Review Article

Available online through
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IJRAP
ISSN 2229-3566

A REVIEW ON HEPATOPROTECTIVE ACTIVITY OF SILYMARIN

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Received on: 10/01/2011 Revised on: 02/02/2011 Accepted on: 12/02/2011

ABSTRACT

Silymarin is obtained from the seeds of *Silybum marianum* (milk thistle), an edible plant that has been used medicinally from ancient times for the treatment of various liver disorders. It is widely prescribed by herbalists and has almost no known side effects. The plant is native to the Mediterranean and grows throughout Europe, North America, India, China, South America, Africa and Australia. The flavonoid silymarin and one of its structural components, silybin (silybinin), are substances with documented hepatoprotective activities, hence, they have been reported to be effective in liver diseases, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis, and cirrhosis and alcoholic liver diseases. Silymarin has been reported to inhibit the hepatotoxin binding to receptor sites on the hepatocyte membrane; reduce the glutathione (GSH) oxidation to enhance its level in the liver and intestine; and stimulate the ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration. Overall, silymarin possesses antioxidant, immunomodulatory, anticancer, antiinflammatory, antihepatoxic and some other pharmacological activities. Its effectiveness against multiple disorders makes it a very promising drug of natural origin.

KEYWORDS: Hepatoprotective activity, liver disorders, *Silybum marianum* (milk thistle), silymarin.

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INTRODUCTION

Silymarin is a flavonolignan (polyphenolic fraction) extracted from the seeds (and may also from the fruits) of *Silybum marianum* (milk thistle or bank thistle) plant, belonging to Compositae family. Silymarin mainly contains three flavonolignan isomers, i.e., silybin (or silybinin), silydianin and silychristin[1-8]. Some reporters[4,7] also elucidated that silymarin consists of four flavonolignan isomers, viz., silybin, isosilybin, silydianin and silychristin with an empirical formula $C_{25}H_{22}O_{10}$, and the structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory actions[7]. Of all the isomers that constitute silymarin, silybin is the most active. Silymarin and silybin have been found to provide cytoprotection and above all, hepatoprotection[3-7]. Silymarin has been found to cure various liver disorders as it has established the efficacy in restoration of liver function and regeneration of liver cells[1-17]. It antagonized the toxin (alpha-aminitine) of *Amanita phalloides* and provided hepatoprotection against toxicity caused by phalloidine, galactosamine, paracetamol, carbon tetrachloride, thioacetamide and halothane[1-7]. Silymarin has also protected the hepatocytes from injury due to poisoning, ischaemia, radiation, iron overload and viral hepatitis, so it is included in the pharmacopoeia of many countries, and is often used as supportive therapy in food poisoning by fungi and in chronic liver disorders such as steatosis and alcohol-related liver disease[7].

It has been further pointed out that silymarin is used medicinally to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis, cirrhosis and alcoholic liver diseases. It is also effective in certain cancers. Its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane; reduction of glutathione (GSH) 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[5]. Silymarin 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 possesses antioxidative, anti-inflammatory, antifibrotic, antilipid peroxidative, membrane stabilizing and liver regenerating activities/ mechanisms. Its clinical uses in humans comprise therapy in alcoholic liver diseases, liver cirrhosis, *Amanita* mushroom poisoning, viral hepatitis, toxic and drug-induced liver diseases[6].

With the above backgrounds, this review has been putforth to focus on hepatoprotective activities, including mechanism of action of silymarin, which can make a breakthrough as a new approach against various liver diseases. Recognition of silymarin derivatives opens new ways for its application in the remedy of liver disorders.

SPECIFIC REPORTS ON HEPATOPROTECTIVE ACTIVITY OF SILYMARIN

The hepatoprotective/hepatogenic activity of silymarin or extracts of *S. marianum* xenobiotic intoxication and fungal intoxication has been reported by several workers[4-5,7]. Silymarin was found to completely neutralize the hepatotoxic effect of various agents as evidenced by significant reduction in prolongation of hexobarbitol sleeping time and incresed serum levels of transaminases and sorbitol dehydrogenase at the dose of 100 mg/kg, iv against carbon tetrachloride (0.15 ml/kg, oral) poisoning in rat. Similarly, a 100% protection by silymarin (50 mg/kg, iv) against phalloidine (3 mg/kg, ip) hepatotoxicity and a marked hepatoprotective effect of silymarin (75 mg/kg, iv) in hepatotoxicity induced by alpha-aminitine (0.5 mg/kg, ip) in mouse were recorded. A significant reduction and restoration of the activity of serum transaminases was also achieved after administration of silymarin during praseodymium and galactosamine induced hepatotoxicity[1-2]. The hepatogenic effects of the aqueous extract (AqE) and petroleum ether extract (PEE) of *S. marianum* seeds were studied by Pandey[2] and found that these extracts at the dose of 1000 mg/kg body weight, orally, daily from 3rd to 7th day of the experiment produced beneficial results against paracetamol (500 mg/kg, orally, once on 1st day) induced hepatotoxicity. The AqE and PEE of *S. marianum* seeds significantly ($P<0.05$) improved the paracetamol altered activities of serum alkaline phophatase (SAP) and serum arginase (SARG)[2,13]; SGOT and SGPT[2,15]; and serum proteins[2,16]; and also caused the regeneration of hepatic tissues in albino rats[2,14]. The normalization and regeneration of liver tissues were also produed by *S. marianum* PEE in albino mice[2,13,17]. Silymarin has been found to protect the rats from hepatotoxicity caused by carbon tetrachloride (0.75 ml/kg, oral)[18]; while different doses of this drug (3-20 mg/kg, orally, daily for 7 days) also showed hepatoprotection against thioacetamide (200 mg/kg, sc) induced hepatic damage in rat[2,19].

Silymarin has been shown to prevent carbon tetrachloride-induced lipid peroxidation and hepatotoxicity. Silybinin preserved the functional and structural integrity of hepatocyte membranes by preventing alterations of their phospholipid structure produced by carbon tetrachloride, and by restoring SAP and gamma glutamyl transpeptidase (GGT) activities[20]. Silymarin protects rat liver mitochondria and microsomes *in vitro* against the formation of lipid peroxides induced by various agents[21]. Silymarin affords hepatoprotection against specific injury induced by microcystin (a hepatotoxin), paracetamol, halothane and alloxan in several experimental models. Data obtained in experimental models of hepatic injury have shown that silymarin is able to normalize the carbon tetrachloride increased plasma lipids and to antagonise the reduction in serum free fatty acids induced by thioacetamide; and during paracetamol induced hepatic injury in rats, silymarin improved the low-density lipoprotein (LDL) binding to hepatocytes, an important factor for the reduction of LDL in plasma. The increase in protein synthesis was induced by silybinin only in injured livers (not in healthy controls). 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, and thereby protected the hepatic structures, liver glucose stores and enzyme activity *in vivo*. A dose of 15 mg/kg of silymarin was administered intravenously 60 minutes before intraperitoneal administration of a lethal dose of phalloidine which was able to protect the dogs, rabbits, rats and mice (100% survival). Histochemical and histoenzymological studies have shown that silymarin, administered 60 minutes before or no longer than 10 minutes after induction of acute intoxication with phalloidine, is able to neutralize the effects of the toxin and to modulate hepatocyte function. 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[4-5,7].

Dixit et al. cited that silymarin provided protection from phenylhydrazine-induced liver glutathione depletion and lipid superoxidation in rat. The authors further stated that tert-butyl hydroperoxide induced the microsomal lipid peroxidation and has been used as the model in different

studies demonstrating the protective effect of silymarin. Silymarin also inhibited the oxygen consumption by rat microsomes and showed the reduced enzyme loss and morphological alterations in neonatal rat hepatocytes. The inhibition of lipid peroxidation by silymarin-perfused rat hepatocytes was also shown. Silymarin reduced the enzyme loss and morphological alterations induced by erythromycine in neonatal rat hepatocytes as the model. Using the model of microcystin which produced the acute hepatotoxicity in mice and rats, the neutralization of microcystin's lethal effects and pathological alterations by silymarin was also demonstrated[5]. Furthermore, the hepatoprotective activity of silymarin has also been demonstrated by various researchers from all over the world against partial hepatectomy models and toxic models in experimental animals after administration of acetaminophen (paracetamol), carbon tetrachloride, ethanol, galactosamine and *A. phalloides* toxin[7]. The rats with partial hepatectomy, where 70% of liver was removed, when subjected to silymarin pretreatment showed increased synthesis of DNA, RNA, protein and cholesterol, suggesting the liver regeneration[22]. Silymarin when compared with various polyherbal formulations in carbon tetrachloride induced hepatotoxicity in rats has led to complete normalization of elevated transaminases levels[23]. Silymarin treatment protected completely against harmful increase in the membrane ratios of cholesterol phospholipids and sphingomyelin:phosphatidylcholine in rats with carbon tetrachloride induced cirrhosis[20]. The rats with chronic carbon tetrachloride induced liver damage were treated with silymarin (50 mg/kg, orally for 5 days). The collagen content in livers of animals pre-treated with carbon tetrachloride was increased approximately four-fold which prevented the cirrhotic changes in rats and reduced the liver collagen content by 55%[24]. Silymarin has also been studied for its protective action against acetaminophen (an analgesic, antipyretic and antiinflammatory drug) induced centrilobular hepatic necrosis in animal models. *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[7]. A comparative study of andrographolide and silymarin on acetaminophen induced cholestasis has produced the dose dependent cholestatic and anticholestatic effects of these drugs[25]. Silymarin and andrographolide were compared in experimental toxic models of carbon tetrachloride and paracetamol in mice. Silymarin when given to mice @ 100 mg/kg, ip for 7 days, led 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 the paracetamol induced hepatotoxicity. Conclusively, this study suggested that silymarin elicit the hepatoprotection by preventing hepatic cell necrosis or by hepatic cell regeneration[26]. Silybin dihemisuccinate (a soluble form of silymarin) protected the rats against liver glutathione depletion and lipid peroxidation induced by acute acetaminophen hepatotoxicity and showed potential benefits of silymarin as an antidote[27].

The hepatoprotective activity of silymarin against ethanol (ethyl alcohol) induced damage has been been demonstrated in tested animals as evidenced from the improvements in some liver function tests such as SGOT, SGPT and gamma glutamyl transferase[28]. Galactosamine produced liver damage, with histopathological changes resembling human viral hepatitis has been also been treated with the administration of silymarin[29]. The oxidative stress due to increased hepatic lipid peroxidation is the major mechanism of iron induced hepatotoxicity. Pretreatment in rats with silymarin reduced the iron induced increase in lipid peroxidation and levels of serum enzymes, as also noted in *Withania somnifera,* indicating their hepatoprotective action[30]. Silymarin was 100% effective in preventing liver toxicity when given as pretreatment or upto 10 minutes after *A. phalloides* poisoning in mice. Severe liver damage and resultant death was avoided when silymarin was administered within 24 hr. In a study with dogs, none died when given silymarin 5-24 hr after ingesting an LD_{50} of *A. phalloides* (85 mg/kg). Liver enzymes and liver biopsies showed significant protective effect of silymarin post-treatment. Silymarin was also found to protect the liver tissues from injury caused by ischaemia, radiation and viral hepatitis[7]. Silymarin also protected against Fumonisin B_1 (a mycotoxin produced by *Fusarium verticillioides* found on corn and corn-based foods) liver damage by inhibiting biological functions of free sphingoid bases and increasing cellular regeneration[31].

MECHANISM OF ACTION OF SILYMARIN

As a hepatoprotective drug, silymarin has been reported to possess multiple mechanism of actions against different hepatotoxic agents. The antioxidant property and cell regenerating functions as a result of increased protein synthesis are considered as most important actions[32]. Silymarin or *S. marianum* has the antioxidant activity. Free radicals, including superoxide radical,

hydroxyl radical (.OH), hydrogen peroxide (H^2O_2) and lipid peroxide radicals have been implicated in liver diseases[33]. 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[34]. 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[35]. 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[20]. The stimulation of protein synthesis is an important step in the repair of hepatic injury, and is essential for restoring the structural proteins and enzymes damaged by hepatotoxins. Overall, the hepatoprotection provided by silymarin appears to rest on four actions: (a) activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of GSH; (b) ability to regulate the membrane permeability and to increase membrane stability in the presence of xenobiotic damage; (c) capacity to regulate the nuclear expression by means of a steroid-like effect; and (d) inhibition of the transformation of stellate hepatocytes into myofibroblasts, which are collagen fibres leading to cirrhosis. Silymarin and silybinin inhibit the absorption of toxins, such as phalloidine or beta-amanitine, preventing them from binding to the cell surface and inhibiting membrane transport systems. Further, silymarin and silibinin, by interacting with the lipid component of cell membranes, may influence their chemical and physical properties[3-5,7].

Well documented scavenging activity of silymarin and silybinin may explain the protection afforded by these substances against hepatotoxic agents. Silymarin and silybinin can exert their actions by acting as free radical scavengers and interrupting the lipid peroxidation processes involved in the hepatic injury produced by toxic agents. Both these 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. Silybinin 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[7,9]. Silymarin works by acting as an antioxidant that prevents chain rupture. 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, silybinin produced a significant increase in the formation of ribosomes and in DNA synthesis, as well as an increase in protein synthesis. Silymarin can inhibit the hepatic cytochrome P450 (CYP) detoxification system (phase I metabolism). It has been shown recently in mice that silybinin is able to inhibit numerous hepatic CYP enzyme activities. This effect could explain some of the hepatoprotective activities 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. In addition, silymarin, together with other antioxidant agents, could contribute towards protection against free radicals generated by enzymes of the CYP system[7].

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