Silymarin: A Comprehensive Review

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ABSTRACT
Silymarin, a flavonolignan from the seeds of 'milk thistle' (Silybum marianum), has been widely used from ancient times because of its excellent hepatoprotective action. It is a mixture of mainly three flavonolignans, viz, silybin, silidianin and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis and cirrhosis and alcoholic liver diseases. It has also been reported to be effective in certain cancers. Its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane; reduction of glutathione oxidation to enhance its level in the liver and intestine; antioxidant activity and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration. It is orally absorbed but has very poor bioavailability due to its poor water solubility. This review focuses on the mechanism of action, pharmacokinetics, pharmacodynamics, various pharmacological activities and toxicity of silymarin. The nontraditional use of silymarin may make a breakthrough as a new approach to protect other organs in addition to liver.

KEYWORDS: Hepatoprotection, herbal drugs, milk thistle and silymarin.

INTRODUCTION
Flavonoids belong to the family of the benzo gamma-pyrones. More than 4000 different flavonoids are currently known; they are ubiquitous not only in the plant kingdom, where they are particularly abundant in the photosynthetic cells of higher plants, but also in the animal kingdom. For centuries they have been attributed numerous therapeutic properties and many have been used as popular therapeutic remedies. Compounds such as quercetin, taxifolin and silymarin have been used as active ingredients, both alone and as components of complex chemical preparations. Silymarin is a flavonolignan that has been introduced fairly recently as a hepatoprotective agent. It is extracted from the seeds and fruit of Silybum marianum (Compositae) and in reality is a mixture of three structural components: silibinin, silydianine and silychristine. The structure of the constituents of silymarin was clarified in the 1960s (Figure 1) (1, 2). The main chemical difference between silymarin and other flavonoids is that its isomers are substituted by a coniferyl alcohol group. Of the three isomers that constitute silymarin, silibinin is the most active (3, 4). From a medical point of view, silymarin and silibinin have been found to provide cytoprotection and above all, hepatoprotection (2, 5). Silymarin is used for the treatment of numerous liver disorders characterised by degenerative necrosis and functional impairment (3). Furthermore, it is able to antagonise the toxin of Amanita phalloides (6, 7) and provides hepatoprotection against poisoning by phalloidin (8) galactosamine (9) thioacetamide (10) halothane (11) and carbon tetrachloride (12). The compound also protects hepatocytes from injury caused by ischaemia, radiation, iron overload and viral hepatitis (13). Silymarin is included in the pharmacopoeia of many countries under the trademark Legalon™ or Hepatron™ and is often used as supportive therapy in food poisoning due to fungi and in chronic liver disorders, such as steatosis (14) and alcohol-related liver disease (15).

CHEMISTRY OF SILYMARIN
Silymarin is extracted from the dried seeds of milk thistle plant, where it is present in higher concentrations than in other parts of the plant (16). The active principle was first isolated and chemically characterized during 1968-1974. Later the biochemical effects of silymarin on RNA, protein and DNA synthesis was reported by Sonnenbichler and Zetl (17). Silymarin is a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin with an empirical formula C_{25}H_{22}O_{10} (Fig.1). The structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory actions. Among the
isomers silybin is the major and most active component and represents about 60-70 %, followed by silychristin (20%), silydianin (10%), and isosilybin (5%) (18). Silipide (IdB1016) is the silybin - phosphatidylcholine complex which ensures a large increase in the bioavailability of silybin (19).

MECHANISM OF ACTION
The preclinical studies using different hepatotoxic substances showed that silymarin has multiple actions as a hepatoprotective agent. The antioxidant property and cell-regenerating functions as a result of increased protein synthesis are considered as most important (20).

(i) Antioxidant properties: Free radicals, including the superoxide radical, hydroxyl radical (OH), hydrogen peroxide (H2O2) and lipid peroxide radicals have been implicated in liver diseases (21). These reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to xenobiotics (22). The mechanism of free radical damage include ROS- induced peroxidation of polyunsaturated fatty acid in the cell membrane bilayer, which causes a chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane lipids and proteins. Subsequently cell contents including DNA, RNA, and other cellular components are damaged (23). The cytoprotective effects of silymarin are mainly attributable to its antioxidant and free radical scavenging properties. Silymarin can also interact directly with cell membrane components to prevent any abnormalities in the content of lipid fraction responsible for maintaining normal fluidity (24).

(ii) Stimulation of protein synthesis: Silymarin can enter inside the nucleus and act on RNA polymerase enzymes resulting in increased ribosomal formation. This in turn hastens protein and DNA synthesis (25). This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver.

(iii) Anti-inflammatory actions: The inhibitory effect on 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis is a pivotal pharmacological property of silymarin. Leukotriene (B4) synthesis was reduced while prostaglandin (E2) synthesis was not affected at higher concentrations of use of silibinin (26). A study which evaluated the action of silibinin in isolated Kupffer cells indicated a strong inhibitory effect on leukotriene B4 (LTB4) formation with the IC50 value of 15 μmol/L. But no effect was observed on tumour necrosis factor-alpha (TNF-α) formation (27). The NF-kB is a key regulator of inflammatory and immune reactions. Silymarin is found to suppress both NF-kB DNA binding activity and its dependent gene expression induced by okadaic acid in the hepatoma cell line HEP G2. But the NF-kB activation induced by TNF-α was not affected by silymarin, demonstrating a pathway dependent inhibition by silymarin (28).

(iv) Antifibrotic action: Liver fibrosis can result in remodeling of liver architecture leading to hepatic insufficiency, portal hypertension and hepatic encephalopathy. These processes involve complex interplay of cells and mediators (29). In the initial phase there will be proliferation of hepatic parenchymal cells. The conversion of hepatic stellate cells (HSC) into myofibroblast is considered as the central event in fibrogenesis. Silymarin inhibits NF-kB and also retards HSC activation. It also inhibits protein kinases and other kinases involved in signal transduction and may interact with intracellular signaling pathways (30).

PHARMACOKINETICS
Silymarin is not soluble in water and is usually administered in capsules as a standard extract (70 to 80% silymarin). Absorption after oral administration is rather low, with recovery in the bile in rats ranging from 2 to 3%. Peak plasma concentrations are achieved in 4 to 6 hours, both in animals and in humans. Silymarin is mainly excreted in the bile and, to a lesser degree, in the urine. Its elimination half-life ranges from 6 to 8 hours (31-33). However, other authors (34) reported plasma levels of 500 mg/L (as silibinin) 90 minutes after oral administration of 200 mg/kg of silymarin or of purified 3. marianum extract in mice.

Silibinin and other components of silymarin are rapidly conjugated with sulfate and glucuronic acid in the liver. The conjugates pass into the plasma and into the bile, where they are found in amounts corresponding to 80% of the total dose administered. A negligible portion is eliminated in the urine. These findings suggest the existence of enterohepatic circulation, intestinal absorption, conjugation in the liver, excretion in the bile, hydrolysis by the intestinal flora and reuptake in the intestine (35).

PHARMACODYNAMICS
1) Antioxidant properties: Flavonoids usually possess good antioxidant activity. The water-soluble dehydrosuccinate sodium salt of silibinin is a powerful inhibitor of the oxidation of linoleic acid-water emulsion catalysed by Fe(2+) salts (36). It also inhibits in a concentration-dependent way the microsomal peroxidation produced by NADPH - Fe(3+) -ADP, a well known experimental system for the formation of hydroxy radical (37). In studies performed in rat hepatic microsomes, it has been demonstrated that lipid peroxidation produced by Fe(3+)/ascorbate is inhibited by silibinin dihemisuccinate; the inhibition is concentration-dependent (38, 39). It has been shown that silymarin is as active as quercetin and dihydroquercitin, and more active than quercitrin, in terms of antiperoxidation activity, independent of the experimental model used to produce peroxidation (40). It has recently been reported that in rat hepatocytes treated with tert-butyl hydroperoxide (TBH), silymarin reduces the loss of lactate dehydrogenase (LDH), increases oxygen consumption, reduces the formation of lipid peroxides and increases the synthesis of urea in the perfusion medium. Furthermore, silymarin is able to antagonise the increase in Ca2+ produced by TBH, reducing ion levels down to below 300 nmol/L. The protective effect of silymarin is mediated by the inhibition of lipid peroxidation and the modulation of hepatocyte Ca2+ content seems to play a crucial role (41).

2) Protective Effects in Models of Oxidative Stress: Oxidative stress is defined as structural and/or functional injury produced in tissues by the uncontrolled formation of pro-oxidant free radicals. Oxidative stress usually develops
when the pro-oxidant action of an inducer exceeds the anti-oxidant capacity of the cell defence system, altering its homeostatic capacity. Numerous substances induce oxidative stress, including carbon tetrachloride, THB, ethanol, paracetamol (acetaminophen) and phenylhydrazine. It has been shown in rats that silibinin protects neonatal hepatocytes from cell damage produced by erythromycin, amitriptyline, norriptrylamine and THB (42). Erythrocytes obtained from rats treated with silymarin exhibited high resistance against the haemolysis produced by phenylhydrazine (43, 44) and the lysis induced by osmotic shock (45). This suggests that silymarin may act by increasing the stability of the erythrocyte membrane. The cytoprotective activity of silymarin has also been shown in hepatocytes of rats subjected to osmotic stress produced by hypotonic saccharose solutions (46). The perfused liver is a valid experimental model for the evaluation of the effect of substances that induce oxidative stress and of the protection provided by scavengers. Using this experimental model, it has been shown that phenylhydrazine produces an increase in oxygen consumption in rat liver in vitro and in the release of thiobarbituric acid reactive substances (TBARS) in the perfusate (47). This stress is associated with a reduction in the amount of reduced glutathione (GSH) in the liver; GSH exerts important protective activity against chemically induced oxidative stress (48, 49). Using liver from rats pretreated in vitro with silibinin 50 mg/kg intravenously, a significant reduction in the oxygen consumption stimulated by phenylhydrazine and in the release of TBARS was observed, without any changes in GSH levels (43, 47). The antioxidant effect of silibinin was observed in rats with acute intoxication caused by ethanol (45, 48) or paracetamol (50) which are peroxidation inducers that produce marked GSH depletion in the liver. Treatment with silymarin or silibinin was able to protect animals from oxidative stress produced in the liver by ethanol or paracetamol (48, 50, 51). Furthermore, it has been reported that treatment with silibinin attenuates the increase in plasma levels of AST, ALT and gammaglutamyl transpeptidase (GGT) observed after intoxication by paracetamol (45). The hepatoprotective activity of silibinin has also been studied in rats with liver cirrhosis induced by the long-term administration of carbon tetrachloride. Muriel & Mourelle (52) have shown that silibinin preserves the functional and structural integrity of hepatocyte membranes by preventing alterations of their phospholipid structure produced by carbon tetrachloride and by restoring alkaline phosphatase and GGT activities. Another interesting property of silibinin and silymarin is their role as regulators of the content of GSH in various organs. In rats treated with silibinin intravenously or silymarin intraperitoneally, a significant increase in the amount of the GSH contained in the liver, intestine and stomach was found, whereas there were no changes in the lungs, spleen and kidneys (53).

3) Activity against Lipid Peroxidation:
Lipid peroxidation is the result of an interaction between free radicals of diverse origin and unsaturated fatty acids in lipids. Lipid peroxidation involves a broad spectrum of alterations and the consequent degeneration of cell membranes may contribute towards the development of other disorders of lipoprotein metabolism, both in the liver and in peripheral tissues. Silymarin appears to act as an antioxidant not only because it acts as a scavenger of the free radicals that induce lipid peroxidation, (38, 54) but also because it influences enzyme systems associated with glutathione and superoxide dismutase (53). It has been shown that all the components of silymarin inhibit linoleic acid peroxidation catalysed by lipoxigenase (55) and that silymarin protects rat liver mitochondria and microsomes in vitro against the formation of lipid peroxides induced by various agents (56).

4) Effects on Liver Lipids:
The influence of silymarin on cellular permeability is closely associated with qualitative and quantitative alterations of membrane lipids (both cholesterol and phospholipids) (52, 57, 58). This suggests that silymarin may also act on other lipid compartments in the liver; this may influence lipoprotein secretion and uptake. It has been shown that silymarin and silibinin reduce the synthesis and turnover of phospholipids in the liver of rats. Furthermore, silibinin is able to neutralise two effects of ethanol in rats: the inhibition of phospholipid synthesis and the reduction in labelled glycerol incorporation into lipids of isolated hepatocytes (59, 48, 46). In addition, silibinin stimulates phosphatidylcholine synthesis and increases the activity of cholinephosphate cytidylytransferase in rat liver both in normal conditions and after intoxication by galactosamine (61). Data on the influence of silymarin on triglyceride metabolism in the liver are scanty. It is known that in rats silibinin is able to partly antagonise the increase in total lipids and triglycerides produced in the liver by carbon tetrachloride (62) and probably to activate fatty acid β-oxidation (45). It has also been suggested that silymarin may diminish triglyceride synthesis in the liver (59). Letteron et al (38) studied the mechanisms of action of silymarin that provide protection against lipid peroxidation and the hepatotoxicity of carbon tetrachloride in mice and came to the conclusion that silymarin works by reducing metabolic activation by carbon tetrachloride and by acting as an antioxidant that prevents chain rupture authors have shown that silymarin affords hepatoprotection against specific injury induced by microcystin (a hepatotoxin), paracetamol, halothane and alloxan in several experimental models (63-66).

5) Effects on Plasma Lipids and Lipoproteins:
The administration of silymarin reduces plasma levels of cholesterol and low-density lipoprotein (LDL) cholesterol in hyperlipidaemic rats, whereas silibinin does not reduce plasma levels of cholesterol in normal rats; however, it does reduce phospholipid levels, especially those transported in LDL (59). Data obtained in experimental models of hepatic injury have shown that silymarin is able to normalise the increase in plasma lipids observed after administration of carbon tetrachloride and to antagonise the reduction in serum free fatty acids induced by thioacetamide. In the experimental model of hepatic injury produced by thioacetamide, silymarin did not appear to be able to normalise the reduction in triglycerides in serum. In the experimental model of hepatic injury produced by paracetamol in rats, it was evident that
silymarin improves LDL binding to hepatocytes, an important factor for the reduction of LDL in plasma (59).

6) Stimulation of Liver Regeneration:
One of the mechanisms that can explain the capacity of silymarin to stimulate liver tissue regeneration is the increase in protein synthesis in the injured liver. In vivo and in vitro experiments performed in the liver of rats from which part of the organ had been removed, silybin produced a significant increase in the formation of ribosomes and in DNA synthesis, as well as an increase in protein synthesis (67). Interestingly, the increase in protein synthesis was induced by silybin only in injured livers, not in healthy controls (68). The mechanism whereby silybin stimulates protein synthesis in the liver has not been defined; it may be the physiological regulation of RNA polymerase I at specific binding sites, which thus stimulates the formation of ribosomes (69). In rats with experimental hepatitis caused by galactosamine, treatment with intraperitoneal silymarin 140 mg/kg for 4 days completely abolished the inhibitory effect of galactosamine on the biosynthesis of liver proteins and glycoproteins (70). These data support the results of previous experiments in a similar model of acute hepatitis in the rat, in which silymarin protected hepatic structures, liver glucose stores and enzyme activity in vivo from injury produced by galactosamine (71). The capacity of silymarin to stimulate protein synthesis has also been studied in neoplastic cell lines, in which no increase in protein synthesis, ribosome formation or DNA synthesis has been found after treatment with silymarin (68).

7) Effects during Experimental Intoxication with Amanita phalloides:
The therapeutic activity of silymarin against mushroom poisoning is worthy of particular attention. The hepatoprotective properties of silymarin have been tested in dogs, rabbits, rats and mice. A dose of 15 mg/kg of silymarin was administered intravenously 60 minutes before intraperitoneal administration of a lethal dose of phalloidin, and was able to protect all animal species tested (100% survival) from the action of the toxin. When it is injected 10 minutes after phalloidin, silymarin affords similar protection only at doses of 100 mg/kg. The longer the time that has elapsed after administration of the toxin, the less effective the drug becomes and after 30 minutes it is no longer effective even at high doses.

Histochemical and histoenzymological studies have shown that silymarin, administered 60 minutes before or no longer than 10 minutes after induction of acute intoxication with phalloidin, is able to neutralise the effects of the toxin and to modulate hepatocyte function (72, 73). Similar results were obtained in dogs treated with sublethal oral doses of A. phalloides, in which hepatic injury was monitored by measuring enzymes and coagulation factors. Amongst the numerous substances tested (prednisolone, cytochrome C, benzylpenicillin, silymarin), only benzyl-penicillin (1000 mg/kg intravenous infusion after 5 hours) and silymarin (50 mg/kg intravenous infusion after 5 hours and 30 mg/kg after 24 hours) were able to prevent the increase in hepatic enzymes and the fall in coagulation factors induced by experimental intoxication (74).

The cyclopeptides of fungi of the genus Amanita, including amatoxins and fallotoxins, are captured by hepatocytes through the sinusoidal system, which is also involved in the mediation of liver uptake of biliary salts. It has been demonstrated that silibinin is able to inhibit uptake of amanitin in isolated preparations of hepatocyte membranes, and the same effect has been shown for taurocholate, antamanide, prednisolone and phalloidin. The effect of silibinin appears to be competitive (75). Recently, the role of tumour necrosis factor-α (TNF-α) in hepatic injury produced by a-amanitin has been investigated in primary cultures of rat hepatocytes. At a concentration of 0.1 µmol/L, the toxin inhibits RNA and protein synthesis within 12 hours, but cytotoxicity appears only much later (36 hours). TNF-α is not indispensible for the development of cytotoxicity, but exacerbates it and markedly increases lipid peroxidation. The addition of silibinin at a concentration of 25 µmol/L to the culture medium prevented the effects of TNF-α (50µg/L).

8) Anti-Inflammatory and Anticarcinogenic Properties:
A significant anti-inflammatory effect of silymarin has been described in liver tissue. Studies have shown that silymarin exerts a number of effects, including inhibition of neutrophil migration, inhibition of Kupffer cells, marked inhibition of leukotriene synthesis and formation of prostaglandins (69, 76-78). The protection afforded by silymarin against carcinogenic agents has been studied in various experimental animal models. A series of experiments have been performed in nude mice with nonmelanoma skin cancer produced by UVB radiation, studying its initiation, promotion and complete carcinogenesis. In all the stages studied, silymarin applied onto the skin at different doses appeared to reduce significantly the incidence, multiplicity and volume of tumours per animal. Furthermore, in a short-term experiment (using the same experimental model), the application of silymarin significantly reduced apoptosis, skin oedema, depletion of catalase activity and induction of cyclo-oxygenase and ornithine decarboxylase activity. This effect provides protection against photocarcinogenesis (79). Similar results were also obtained in the model of skin carcinogenesis produced by chemical carcinogenic agents in carcinogenesis-sensitive (SENCAR) mice (80, 81). The molecular bases of the anti-inflammatory and anticarcinogenic effects of silymarin are not yet known; they might be related to the inhibition of the transcription factor NF-kB, which regulates the expression of various genes involved in the inflammatory process, cytoprotection and carcinogenesis (82-84). It has also been hypothesised that silymarin may act by modulating the activation of regulating substances of the cellular cycle and of mitogen-activated protein kinase (85).

9) Antifibrotic effects:
Stellate hepatocytes have a crucial role in liver fibrogenesis. In response to fibrogenic influences (for example protracted exposure to ethanol or carbon tetrachloride), they proliferate and transform into myofibroblasts responsible for the deposition of collagen fibres in the liver. Recently, the effects
of silibinin on the transformation of stellate cells into myofibroblasts have been investigated. The results have shown that silibinin, at a concentration of 100μmol/L, reduces the proliferation of stellate cells isolated from fresh liver of rats by about 75%, reduces the conversion of such cells into myofibroblasts, and down regulates gene expression of extracellular matrix components indispensable for fibrosis (86). Furthermore, it has been demonstrated that silymarin improves hepatic fibrosis in vivo in rats subjected to complete occlusion of the biliary duct, a manoeuvre that causes progressive hepatic fibrosis without inflammation. Silymarin, administered at a dosage of 50 mg/kg/day for 6 weeks, is able to reduce fibrosis by 30 to 35% as compared with controls. A dose of 25 mg/kg/day is not effective (87). Colchicine and silymarin, administered at a dose of 50 mg/kg orally for 35 days, were able to prevent completely all the alterations induced by carbon tetrachloride in rats (peroxidation of lipids, Na+, K+ and Ca2+-ATPase), except for the hepatic content of collagen, which was reduced only by 55% as compared with controls; moreover, alkaline phosphatase and ALT were unchanged as compared with controls. In the group of rats treated with silymarin, the loss of glycogen was inhibited completely (88).

10) Inhibition of Cytochrome P450

Silymarin can inhibit the hepatic cytochrome P450 (CYP) detoxification system (phase I metabolism). It has been shown recently in mice that silibinin is able to inhibit numerous hepatic CYP enzyme activities (89). Whereas other researchers have not detected any effect of silymarin on the CYP system (90-92). This effect could explain some of the hepatoprotective properties of silymarin, especially against the intoxication due to A. phalloides. The Amanita toxin becomes lethal for hepatocytes only after having been activated by the CYP system. Inhibition of toxin bioactivation may contribute to the limitation of its toxic effects. Additionally, silymarin, together with other antioxidant substances, could contribute towards protection against free radicals generated by enzymes of the CYP system.

II) Overview of Mechanisms of Action:

The hepatoprotection provided by silymarin appears to rest on four properties:

- Activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of GSH;
- Ability to regulate membrane permeability and to increase membrane stability in the presence of xenobiotic damage;
- Capacity to regulate nuclear expression by means of a steroid-like effect; and
- Inhibition of the transformation of stellate hepatocytes into myofibroblasts, which are collagen fibres leading to cirrhosis. Silymarin and silibinin inhibit the absorption of toxins, such as phalloidin or α-amanitin, preventing them from binding to the cell surface and inhibiting membrane transport systems. Furthermore, silymarin and silibinin, by interacting with the lipid component of cell membranes, can influence their chemical and physical properties. Studies in erythrocytes, mast cells, leucocytes, macrophages and hepatocytes have shown that silymarin renders cell membranes more resistant to lesions (figure 2) (45,93,69). Furthermore, the well documented scavenging activity of silymarin and silibinin can explain the protection afforded by these substances against hepatotoxic agents. Silymarin and silibinin may exert their action by acting as free radical scavengers and interrupting the lipid peroxidation processes involved in the hepatic injury produced by toxic agents. Silymarin and silibinin are probably able to antagonise the depletion of the two main detoxifying mechanisms, GSH and superoxide dismutase (SOD), by reducing the free radical load, increasing GSH levels and stimulating SOD activity. Furthermore, silibinin probably acts not only on the cell membrane, but also on the nucleus, where it appeared to increase ribosomal protein synthesis by stimulating RNA polymerase I and the transcription of rRNA (68-70). The stimulation of protein synthesis is an important step in the repair of hepatic injury and is essential for restoring structural proteins and enzymes damaged by hepatotoxins (45, 93). See Figure 2

**Figure 2: Mechanism of action of silymarin as proposed by Valenzuela and Garrido (45)**

**TOXICITY**

The acute toxicity of silymarin has been studied in mice, rats, rabbits and dogs after intravenous infusion. The 50% lethal dose (LD50) values are 400 mg/kg in mice, 385 mg/kg in rats and 140 mg/kg in rabbits and dogs. However, these values are only approximate, as they depend on the infusion rate. When the compound is given by slow infusion (over 2 to 3 hours), values of 2 g/kg may be recorded in rats. After oral administration tolerance is even higher, with values over 10 g/kg. In the event of acute intoxication, the cause of death seems to be cardiovascular failure (94). Similar results have also been obtained by Vogel et al (95). Other experiments to assess the acute toxicity of silymarin were performed in beagle dogs, rabbits, Wistar rats and NMRI mice after an intravenous bolus dose. Silymarin was used as the hemisuccinate sodium salt and the animals were kept under observation for 14 days. The LD50 was 1050 and 970 mg/kg in male and female mice, respectively, and 825 and 920 mg/kg in male and female rats, respectively. The mean lethal dose for rabbits and the maximum tolerated dose in dogs were calculated to be about 300 mg/kg (72). These data demonstrate that the acute toxicity of silymarin is very low. Similarly, its subacute and chronic toxicity are very low; the compound is also devoid of embryotoxic potential (96).

**EPERIMENTAL PHARMACOLOGY**

Hepatoprotective activity of silymarin has been demonstrated by various researchers from all over the world against partial
hepatectomy models and toxic models in experimental animals by using acetaminophen, carbon tetrachloride, ethanol, D-galactosamine and Amanita phalloides toxin.

1) Hepatectomy: Rats with partial hepatectomy, where 70 percent of the liver is removed, when subjected to silymarin pretreatment showed increased synthesis of DNA, RNA, protein and cholesterol suggesting the regeneration of liver (97,98). Interestingly, the increased protein synthesis was found in damaged livers with partial hepatectomy, but not in the respective controls (99). The mechanism of increased protein synthesis is not known, but probably silymarin initiates a physiologic regulator, so the silybin fits in to a specific binding site on the polymerase, thus stimulating ribosome formation (100). Probably silymarin is able to enter the nucleus and specifically stimulate RNA polymerase I, owing to its structural similarity to steroids. Silymarin has been found to suppress nuclear factor kappa B (NF-κ-B) DNA binding activity and its dependent gene expression.

2) Carbon tetrachloride: Among various chemical agents, carbon tetrachloride (CCl₄) has been thoroughly studied for its hepatotoxic properties (101). Various hepatoprotective agents have been studied to observe the beneficial effects against the chemically induced liver injury produced by carbon tetrachloride (102). Silymarin when compared with various polyherbal formulations in CCl₄ induced hepatotoxicity in rats has led to complete normalization of elevated transaminases levels (103). Muriel and Mourelle found that silymarin treatment protected completely against harmful increase in the membrane ratios of cholesterol phospholipids and sphingomyelin: phosphatidyleholine in rats with carbon tetrachloride induced cirrhosis (104). Rats with chronic CCl₄ induced liver damage were treated with oral silymarin, 50 mg/kg administered for 5 days. Collagen content in livers of animals pre-treated with CCl₄ was increased approximately four-fold in comparison to control. It prevented the cirrhotic changes in rats. It also reduced liver collagen content by 55% (105).

3) Acetaminophen: Acetaminophen is an analgesic and antipyretic agent known to cause centrilobular hepatic necrosis at toxic doses. Silymarin has been studied for its protective action against acetaminophen induced toxicity in animal models. Ramellini & Meldolesi in their in vitro studies on rat hepatocyte showed that silymarin treatment normalized the elevated biochemical parameters of liver and serum, caused by acetaminophen, by its stabilizing action on plasma membrane (106). A comparative study of andrographolide and silymarin on acetaminophen induced cholestasis has produced the dose dependent cholestatic and anticholestatic effects of these drugs (107). In our laboratory, the hepatoprotective herbal drugs silymarin and andrographolide were compared in experimental toxic models of carbon tetrachloride and paracetamol in mice. The hepatoprotective effects of silymarin were studied on various parameters like macroscopic appearance, microscopic observation, and its mechanism of action. Silymarin when given to mice in a dose of 100 mg/kg i.p for 7 days, lead to a robust growth of liver and the weight of the liver tissue was more than twice that of the carbon tetrachloride treated group. It also reduced and restored the phenobarbitone induced sleeping time in paracetamol as well as carbon tetrachloride models. Further silymarin prevented hepatic cell in 87.5 % of animals when subjected to paracetamol induced hepatotoxicity. However, silymarin gave a small percentage of protection (only 16%) against carbon tetrachloride induced hepatic necrosis. From this study, it has been concluded that silymarin showed histopathological evidence of hepatoprotection by preventing hepatic cell necrosis or by hepatic cell regeneration (108). Silybin dihemisuccinate, a soluble form of flavonoid of silymarin, has protected rats against liver glutathione depletion and lipid peroxidation induced by acute acetaminophen hepatotoxicity and showed potential benefits of silymarin as an antidote (109). With relatively high doses (0.05 mmol/ l), silymarin has been shown to reduce acetaminophen enhanced CYP 2El mediated cytotoxicity of methotrexate in human hepatocytes (110). In vitro experiments with kidney cells damaged by paracetamol, cisplatin and vincristine have demonstrated that administration of silybin before and after the drug-induced injury can lessen or avoid the toxic effects (111).

4) Ethanol: Acute and chronic administration of ethanol produces a drastic decrease in the hepatic content of reduced glutathione (GSH); an important biomolecule against chemically induced cytotoxicity (112). The hepatoprotective activity of silymarin against ethanol-induced damage has been tested in different animals. The administration of ethanol has reduced a marked increase in serum alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (γ-GT) levels, with a disturbance in reduced and oxidized glutathione ratio. The group which received silymarin did not show any significant changes in these parameters, showing its protective role against ethanol (113).

5) Galactosamine: Galactosamine produces liver damage, with histopathological changes resembling human viral hepatitis (112). Galactosamine administration in rats produced cholestasis, due to inhibition of the synthesis of bile acids and also their conjugation with proteins or to damage in the biliary system. Saraswat et al (114) reported the significant anticholestatic effect of silymarin in comparison to andrographolide. The effects of silymarin in normalizing elevated serum transaminases and alkaline phosphatas have been shown in isolated rat hepatocytes, which are inferior to C-I-1, a herbal protein from Cajanus indicus (115).

6) Iron: Iron overload is associated with liver damage, characterized by massive iron deposition in hepatic parenchymal cells, leading to fibrosis and eventually to hepatic cirrhosis (116). The oxidative stress due to increased hepatic lipid peroxidation is the major mechanism of iron induced hepatotoxicity. Pretreatment in rats with silymarin educated iron induced increase in lipid peroxidation and levels f serum enzymes, as also noted in Wistar somniferia indicating their hepatoprotective action (117).

7) Amanita phalloides toxin: In mice, silymarin was 100 % effective in preventing liver toxicity if given as pretreatment or up to 10 min after Amanita toxin poisoning. Severe liver damage and resultant death was avoided if silymarin was
administered within 24 h (118). In a study with dogs, none died when given silymarin 5-24 h after ingesting an LD50 of Amanita phalloides (85 mg/kg) compared with 33 % in controls. Liver enzymes and liver biopsies showed significant protective effect of silymarin posttreatment (119). Silymarin has also been found to protect liver cells from injury caused by ischaemia (120), radiation (121) and viral hepatitis.

**CONCLUSION**

The excellent hepatoprotective activity of silymarin, besides its immunomodulatory, antioxidant and anti-inflammatory activities, as evident by a number of studies cited above, makes it a very promising drug of natural origin. Its good safety profile, easy availability and low cost are added advantages. It has established efficacy in the restoration of liver function and regeneration of liver cells. It may prove superior to polyherbal formulations in the near future because of its better standardization, quality control and freedom from microbial and metal contamination. Silymarin may make a breakthrough as a new approach to protect other organs in addition to liver.

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