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REVIEW

Application of silymarin in human and animal medicine

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Abstract: Silymarin, the polyphenolic fraction from Milk Thistle (*Silybum marianum*) and its main component silybinin, are used almost exclusively for hepatoprotection in humans. Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals. It effects, by antioxidative, anti-inflammatory, antifibrotic, anti-lipid peroxidative, membrane stabilizing and liver regenerating mechanisms. Its clinical applications in humans comprise therapy in alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxic and drug-induced liver diseases. Recognition of new silymarin derivatives opens new ways for its application in liver therapy. Silymarin application in veterinary medicine is also reviewed.

Key words: Milk Thistle, sylimarin, hepatoprotection

INTRODUCTION

Seeds of *Silybum marianum* (L.) Gearthn, Milk Thistle have been used for more than 2,000 years to treat a range of liver and gallbladder disorders, including hepatitis, cirrhosis and icterus. More recently, the protection of liver against poisoning from chemical and environmental toxins, including snake bites, insect stings, mushroom poisoning (*Amanita phalloides*) and alcohol abuse are pronounced. *Silybum marianum* is a member of the aster family (Asteraceae or Compositae) which encompasses daisies and thistles, including the common thistle and artichoke. The plant itself, with large purple flowering heads, grows as a stout thistle in rocky soils. The leaves are characterized by milky veins from which the plant derives its name. It is one of the oldest and most thoroughly researched plants in the treatment of the liver diseases. This medical plant has been used from ancient times – Theoprastus (4th century BC) was probably the first to describe it under the name "Pternix" [1,2].

The active extract of milk thistle is a mixture of the flavonolignans, containing approximately 70-80% of the silymarin and 20-30% of a chemically undefined fraction, comprising polymeric and oxidized polyphenolic fraction (PP).

The main component of the silymarin is silybinin, which is a mixture of 2 diastereomers, A and B (1:1 proportion) (Fig.1). Other flavonolignans in silymarin, namely isosilibin, dehydrosilibin, silychristin, silydianin and taxifolin, are all mainly found in the seeds of *Silybum marianum* [1, 2].

PHARMACOLOGY AND TOXICOLOGY

Silymarin is insoluble in water and usually administered in capsule form with a standard extract containing 70-80% silymarin. Pharmacokinetic studies have shown that there is low absorption of silybinin after an oral dose. Peak plasma concentrations are reached after 2 h and the elimination $t_{1/2}$ is 6-8 h. Elimination takes place in the urine (3-8%) after an oral dose, while 20-40% is recovered from the bile as glucuronide and sulfate conjugates. Silybinin reaches peak levels in bile within 2-9 h, and biliary excretion continues for 24 h after a single dose [3, 4].

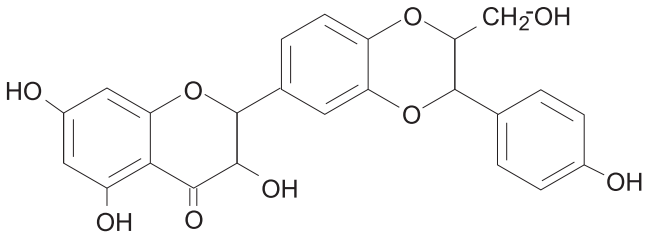

Figure 1 Chemical structure of silybinin.

The acute toxicity of silymarin has been studied in mice, rats, rabbits and dogs after intravenous infusion. The LD_{50} values are reported as 400 mg/kg b.w. in mice, 385 mg/kg b.w. in rats and 140 mg/kg b.w. in rabbits and dogs. However, these values are only approximate as they depend on the infusion rate. When the compound is administered by slow infusion, values of 2 g/kg b.w. may be recorded in rats. After oral administration, tolerance is even higher, with values ranging over 10 g/kg b.w. In the cases of acute intoxication, the cause of death seems to be cardiovascular failure. Other experiments to assess the acute toxicity of silymarin were performed in beagle dogs, rabbits, rats and mice after an intravenous bolus dose. Silymarin was used as hemisuccinate sodium salt and the animals kept under observation for 14 days. The LD_{50} was 1,050 and 970 mg/kg b.w. in male and female mice, respectively, and 825 and 920 mg/kg b.w. 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 b.w. [3, 4].

The acute, subacute and chronic toxicity of silymarin is very low. Moreover, silymarin is also devoid of embryotoxic potential.

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Since silymarin has a relatively poor intestinal absorption, efforts have been made to increase its oral bioavailability by combining its active component, silybinin, with phosphatidylcholine, with liposomes containing variables amount of cholesterol and phospholipids, or with lipid microspheres formed by an internal oils core, surfactans (e.g. soybean lecithin) and different co-surfactants (e.g. propylene glycol) [5, 6, 7, 8].

All these formulations show higher oral bioavailability and facilitate passive targeting to the liver, thus conferring greater pharmacological activity compared with pure silybinin or silymarin.

INTERACTION

In vitro studies showed that silymarin in higher concentrations has an inhibitory effect on both phase I and II drug metabolizing enzymes [9, 10].

The CYP 3A4, CYP 2D6 and CYP 2C9 are the major enzymes inhibited by this flavonolignan, although the concentrations obtained from plasma at pharmacological doses are comparatively much less (about 0.5 μM) compared to that needed for the inhibition of cytochrome enzymes (about 10 μM) [9, 11]. Recent reports suggest that silymarin is a potent inhibitor of hepatic UDP glucuronosyltransferase1A1 (UGT1A1), but its clinical significance is not known [12, 13]. However, clinicians should take appropriate precautions while prescribing co-administered drugs which are metabolized by similar mechanisms [9, 10, 11]. Enhanced glucuronidation is an important phase II of liver detoxification pathway.

Glucuronic acid is conjugated with toxins to facilitate their elimination from the body via bile. Silymarin may similarly facilitate the bilirubin conjugation with glucuronic acid, or inhibit γglucuronidase enzyme from the toxic pathogenic intestinal bacteria. This may be of help in patients with jaundice. Silymarin and related flavonolignans displayed inhibition of the catalytic activities of cytochrome P450 isoenzymes *in vitro* in concentrations greatly exceeding those physiologically attainable, and due to low solubility of silybinin it is virtually impossible to reach such toxic concentrations *in vivo*. Therefore, these findings imply that no adverse effects of silymarin in terms of drug interactions should be expected [11-14].

MECHANISM OF ACTION

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 [13, 15].

The protection provided by silymarin appears to rest on 4 properties (Fig. 2):

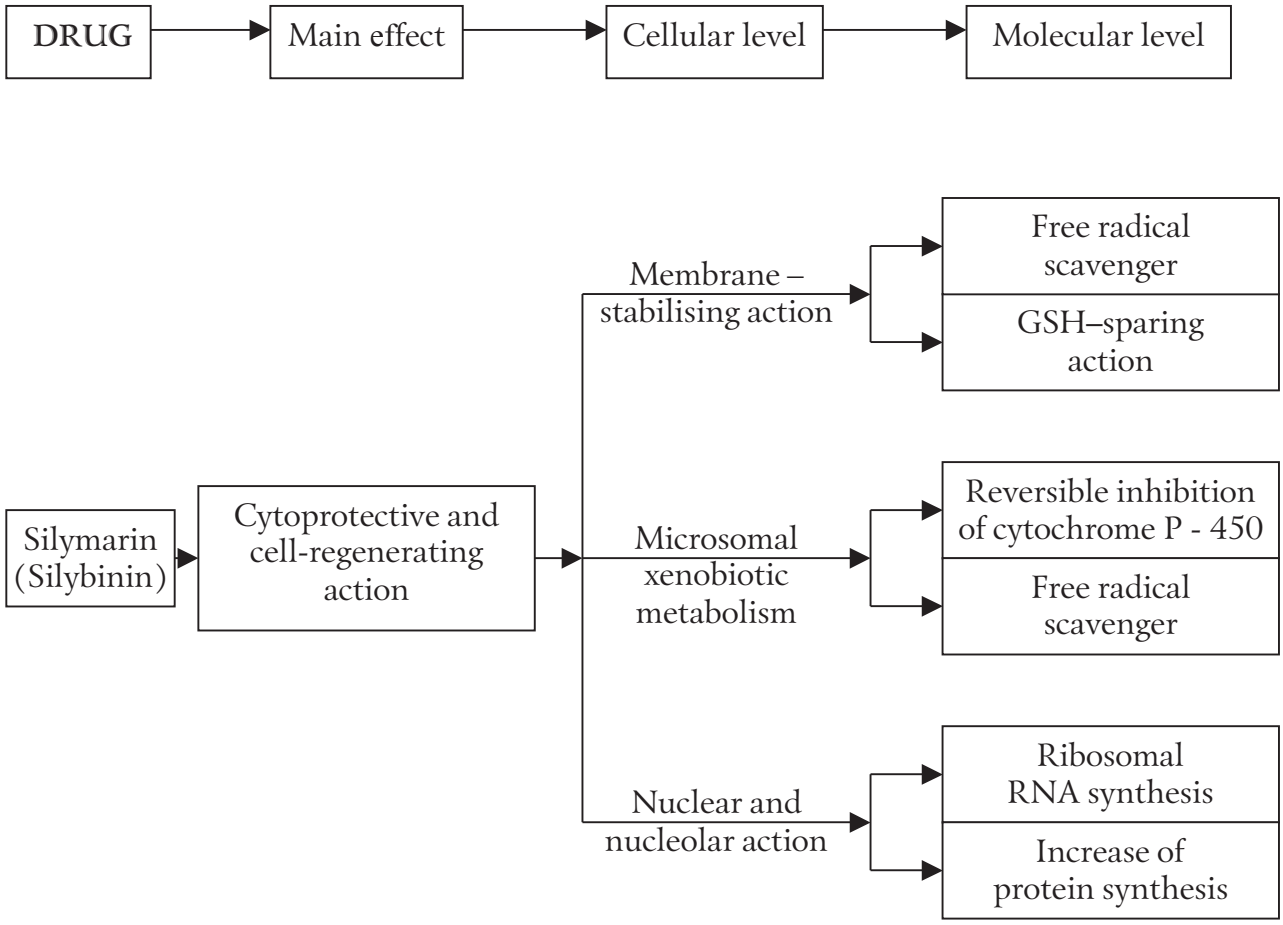

Figure 2 Mechanism of action of silymarin as proposed by Valenzuela and Garrido [45].

1. Activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of GSH.
2. Ability to regulate membrane permeability and to increase membrane stability in the presence of xenobiotic damage.
3. Capacity to regulate nuclear expression by means of a steroid-like effect.
4. Inhibition of the transformation of stellate hepatocytes into myofibroblasts, which are responsible for the deposition of collagen fibres leading to cirrhosis.

Flavonoids usually possess good antioxidant activity. Free radicals, including the superoxide radical, hydroxyl radical, hydrogen peroxide and lipid peroxide radicals have been implicated in liver diseases [16]. These reactive oxygen species (ROS) are produced in the body as a result of biochemical processes in the body, and as a result of increased exposure to xenobiotics [17]. 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. Silymarin is probably able to antagonize the depletion of the 2 main detoxifying mechanisms – GSH and superoxide dismutase (SOD), by reducing the free radical load, increasing GSH levels and stimulating SOD activity. Subsequently, the cell contents, including DNA, RNA and other cellular components, are damaged [18].

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 [19].

Stimulation of protein synthesis. Silymarin can enter into the nucleus and act on RNA polymerase I enzymes and the transcription of rRNA, resulting in increased ribosomal formation. This in turn hastens protein and DNA synthesis [20], which enhances the biosynthetic apparatus in the cytoplasm, thus leading to an increase in the synthesis rate of both structural and functional proteins. At least conceptually, this stimulation may enable cells to counteract the loss of transporters and enzymes occurring under many pathological conditions. This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver.

Anti-inflammatory actions. The inhibitory effect on the 5-lipoxygenase pathway, resulting in inhibition of leukotriene synthesis, is a pivotal pharmacological property of silymarin. Leukotriene (B_4) synthesis was reduced while prostaglandin (E_2) synthesis was not affected at higher concentrations of silybinin [21]. A study which evaluated the action of silybinin in isolated Kuppfer cells indicated a strong inhibitory effect on leukotriene B4 formation with the IC_{50} value of 15 μM. But no effect was observed on the tumor necrosis factor – alpha (TNF-α) formation [22]. The NF-κB is a key regulator of inflammatory and immune reactions. Silymarin is found to suppress both NF-κB DNA binding activity and its dependent inhibition by silymarin [23]. The results of an *in vivo* study in male BALB/c mice treated with silymarin suggested that parenteral exposure to silymarin results in suppression of T-lymphocytes at low doses, and stimulation of inflammatory process at higher doses. Thus the ability of the immune system against bacterial infection will increase at higher doses, and may be an additional therapeutic application of this flavonoid mixture [24].

Antifibrotic effects. Liver fibrosis can result in remodeling of liver architecture, leading to hepatic insufficiency, portal hypertension and hepatic encephalopathy. These processes involve a complex interplay of cell and mediators [21, 25]. 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. This diminishes expression of the profibrogenic procollagen alpha I and the tissue inhibitor of matalloproteinases-1, most likely by down-regulation of pro-fibrogenic cytokine, TGF-β1. Kupffer cells promote stellate-cell proliferation and activation, and the counteracting action of silimarin at this level may play a key role. When administered at concentrations reached in plasma after clinical doses, silibinin inhibits production of mediators in Kupffer cells involved in stellate-cell activation, such as reactive oxygen species and leukotrienes. Inhibition of leukotriene production is due to inhibition of 5-lipoxygenase, the enzyme that catalyzes leukotriene formation from arachidonic acid. Interestingly, 5-lipoxygenase inhibition was shown to lead to Kupffer cell growth arrest and apoptosis. Production of leukotrienes by granulocytes was also inhibited by silybinin. Silymarin inhibits NF-κB and also retards HSC activation. It also inhibits protein kinases and other kinases involved in signal transduction and may interact with intracellular signaling pathways [21, 22, 25, 26].

Drug and toxin related liver damage. Hepatocellular injury due to drugs seems to be the primary event. This is rarely due to the drug itself and a toxic metabolite is usually responsible. The drug metabolizing enzymes activate chemically stable groups to produce electrophilic metabolites. These potent agents bind covalently to liver molecules such as proteins and fatty acids which are essentials to the life of the hepatocyte and necrosis ensues. This follows exhaustion of intracellular substances such as glutathione, which are capable of preferentially conjugating with an unpaired electron and are produced by oxidative reaction of cytochrome P450. This free radical can also bind covalently to proteins and to unsaturated fatty acids of cell membrane damage. The end result is hepatocyte death related to the failure to pump calcium from the cytosol and to depressed mitochondrial function [15, 18, 26, 27].

Silymarin inhibits the uptake of α-amanitin, the amatoxin from the poisonous mushoroom Amanita phalloides, by competing with its basolateral transport system. This blocks entry of the amatoxin into the hepatocyte and prevents inhibition of RNA polymerase II and the concomitant blockage of protein synthesis [20, 16, 27, 28].

Silymarin has a regulatory action on cellular and mitochondrial membrane permeability in association with an increase in membrane stability against xenobiotic injury [26]. It can prevent the absorption of toxins into the hepatocytes by occupying the binding sites as well as inhibiting many transport proteins at the membrane [27]. These actions, together with the antiperoxidative property, make silymarin a suitable candidate for the treatment of iatrogenic and toxic liver diseases.

In *in vitro* studies, silymarin was a potent inhibitor of cyclic AMP (cAMP)-phosphodiesterase [28]; thus, an increase in the hepatic cAMP levels induced by silymarin is likely and may act as a second messenger of some beneficial effects of silymarin (stabilization of cellular membranes).

Silymarin exerts strong anticancer activity against human HCC (hepatocellular carcinoma) cells by modulating cell cycle and associated proteins, causing growth inhibition as well as apoptotic death, as it does for many other human cancer cells (prostate, skin, kidney, colon). Silybinin caused G_1 arrest in HepG2 and both G_1 and G_2-M arrests in Hep3B cells. Furthermore, it strongly inhibited CDK2, CDK4 and CDC2 kinase activity in these HCC cells [29]. These results support the clinical usefulness of silybinin against hepatocellular carcinoma in addition to its known clinical efficacy as an antihepatotoxic agent.

USE IN VETERINARY MEDICINE

Plant preparations from *S. marianum* have indisputable applications in veterinary medicine. Silymarin preparations are used either as feed supplement intended for improving animal health and their productivity, or for therapeutic purposes [30, 31].

Dairy cows experience moderate to severe fatty liver at calving, which impairs liver function and consequently leads to ketosis seriously compromising their health and milk production. Severity of the peripartum fatty liver can be alleviated by using hepatoprotective agents such as silymarin [30, 31].

Seeds of milk thistle were used as a feed supplement for dairy cows with very promising results. It decreased of the ketonuria in cows fed with milk thistle meal. Milk production in the cows of control groups decreased during the trial, but in the test cows it was higher by 3.4-7.7%, in comparison with the milk yield at the beginning of the trials [32]. Differences in metabolism parameters and milk production in the test cows were observed even a fortnight after the diet ceased to contain milk thistle seed. The cows fed with silymarin (10 g per day) showed a quicker onset of the peak of milk production, which was one week sooner than in the control group, and with better overall milk yield [31]. No changes in the milk composition nor silymarin effects on liver histology and biochemistry were observed [32]. Silymarin influences positively the metabolic pattern of the liver enzymes during the lactation onset in cows [33, 34].

Silymarin can lower but not completely block aflatoxin M_1 (AFM_1 – a major metabolite of AFB_1) excretion in cow milk [35].

Silymarin decreases the toxicity of aflatoxin B_1 in broiler chicks, as demonstrated by serum alanine amino transferase (ALT) activity, liver histology, feed intake and body weight gain [36]. These findings suggest that silymarin might be used in chickens to prevent the toxic effects of AFB_1 originating from contaminated feed. Effects of silymarin on higher body weight and increased hatchability were demonstrated in chickens and turkeys. It also prevented excessive adiposis in birds. Hepatoprotective effects of silymarin were also proved by biochemical and histopathological examinations [30, 37].

Silybum marianum, together with other plants (*Melissa oficinals*, *Mentha piperita*, *Urtica dioica*, *Thymus Vulgaris*, *Agropyron repens*, *Allium*, *Capsicum annum*, *Origanum maiorana*, *Coriandrum sativum* and *Taraxacum vulgare*), was used as a substitute for fodder antibiotics in pigs. The experimental group had better body weight gains compared with the control group (no supplements), and 6.1% better compared with the group receiving antibiotics [30, 38].

Silymarin is also applied in veterinary medicine for the treatment of hepatic disorders of various etiology. An interesting application was reported in the treatment of sheep intoxication by sawfly (*Arge pullata*) larvae [38]. Ruminantes ingesting sawfly larvae (*A. pullata* or *Lophyrotoma interrupta*) developed massive liver necrosis and degeneration of the kidney tubules. This intoxication is caused by a toxic octapeptide lophyrotomin contained in the larvae which is structurally similar to the toxic cyclic peptides from Amanita mushorooms. [40]. Therefore, it was suggested using the same treatment for this intoxication, e.g. the application of silymarin. This approach proved to be very successful, suggesting a similar mechanism of intoxication by these peptides [39].

A series of studies report the use of silymarin in the treatment of various hepatic disorders in dogs – all with mostly positive results [40-42]. The clinical results were corroborated by biochemical data [43]. Silymarin was used as an adjunct therapy in the treatment of canine Giardia parasitosis, where metronidazol used as an antibiotic of choice often causes hepatic disorders [44]. Dogs treated with silymarin had normal serum indicators of liver inflammation compared with the positive control group in which these indicators significantly increased.

A summary of the silymarin data enables the conclusion that there are multiple beneficial actions of the flavonolignant. This is mainly due to its hepatoprotective action and to stimulation of protein synthesis, regulation of biomembranes functions, anti-inflammatory and anti-carcinogenic activity, which are also well documented. Silymarin is recommended for both veterinary treatment and as a protective agent in intensive animal production.

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