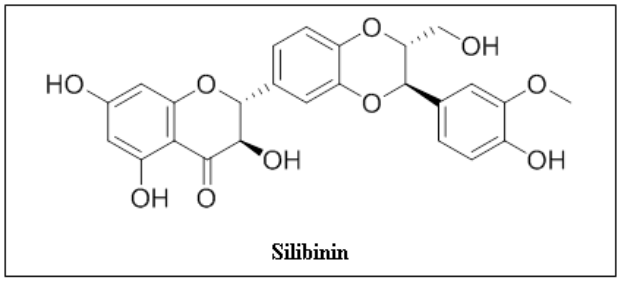
For centuries, milk thistle extracts have been employed as a traditional "liver tonic".[1] Recently, the active component in milk thistle extracts, silibinin, has undergone *in vitro* and *in vivo* research to assess its advantageous impact on liver disease.[2] Silibinin elevates antioxidant levels and enhances outcomes in liver diseases caused by oxidative damage. Additionally, silibinin administration has been linked to safeguarding the liver against toxins and reducing hepatic inflammation and fibrosis.[3,4]

Silibinin's anti-inflammatory effects go beyond inhibiting reactive oxygen species-dependent mechanisms. Many of its anti-inflammatory properties result from nuclear DNA/RNA-mediated effects, suppressing NF-κB translocation and binding.[5,6] Silibinin also hinders the expression of tumor necrosis factor α (TNF), TNF receptor 1, TNF receptor 1-associated apoptosis-ligand, and hepatic apoptosis, along with elevated liver enzyme activity induced by fumonisin treatment in mice. In a model of acute hepatitis in mice, Silibinin significantly inhibits TNF and interleukin 4 expression.[7] Additionally, it strongly inhibits the 5-lipooxygenase pathway and leukotriene formation in Kupffer cells *in vitro*. Silibinin, under lipopolysaccharide (LPS) stimulation, inhibits inducible nitric oxide synthase expression *in vitro*. Various *in vitro* studies reveal a

reduction in monocyte chemoattractant protein 1 due to interleukin (IL) 1β stimulation in stellate cells and hepatocytes. Furthermore, silibinin administration decreases IL-1β and prostaglandin E2 in vivo, improving survival in a mouse LPS sepsis model. Recent evidence suggests that silibinin inhibits selectin adhesion molecule expression, crucial for leukocyte migration.[8-12]

Silibinin exhibits antifibrotic effects in the liver by impeding the conversion of hepatic stellate cells to myofibroblasts, limiting cell signalling.[13] It decreases stellate cell DNA synthesis, proliferation, migration, and fibrous tissue production. In a rat hepatic injury model, oral administration of silibinin reduces liver collagen concentration by up to 55%. Silibinin also decreases collagen, procollagen III, procollagen α1, and profibrogenic mRNA expression by 30% in rats with biliary obstruction.[14] In a long-term study with baboons, ethanol and silibinin administration results in lower hepatic collagen type I concentrations, procollagen mRNA, and reduced incidence of alcohol-induced hepatic fibrosis and cirrhosis compared to the control group.[15] Combining milk thistle extracts with praziquantel in an *in vivo* model of schistosomal liver fibrosis reduces markers of inflammation and fibrosis.[1]

Silibinin's hepatoprotective effects in liver disease may stem from enhanced protein synthesis mechanisms. In an *in vivo* study, silibinin selectively increased DNA synthesis in partially hepatectomized rats, supporting hepatic regeneration and repair after toxic and inflammatory insults.[16] Another hepatoprotective effect involves preventing estrogen- and taurolithocholate-induced cholestasis by up-regulating the bile salt export pump. Silibinin administration leads to a dose-dependent increase in bile flow (choleresis), primarily due to stimulating bile salt synthesis *in vivo* in rats.[17]



Silibinin

**2.1 Protective effects of silibinin in drug induced hepatotoxicity**

**2.1.1. Silibinin in Dacarbazine induced hepatotoxicity**

In the study by Durymanov *et al.* the use of pre-treatment with PLGA/Silibinin (SBN) nanoparticles to mitigate hepatotoxicity induced by Dacarbazine was investigated in mice.[18] Different formulations were administered to the mice, and the expression level of antioxidant and phase II liver enzymes was assessed. Statistical analysis was performed using one-way analysis of variance (ANOVA) with post-hoc Tukey's test or Dunnett's t-test. After the therapeutic experiment with Dacarbazine, the mice were evaluated for hepatoprotective effects of SBN formulations. Elevated levels of ALT, AST, and bilirubin in blood serum upon Dacarbazine treatment were observed, which were decreased by pre-treatment with both free SBN and PLGA/SBN formulations.[19,20]

This study emphasized that drug-induced hepatotoxicity is often idiosyncratic and associated with the production of free radicals, oxidative stress, and inflammatory reaction. The study concluded that the developed intravenous PLGA/SBN nanoparticle formulation displayed a controlled release profile, enhanced induction of phase II enzymes, and improved protective properties in Dacarbazine-induced hepatotoxicity compared to free SBN. This mitigation of liver injury was achieved even after a single injection of PLGA/SBN nanoparticles, likely due to better bioavailability of encapsulated SBN for liver tissue.

Thus the study demonstrated the potential of the intravenous PLGA/SBN nanoparticle formulation in mitigating Dacarbazine-induced hepatotoxicity in mice, highlighting its controlled release profile and improved protective properties compared to free SBN. The findings suggest the therapeutic promise of this nanoparticle formulation in addressing drug-induced liver injury.

**2.1.2. Silibinin in N-nitrosodimethylamine-induced hepatotoxicity**

The study by Harrison and group focused on the use of Silibinin (SBN) to alleviate N-nitrosodimethylamine (DMN)-induced glutathione dysregulation and hepatotoxicity in Wistar albino rats.[21] DMN was administered to induce hepatotoxicity and oxidative stress in the rats, and different treatment groups were established, including rats sacrificed at the end of the DMN treatment, rats left without treatment after the last DMN dose, and rats treated with SBN for two weeks.

Biochemical assays and histopathology were conducted to assess liver function and tissue changes. The study found significant increases in the activities of marker enzymes of liver toxicity in the serum, attributed to the leakage of these enzymes from the damaged cells due to loss of structural and functional integrity.[22-26] Statistical analysis was performed using one-way ANOVA and Tukey's multiple comparison tests. The discussion emphasized the hepatotoxicity induced by intermittent DMN administration, leading to hepatocellular necrosis, inflammatory changes, and fibrosis in the rats.

In conclusion, the study demonstrated the potential of SBN in mitigating DMN-induced hepatotoxicity and glutathione dysregulation in rats. The findings underscore the significance of SBN in protecting against liver damage induced by DMN, and further research in

this area may provide insights into potential therapeutic applications for hepatic conditions.

**2.1.3. Silibinin in zidovudine-induced hepatotoxicity**

In a recent study by Raghu and co-workers, researchers investigated the potential of silibinin in mitigating liver toxicity and oxidative stress induced by prolonged zidovudine (AZT) treatment in rats.[27] The research focused on the impact of AZT administration on liver function, oxidative stress, and lipid levels, as well as the protective effects of concurrent SBN treatment. The study involved administering AZT and SBN to Wistar albino rats over a period of 45 days, with various biochemical and histopathological analyses conducted to assess liver function and tissue changes. AZT treatment led to significant increases in liver enzymes and markers of oxidative stress, as well as indications of hyperlipidemia in the serum.[28,29] Histopathological examination revealed substantial liver tissue changes in the rats receiving AZT alone, including cellular enlargement, inflammatory alterations, and increased sinusoidal spaces. However, simultaneous treatment with SBN alongside AZT demonstrated significant protection against hepatotoxicity, oxidative stress, and hyperlipidemia induced by AZT. This protective effect was attributed to the hepatoprotective, membrane-stabilizing, antioxidant, and free radical scavenging properties of SBN. The experimental protocol, sample collection, and preparation methods employed were conducted in accordance with established procedures, and the study findings were subjected to statistical analysis to assess the significance of the results. The histopathological evaluations of liver tissues further supported the protective effects of SBN when administered alongside AZT, as indicated by the reduction in cellular hypertrophy, sinusoidal space, and inflammatory cell infiltration compared to AZT alone-treated rats. Overall, the study provides valuable insights into the potential therapeutic role of SBN in mitigating AZT-induced liver toxicity and oxidative stress, offering promising avenues for further research in this area.

**2.1.4. Silibinin in zidovudine and isoniazid induced hepatotoxicity**

In this study by Raghu *et al.*, the authors investigated the potential hepatotoxic effects of the combined regimen of the anti-retroviral drug zidovudine (AZT) and isoniazid (INH), commonly administered to HIV/AIDS patients with TB co-infection, and explored the protective effects of silibinin (SBN) against AZT+INH-induced liver toxicity in rats.[30] The research involved the administration of AZT alone, INH alone, AZT+INH, SBN alone, and SBN+AZT+INH to separate groups of rats over a 45-day period. The control group received saline/propylene glycol. Various biochemical and histopathological analyses were performed to assess liver function, oxidative stress, and tissue changes. The results revealed that INH alone and AZT+INH induced significant liver injury, as evidenced by increased marker enzyme activities, bilirubin levels, oxidative stress parameters, and histopathological changes in liver tissues.[31-34] However, rats receiving SBN alongside AZT+INH showed significant protection against hepatotoxicity, oxidative stress, and histopathological alterations induced by the drug combination. This suggests that SBN possesses hepatoprotective and antioxidant properties against AZT+INH-induced liver toxicity. The study provides valuable insights into potential therapeutic strategies for mitigating the hepatotoxic effects of AZT+INH in HIV/AIDS patients with TB co-infection. Further investigation into the use of SBN, potentially in combination with other agents to improve its therapeutic efficiency, is warranted. The study utilized robust experimental protocols, including the use of a suitable animal model, detailed experimental procedures, and appropriate statistical analysis. The significance of the results was demonstrated through meticulous biochemical and histopathological evaluations, lending credibility to the findings and their potential implications for clinical practice.

This research contributes to addressing the challenging issue of hepatotoxicity associated with AZT+INH therapy in HIV/AIDS patients with TB co-infection, providing a foundation for further exploration of SBN as a potential protective intervention in this context.

**2.1.5. Silibinin in Itraconazole-induced Hepatotoxicity**

A study by Sozen and group aimed to assess the protective effects of Silibinin (SIL) in Wistar Albino rats with liver damage induced by Itraconazole (ITZ) administration.[35] ITZ, a member of the triazole group of antifungals, is known for its potent antifungal properties but has been associated with serious hepatotoxic events. The latest research has highlighted the potential role of certain drugs, such as ITZ, in inducing cellular oxidative stress response in pathogens, leading to increased microorganism death rates. The study used thirty-two adult female Wistar Albino rats, which were divided into four groups: Control (CTL), SIL, ITZ, and ITZ + SIL. The rats received specific oral treatments for 14 days, and various biochemical parameters were measured at the end of the experiment. These parameters included serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), myeloperoxidase (MPO), nitric oxide (NO), superoxide dismutase (SOD), and glutathione peroxidase (GSH Px). Additionally, comet assay was performed to assess DNA damage. Histopathological evaluation of the liver tissue was conducted, focusing on parameters such as hepatocyte degeneration, ductular reaction, bile duct plugs, inflammation, multinuclear giant cell formation, xanthomatous cell presence, apoptotic cells, and necrosis. The findings revealed that ITZ administration resulted in significant hepatocyte degeneration, ductular reaction, bile duct plugs, inflammation, apoptotic cells, and trends in multinuclear giant cell formation. However, the co-administration of ITZ and SIL led to a reduction

in portal/periportal inflammation and apoptosis of parenchymal hepatocytes. Moreover, SIL partially improved hepatocyte degeneration and multinuclear giant cell formation.

The study utilized statistical analysis to evaluate the significance of the differences observed between the groups. The results indicated that SIL had a protective effect on the liver, mitigating the detrimental effects induced by ITZ administration. In summary, the study demonstrated the potential hepatoprotective effects of Silibinin in rats with ITZ-induced liver damage. The combination of ITZ and SIL showed promise in ameliorating liver injury, highlighting the potential for Silibinin as a protective agent against drug-induced hepatotoxicity.

**2.1.6. Silibinin in Methotrexate-induced hepatotoxicity in rats**

The study by Yanaşoğlu *et al.* aimed to explore the potential hepatoprotective properties of silibinin and its impact on oxidative stress markers and cytokines in the context of high-dose methotrexate-induced hepatotoxicity in rats.[36] The study involved the random allocation of rats into five groups, with methotrexate administered intraperitoneally (20 mg/kg) on the first day in all groups except the control. Silibinin was then injected for five consecutive days in varying doses (25, 50, and 100 mg/kg/day) to different groups in conjunction with methotrexate. Blood and liver samples were collected on the sixth day for further analysis. Assessments included measurements of serum total antioxidant capacity, total oxidant status, total thiol, native thiol, alanine aminotransferase, aspartate transaminase, bilirubin, albumin, tumor necrosis factor-alpha, and interleukin-10 levels. Additionally, a histopathological evaluation of liver tissues was conducted. Methotrexate administration led to a reduction in total antioxidant capacity and an increase in the disulfide/total thiol ratio. Histopathological examination revealed heightened hepatic damage, with notable inflammatory cell infiltration following methotrexate administration. However, the administration of 50 mg/kg/day of silibinin demonstrated a preventive effect against inflammatory cell infiltration. The findings suggested that the administration of 50 mg/kg/day of silibinin may mitigate hepatic damage induced by methotrexate in rats by enhancing antioxidant capacity.

**2.1.7. Silibinin in Cisplatin and taxol induced hepatotoxicity**

Ovarian cancer stands as one of the most fatal malignancies in the realm of gynecological cancers. The combined use of cisplatin and paclitaxel is widely prevalent in clinical settings for treating ovarian cancer. However, prolonged administration of cisplatin and paclitaxel leads to significant drug resistance and hepatotoxicity. Given that silibinin is commonly utilized as an anti-hepatotoxic agent in Europe and Asia, the purpose of the study by Yang *et al.* was to explore whether silibinin could reinstate the responsiveness of drug-resistant human ovarian cancer cells to the combination of cisplatin and paclitaxel and concurrently alleviate drug-induced hepatotoxicity.[37] Normal hepatocyte LO2 cells and A2780/DDP cells were subjected to treatment with silibinin, cisplatin, paclitaxel, and a combination of cisplatin, paclitaxel, and silibinin for a duration of 48 hours. Cell viability was assessed using MTT and long-term proliferation assays, while flow cytometric analysis was utilized to determine apoptosis and cell cycle progression. Additionally, immunofluorescence assays were employed to evaluate DNA damage, and the metastatic activity of A2780/DDP cells was ascertained through a cell adhesion assay. The introduction of silibinin in conjunction with cisplatin and/or paclitaxel heightened the antitumor efficacy of these drugs on A2780/DDP cells, suppressed the cell-matrix adhesion of A2780/DDP, inhibited cell proliferation, and induced apoptosis in A2780/DDP cells. Furthermore, silibinin effectively mitigated cisplatin and/or paclitaxel-induced hepatotoxicity by safeguarding DNA integrity and reinstating the proliferative potential of cisplatin and/or paclitaxel-treated LO2 cells. The findings indicate that silibinin has the potential to restore the responsiveness of cisplatin and paclitaxel in drug-resistant human ovarian cancer cells and mitigate drug-induced hepatotoxicity at the cellular level. This study showcases the ability of silibinin to enhance the antitumor efficacy of cisplatin and/or paclitaxel on cisplatin-resistant human ovarian carcinoma (A2780/DDP) cells and reduce cisplatin and/or paclitaxel-induced hepatotoxicity at the cellular level. If validated in vivo, the combination of silibinin with cisplatin and/or paclitaxel could serve as a beneficial chemotherapeutic approach, particularly for patients with tumors that are resistant to cisplatin.

**2.1.8. Silibinin in Pyrazinamide- and Isoniazid-Induced Hepatotoxicity**

Drug-induced liver injury (DILI) is a common cause for drug removal from the market, especially when hepatotoxicity manifestations are complex and demand a deeper understanding. The study by Goh *et al.* focused on simulating silibinin's clinical roles in the prevention, treatment, and recovery from hepatotoxicity induced by HRZE (a combination of antitubercular drugs isoniazid, rifampicin, pyrazinamide, and ethambutol) using an *in vitro* model.[38] The findings revealed that as a rescue agent, silibinin significantly mitigated hepatotoxicity induced by isoniazid within a specific concentration range, termed the "Goldilocks zone," suggesting its potential efficacy at moderate levels of DILI.

The study demonstrated that silibinin's hepatoprotective effect stemmed from two main aspects. Firstly, it reduced intracellular levels of oxidative stress and damage to intracellular and mitochondrial targets, thereby

decreasing apoptotic activity. Secondly, silibinin induced the Nrf2–ARE-related protein expression, enhancing endogenous proteins that protect cells from oxidative damage. Notably, the study highlighted the need for carefully titrating silibinin's dose to optimize hepatoprotection and minimize potential side effects. However, it was observed that silibinin was not effective as a prophylactic or recovery agent, suggesting limitations in preventing HRZE-induced (rifampicin and isoniazid used in combination with pyrazinamide and ethambutol) hepatotoxicity and aiding in the recovery process. The study also indicated the potential role of silibinin in reducing stellate cell migration, which is vital in liver diseases involving fibrotic activity, injury, and regeneration. Future research directions were proposed to further characterize silibinin's role in recovery, including the use of co-cultures to mimic paracrine responses and personalize regimens based on patients' conditions. The findings also underscored ethical considerations in clinical practice and the need for further clinical trials to investigate silibinin's hepatoprotective effect, especially in moderate-to-high DILI.

Additionally, the study revealed that silibinin protected against apoptosis induced by isoniazid and pyrazinamide and reduced intracellular oxidative stress, emphasizing its safety and potential for further development.

### 2.2. Protective effects of silibinin in chemicals and poison induced hepatotoxicity

#### 2.2.1 Silibinin Against Diazinon Induced Hepatotoxicity

The study by Beydilli *et al.* investigated the effects of Diazinon (DI) exposure on liver function and the potential protective role of Silibinin in female 12-week-old Wistar albino rats.[39] DI, an organophosphorous pesticide, is known to induce oxidative stress and renal dysfunction. The experiment involved four groups of rats, including a control group, a DI group, a Silibinin group, and a DI + Silibinin group. Blood and liver samples were collected and analyzed for various biochemical markers.

The results showed significantly increased levels of ALT, AST, NO, and MPO in the DI group compared to the control group, indicating liver damage.[40-42] However, the DI + Silibinin group exhibited decreased levels of these markers, suggesting a protective effect of Silibinin against DI-induced liver damage. Additionally, histopathological examination revealed significant tissue damage in the DI group, while the DI + Silibinin group showed improvements in liver structure, particularly in reducing inflammation and vacuolization.

The discussion highlighted the critical role of the liver in detoxification processes and the potential for oxidative stress to cause hepatocellular injury. The study suggested that DI increased reactive oxygen species (ROS) in liver tissue, leading to oxidative stress and liver injury, consistent with previous research. Silibinin was proposed to exert a free radical-eliminating activity and extensive antioxidant effect, potentially reversing the effects of DI-induced oxidative stress. In conclusion, the study emphasized the hepatoprotective effects of Silibinin against DI-induced liver damage in female Wistar albino rats, shedding light on potential therapeutic interventions for pesticide-induced liver injury. The findings suggest that Silibinin could be further explored for its hepatoprotective properties and its potential application in mitigating pesticide-induced liver damage. Overall, the study provided valuable insights into the mechanisms of DI-induced liver damage and the potential protective effects of Silibinin, contributing to the understanding of pesticide toxicity and the development of interventions to mitigate its adverse effects on liver function.

#### 2.2.2 Silibinin in galactosamine/lipopolysaccharide-induced hepatotoxicity

Hashem *et al.* explored the impact of antioxidants, specifically silibinin and vitamin E, in the treatment of D-Galactosamine (D-GalN) and Lipopolysaccharide (LPS)-induced hepatotoxicity in male Albino Wistar rats. The research focused on the role of Apoptosis Signal-Regulating Kinase 1 (ASK1) in the activation of MAP kinase cascades and its implication in oxidative stress-related diseases, including hepatic disorders.[44,45] ASK1, activated by various stressors such as ROS and LPS, triggers the MAP kinase kinase (MAPKK) pathway, resulting in cell death, inflammation, and differentiation. The study investigated the potential of silibinin and vitamin E as antioxidants to modulate the ASK1-p38 MAPK pathway by deactivating ASK1, thus blocking downstream effector signaling and alleviating hepatotoxicity. Methods included the administration of D-GalN/LPS, followed by treatment with silibinin and vitamin E in curative and prophylactic regimens. Biochemical analysis of serum and liver samples was performed to measure markers such as ALT, AST, TBARS, GSH, CAT, and SOD, while histopathological examination provided insights into liver tissue changes. The results indicated that silibinin and vitamin E acted as antioxidants, deactivating ASK1 and increasing the levels of Trx1, TrxR1, and PP5. This mechanism was pivotal in blocking downstream effector signaling, particularly p38 MAPK, and mitigating hepatotoxicity.

In conclusion, the study provided new insights into the mechanisms of ASK1 deactivation and the precise downregulation of downstream signaling kinase p38 MAPK in D-GalN/LPS-induced hepatotoxicity. The findings highlighted the potential therapeutic roles of silibinin and vitamin E as antioxidants in modulating the ASK1-p38 MAPK pathway, ultimately contributing to the alleviation of hepatotoxicity associated with oxidative stress-related liver diseases. The comprehensive approach of the research shed light on potential avenues for antioxidant-based interventions in hepatic disorders.

**2.2.3. Silibinin in Abrin induced hepatotoxicity**

Abrin, derived from the seeds of Abrus precatorius, is known for its high toxicity, surpassing that of ricin, and is recognized as a potent bio-warfare agent. Despite its significant impact, the precise mechanism of abrin-induced liver damage remains unclear. Silibinin, renowned for its antioxidant, anti-inflammatory, and hepatoprotective properties, has not been studied for its therapeutic potential in abrin toxicity. Consequently, a study by Saxena *et al.* aimed to elucidate the mechanisms involved and assess the protective role of silibinin against abrin-induced liver damage.[46]

The experiment involved evaluating various parameters associated with liver function, oxidative stress, inflammation, Fas pathway activation, and liver histopathology in BALB/c mice following exposure to abrin. The results revealed that abrin exposure led to hepatotoxicity, oxidative stress, inflammation, histopathological alterations, and increased Fas pathway signaling. However, administration of silibinin improved the survival of abrin-exposed mice by reducing serum liver enzymes and restoring antioxidant capacity. Furthermore, silibinin demonstrated the ability to attenuate abrin-induced inflammation and inhibit the Fas pathway. This study marks the first documentation of the hepatoprotective potential of silibinin against abrin toxicity.

**2.3. PROTECTIVE EFFECTS OF SILIBININ IN HEAVY METAL INDUCED HEPATOTOXICITY**

**2.3.1. Silibinin in Cadmium induced hepatotoxicity**

The study by Srinivasan *et al.* focused on the impact of cadmium (Cd), an environmental toxin that particularly affects the liver and kidneys in humans.[47] Rats were subcutaneously administered with Cd for three weeks, resulting in a significant increase in serum transaminases, alkaline phosphatase, and lactate dehydrogenase activities, accompanied by elevated lipid peroxidation and decreased levels of antioxidants in the liver. The oral administration of silibinin (SB) at a dose of 80 mg/kg body weight effectively normalized hepatic enzyme activities, reduced lipid peroxidation, and restored antioxidant defense in the liver, as opposed to lower doses of SB (20 and 40 mg/kg body weight). Histopathological analysis further corroborated these findings, concluding the potential of SB in mitigating Cd-induced hepatic injury.

The study also delves into the detailed impacts of Cd toxicity on vital hepatic enzymes activities, such as AST, ALT, ALP, and LDH, as well as the efficacy of SB in mitigating these effects. Additionally, it highlights the role of SB in reducing oxidative damage, enhancing enzymic and non-enzymic antioxidant levels, and chelating Cd in the liver, ultimately contributing to the protection of liver architecture and function in Cd-intoxicated rats.

Overall, the study suggests that SB may offer a beneficial intervention to mitigate the toxic effects of Cd-induced liver damage, primarily due to its antioxidant properties, free radical scavenging ability, and metal chelating activities. However, further research is warranted to fully elucidate the specific mechanisms through which SB protects against Cd-induced toxicity in experimental rats.

**2.3.2. Arsenic induced hepatotoxicity**

Arsenic (As) compounds are widely recognized as environmental toxicants and human carcinogens, presenting a significant global health challenge. Silibinin (SB), a key flavonolignan found in milk thistle of Silybum marianum, has been identified as possessing antioxidant properties and as a metal chelator due to the arrangement of its functional groups. Despite these attributes, its potential in mitigating arsenic-induced toxicity in experimental animals had not been investigated. Thus, a study by Muthumani and group aimed to elucidate the potential mitigating role of silibinin against arsenic-induced hepatotoxicity in rats.[48] In the study, rats were orally administered with arsenic alone (at a dosage of 5 mg/kg body weight/day) and in combination with silibinin (at a dosage of 75 mg/kg body weight/day) over a period of four weeks. Hepatotoxicity was assessed through increased activities of serum hepatospecific enzymes, including aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transferase, lactate dehydrogenase, and total bilirubin.[49] Additionally, elevated levels of lipid peroxidative markers such as thiobarbituric acid reactive substances, lipid hydroperoxides, protein carbonyl content, and conjugated dienes further indicated hepatotoxicity induced by arsenic. Furthermore, the toxic effects of arsenic were reflected in the significantly reduced activities of membrane-bound ATPases and enzymatic antioxidants (e.g., superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase, and glucose-6-phosphate dehydrogenase), as well as nonenzymatic antioxidants like reduced glutathione, total sulfhydryl groups, and vitamins C and E. The administration of silibinin resulted in a significant reversal of the toxic effects induced by arsenic in hepatic tissue. These observations were reinforced by the reduction of DNA damage in hepatocytes, as well as histopathological examinations of the liver, collectively suggesting a potential protective effect of silibinin against arsenic-induced hepatotoxicity in rats. In conclusion, the study highlighted the potential of silibinin as a protective agent against arsenic-induced hepatotoxicity in rats, by effectively mitigating the toxic effects and oxidative damage induced by arsenic. These findings contribute to the growing understanding of potential therapeutic interventions for arsenic-induced health issues and underline the promising role of silibinin in this context.

### 2.3.3. Silibinin in Ethanol- or acetaldehyde induced liver damage

Alcoholic liver disease is a significant contributor to liver injury, necessitating effective preventative and treatment strategies. The study by Song and co-workers investigates the role of silibinin in mitigating ferroptosis, a form of cell death induced by ethanol or acetaldehyde, in hepatic cells.[50] The research utilized human carcinomatous liver HepG2 cells and immortalized liver HL7702 cells to explore the protective effects of silibinin. The findings indicate that ethanol or acetaldehyde treatment led to ferroptosis in the cells, characterized by heightened reactive oxygen species (ROS) stress and elevated iron levels. Silibinin was observed to counteract oxidative stress and reduce iron levels, effectively rescuing the cells from ferroptosis. Moreover, silibinin reversed the ethanol- or acetaldehyde-induced impairment of nuclear receptor co-activator 4 (NCOA4)-dependent autophagic degradation of ferritin, a protein responsible for iron storage. Additionally, silibinin restored PINK1 and Parkin-mediated mitophagy, which was hindered by ethanol or acetaldehyde exposure. The study further employed inhibitors for apoptosis, necroptosis, and ferroptosis to determine the type of cell death involved in ethanol- or acetaldehyde-treated cells. The results showed that all inhibitors increased cell viability, with ferroptosis inhibitors notably demonstrating efficacy. The discussion within the study emphasizes the biological significance of iron ions in organisms and highlights the potential damage caused by excess free reactive iron ions.[51,52] The study underscores ferroptosis as a novel form of programmed cell death in alcoholic liver disease, attributing hepatic iron ion accumulation to its manifestation. In conclusion, the research elucidates that ethanol and acetaldehyde promote ferroptosis through autophagic degradation of ferritin and reduction in mitophagy, ultimately leading to lipid peroxidation. Silibinin was found to reverse ferroptosis induced by ethanol or acetaldehyde, offering insights into potential therapeutic approaches for alcoholic liver injury. These findings provide valuable knowledge regarding the pathological mechanisms underlying alcoholic liver disease and present promising avenues for therapeutic intervention.

## 3. CONCLUSION

In conclusion, the comprehensive review delves into the remarkable potential of Silibinin, a flavonolignan derived from milk thistle, as a formidable guardian against hepatotoxicity—a pervasive health challenge on a global scale. Silibinin's multifaceted protective effects on the liver are elucidated through a meticulous exploration of its impact on drug-induced, chemical-induced, and metal-induced hepatotoxic insults.

The multifaceted actions of Silibinin underscore its promise as a versatile intervention in the realm of hepatoprotection. As we navigate the complexities of hepatotoxicity, Silibinin stands out as a beacon of hope, offering a holistic and effective approach to safeguarding liver health. This review not only consolidates existing understanding but also paves the way for further exploration of Silibinin's therapeutic potential, emphasizing its crucial role in the ongoing pursuit of liver health and well-being.

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