Abstract and Introduction

Abstract

The flavonoid silymarin and one of its structural components, silibinin, are substances with documented hepatoprotective properties. Their mechanisms of action are still poorly understood. However, the data in the literature indicate that silymarin and silibinin act in four different ways: (i) as antioxidants, scavengers and regulators of the intracellular content of glutathione; (ii) as cell membrane stabilisers and permeability regulators that prevent hepatotoxic agents from entering hepatocytes; (iii) as promoters of ribosomal RNAsynthesis, stimulating liver regeneration; and (iv) as inhibitors of the transformation of stellate hepatocytes into myofibroblasts, the process responsible for the deposition of collagen fibres leading to cirrhosis. The key mechanism that ensures hepatoprotection appears to be free radical scavenging. Anti-inflammatory and anticarcinogenic properties have also been documented.

Silymarin is able to neutralise the hepatotoxicity of several agents, including *Amanita phalloides*, ethanol, paracetamol (acetaminophen) and carbon tetrachloride in animal models. The protection against *A phalloides* is inversely proportional to the time that has elapsed since administration of the toxin. Silymarin protects against its toxic principle -amanitin by preventing its uptake through hepatocyte membranes and inhibiting the effects of tumour necrosis factor- , which exacerbates lipid peroxidation.

Clinical trials have shown that silymarin exerts hepatoprotective effects in acute viral hepatitis, poisoning by *A phalloides*, toxic hepatitis produced by psychotropic agents and alcohol-related liver disease, including cirrhosis, at daily doses ranging from 280 to 800mg, equivalent to 400 to 1140mg of standardised extract. Hepatoprotection has been documented by improvement in liver function tests; moreover, treatment with silymarin was associated with an increase in survival in a placebo-controlled clinical trial in alcoholic liver disease.

Pharmacokinetic studies have shown that silymarin is absorbed by the oral route and that it distributes into the alimentary tract (liver, stomach, intestine, pancreas). It is mainly excreted as metabolites in the bile, and is subject to enterohepatic circulation. Toxicity is very low, the oral 50% lethal dose being 10 000 mg/kg in rats and the maximum tolerated dose being 300 mg/kg in dogs. Moreover, silymarin is devoid of embryotoxic potential.

In conclusion, silymarin is a well tolerated and effective antidote for use in hepatotoxicity produced by a number of toxins, including *A phalloides*, ethanol and psychotropic drugs. Numerous experimental studies suggest that it acts as a free radical scavenger, with other liver-specific properties that make it a unique hepatoprotective agent.

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 the most well known compound of the flavonoids, thanks to its well defined therapeutic properties. 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]

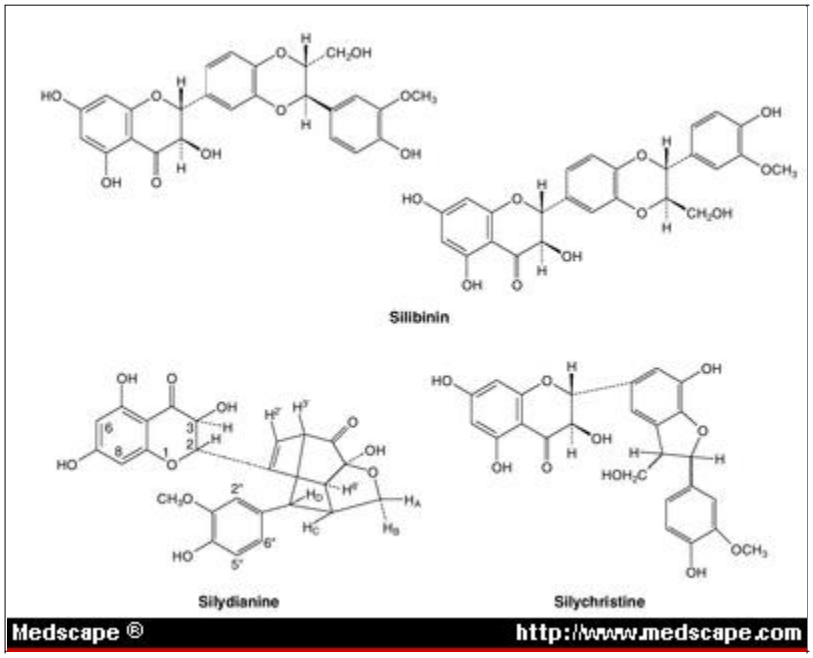

Figure 1. The three structural components of silymarin: silibinin, silydianine, and silychristine.

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]

1. Pharmacodynamics

1.1 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.[15] It also inhibits in a concentration-dependent way the microsomal peroxidation produced by NADPH-Fe^{2+} -ADP, a well known experimental system for the formation of hydroxy radicals.[16] In studies performed in rat hepatic microsomes, it has been demonstrated that lipid peroxidation produced by Fe(III)/ascorbate is inhibited by silibinin dihemisuccinate; the inhibition is concentration-dependent.[17,18]

It has been shown that silymarin is as active as quercetin and dihydroquercetin, and more active than quercitrin, in terms of antiperoxidant activity, independent of the experimental model used to produce peroxidation.[19]

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 Ca^{2+} 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 Ca^{2+} content seems to play a crucial role.[20]

1.2 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, TBH, ethanol, paracetamol (acetaminophen) and phenylhydrazine. It has been shown in rats that silibinin protects neonatal hepatocytes from cell damage produced by erythromycin, amitriptyline, nortriptyline and TBH.[21]

Erythrocytes obtained from rats treated with silymarin exhibited high resistance against the haemolysis produced by phenylhydrazine[22,23] and the lysis induced by osmotic shock.[1] 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.[24]

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.[25] 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.[26,27] Using liver from rats pretreated *in vivo* with silibinin 50 mg/kg intravenously, a significant reduction in the oxygen consumption stimulated by phenylhydralazine and in the release of TBARS was observed, without any changes in GSH levels.[22,25]

The antioxidant effect of silibinin was observed in rats with acute intoxication caused by ethanol[1,26] or paracetamol,[28] 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.[2,26,28] 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.[1]

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[29] 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 (Table 1).[30]

Table 1. Experimental studies on the hepatoprotective action of silymarin and silibinin in xenobiotic intoxication

Agent	Experimental model	Silymarin or silibinin action	References

Phenylhydrazine	Rat erythrocytes	Inhibition of haemolysis and lipid peroxidation	Valenzuela et al.[22]
	Rat liver	Protection from liver glutathione depletion and lipid superoxidation	Valenzuela et al.[23]
tert-Butyl hydroperoxide	Rat microsomes Neonatal rat hepatocytes	Inhibition of O_2 consumption Reduction of enzyme loss and morphological alterations	Valenzuela & Guerra[16] Davila et al.[21]
		Inhibition of lipid peroxidation	Farghali et al.[20]
	Perfused rat hepatocytes		
CCl4	Acute intoxication in mice and rats	Prevention of lipid peroxidation and hepatotoxicity	Letteron et al.[31] Muriel & Mourelle[29]
	Cirrhosis in rats	Protection	Mourelle & Franco[40];
	Chronic intoxication in rats	Prevention of chronic liver damage	Mourelle et al.[12] Muriel & Mourelle[29] Favari & Perez Alvarex[59]
Ethanol	Acute intoxication in rats	Neutralisation of lipid peroxidation	Valenzuela et al.[26]
	Chronic intoxication in rats	Reduction in liver alterations	Valenzuela & Garrido[1] Campos et al.[28] Platt & Shnorr[32]
Halothane	Acute intoxication in rats	Protection from liver toxic effects	Siegers et al.[11]
Thioacetamide	Acute intoxication in rats	Antihepatotoxic effects Hepatoprotection	Schriever et al.[41]
	Chronic intoxication in rats		Hahn et al.[33]
Galactosamine	Acute hepatitis in rats	Protective effects	Barbarino et al.[9]
	Perfused rat hepatocytes	Inhibition of lipid peroxidation	Farghali et al.[20]
	Experimental hepatitis in rats	Inhibition of toxic effects on protein synthesis	Tyutyulkova et al.[34]
Paracetamol	Acute intoxication in mice/rats	Protective effects, reduction in lipid peroxidation and glutathione depletion	Muriel et al.[35] Campos et al.[28]

Erythromycin estolate	Neonatal rat hepatocytes	Reduction of enzyme loss and morphological alterations	Davila et al.[21]
Amitriptyline		Reduction of enzyme loss and morphological alterations	
Nortriptyline		Reduction of enzyme loss and morphological alterations	
Microcystin	Acute hepatoxicity in mice/rats	Neutralisation of lethal effects and pathological alterations	Mereish et al.[36]

1.3 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,[17,31] but also because it influences enzyme systems associated with glutathione and superoxide dismutase.[30]

It has been shown that all the components of silymarin inhibit linoleic acid peroxidation catalysed by lipoxygenase[37] and that silymarin protects rat liver mitochondria and microsomes *in vitro* against the formation of lipid peroxides induced by various agents.[38]

1.4 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).[29,39,40] 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.[14,27,32] In addition, silibinin stimulates phosphatidylcholine synthesis and increases the activity of cholinephosphate cytidyltransferase in rat liver both in normal conditions and after intoxication by galactosamine.[41]

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[12] and, probably, to activate fatty acid ß-oxidation.[1] It has also been suggested that silymarin may diminish triglyceride synthesis in the liver.[14]

Letteron et al.[31] 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.

Other authors have shown that silymarin affords hepatoprotection against specific injury induced by microcystin (a hepatotoxin), paracetamol, halothane and alloxan in several experimental models (Table 1).[11,35,36,42]

Table 1. Experimental studies on the hepatoprotective action of silymarin and silibinin in xenobiotic intoxication

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	Chronic intoxication in rats		Hahn et al.[33]
Galactosamine	Acute hepatitis in rats	Protective effects	Barbarino et al.[9]
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Paracetamol	Acute intoxication in mice/rats	Protective effects, reduction in lipid peroxidation and glutathione depletion	Muriel et al.[35] Campos et al.[28]
Erythromycin estolate	Neonatal rat hepatocytes	Reduction of enzyme loss and morphological alterations	Davila et al.[21]
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Nortriptyline		Reduction of enzyme loss and morphological alterations	
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1.5 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.[14]

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.[14]

1.6 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 *in vivo* and *in vitro* experiments performed in the liver of rats from which part of the organ had been removed, silibinin produced a significant increase in the formation of ribosomes and in DNA synthesis, as well as an increase in protein synthesis.[43] Interestingly, the increase in protein synthesis was induced by silibinin only in injured livers, not in healthy controls.[44] The mechanism whereby silibinin 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.[13] 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.[34]

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.[9]

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.[44]

1.7 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.[6,7]

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 (Table 2).[45]

Table 2. Experimental studies on the hepatoprotective action of silymarin and silibinin in fungal intoxication

Agent	Experimental model	Dose (intravenous) of silymarin or silibinin (mg/kg)	Action	References
Phalloidin, -amanitin	Acute intoxication in mice	50-150	Protection and cure	Choppin & Desplaces[7]
Phalloidin, -amanitin	Acute intoxication in mice/rats/rabbits/dogs	15 at 60 min before phalloidin	Protection	Desplaces et al.[6]
		100 at 10 min before phalloidin	Protection	
Sublethal doses of *A. phalloides*	Acute intoxication in dogs	50 at 5h and 30 at 24h	Prevention of increase in liver enzymes and of reduction in coagulation factors	Floersheim et al.[45]
Phalloidin	Acute intoxication in mice	12.5-100	Protection	Vogel & Trost[8]

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.[2]

Recently, the role of tumour necrosis factor- (TNF) in hepatic injury produced by -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 is not indispensable for the development of cytotoxicity, but exacerbates it and markedly increases lipid peroxidation. The addition of silibinin at a concentration of 25µmol/L to the culture medium prevented the effects of TNF (50µg/L)

1.8 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.[13,47-49]

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.[50] Similar results were also obtained in the model of skin carcinogenesis produced by chemical carcinogenic agents in carcinogenesis-sensitive (SENCAR) mice.[51,52]

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-κB, which regulates the expression of various genes involved in the inflammatory process, in cytoprotection and carcinogenesis.[53-55] 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.[56]

1.9 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 downregulates gene expression of extracellular matrix components indispensable for fibrosis.[57]

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.[58]

Colchicine and silymarin, administered at a dose of 50 mg/kg orally for 55 days, were able to prevent completely all the alterations induced by carbon tetrachloride in rats (peroxidation of lipids, Na^+ ,K^+ -and Ca^{2+} -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.[59]

1.10 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,[60] whereas other researchers have not detected any effect of silymarin on the CYP system.[61-63]

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.

1.11 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 responsible for the deposition of collagen fibres leading to cirrhosis.

Silymarin and silibinin inhibit the absorption of toxins, such as phalloidin or -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).[1,2,13]

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.[13,34,44] 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.[1,2]

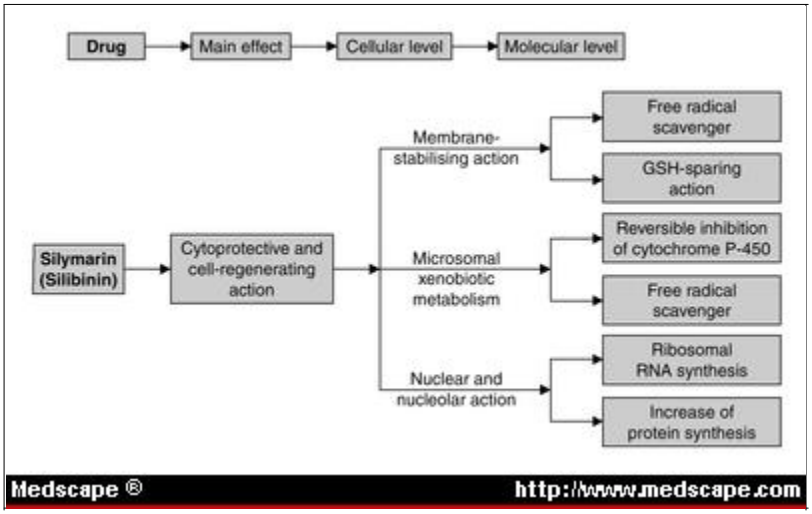

Figure 2. Mechanism of action of silymarin as proposed by Valenzuela and Garrido.[1]

2. 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 animal sand 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.[64-66] However, other authors[67] reported plasma levels of 500 mg/L (as silibinin) 90 minutes after oral administration of 200 mg/kg of silymarin or of purified *S 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. An egligible 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.[69]

The presence of this cycle makes the study of the intestinal absorption of the natural products very difficult. However, the use of labelled silibinin in the rat has made it possible to show that intestinal absorption of a dose of 20 mg/kg amounts to about 35%. Peak radioactivity is found in the plasma 30 minutes after ingestion.[3]

In 1975, Bülles et al.[68] showed that silibinin is excreted mainly unmodified in the urine after oral or intravenous administration of silibinin *N*-methylglucamine (2 to 120 mg/kg and 20 mg/kg as silibinin, respectively) in the rat, whereas in the bile it is excreted as metabolites, independently of the route of administration. Silibinin is excreted in minimal quantities in the urine during the 48 hours following oral (2 to 5%) or intravenous (8%) administration. On the contrary, biliary excretion is fairly high during the same period (about 40 to 45% after oral administration of up to 20 mg/kg, and about 80% after intravenous administration). The ratio between dose and quantity excreted in the bile is linear for doses up to 20 mg/kg. Study of the kinetics of biliary excretion after oral administration of 20 mg/kg showed that peak excretion occurs 1 hour after administration. Similar results have been reported by Mennicke.[69]

Tissue distribution of silibinin was studied in SENCAR mice (6 to 7 weeks old) after oral administration of 50 mg/kg.[70] Peak concentrations of free silibinin were recorded after 0.5 hours in the liver, lungs, stomach and pancreas, with values of 8.8 ± 1.6, 4.3 ± 0.8, 123 ± 21, 5.8 ± 1.1µg/g of tissue, respectively. In the skin and prostate, peak concentrations of silibinin were 1.4 ± 0.5 and 2.5 ± 0.4µg/g, respectively, and were reached 1 hour after administration. With regard to sulfate conjugates and ß-glucuronides of silibinin, excluding the lungs and stomach, in which peak values were reached after 0.5 hours, all the other tissues analysed exhibited peak tissue concentrations after 1 hour. The concentrations of free and conjugated silibinin diminished exponentially after 0.5 or 1 hour, with an elimination half-life of 57 to 127 minutes for the free portion and 45 to 94 minutes for the conjugated portion. Assessment of the effects of silibinin 100 and 200 mg/kg/day orally on phase II enzymes revealed a dose-and time-dependent increase in glutathione-S-transferase and quinone reductase activity that was moderately or markedly significant in the liver, lungs, stomach, skin and small intestine.[70]

3. 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.[3] Similar results have also been obtained by Vogel et al.[5]

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.[6]

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.[33]

4. Therapeutic Activity

The results of most clinical trials with the thistle (*Silybum marianum*) extract, silymarin, are difficult to interpret because of a variety of reasons: small sample size, variability in type and severity of the liver disorders, heterogeneous dosages, inconsistent use of a control group, poorly defined objectives. Furthermore, the intrinsic capacity of the liver to improve after exposure to hepatotoxins was not always taken into consideration.

4.1 Acute Viral Hepatitis

The results of double-blind clinical trials in patients with acute viral hepatitis indicate that therapy with silymarin reduces complications, reduces the duration of hospital stay and promotes recovery. In patients with acute hepatitis randomly allocated to receive silymarin 140mg or placebo three times daily for at least 3 weeks, the proportion of patients in whom AST normalised was much higher in the treated group (82%) than in controls (52%). The percentage of patients in whom bilirubin normalised was 40% in the active treatment group versus 11% in the control group.[71]

4.2 Hepatitis Induced by Toxins or Drugs

It has been shown that silymarin reduces the hepatic injury produced by poisoning with *A phalloides*, phenothiazines and butyrophenones in humans.[13] Generally, the mortality rate among patients poisoned with *A phalloides* and treated with various drugs, except silymarin, ranges from 22 to 40% in adults, and is higher in children.[71]

In a retrospective study performed in patients with intoxication caused by *A phalloides*, the severity of hepatic injury was found to be closely related to the time that had elapsed between ingestion and treatment with silibinin: the shorter the interval, the less severe the injury.[72] Silibinin was administered by intravenous infusion at a mean dosage of 33 mg/kg/day for on average 81.6 hours. All the 18 patients included in the study survived, except one who had taken a high dose to commit suicide. Other treatments were not related to reduction in liver injury.

Patients exposed long term to organic phosphates and treated with silymarin for 1 month did not exhibit any improvement in liver function as compared with controls, although serum levels of pseudocholinesterase were considerably increased. This may reflect blockage of toxin anti-cholinesterase activity by silymarin.[73]

The few studies on silymarin in toxic hepatitis in the literature have yielded positive results. An interesting clinical trial was performed in patients being treated with psychotropic agents (phenothiazines or butyrophenones). Patients were subdivided into two groups: in the first group treatment was discontinued, and in the other group treatment was continued at the same dose. The groups were further subdivided into two subgroups: one was given silymarin 800mg daily for 90 days, the other was given placebo. The results showed that silymarin is able to improve liver function, independently of the discontinuation of psychotropic therapy.[74] Similar results were obtained by other authors.[71]

4.3 Chronic Hepatitis and Cirrhosis

In a clinical trial performed in 170 patients with a positive biopsy for cirrhosis, followed up for 2 to 6 years and given oral silymarin 140mg three times daily (87 patients, of whom 46 had cirrhosis due to alcohol abuse) or placebo (83 patients, of whom 45 had cirrhosis due to alcohol abuse) the mean survival rate after 4 years was significantly ($p = 0.036$) higher in patients treated with silymarin (58 ± 9%) as compared with those treated with placebo (39 ± 9%), whereas no significant difference was found in biochemical markers. Analysis of subgroups revealed that treatment was more effective in patients with alcohol-related cirrhosis ($p = 0.01$) and in groups of patients with nonalcoholic cirrhosis, whereas it was ineffective in patients with class B or C portal hypertension.[15]

Another two interesting studies are reported in the review by Flora et al.[71] The first study was performed in 2637 patients with chronic liver disease, treated with high doses of silymarin (560 mg/day) for 8 weeks. Resolution of subjective symptoms was achieved in 63% of cases; AST diminished on average by 36%, ALT by 34% and GGT by 46%. Furthermore, the investigators reported a reduction in hepatomegaly upon palpation. The second study was performed under double-blind conditions in patients with persistent or aggressive chronic hepatitis, with or without cirrhosis, monitored for 3 to 12 months and treated with silymarin. Treatment did not produce any signs of improvement in liver function; however, histological examination revealed an improvement in portal inflammation, parenchymal alterations and necrosis.

4.4 Alcohol-Related Liver Disease

A randomised clinical trial was performed in patients with moderate alcohol-related liver disease (ALT and AST <200 U/ml) and persistent abnormalities of liver function after abstinence from alcohol for at least 1 month. Patients were treated with silymarin 420 mg/day or with placebo for 4 weeks. At the end of the study period, mean AST and ALT levels diminished by 30.1% and

40.8%, respectively, in patients treated with silymarin who had completed the study as compared with increases of 5.4% and 2.8%, respectively, in patients treated with placebo (p □0.001). There was no significant difference in bilirubin levels.[75]

Not all the clinical trials performed with silymarin in this indication have yielded positive results.[76] The results of a recent randomised double-blind study in 125 patients with histologically documented alcohol-related cirrhosis did not show any significant benefit on survival after 2 years of treatment with silymarin 450 mg/day by the oral route.[77]

4.5 Dosage and Administration

In the clinical trials described, the daily oral dose of silymarin used ranged from 280 to 800mg. This is equivalent to 400 to 1140mg of standardised extract containing 70% silymarin. The recommended dosage for active disease is 140mg of silymarin (200mg of extract) three times daily. If the preparation silipide (silymarin-phosphatidylcholine) is used, 100mg three times daily is the appropriate dosage.[71] At higher dosages (>1500 mg/day) silymarin may have a laxative effect due to an increase in secretion and bile flow.[13] Moderate allergic reactions have also been reported.[13,66]

5. Antioxidant and Hepatoprotective Effects of Other Flavonoids

Flavonoids are a large group of phenolic compounds ubiquitously distributed in the plant kingdom. More than 4000 flavonoids belonging to different classes have been identified so far. They are important for the normal growth, development and defence of plants. Important constituents of the human diet, they are present in fruit and vegetables. Multiple biological effects of flavonoids have been described, including anti-inflammatory, antiallergic, antihaemorrhagic, anti-mutagenic, antineoplastic and hepatoprotective activities.[78] The biological and pharmacological effects of flavonoids in mammals are assumed to result mainly from two properties: modulation of certain enzymes (hexokinase, aldose reductase, phospholipase C, protein kinase C, cyclo-oxygenase, lipoxygenase, myeloperoxidase, NADPH oxidase and xanthine oxidase) and their antioxidant activity. The various flavonoids, however, vary greatly in their efficacy, and a single flavonoid can inhibit one enzyme at a certain concentration while inhibiting another enzyme at a 100-fold higher concentration.[46,47]

Some flavonoids, including quercetin and silibinin, can protect cells and tissues against the effects exerted by reactive oxygen species. Their antioxidant activity results from the scavenging of free radicals and other oxidising intermediates, from the chelation of iron or copper ions, and from inhibition of oxidases.[79] As discussed in the preceding sections, flavonoids from *Silybum marianum* have been widely used for the treatment of liver disorders. In experimental animal models they were demonstrated to exert not only a positive effect on intact liver cells or cells not yet irreversibly damaged, but also to stimulate their regenerative capacity after partial hepatectomy.[41]

Antihepatotoxic activity was also demonstrated for kolaviron, a defatted alcoholic extract of the seeds of *Garcinia kola*, and for *Garcinia* biflavonones, in mice intoxicated with phalloidin.[80] Other flavonoids extracted from *Baccharis trimera* were reported to protect mice from hepatic damage; hispidulin appeared to be the most active compound, and quercetin, luteolin, nepetin and apigenin were less active or inactive.[81] Quercetin, however, was demonstrated to exert some ameliorative effects on tissue damage induced by cigarette smoke[78] and to reduce the cytotoxic effect of T-2 mycotoxin.[82] Gossypin and hydroxyethyl rutosides significantly reduced the toxic effect of dermal application of sulphur mustard on hepatic lipid peroxidation in mice. Moreover, it increased the survival rate of the animals.[83]

6. Conclusions

The biochemical mechanism or mechanisms of the flavonoid silymarin have not been completely established. However, the results of numerous experimental studies strongly suggest that its hepatoprotective effects are mainly due to free radical scavenging. This property is reflected by the membrane stabilisation and GSH modulation that it produces. Silymarin exerts other important effects, which include liver-specific actions:

hepatocyte membrane stabilisation and permeability regulation, stimulation of ribosomal RNA synthesis promoting liver regeneration, and the prevention of the transformation of stellate hepatocytes into myofibroblasts, which are responsible for the deposition of collagen fibres. These properties afford effective protection against the hepatotoxic effects of a number of xenobiotic compounds, including *Amanita phalloides* toxins, ethanol and psychotropic compounds, which has been documented in clinical trials in terms of improvement of liver function and, in the case of ethanol, increase in survival.

Pharmacokinetic and toxicity studies have not disclosed any issues that could limit the therapeutic use of silymarin.

In conclusion, silymarin is a well tolerated and effective antidote for use in cases of hepatotoxicity produced by a number of hepatotoxic agents, including *Amanita phalloides*, ethanol and psychotropic drugs. Numerous experimental studies suggest that it acts as a free radical scavenger with other liver-specific properties that make it a unique hepatoprotective agent.

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Acknowledgments

The authors wish to thank Madaus S.R.L., Bozen, Italy, for funding used to assist in the preparation of this manuscript.

Reprint Address

Correspondence and offprints: Dr F. Fraschini, Department of Pharmacology, University of Milan, Via Vanvitelli 32, Milan, 20129, Italy.

Clin Drug Invest. 2002;22(1)