

Uncovering Silibinin's Protective Effects on the Liver: A Concise Review

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Abstract Hepatotoxicity, characterized by liver damage due to various agents, poses a significant health challenge worldwide. Silibinin (SB), a flavonolignan extracted from the milk thistle (*Silybum marianum*), has garnered attention for its potential hepatoprotective properties. This review provides a thorough examination of SB's efficacy in mitigating hepatotoxicity induced by drugs, chemicals, metals and toxins. SB's anti-inflammatory properties extend beyond just inhibiting reactive oxygen species. It also encompasses effects mediated by nuclear RNA/DNA, which work to suppress NF- κ B binding and translocation. SB demonstrates notable efficacy in countering drug-induced hepatotoxicity, inhibiting tumor necrosis factor α (TNF) expression and associated apoptotic pathways. Moreover, it attenuates the impact of chemical insults, exemplified by its ability to suppress TNF and interleukin 4 expressions in acute hepatitis models. SB's influence on the leukotriene formation and 5-lipoxygenase (LOX) pathway, as well as its inhibitory effects on inducible nitric oxide synthase expression, further contributes to its protective role against various chemical-induced hepatotoxic insults. This comprehensive review synthesizes current knowledge on SB's protective effects in hepatotoxicity, providing insights into its potential as a therapeutic agent against diverse challenges posed by drug, chemical, and metal-induced liver damage. The multifaceted actions of SB underscore its promise as a versatile and effective intervention in the realm of hepatoprotection.

Keywords Hepatotoxicity, Silibinin, Drug Induced Hepatotoxicity, Milk Thistle

1. Introduction

For generations, extracts from milk thistle have served as a customary remedy for liver health [1]. Contemporary research, both in laboratory tests and animal studies, has explored the beneficial effects of Silibinin (SB, **Fig. 1**), the key compound found in these extracts, on liver disease [2]. SB has been shown to boost levels of antioxidants and improve conditions in liver diseases that stem from oxidative harm. Furthermore, treatment with SB is associated with protective actions against liver toxins and has been found to help reduce liver inflammation and the development of fibrous tissue [3,4]. Some of the other indications of SB are enlisted in **Table 1**.

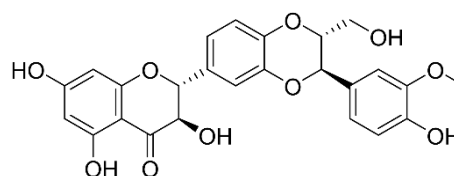


Figure 1. Chemical structure of silibinin

SB's anti-inflammatory properties go beyond the suppression of mechanisms dependent on reactive oxygen species (**Fig. 2**). Its anti-inflammatory properties stem from nuclear DNA/RNA-mediated effects, which suppress the translocation and binding of NF-Kb [11,12]. SB also inhibits the expression of tumor necrosis factor α (TNF),

TNF receptor 1, TNF receptor 1-associated apoptosis-ligand, and hepatic apoptosis. Additionally, it reduces elevated liver enzyme activity induced by fumonisin treatment in mice. In a mouse model of acute hepatitis, SB significantly inhibits TNF and interleukin 4 expression [13]. Furthermore, it shows potent inhibition of the 5-LOX pathway and the formation of leukotrienes in Kupffer cells *in vitro*. It also inhibits the expression of inducible nitric oxide synthase under lipopolysaccharide (LPS) stimulation *in vitro*. *In vitro* studies have shown a reduction in monocyte chemoattractant protein 1 due to interleukin (IL) 1 β stimulation in stellate cells and hepatocytes. Furthermore, *in vivo*, SB reduces IL-1 β and prostaglandin E2 levels, leading to improved survival in a mouse model of LPS sepsis. Newer findings also indicate that SB suppresses selectin adhesion molecule expression, which plays a crucial role in leukocyte migration [14-18].

The liver's antifibrotic effects are evidenced by SB, which inhibits the differentiation of hepatic stellate cells into myofibroblasts, thus restricting cellular signaling [19]. It decreases migration, proliferation, fibrous tissue production, and stellate cell DNA synthesis. In a rat hepatic injury model, oral administration of SB reduces liver collagen concentration by up to 55%. In rats with biliary obstruction, SB also reduces the expression of procollagen α 1, procollagen III, collagen, and profibrogenic mRNA by 30% [20]. During a long-term study involving baboons, the

administration of ethanol and SB leads to decreased levels of hepatic procollagen mRNA, collagen type I and a lower occurrence of hepatic fibrosis induced by alcohol and cirrhosis compared to the control group [21]. Furthermore, the combination of milk thistle extracts with praziquantel in an *in vivo* model of schistosomal liver fibrosis results in the reduction of markers of fibrosis and inflammation [1].

The protective effects of SB in hepatic disease could potentially stem from improved protein synthesis mechanisms. In an *in vivo* investigation, partly hepatectomized rats showed a selective increase in DNA synthesis after receiving SB, providing support for regeneration of hepatocytes and repair after inflammatory and toxic insults [22]. Additional defensive effect involves preventing tauroolithocholate and cholestasis induced by estrogen resulting in up-regulating the bile salt export pump. Administering SB results in a dose-dependent rise in bile flow (choleresis) in rats, mainly attributed to the stimulation of bile salt production *in vivo* [23].

This thorough review consolidates existing information about the hepatoprotective effects of SB, offering understanding into its potential as a therapeutic solution against the various complexities associated with drug, chemical, toxins and metal-induced liver injuries (**Fig. 3**). The diverse capabilities of SB emphasize its potential as a versatile and successful intervention within the field of hepatoprotection.

Table 1. Some of the principal indications of SB

Indication	Description	Mechanism of Action	Evidence/Study References	Clinical Relevance	References
Liver Diseases (e.g., Cirrhosis)	Used for the treatment of liver diseases including cirrhosis	Antioxidant activity, inhibition of lipid peroxidation, stabilization of cell membranes	Meta-analysis of clinical trials shows improvement in liver function markers.	Helps reduce liver damage and improve liver function.	Abenavoli, et al. (2018) [5]
Cancer	Investigated for anticancer properties	Induces apoptosis, inhibits cell proliferation, modulates signaling pathways (e.g., STAT3)	Several preclinical studies demonstrate anti-proliferative effects in prostate and skin cancer cell lines.	Potential therapeutic agent in cancer treatment, under investigation.	Ting et al. (2013) [6]
Diabetes	Evaluated for blood sugar regulation	Enhances insulin sensitivity, reduces oxidative stress	Clinical trials indicate improvement in blood glucose levels and insulin resistance.	May support diabetes management alongside conventional treatment.	Huseini, et al. (2006)[7]
Neuroprotection	Explored for protective effects in neurodegenerative diseases	Anti-inflammatory, reduces oxidative stress, prevents neuronal damage	Animal studies and early clinical trials suggest neuroprotective effects, particularly in Alzheimer's disease.	Potential for preventing or slowing neurodegenerative diseases.	Bai et al. (2019) [8]
Kidney Diseases	Used in nephrotoxicity prevention and treatment	Antioxidant, anti-inflammatory	Animal studies show protective effects against drug-induced nephrotoxicity (e.g., cisplatin).	May help protect kidney function in patients undergoing chemotherapy.	Bokemeyer et al. (2010) [9]
Hypertension	Used in blood pressure, and ventricular hypertrophy	Antioxidant	Studies in rat models reduced blood pressure, left ventricular hypertrophy and arrhythmias	Potential for preventing hypertension	Chen et al. (1993) [10]

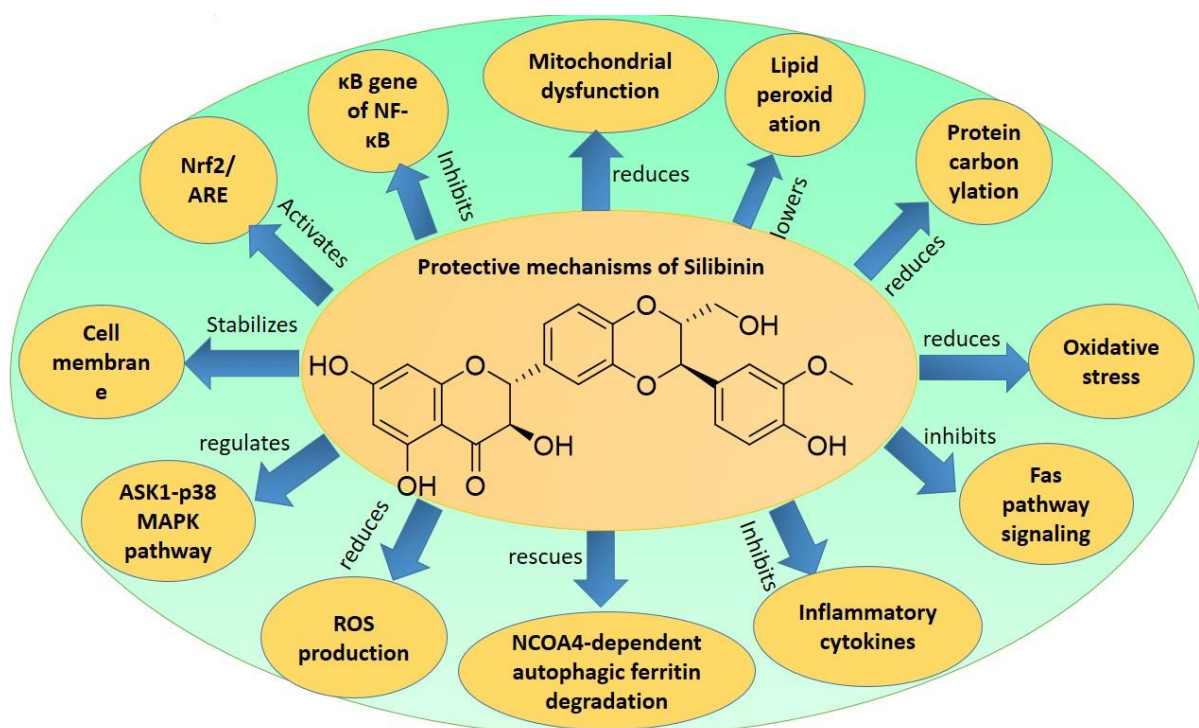


Figure 2. Various protective mechanisms of Silibinin

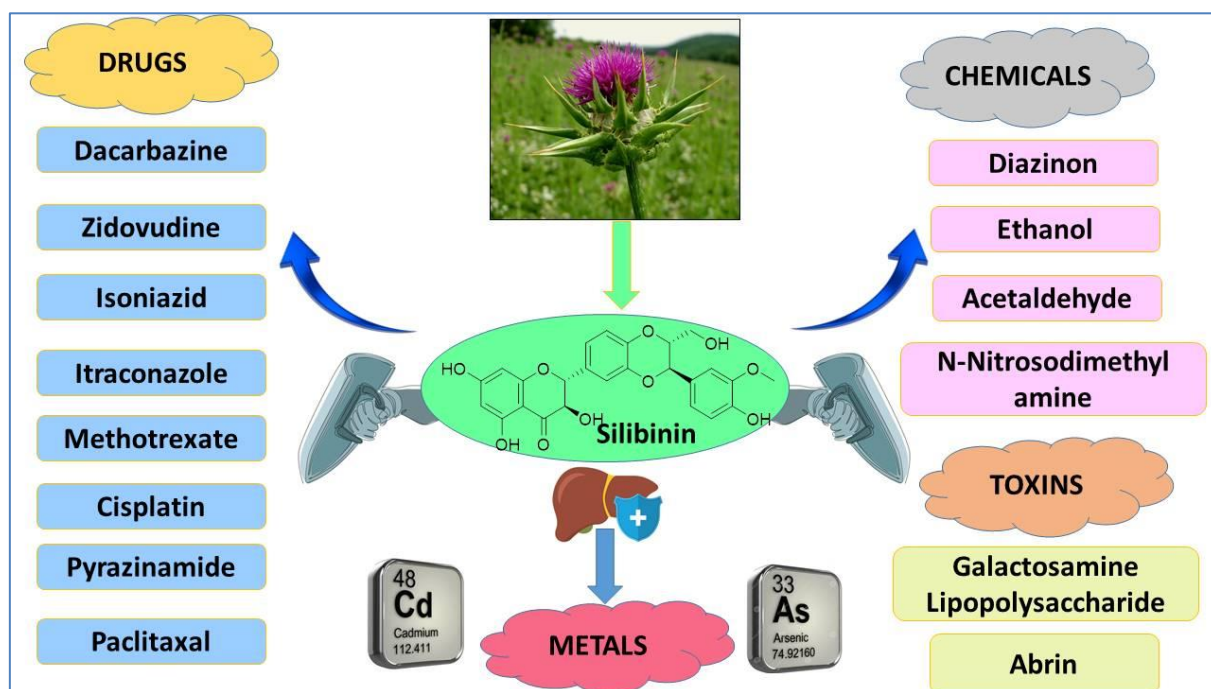


Figure 3. Silibinin: Safeguarding the Liver from Drug, Chemical, Toxin, and Metal-Induced Hepatotoxicity

2. Protective Effects of SB in Drug Induced Hepatotoxicity

2.1. Silibinin in Dacarbazine Induced Hepatotoxicity

Dacarbazine is a chemotherapy medication employed in the treatment of different cancer types, such as Hodgkin's

lymphoma and non-Hodgkin's lymphoma [24]. It works by interfering with the production of DNA in cancer cells, which leads to their death. However it is associated with various side effects like acute hepatic necrosis. In a research conducted by Durymanov *et al.* [24], the use of pre-treatment with Poly (lactic-co-glycolic acid) PLGA/SB nanoparticles to alleviate Dacarbazine-induced

hepatotoxicity in mice was investigated. Phase II hepatic enzyme expression and antioxidant expression were measured in the mice that were given various formulations. Following a Dacarbazine-based treatment trial, the mice's ability to protect their livers was assessed by the use of SB formulations. Following dacarbazine treatments, the study found that serum levels of ALT, AST, and bilirubin were raised. However, these elevated levels were reduced by pre-treatment with both free SB and PLGA/SB formulations [25, 26].

Comparing the intravenous PLGA/SB nanoparticle formulation to unbound SB, it demonstrated a regulated release pattern and enhanced phase II enzyme induction, offering better protective qualities against Dacarbazine-induced hepatotoxicity. The study highlighted the fact that liver damage caused by drugs is often a unique occurrence and is linked to the generation of free radicals, oxidative stress, and an inflammatory reaction. This improvement in liver damage was noticed even with a single PLGA/SB nanoparticle injection; this may be related to the encapsulated SB's increased bioavailability for liver tissue.

2.1.1. Mode of Action in Dacarbazine Induced Hepatotoxicity

Silibinin has been shown to stimulate the Nrf2/ARE pathway within the hepatocytes. This pathway is crucial for cellular defense against oxidative stress by regulating the expression of antioxidant and detoxifying enzymes. Apart from this, in this study, analysis of different SBN formulations on AML12 hepatocytes revealed increased expression levels of Nrf2-driven enzymes, including glutathione reductase and glutathione-S-transferases A3 and P1. These enzymes are involved in detoxification processes and antioxidant defense mechanisms. SB treatment resulted in the upregulation of SOD2 expression, despite it not being directly regulated by Nrf2. SOD2, a mitochondrial antioxidant enzyme, plays a crucial role in scavenging superoxide radicals and maintaining cellular redox balance. The highest impact on the expression of GSTP1, glutathione reductase, and SOD2 was noted after 48 hours of exposure to both unbound SBN and PLGA/SBN formulations. This suggests a time-dependent response to SBN treatment, with maximum activation of antioxidant defense mechanisms occurring after prolonged exposure. Measurement of glutathione-S-transferase activity indicated maximal values at 48 hours, consistent with the observed upregulation of GSTP1 expression.

2.2. Silibinin in Zidovudine-Induced Hepatotoxicity

Zidovudine (AZT) is an antiretroviral medication employed in the management of HIV. However, it can cause liver damage, known as hepatotoxicity, in some cases. In a recent study by Raghu and co-workers, the potential of SB in mitigating liver toxicity and oxidative stress induced by prolonged AZT treatment in rats was

examined [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 SB treatment. The study involved administering AZT and SB 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 oxidative stress markers, as well as indications of hyperlipidemia in the serum [28,29]. Histopathological examination revealed substantial hepatic tissue changes in the rats receiving only AZT, including cellular enlargement, inflammatory alterations, and increased sinusoidal spaces. However, simultaneous treatment with SB alongside AZT demonstrated significant protection from liver toxicity, hyperlipidemia, and oxidative stress induced by AZT. The reduction in effect was attributed to the hepatoprotective, membrane-stabilizing, antioxidant, and free radical scavenging qualities of SB, which contributed to its protective properties. The experimental protocol, sample collection, and preparation methods employed were conducted in accordance with established procedures, and the study results underwent statistical analysis to evaluate the significance of the findings. The histopathological evaluations of liver tissues further supported the protective effects of SB when administered alongside AZT, as evidenced by the decrease in sinusoidal space, cellular hypertrophy, and infiltration of inflammatory cells when compared to rats treated solely with AZT. Overall, the study provides valuable insights into the potential therapeutic role of SB in mitigating AZT-induced liver toxicity and oxidative stress, offering promising avenues for further research in this area.

2.2.1. Mode of Action Zidovudine-Induced Hepatotoxicity

SB has demonstrated the ability to suppress the κ B motif of NF- κ B DNA-binding activity and its gene expression in liver cells. Additionally, it prevents the translocation of NF- κ B (p65 protein phosphorylation) into the nucleus, without affecting its DNA-binding capacity. This indicates that simultaneous treatment with SBN and AZT might mitigate liver damage by restraining the expression of the NF- κ B transcription factor. However, additional research is advised to reveal the exact mechanism by which SB inhibits this signal transduction pathway against AZT-induced liver toxicity.

2.3. Silibinin in Zidovudine and Isoniazid Induced Hepatotoxicity

Raghu et al. [30] also examined the potential hepatotoxic effects of the concurrent administration of the AZT and isoniazid (INH), a common regimen for HIV/AIDS patients with TB co-infection and explored the shielding properties of SB against liver toxicity induced by the combination of AZT and INH in rats. The study involved

the administration of AZT alone, INH alone, AZT+INH, SB alone, and SB+AZT+INH to separate groups of rats over a 45-day period. The control group was administered saline/propylene glycol. Various biochemical and histopathological analyses were performed to assess liver function, oxidative stress, and tissue changes. The findings indicated that both INH alone and the combination of AZT and INH caused notable hepatic impairment, as evidenced by amplified marker enzyme activities, bilirubin levels, oxidative stress parameters, and alterations in histopathology of liver tissues [31-34]. However, rats receiving SB alongside AZT+INH showed significant protection against oxidative stress, hepatotoxicity and histopathological alterations induced by the drug combination. This research proposed to address 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 SB as a potential protective intervention in this context.

2.3.1. Mode of Action Zidovudine and Isoniazid Induced Hepatotoxicity

SB demonstrates a liver-protective action by reducing the elevation of liver toxicity marker enzymes, bilirubin, and protein in the blood, as well as lowering the enzyme activities in liver tissue. Moreover, SB helps in reducing oxidative stress by inhibiting the rise of lipid peroxidation (LPO) and carbonyl content, and by decreasing the levels of catalase (CAT) and protein thiol. Additionally, it combats hyperlipidemia by reversing the increase in plasma triglycerides (TGs) and free fatty acids (FFAs) towards healthy levels, particularly when used alongside AZT.

2.4. Silibinin in Itraconazole-Induced Hepatotoxicity

Itraconazole (ITZ), a member of the triazole group of antifungals, is known for its potent antifungal properties but has been associated with serious hepatotoxic events. Sozen and group conducted a study with the objective of evaluating the protective effects of SB in Wistar Albino rats that experienced hepatic damage induced by the administration of ITZ [35]. ITZ triggers a cellular oxidative stress response in pathogens, resulting in elevated rates of microorganism mortality. The investigation involved Wistar Albino rats, which were segregated into four groups: Control (CTL), SB, ITZ, and ITZ + SB. The rats received specific oral treatments for 14 days, and biochemical parameters were assessed upon completion of the experiment. The parameters comprised serum concentrations of ALT, AST, superoxide dismutase (SOD), nitric oxide (NO), myeloperoxidase (MPO), 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, formation of multinuclear giant cell, 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, concurrent administration of ITZ and SB led to parenchymal hepatocytes apoptosis and a decrease in portal/periportal inflammation. The outcomes showed that SB had a protective effect on the liver, mitigating the detrimental effects induced by ITZ administration.

2.4.1. Mode of Action Itraconazole-Induced Hepatotoxicity

ITZ leads to production of ROS, resulting in damage of DNA and liver injury. SB combats this inflammatory process by reducing the MPO pathway and suppressing the generation of free radicals. It serves a vital function in stopping ROS production, boosting the activities of antioxidant enzymes, decreasing MPO activity in hepatic inflammation, inducing cytochrome P450 isoenzyme CYP3A4, and fostering cellular resistance against oxidative damage induced by ITZ. Additionally, SB partially ameliorated hepatocyte degeneration and the formation of multinuclear giant cells.

2.5. Silibinin in Methotrexate-Induced Hepatotoxicity in Rats

Methotrexate (MTX), an anticancer drug, is widely recognized for inducing increases in serum aminotransferase levels, and prolonged treatment has been linked to the development of fibrosis, fatty liver disease, and perhaps cirrhosis [36]. Yanaşoğlu *et al.* [36] conducted a study with the objective of investigating the potential hepatoprotective properties of SB and its influence on oxidative stress markers and cytokines in the setting of hepatotoxicity induced by high-dose MTX in rats. 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. SB was then injected for five consecutive days in varying doses (25, 50, and 100 mg/kg/day) to different groups in conjunction with methotrexate. On the sixth day, blood and liver samples were gathered for subsequent analysis. Various assessments were conducted, including measurements of serum total antioxidant capacity, native thiol, total thiol, total oxidant status, aspartate transaminase, alanine aminotransferase, albumin, bilirubin, interleukin-10, and tumor necrosis factor-alpha levels. Furthermore, a histopathological examination of hepatic tissues was done. MTX administration led to a reduction in total antioxidant capacity and an increase in the total thiol/disulfide ratio. Histopathological examination revealed heightened hepatic damage, with notable inflammatory cell infiltration following methotrexate administration.

However, the administration of 50 mg/kg/day of SB demonstrated a preventive effect against inflammatory cell infiltration. The findings suggested that the administration of 50 mg/kg/day of SB may mitigate liver injury induced by MTX in rats by enhancing antioxidant capacity.

2.5.1. Mode of Action Methotrexate-Induced Hepatotoxicity

SB exerts its mechanism of action through potent antioxidant effects, as evidenced by its ability to reverse the decline in serum total antioxidant capacity (TAC) and restore disrupted thiol/disulfide homeostasis caused by MTX. Notably, the study highlighted that SB exhibited enhanced antioxidant activity, especially at doses of 50-100 mg/kg/day. Despite MTX's known modulation of inflammatory cytokines such as TNF- α and IL-10, it was found that high MTX doses did not significantly impact these cytokine levels on treatment with SB. Surprisingly, MTX therapy resulted in decreased serum ALP and ALT levels across all the groups, whereas bilirubin and AST levels remained comparable to controls, indicating variable responses in different studies. Additionally, serum albumin levels decreased in both MTX and SB-treated groups, potentially influenced by MTX's nephrotoxic side effects.

2.6. Silibinin in Cisplatin and Paclitaxel Induced Hepatotoxicity

Ovarian cancer ranks among the most fatal gynecological malignancies. The concurrent utilization of cisplatin and paclitaxel is extensively utilized in clinical settings for treating ovarian cancer; however, prolonged administration of these drugs frequently results in drug resistance and liver toxicity. In the study conducted by Yang *et al.* [37], the aim was to investigate whether SB could restore the sensitivity of drug-resistant human ovarian cancer cells to the combination of paclitaxel and cisplatin while simultaneously alleviating drug-induced liver damage.

The research involved the treatment of healthy liver A2780/DDP cells and LO2 cells with SB, cisplatin, paclitaxel, and a combination of these drugs for 48 hours. MTT and long-term proliferation tests were used to evaluate the viability of the cells, while flow cytometric analysis was employed to determine apoptosis and cell cycle progression. In addition, immunofluorescence assays were used to evaluate DNA damage, and the metastatic activity of A2780/DDP cells was determined through a cell adhesion assay.

The results indicated that the combination of SB with cisplatin and/or paclitaxel significantly improved the anti-tumor effectiveness of these drugs on A2780/DDP cells. This combination reduced cell-matrix adhesion, inhibited cell proliferation, and triggered apoptosis in A2780/DDP cells. Additionally, SB demonstrated

protective effects against cisplatin and/or paclitaxel-induced liver damage by preserving DNA integrity and restoring the proliferative capacity of LO2 cells treated with these drugs.

These results propose that SB may be able to lessen drug-induced hepatic damage at the cellular level and restore drug sensitivity to cisplatin and paclitaxel in drug-resistant human ovarian cancer cells. The study highlights the ability of SB to enhance the anti-tumor effectiveness of cisplatin and/or paclitaxel on cisplatin-resistant human ovarian carcinoma (A2780/DDP) cells and reduce cisplatin and/or paclitaxel-induced liver damage at the cellular level. If validated *in vivo*, the combination of SB with cisplatin and/or paclitaxel could prove to be a valuable chemotherapeutic approach, particularly for patients with tumors resistant to cisplatin.

2.6.1. Mode of Action in Cisplatin and Paclitaxel Induced Hepatotoxicity

SB acts as an intracellular ROS scavenger, successfully lowering the mortality of hepatocyte cells caused by drugs and restoring proliferative ability by defending DNA from toxic injury caused by cisplatin and/or taxol through enhanced DNA repair mechanisms.

2.7. Silibinin in Pyrazinamide- and Isoniazid-Induced Hepatotoxicity

Drug-induced liver injury (DILI) frequently leads to the withdrawal of drugs from the market, particularly when there is a complex presentation of hepatotoxicity that requires thorough investigation. The study by Goh *et al.* [38] focused on simulating SB'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. The findings revealed that as a rescue agent, SB 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 SB's liver protective effect stemmed from mainly two aspects. Initially, it lowers intracellular oxidative stress levels and minimizes damage to intracellular and mitochondrial structures, resulting in reduced apoptotic activity. Secondly, SB stimulates the expression of Nrf2-ARE-related proteins, boosting the production of endogenous proteins that safeguard cells against oxidative harm. Notably, the study highlighted the need for carefully titrating SB's dose to optimize hepatoprotection and minimize potential side effects. However, it was observed that SB 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 SB 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 SB'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 SB's hepatoprotective effect, especially in moderate-to-high DILI.

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

2.7.1. Mode of Action in Pyrazinamide- and Isoniazid-Induced Hepatotoxicity

The liver protective effect of SB is attributed to its ability to reduce intracellular oxidative stress, minimize damage to intracellular and mitochondrial structures, and subsequently decrease apoptotic activity. Additionally, SB stimulates the expression of Nrf2–ARE-related proteins, enhancing the production of endogenous proteins that protect cells against oxidative harm. While SB was not effective as a prophylactic or recovery agent against HRZE-induced hepatotoxicity, it demonstrated potential in reducing stellate cell migration, crucial in liver diseases involving fibrotic activity, injury, and regeneration.

3. Protective Effects of Silibinin in Chemicals and Poison Induced Hepatotoxicity

3.1. Silibinin against Diazinon Induced Hepatotoxicity

Diazinon (DI), an organophosphorous pesticide is a widely used herbicide that can cause hepatotoxicity, or liver damage, in humans and animals [39]. Exposure to high levels of diazinon can cause symptoms such as nausea, vomiting, abdominal pain, jaundice, and liver failure. Although the precise mechanism underlying diazinon-induced hepatotoxicity is unknown, it is thought to entail inflammation and oxidative stress in the liver. The study by Beydilli *et al.* [39] examined the effects of DI exposure on liver function and the potential protective role of SB in female 12-week-old Wistar albino rats. The experiment involved four groups of rats, including a control group, a DI group, a SB group, and a DI + SB group. Samples of blood and liver were gathered and examined for a range of biochemical indicators. 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 + SB group exhibited decreased levels of these markers, suggesting a protective effect of SB against DI-induced

liver damage. Additionally, histopathological examination revealed significant tissue damage in the DI group, while the DI + SB group showed improvements in liver structure, particularly in reducing inflammation and vacuolization. SB was proposed to exert a free radical-eliminating activity and extensive antioxidant effect, potentially reversing the effects of DI-caused oxidative damage. In inference, the study emphasized the hepatoprotective effects of SB against damage to the liver caused by DI in female Wistar albino rats, shedding light on potential therapeutic interventions for pesticide-induced liver injury. The findings suggest that SB could be further explored for its hepatoprotective properties and its potential application in mitigating pesticide-induced liver damage.

3.1.1. Mode of Action against Diazinon Induced Hepatotoxicity

Diazinon disrupts mitochondrial membrane transportation in rat liver by binding extensively to biological membranes, particularly phospholipid bilayers. SB exerts its protective effects by targeting the polar head group of phospholipids in cellular membranes, acting as a shield against lipid peroxidation. This natural compound demonstrates membranotropic behavior, forming robust bonds with hepatocellular membranes and aiding in the maintenance of cellular integrity. Its antioxidative mechanism contributes significantly to metabolic and cell-regulating actions, playing a pivotal role in its hepatoprotective effects against oxidative damage and free radicals, highlighting silibinin's potential as a valuable therapeutic agent in liver health.

3.2. Silibinin in Galactosamine/ Lipopolysaccharide-Induced Hepatotoxicity

Galactosamine is a sugar that occurs naturally in the body and aids in the metabolism of fats. It appears to be related with hepatotoxicity, which is liver damage, in some people [43]. Lipopolysaccharides, also known as LPS, are found on the outer surface of bacteria. Studies have shown that LPS can increase the threat of liver damage in people with certain genetic makeup. Additionally, exposure to LPS can exacerbate existing liver disease. Hashem *et al.* [43] explored the impact of antioxidants, specifically SB 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 [38]. ASK1, stimulated by various stressors like ROS and LPS, triggers the MAP kinase kinase (MAPKK) pathway, resulting in cell death, inflammation, and differentiation. The research examined the ability of SB and vitamin E as antioxidants to regulate the ASK1-p38

MAPK pathway by inhibiting ASK1, thus interrupting downstream signaling and mitigating liver damage. The methods involved administering D-GalN/LPS, then treating with SB and vitamin E in both curative and prophylactic protocols. 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 SB 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 summary, the study offered fresh perspectives on the deactivation of ASK1 and the specific reduction of downstream signaling kinase p38 MAPK in D-GalN/LPS-induced liver damage. The findings highlighted the potential therapeutic roles of SB 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.

3.2.1. Mode of Action in Galactosamine/ Lipopolysaccharide-Induced Hepatotoxicity

The mode of action of SB involved targeting the ASK1-p38 MAPK pathway to counteract oxidative stress-induced hepatotoxicity. SB treatment exhibited a significant reduction in ASK1 and p38 MAPK levels by upregulating the gene expressions of Trx1, TrxR1, and PP5, thereby ameliorating oxidative stress levels.

3.3. Silibinin in Ethanol- or Acetaldehyde Induced Liver Damage

Ethanol and its major toxic metabolite acetaldehyde associated with alcoholic liver disease is a significant contributor to liver injury, necessitating effective preventative and treatment strategies [44]. The research conducted by Song and group explores the impact of SB on alleviating ferroptosis, a form of cell death caused by acetaldehyde or ethanol in liver cells. [45]. The research utilized human carcinomatous liver HepG2 cells and immortalized liver HL7702 cells to explore the protective effects of SB. The findings indicated that ethanol or acetaldehyde treatment led to ferroptosis in the cells, characterized by amplified reactive oxygen species (ROS) stress and elevated iron levels. SB was observed to counteract oxidative stress and reduce iron levels, effectively rescuing the cells from ferroptosis. Moreover, SB 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, SB restored PINK1 and Parkin-mediated mitophagy, which

was hindered by ethanol or acetaldehyde exposure. In addition, the study utilized inhibitors targeting apoptosis, necroptosis, and ferroptosis to identify the specific type of cell death occurring in cells treated with acetaldehyde or ethanol. The findings revealed that all inhibitors enhanced cell viability, with notable effectiveness shown by the ferroptosis inhibitors. 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 [46, 47]. 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 summary, the study reveals that acetaldehyde and ethanol promote ferroptosis through the autophagic deterioration of ferritin and decreased mitophagy, resulting in lipid peroxidation. SB was observed to counteract ferroptosis triggered by ethanol or acetaldehyde, providing valuable insights into possible beneficial approaches for alcoholic hepatic damage.

3.3.1. Mode of Action in Ethanol- or Acetaldehyde Induced Liver Damage

The mode of action of SB involves mitigating oxidative stress and decreasing iron levels in cells exposed to ethanol or acetaldehyde, thus disrupting the cascade of ferroptosis induction. Additionally, SB counteracts ethanol- or acetaldehyde-induced mitophagy arrest mediated by PINK1 and Parkin, rescues NCOA4-dependent autophagic degradation of ferritin, and has a crucial role in preventing ferroptosis.

3.4. Silibinin in N-Nitrosodimethylamine-Induced Hepatotoxicity

N-nitrosodimethylamine (DMN) is a chemical compound that is often found in tobacco smoke, foods, and medicines. Long-term exposure to DMN has been linked to an augmented danger of liver cancer and hepatotoxicity. The study by Harrison and group focused on the use of SB to alleviate DMN-induced glutathione dysregulation and hepatotoxicity in Wistar albino rats [48]. DMN was administered to induce oxidative stress and hepatotoxicity 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 SB for two weeks.

Biochemical assays and histopathology were conducted to assess liver function and tissue changes. The study found significant increases in the activity of marker enzymes indicating liver damage in serum, attributed to the leakage of these enzymes from the damaged cells due to loss of structural and functional integrity [49-52]. The study emphasized the hepatotoxicity induced by intermittent DMN administration, leading to hepatocellular necrosis, inflammatory changes, and fibrosis in the rats.

3.4.1. Mode of Action in

N-Nitrosodimethylamine-Induced Hepatotoxicity

SB was observed to enhance protein synthesis by activating RNA polymerase enzymes in liver cells, potentially explaining the reversal of reduced protein amounts in the serum and rat livers treated with DMN and SB. Furthermore, the restoration of depleted GSH and antioxidant vitamins in SB-treated rats affected by DMN may be attributed to the antioxidant and membrane-stabilizing properties of SBN. It has been proposed that SB could counteract toxic effects by binding to receptor sites on hepatocellular membranes and maintaining GSH/GSSG homeostasis, thereby contributing to the protection of liver cells against the hepatotoxicity induced by DMN through its antioxidant, free radical scavenging, membrane-binding, and stabilizing characteristics.

3.5. 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. While abrin-induced liver damage has a significant impact, the exact mechanism remains unclear. SB, known for its antioxidant, anti-inflammatory, and hepatoprotective properties, has not been researched for its potential therapeutic use in abrin toxicity. Consequently, a study by Saxena *et al.* [53] aimed to elucidate the mechanisms involved and assess the defending role of SB against abrin-induced liver toxicity.

The trial involved assessing various indicators related to liver function, oxidative stress, inflammation, Fas pathway activation, and liver histopathology in BALB/c mice after being exposed to abrin. The findings demonstrated that abrin exposure resulted in liver damage, oxidative stress, inflammation, histological changes, and heightened Fas pathway activity. However, the administration of SB enhanced the survival of abrin-exposed mice by reducing serum liver enzymes and restoring antioxidant capacity. Additionally, SB exhibited the ability to diminish abrin-induced inflammation and suppress the Fas pathway. This investigation represented the first documentation of SB's potential to protect the liver from abrin toxicity.

3.5.1. Mode of Action in Abrin Induced Hepatotoxicity

Abrin intoxication led to hepatotoxicity, oxidative stress, inflammation, altered histopathology, and increased Fas pathway signaling. SB improved the survival of abrin-exposed mice by decreasing serum liver enzymes and restoring the antioxidant capacity. Furthermore, silibinin inhibited abrin-induced inflammation and Fas pathway signaling.

4. Protective Effects of Silibinin in Heavy Metal Induced Hepatotoxicity

4.1. Silibinin in Cadmium Induced Hepatotoxicity

The study by Srinivasan *et al.* [54] focused on the impact of cadmium (Cd), an environmental toxin that particularly affects the liver and kidneys in humans. Rats received subcutaneous administration of Cd for three weeks, resulting in a notable increase in serum transaminases, alkaline phosphatase, and lactate dehydrogenase activities, accompanied by heightened lipid peroxidation and reduced levels of antioxidants in the liver. The oral administration of SB at a dosage of 80 mg/kg body weight effectively normalized hepatic enzyme activities, decreased lipid peroxidation, and reinstated antioxidant defense in the liver, in contrast to lower doses of SB (20 and 40 mg/kg body weight). Histopathological analysis further supported these findings, affirming the potential of SB in alleviating 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 enzymatic and non-enzymatic antioxidant levels, and chelating Cd in the liver, ultimately contributing to the protection of liver architecture and function in Cd-intoxicated rats.

In general, the research indicates that SB could provide a valuable intervention to alleviate the harmful impacts of Cd-induced liver damage, primarily owing to its antioxidant properties, capacity to scavenge free radicals, and metal chelating activities. However, additional investigation is necessary to comprehensively understand the precise mechanisms by which SB safeguards against toxicity induced by Cd in rat experimental model.

4.1.1. Mode of Action in Cadmium Induced Hepatotoxicity

Administration of silibinin (SBN) to cadmium-treated rats leads to a significant decrease in lipid peroxides, attributed to SB'S ability to scavenge free radicals, indicating its bioactivity in directly reacting with various reactive oxygen species (ROS). SB does this by chelating cadmium and controlling the endogenous intake of non-enzymatic antioxidants. SB exhibits the antioxidant capabilities of thiol compounds, including reducing power and metal ion chelating actions, as well as an increase in intracellular redox potential, which regulates transcription factor activity and modulates cellular processes. Furthermore, the active locations of SB's C-20 hydroxyl groups may help to improve tissue thiol pools, thereby lowering Cd-induced oxidative risks and increasing antioxidant status in rats treated with SB.

4.2. Silibinin in Arsenic Induced Hepatotoxicity

Arsenic (As) compounds are widely recognized as environmental hazards and human carcinogens, presenting a significant global health issue. SB 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. Therefore, Muthumani and colleagues conducted a study with the objective of exploring the potential protective role of SB against arsenic-induced liver toxicity in rats [55]. In the study, rats were orally administered with arsenic alone (at a dosage of 5 mg/kg body weight/day) and in combination with SB (at a dosage of 75 mg/kg body weight/day) over a period of four weeks. The evaluation of liver damage included measuring elevated levels of specific liver enzymes in the serum, namely ALT, AST, ALP, lactate dehydrogenase, gamma total bilirubin and glutamyl transferase [56-59]. The presence of increased levels of lipid peroxidation markers, such as thiobarbituric acid reactive substances, lipid hydroperoxides, protein carbonyl content, and conjugated dienes, indicated arsenic-induced liver damage. Furthermore, arsenic's negative effects were demonstrated by significantly decreased activity of membrane-bound ATPases and enzymatic antioxidants (including superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase, and glucose-6-phosphate dehydrogenase), as well as non-enzymatic antioxidants such as reduced glutathione, total sulfhydryl groups, and vitamins C and E. The administration of SB resulted in a substantial reversal of the toxic effects caused by arsenic in the liver tissue. These findings were corroborated by the reduction of DNA damage in hepatocytes and histopathological assessments of the liver, collectively indicating a potential protective effect of SB against arsenic-induced liver toxicity in rats. In conclusion, the study highlighted SB's potential 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 SB in this context.

4.2.1. Mode of action in Arsenic Induced Hepatotoxicity

δ -Aminolevulinic acid dehydratase (ALAD) is an enzyme with sulfhydryl groups that aid in the asymmetrical condensation of two molecules of aminolevulinic acid (ALA-substrate) to produce prophobilinogen during heme synthesis. The exposure to arsenic causes a significant reduction in ALAD levels in the bloodstream and liver, possibly attributed to the strong binding affinity of arsenic for sulfhydryl groups, which could potentially inhibit ALAD activity. The

administration of SB to arsenic-intoxicated rats resulted in a marked reduction in liver tissue lipid peroxidation and maintenance of membrane-bound enzyme activities. This protective effect of SB is attributed to its capability to shield SH groups from oxidative damage by inhibiting membrane lipid peroxidation and stabilizing the membranes.

5. Conclusions

Therefore, we can conclude that SB, a flavonolignan obtained from milk thistle, is a powerful protector against liver damage, which is a prevalent health issue worldwide. SB'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 SB underscore its promise as a versatile intervention in the realm of hepatoprotection. As we navigate the complexities of hepatotoxicity, SB 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 SB's therapeutic potential, emphasizing its crucial role in the ongoing pursuit of liver health and well-being.

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Conflict of Interest

The authors have no conflict of interest to declare.

List of Abbreviations

SB	Silibinin
NF-Kb	Nuclear factor kappa B
TNF	Tumor necrosis factor α
LPS	Lipopolysaccharide
IL-1B	Cytokine interleukin – 1B
PLGA/SB	Poly [lactic – co glycolic acid]
DMN	N- nitrosodimethylamine
AZT	Zidovudine
INH	Isoniazide
TB	Tuberculosis
ITZ	Itraconazole

CTL	Cytotoxic T lymphocytes
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
MPO	Myeloperoxidase
NO	Nitric acid
SOD	Superoxide dismutase
GSHpx	Glutathione peroxidase
DILI	Drug induced liver injury
HRZE	Isoniazide + Rifampicin +Pyrazinamide + Ethambutol
Nrf2-ARE	Nuclear factor erythroid 2 – related factor 2
DI	Diazinon
ALT	Alanine transaminase
D-GalN	D – galactosamine
ASK1	Apoptosis signal regulating kinase 1
ROS	Reactive oxygen species
MAPKK	Mitogen activated protein [MAP] kinase kinase [MAPKK]
TBARS	Thiobarbituric acid reactive substance
GSH	Glutathione
CAT	Catalase
Trx1	Thioredoxin
TrxR1	Thioredoxin reductase 1
PP5	Polypropylene
BALB/C	Bagg albino
Cd	Cadmium
ALP	Alkaline phosphatase
LDH	Lactate dehydrogenase
As	Arsenic
HepG2	Human liver cancer cell line
HL7702	Human hepatocyte
NCOA4	Nuclear receptor coactivator 4
PINK1	PTEN – induced kinase 1

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