Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches

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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 various pharmacological activities of silymarin including the clinical trials. For the first time, the review also looks at the formulation work that has been done to enhance its solubility, so as to increase its bioavailability and thus, its hepatoprotective action.

KEY WORDS: Bioavailability, drug delivery, hepatoprotection, milk thistle, silymarin

Silymarin is obtained from *Silybum marianum* (milk thistle), an edible plant that has been used medicinally for centuries as a herbal medicine for the treatment of liver-related 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 and North America.[1,2] It also grows in India, China, South America, Africa, and Australia. This herb is approved for sale in Canada in 70 different products[3] and generates an annual business of $180 million in Germany alone.[1]

Silymarin is a polyphenolic flavonoid, extracted using 95% ethanol, from the seeds of the milk thistle. The plant consists of approximately 70-80% of the silymarin flavonolignans and approximately 20-30% of a chemically undefined fraction, comprising mostly polymeric and oxidized polyphenolic compounds. The most prevalent component of the silymarin complex is silybin (50-60% of silymarin), which is the most active photochemical and is largely responsible for the claimed benefit of the silymarin. Besides silybin, which is a mixture of two diastereomers (A and B) in approximately 1:1 proportion, considerable amounts of other flavonolignans are present in the silymarin complex, namely silychristin (20%), silydianin (10%), isosilybin (5%), dehydrosilybin, and a few flavonoids, mainly taxifolin. The seeds also contain betaine, trimethylglycine, and essential fatty acids that may contribute to silymarin's hepatoprotective and anti-inflammatory effects.[1,2,4-6]

Pharmacokinetics

Silymarin is not soluble in water and is usually administered in an encapsulated form.[1] Silymarin is absorbed when given orally. Peak plasma concentration is achieved in 6-8 h. The oral absorption of silymarin is only about 23-47%, leading to low bioavailability of the compound; it is administered as a standard extract (70-80% silymarin). After oral administration the recovery in bile ranges from 2-3%. Silybin and the other components of silymarin are rapidly conjugated with sulfate and glucuronic acid in the liver and excreted through the bile.[2,7-9] The poor water solubility and bioavailability of silymarin led to the development of enhanced formulations; e.g., silipide (Siliphos®), a complex of silymarin and phosphatidylcholine that is ten times more bioavailable than silymarin;[10] an inclusion complex formed between silymarin and β-cyclodextrin, which is approximately 18 times more soluble than silymarin.[11] There have been reports of silybin glycosides that have better solubility and stronger hepatoprotective activity.[12]

Toxicity

Studies on the acute toxicity of silymarin after intravenous infusion have been carried out in mice, rats, rabbits, and dogs. The LD_{50} values were 400 mg/kg (mice), 385 mg/kg (rats), and 140 mg/kg (rabbits and dogs). Depending on the infusion rate these values vary. With slow infusion (over 2-3 h) the LD_{50} was 2 g/kg in rats and after oral administration it was 10 g/kg.[13]

Intravenous bolus dose of silymarin as the hemisuccinate sodium salt has also been used to carry out acute toxicity studies in beagle dogs, rabbits, Wistar rats, and NMRI mice. The LD_{50} was 1050 mg/kg (male mice), 970 mg/kg (female mice), 825 mg/kg (male rats), 920 mg/kg (female rats), and 300 mg/kg (rabbits, dogs).[14] These data demonstrate that the acute toxicity of silymarin is very low. Similarly, its subacute and chronic toxicity are also very low.[12,15]

Pharmacology of Silymarin

Hepatoprotective activities

Various experimental studies using compounds that directly or indirectly cause liver damage have been carried out to demonstrate the hepatoprotective action of silymarin in xenobiotic intoxication and fungal intoxication.

***Carbon tetrachloride*:** Carbon tetrachloride is known for its hepatotoxic properties and many hepatoprotective agents have been tested against it. Silymarin has been shown to prevent carbon tetrachloride-induced lipid peroxidation and hepatotoxicity.[16,17] This effect of silymarin is attributed to its ability to normalize the levels of the transaminases that are elevated in hepatotoxicity.[18] Silymarin has been shown to protect harmful increase in the membrane ratios of cholesterol: phospholipids and sphingomyelin: phosphatidylcholine, thus providing protection from carbon tetrachloride-induced cirrhosis in rats.[19] Silymarin has also been found to reduce the increased collagen content in the carbon tertrachloride-induced chronic liver damage.[17,20,21]

***Phenylhydrazine*:** Valenzuela *et al.*, using a rat erythrocytic model, showed that silymarin inhibited the haemolysis and lipid peroxidation caused by phenylhydrazine.[22] The authors, in a separate study using rat liver, showed that silymarin provided protection from phenylhydrazine-induced liver glutathione depletion and lipid superoxidation.[23]

***tert-Butyl hydroperoxide*:** tert-butyl hydroperoxide has been found to induce microsomal lipid peroxidation and has been used as the model in different studies demonstrating the protective effect of silymarin. Valenzuela and Guerra[24] demonstrated that silymarin inhibited oxygen consumption by rat microsomes, while Davila *et al.*, showed that silymarin reduced enzyme loss and morphological alterations in neonatal rat hepatocytes.[25] Farghali *et al.*, demonstrated the inhibition of lipid peroxidation by silymarin-perfused rat hepatocytes.[26]

***Ethanol*:** Administration of ethanol produces a decrease in the hepatic content of glutathione (GSH), which is an important biomolecule that affords protection against chemically-induced cytotoxicity.[27] The administration of ethanol enhances the alanine transaminase level (ALT), aspartate transaminase (AST), and gamma glutamyl transferase levels and also makes the reduced glutathione/oxidized glutathione ratio abnormal. Wang *et al.*, showed the protective effect of silymarin against ethanol-induced changes in these parameters.[28] Valenzuela *et al.* showed the neutralization of the lipid peroxidation, using acute intoxication in rats as the experimental model,[29] while Valenzuela and Garrido showed reduction in the liver alterations, using chronic intoxication in rats as the model.[30]

***Halothane*:** Siegers *et al.*, used acute intoxication caused by halothane in hypoxic rat models and demonstrated that silymarin provided protection from the hepatotoxic effects of halothane.[31]

***Thioacetamide*:** Antihepatotoxic effects and hepatoprotection were observed with silymarin in acute and chronic intoxication induced by thioacetamide in rats.[32,33]

***Galactosamine*:** Galactosamine produces liver damage and administration in rats produces cholestatis due to inhibition of bile acid synthesis. An anticholestatic effect of silymarin has been reported by Saraswat *et al.*[34] Datta *et al.*, showed the effect of silymarin in normalizing elevated levels of serum transaminases and alkaline phosphatase in isolated rat hepatocytes with galactosamine-induced damage.[35] Barbarino *et al.*, showed the protective effect of silymarin in acute hepatitis in rats.[36] Inhibition of galactosamine-induced lipid peroxidation was also observed in perfused rat hepatocytes.[37] When experimental hepatitis in rats was used as the model, inhibition of the toxic effects on protein synthesis was also observed.[38]

***Paracetamol*:** Paracetamol is known to cause centrilobular hepatic necrosis in mice/rats. Silymarin, by its stabilizing action on the plasma membrane, has been shown to normalize the paracetamol-induced elevated biochemical parameters in the liver and serum.[39] Muriel *et al.*[40] and Campos *et al.*,[41] used this model to show the protective effects of silymarin in paracetamol-induced lipid peroxidation and glutathione depletion. Renganathan studied the hepatoprotective effect of silymarin in mice and showed that silymarin prevented the hepatic cell necrosis induced by paracetamol in 87.5% of the animals, however it provided protection in only 16% of the animals with hepatic necrosis induced by carbon tetrachloride.[42] He concluded that silymarin shows its hepatoprotective action either by preventing hepatic cell necrosis or by inducing hepatic cell regeneration. Silybin has also been shown to lessen the paracetamol-induced injury of kidney cells.[43]

***Erythromycin estolate*:** Davila *et al.*, using neonatal rat hepatocytes as the model, showed that silymarin reduced the enzyme loss and morphological alterations induced by erythromycin estolate.[44]

***Microcystin*:** This compound produces acute hepatotoxicity in mice/rats. Using this model, Mereish *et al.* demonstrated the neutralization of microcystin's lethal effects and pathological alterations by silymarin.[45]

***Amanita phalloids toxin*:** Administration of this compound produces acute intoxication in mice /rats/rabbits/ dogs. Silymarin at 50-150 mg/kg intravenous dose provided protection and cure.[14] The increase in liver enzymes and reduction in coagulation factor seen with sublethal doses of A. phalloids can be prevented with silymarin.[46] This is evident at 5 h with silymarin given intravenously at a dose of 50 mg/kg and at 24 h with a dose of 30 mg/kg.

Anti-inflammatory/antiarthritic activities

De la Puerta *et al.*;[47] tested the effect of silymarin in different experimental models of acute inflammation ***in vivo***. In carrageenan-induced paw oedema in male Wistar rats, silymarin given orally reduced the food pad abscesses. In xylene-induced ear mouse inflammation, silymarin applied topically showed higher inhibition (44.52%) than indomethacin (35.96%) at the same dose and was more effective when applied

intraperitoneally. Leukocyte migration is a key process in the inflammatory process; silymarin had a greater inhibitory effect on the leukocyte migration induced by carrageenan in mice and produced a dose-dependent inhibition of leukocyte accumulation in inflammatory exudates. Silymarin also reduced the number of migrating neutrophils. However, silymarin was unable to inhibit phospolipase A_2 an enzyme involved in the inflammatory process. It has also been reported that silymarin is able to inhibit the enzymes lipooxygenase[48] and cyclooxygenase in ***in vitro*** assays.[49]

Gupta ***et al***., have shown the anti-inflammatory and antiarthritic activity of silymarin, which is mediated through the inhibition of 5-lipoxygenase.[50] The anti-inflammatory effect was tested on carrageenan-induced oedema, papaya latex-induced oedema, and arachidonic acid (AA)-induced mouse ear oedema. The results indicated that the activity was less marked in the carageenan model but significant in the papaya latex and AA models of inflammation.

In the AA model, a 25 mg/kg P.O. dose of silymarin produced 36.84% inhibition of oedema, which the authors attributed to its inhibitory action on the formation of 5-lipoxygenase and the leukotrienes involved in inflammation.

Antiarthritic activity was tested in mycobacterial adjuvant-induced arthritis in rats. When tested at 6.25, 12.5, and 25 mg/kg P.O. dose, silymarin showed a dose-related inhibition of 14.87, 23.73, and 31.64% on day 13, while boswellic acids employed as the positive control showed 32.91% inhibition. This showed that the silymarin was more effective in cases of developing arthritis compared to developed arthritis.

Clinical Studies

Škottováa ***et al***., investigated currant oil (from ***Ribes nigrum*** L.)-induced modulation of the antihypercholesterolemic and LDL antioxidant effects of silymarin and (the better bioavailable) silibinin–phosphatidylcholine complex (SPC) in rats fed on a high-cholesterol, high-fat diet.[51] They fed rats on a high-cholesterol diet supplemented with 10% of currant oil containing polyunsaturated fatty acids (PUFA, 61.1% of n-6 and 15.4% of n-3) and lower amounts of saturated (SFA, 7.7%) and monounsaturated (MUFA, 14.3%) fatty acids which caused a significant lowering of plasma cholesterol associated with a mild decrease in VLDL-C and an increase in HDL-C, when compared to rats fed on high-cholesterol diet with 10% of lard fat containing low amounts of PUFA (7.7% of n-6 and 0.7% of n-3) and higher amounts of SFA (42.7%) and MUFA (47.5%). However, currant oil feeding led to the increased oxidizability of LDL. It was found that silymarin, but not SPC, was effective in preventing the development of dietary-induced hypercholesterolemia in both dietary fats, with a slightly better result in rats fed the diet containing currant oil. On the other hand, SPC was more effective than silymarin in suppressing LDL oxidizability. The results suggest that the antihypercholesterolemic effect of silymarin in rats fed on a high-cholesterol diet is improved by dietary currant oil, but the currant oil induces an increased oxidizability of LDL. This can be suppressed by improvement of the bioavailability of silibinin, as demonstrated here with the silibinin-phosphatidylcholine complex.

Lirussi ***et al***., investigated silybin–β-cyclodextrin complex in the treatment of patients with diabetes mellitus and alcoholic liver disease. In noninsulin dependent diabetes mellitus (type 2) and associated chronic liver disease, plasma levels of glucose, insulin, and triglycerides are high, with increased lipid peroxidation and reduction in natural antioxidant reserves. It was hypothesized by the authors that a better glucose and lipid metabolism could be achieved by the rebalancing of cell redox levels and amelioration of liver function. They assessed the effect of the silybin–β-cyclodextrin formulation in 60 patients with chronic alcoholic liver disease and concomitant type 2 diabetes mellitus in a double-blind study for 6 months.[52] The parameters measured included fasting and mean daily plasma glucose levels, glycosylated hemoglobin (HbA_{1c}), basal and stimulated C-peptide and insulin levels, and HDL-cholesterol and triglycerides levels, in addition to conventional liver function tests. Insulin sensitivity was estimated by HOMA-IR. Malondialdehyde (MDA) was also measured before and after treatment as an index of oxidative stress. The results showed that the oral administration of silybin-β-cyclodextrin in patients with type 2 diabetes and compensated chronic alcoholic liver disease caused a significant decrease in both glucose and triglyceride plasma levels. These effects were attributed to the recovery of energy substrates with an improved insulin activity and a reduced lipid peroxidation.

Kim ***et al***., studied the comparative bioavailability of Liverman capsule to Legalon capsule (each containing silymarin) and silymarin tablet in 24 healthy Korean volunteers.[53] Each volunteer received each medicine at the silibinin dose of 120 mg in a 3 × 3 crossover study, with a 1-week washout period between the doses. Plasma concentrations of silibinin were monitored by HPLC for a period of 12 h after the administration. $AUC_{0-\infty}$, C_{max}, and t_{max} were obtained from the plasma concentration–time data. After oral administration of each medicine, the pharmacokinetic parameters of silibinin, viz, AUC_{0-12h}, $AUC_{0-\infty}$, C_{max}, and t_{max} were obtained as follows: Legalon capsule (5.59 µg/ml × h, 6.00 µg/ml × h, 1.33 µg/ml, and 1.83 h), silymarin tablet (4.24 µg/ml × h, 4.63 µg/ml × h, 1.13 µg/ml and 2.10 h) and Liverman capsule (13.9 µg/ml × h, 15.1 µg/ml × h, 6.04 µg/ml and 0.875 h). This showed that all the pharmacokinetic parameters were significantly greater for Liverman capsule compared to Legalon capsule and silymarin tablet, indicating that the absorption and the extent of relative oral bioavailability of silibinin after Liverman capsule were significantly faster and greater than that after Legalon capsule and silymarin tablet, respectively.

In another study designed to assess the absorption of silymarin bound to phosphatidylcholine, plasma silybin levels were determined after administration of single oral doses of silymarin-phosphatidylcholine complex and a similar amount of silymarin to nine healthy volunteers.[54] The bioavailability of the silymarin–phosphatidylcholine complex was much greater than that of silymarin in spite of the rapid absorption with both preparations, as indicated by the higher plasma silybin levels at all sampling times after intake of the complex. The authors concluded that complexation with phosphatidylcholine greatly increases the bioavailability of silymarin, probably by facilitating its passage across the gastrointestinal mucosa.

Vailati ***et al***., in their study designed primarily to evaluate the dose-response relationship of phosphatidylcholine-bound silymarin, demonstrated the positive effects of the complex.[55]

In their study, patients with virus- or alcohol-induced chronic hepatitis were given different doses of the complex: twenty patients received 80 mg twice daily, twenty received 120 mg twice daily, and another twenty patients received 120 mg three times daily, for 2 weeks. At all the doses, phosphatidylcholine-bound silymarin produced a statistically significant decrease of mean serum and total bilirubin levels. When used at dosage of 240 or 360 mg per day, similar results were observed with ALT and GGTP liver enzymes. These results indicated the effectiveness of relatively low doses of phosphatidylcholine-bound silymarin in the short-term treatment of hepatitis caused by viruses or alcohol, though the best results are achieved at higher doses.

Mechanism of Action

Silymarin's hepatoprotective effects are purportedly accomplished via several mechanisms; these include:
- Antioxidation[56,57]
- Inhibition of lipid peroxidation[58]
- Stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration[59]
- Enhanced liver detoxification via inhibition of phase I detoxification[60,61]
- Enhanced glucuronidation and protection from glutathione depletion[41]
- Anti-inflammatory effects, including inhibition of leukotriene and prostaglandin synthesis, Kupffer cell inhibition, mast cell stabilization, and inhibition of neutrophil migration[4,34,47,48,58,62]
- Slowing or even reversing of fibrosis by reduction of the conversion of hepatic stellate cells into myofibroblasts[65]
- Anticarcinogenesis by inhibition of cyclin-dependent kinases and arrest of cancer cell growth
- Silymarin is also found to have immunomodulatory effects on the diseased liver[66,67]

Therapeutic Indications

Amanita mushroom poisoning

The most remarkable use of silymarin is in the treatment of mushroom poisoning caused by **Amanita phalloides** (death cap). Many of the **Amanita** species are highly toxic and ingestion results in severe liver damage and death. This mushroom is known to possess two powerful hepatotoxins, namely, amanitin and phalloidin. Silybin given along with benzyl penicillin has been shown to be effective against amanitin poisoning.[68] In animal studies, silymarin given within 10 min after Amanita toxin ingestion completely counteracted the toxic effects and if given within 24 h of toxin ingestion it prevented death and greatly reduced liver damage.[69]

Carducci *et al.*, in a case study, reported that silybin hemisuccinate given by the intravenous route had a favourable effect on a family of four suffering from severe liver damage caused by Amanita mushroom (amatoxin) compared to when they were treated with the standard therapy.[70] Moreover, investigations done after 2 months showed an absence of any morphological alteration in the hepatobiliopancreatic echography, suggesting that silybin may play a significant role in hepatic tissue protection.

Hepatitis

Studies have shown that silymarin is effective in the treatment of both acute and chronic hepatitis. In acute viral hepatitis, administration of silymarin shortened treatment time and lowered the elevated serum bilirubin, AST, and ALT. In patients with acute hepatitis who were given either silymarin (140 mg) or placebo three times daily for three weeks, the proportion of patients whose AST was normalized was much higher in the treated group (82%) than in controls (52%). In patients with chronic hepatitis, 420 mg silymarin per day for six months also yielded improved serum liver enzyme levels.[71]

Alcoholic liver disease and cirrhosis

Silymarin administration has demonstrated normalization of serum liver enzyme and total bilirubin levels in patients with alcoholic liver disease; there was also improvement in liver tissue histology.[72] In patients with cirrhosis, long-term (41 months) administration of silymarin at 420 mg per day resulted in a significant increase in survival compared to a placebo group.[73]

Hypercholesterolemia

In an animal study conducted by Kreeman *et al.*, silymarin given to rats with diet-induced hypercholesterolemia demonstrated an anticholesterolemic effect similar to probucol, with an increase in HDL cholesterol and a decrease in total and biliary cholesterol.[74]

Psoriasis

Abnormally high levels of cAMP and leukotrienes have been observed in patients with psoriasis and normalization of these levels may improve the condition. The effectiveness of silymarin in the treatment of psoriasis may be due to its ability to improve endotoxin removal by the liver, inhibit cAMP phosphodiesterase, and inhibit leukotriene synthesis.[48,75]

Newer Applications

Silybin/silymarin as chemoprotective and anticancer agents

The chemopreventive action of silymarin helps it inhibit the carcinogenic action of many chemicals. The incidence of urinary bladder neoplasms and preneoplastic lesions induced by N-butyl-N-(4-hydroxybutyl) nitrosamine were significantly reduced.[76] Silymarin also significantly inhibited azoxymethane-induced colon carcinogenesis in rats.[77] Skin carcinogenesis induced by benzoyl peroxide or 12-O-tetradecanoylphorbol-13- acetate was also inhibited by silymarin.[78-80]

Neuroprotective and neurotropic activities of silybin/silymarin

Due to its antioxidative activity, silymarin has been found to be useful in treatment and prevention of many neurodegenerative and neurotoxic processes. Wang *et al.*, demonstrated that silymarin could effectively protect dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting the activation of microglia which represents macrophage-like population of brain cells and which act in host defense and tissue repair in the CNS.[81]

Silybin/silymarin in treatment and prevention of gastrointestinal problems

Many gastrointestinal problems can be treated and/or prevented by silybin/silymarin preparations. In the pancreas,

silybin acts mainly as chemoprotectant and can stimulate recovery after intoxication. Alloxane, which causes necrosis of β-pancreatic cells and lack of insulin secretion, causes production of H_2O_2 which produces cellular damage followed by cell death. Silymarin, due to its antioxidant action, has been found to prevent a rise in both plasma glucose and pancreatic lipid peroxidation in the hyperglycemic rats.[82,83]

Silybin/silymarin in treatment and prevention of cardiopulmonary problems

During cancer therapy, the use of cardioprotective drugs, e.g. doxorubicin, is limited by the cardiotoxicity that is known to be mediated by oxidative stress and induction of apoptosis. Silybin, due to its antioxidant effect, can be very effective in such cardioprotective applications. In the study conducted by Chlopèìková *et al.*, the cell membrane stabilizing and radical scavenging potency of silymarin and its isolated components helped to protect cardiomyocytes (rat) against doxorubicin-induced oxidative stress.[84]

Silybin/silymarin in skin protection

Silymarin has been shown to exhibit preventive effects against photocarcinogenesis in various animal tumor models. Topical application of silymarin to mouse skin reduced UVB-induced tumor incidence, multiplicity, and size compared to that in nontreated animals.[85] Silybin inhibited photocarcinogenesis in mice whether applied topically or administered in the diet.[86]

Adverse Effects

As described earlier in the section on toxicity, silymarin has very low toxicity and has been shown to possess a good safety profile. At high doses, a laxative effect is observed due to increased bile secretion and bile flow.[87]

Adverse effects related to the GI tract such as dyspepsia, bloating, nausea, and diarrohoea were reported in 2-10% of patients in a clinical trial.[88] Serious adverse effects, which are rare, include gastroenteritis associated with collapse and allergy.[8]

Bioavailability enhancement of silymarin

The therapeutic indications mentioned above clearly indicates the potential of silymarin as a natural immunomodulator, but the problem with the use of silymarin lies in its poor bioavailability. Due to this reason the dose of silymarin given needs to be large so as to achieve therapeutic plasma levels. Various groups of scientists have used different approaches to address this problem and have succeeded in improving the bioavailability of silymarin.

Blumenthal *et al.*[90] has reported that the variation in silymarin absorption levels is generally between 20-50%. Several reasons have been attributed for this poor bioavailability, e.g., poor enteral absorption,[91,92] degradation by gastric fluid,[90] or its poor solubility.[90,93-95] This necessitates the incorporation of silymarin into a dosage form that can increase its delivery and hence its bioavailability. A number of studies have been reported in literature that were carried out to enhance the delivery of silymarin. These include complexation with cyclodextrin;[11,96] incorporation in solid dispersion,[97,98] which produced a 2-fold enhancement in the bioavailability of silymarin;[98] provision of silymarin in the form of salts of

polyhydroxyphenylchromanones[93] and other more soluble derivatives;[99] or complexation with phospholipid.[51,55,94]

In order to improve the dissolution rate, Soo Woo *et al.* formulated silymarin in the form of a self-microemulsifying drug-delivery system (SMEDDS).[100] Based on pseudo-ternary phase diagram, the optimum formulation was obtained, consisting of 15% silymarin, 10% glyceryl monooleate as the oil phase, 37.5% mixture of polysorbate 20 and HCO-50 (1:1) as the surfactant, and 37.5% transcutol as the cosurfactant, with a surfactant/cosurfactant ratio of 1. The mean droplet size of the oil phase in the microemulsion was 67 nm. The release profile of silybin from the prepared SMEDDS capsule showed a significant increase in drug release over the reference capsule (Legalon®). The percentage release of silybin from SMEDDS was 2.5 times higher than reference after 6 h. The pharmacokinetic evaluation of silymarin showed that there was an approximately 2-fold decrease in the t_{max} from SMEDDS, 8-fold enhancement in the C_{max}, and 3.6 times increase in the AUC, showing that there was a significant increase in the bioavailability of silymarin from SMEDDS over the conventional reference dosage form.

Abrol *et al.* studied the synergistic hepatoprotective effect of silymarin with phospholipids when encapsulated in microspheres to target the liver and compared the formulations with silymarin solution.[101] Various silymarin-loaded emulsions were formulated with soybean oil as the internal oily phase, soya lecithin as surfactant, and Tween 80 and propylene glycol as surfactant. *In vitro* release profile at 50 rpm showed an almost 4-fold increase in the release after 36 h compared to silymarin solution, indicating that the submicron range lipid emulsions help diffusing the drug in a more pronounced manner and thus increase the bioavailability of the drug. *In vivo* evaluation showed that there was significant reduction in phenobarbitone-induced sleep time (used as a measure of hepatoprotective activity) and reduction in enzymes (SGOT and SGPT) in rats receiving the microspheres as compared to those receiving the control or silymarin solution. Histopathological examination showed that there was almost complete protection of liver cells from carbon tetrachloride-induced intoxication with silymarin in lipid emulsion compared to plain lipid emulsion or silymarin solution. The above results show the positive impact of coupling silymarin with phospholipid in a microparticulate delivery system

To overcome the low bioavailablity of silymarin, Arcari *et al.* prepared an inclusion complex of silybin with β-cyclodextrin. This new complex was compared in *in vitro* tests (dissolution rate) and in an *in vivo* test (rat bile elimination) with silybin, silymarin, and one traditional formulation based on silybin. The results showed a dramatic increase in the dissolution rate of the complex (> 90% within 5 min) compared to the silybin, which is practically insoluble (< 5%). The *in vivo* results agree with the dissolution rates; after oral administration of the silybin complex, silybin concentration in the rat bile was nearly 20 times more than after administration of silybin as such or in a traditional formulation. In the last two cases, the silybin concentration was up to 6 times less than after administration of the same amount of silymarin. These data show that the β-cyclodextrin complexation could help in increasing the bioavailability of silybin.[11]

Yanyu *et al.* prepared silybin–phospholipid complexes to

increase the bioavailability of orally administered silymarin and compared the pharmacokinetic characteristics and bioavailability after administration of silybin–phospholipid complex and silybin–N-methylglucamine in rats.[102] With ethanol as the reaction medium, silybin and phospholipid were dissolved in the organic medium and the solvent was removed under vacuum, which resulted in the formation of the complex. The solubility studies showed a 1000-fold increase in the solubility of the silybin complex in water and octanol compared to the solubility of the physical mixture. Dissolution of silybin complex at pH 6.8 was significantly more than at pH 1.2. At the end of 60 min the amount of silybin dissolved was 158.4 mg at pH 6.8 compared to 6.3 mg at pH 1.2. It was found that the rise in pH increased the dissolution of silybin. Rat bioavailability studies showed more than 5-fold enhancement in the bioavailability of silymarin from the phospholipid complex than the silybin–N-methylglucamine.

Yanyu *et al*. prepared silymarin proliposomes to increase the bioavailability of oral silymarin in beagle dogs.[103] The proliposomes were prepared by film-deposition of a silymarin and phospholipid mixture on a mannitol carrier in a round-bottom flask. Dissolution of proliposomes at pH 1.2 and 6.8 showed that the dissolution of silymarin was complete after 20 min, irrespective of the pH of the media. The dissolution of silymarin from the proliposomes was more than that from the control (silymarin-loaded mannitol powder prepared by same method as the proliposomes, but without the phospholipds). The encapsulation efficiency of the formulation was more than 90%, had a mean particle size of 196.4 nm, and was stable for 3 months at 40°C. The pharmacokinetic parameters for silymarin proliposomes and silymarin showed a t_{max} of 30 min for both and C_{max} of 472.62 and 89.78 ng/ml, and AUC of 2606.21 and 697 ng/ml, respectively, demonstrating enhanced GI absorption of silymarin from the proliposomes.

El-Samaligy *et al*. prepared the silymarin hybrid liposomes by reverse evaporation technique, using lecithin, cholesterol, stearyl amine, and tween 20 in a molar ratio of 9:1:1:0.5.[104] The prepared liposomes showed an encapsulation efficiency of 69.22%. Mixing silymarin-loaded liposomes with unloaded ones in 1:1 proportion was useful in the prevention of aggregates, which threaten liposomes stability. This selected formulation was stable with respect to encapsulation efficiency and particle size after 3 months of storage at 4°C. Differential scanning calorimetery and Fourier Transform Infrared spectroscopy gave evidence of silymarin and phospholipid interaction, which can help in enhancing permeation and hence the bioavailability of silymarin. *In vivo* studies using acute carbon tetrachloride administration produced a significant increase in serum SGPT level that was significantly reduced by the liposomal formulation and the silymarin suspension. The liposomal formulation significantly ($P < 0.001$) reduced the SGPT level compared to suspension. Histopathological examination revealed similar effects with silymarin liposomes and suspension in the improvement of the necrosed area induced by carbon tetrachloride.

Wu *et al*. prepared a lipid-based SMEDDS, using ethyl linoleate (oil phase), tween 80 (surfactant), and ethanol (cosurfactant).[105] *In vitro* drug release studies carried out using the dialysis bag method showed the release of silymarin from SMEDDS as incomplete and having sustained characteristics. The relative bioavailability of SMEDDS was enhanced by an average of 1.88-fold and 48.82-fold from silymarin PEG 400 solution and suspension, respectively. The high bioavailability from the SMEDDS was attributed to its promotion of lymphatic transport[106] and the presence of long-chain oil, which promotes lipoprotein synthesis and subsequent lymphatic absorption.

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 may prove superior to polyherbal formulations in the near future because of its better standardization, quality control, and freedom from microbial and metal contamination.

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