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# SILYMARIN-A REVIEW ON THE PHARMACODYNAMICS AND BIOAVAILABILITY ENHANCEMENT APPROACHES

GHOSH A* GHOSH T AND JAIN S
Department of Pharmaceutics, Himalayan Pharmacy Institute,
Majhitar, Rangpo, Sikkim 737136, India.

## ABSTRACT

Silymarin, a flavonolignan from 'milk thistle' (*Silybum marianum*) plant is used from ancient times as a hepatoprotective drug. Along with hepatoprotective action other actions includes antioxidant, anti-lipid peroxidative, antifibrotic, anti-inflammatory, immunomodulatory and liver regenerating. Silymarin has clinical applications in alcoholic liver diseases, liver cirrhosis, *Amanita* mushroom poisoning, viral hepatitis, toxic and drug induced liver diseases, Psoriasis, and in Neuroprotective and neurotropic activity. Though silymarin does not have antiviral properties against hepatitis virus, it promotes protein synthesis, helps in regenerating liver tissue, controls inflammation, enhances glucoronidation and protects against glutathione depletion. The flavonoid silymarin and one of its structural components, silibinin, are substances with documented hepatoprotective properties. They are proved to be a useful for hepatoprotection in hepatobiliary diseases and in hepatotoxicity due to drugs. The non traditional use of silymarin may make a breakthrough as a new approach to protect other organs in addition to liver. But the main drawback of silymarin is its poor solubility therefore different approaches are been taken to enhance the solubility in turn the bioavailability of the drug. In this review we discuss about its Chemistry, Pharmacodynamics, safety, therapeutic efficacy and Bioavailability enhancement approaches used so far. As it is having a good safety profile, better patient tolerability and an effective drug at an affordable price, in near future new derivatives or new combinations of this drug may prove to be useful.

**KEY WORDS** Bioavailability, herbal drug, hepatoprotective, milk thistle, silymarin.

## INTRODUCTION & HISTORICAL ASPECTS

*Silybum marianum*, commonly known as 'milk thistle' (Family: Asteraceae/Compositae) is one of the oldest and thoroughly researched plants in the treatment of liver diseases.[1]It is an annual or bi-annual plant up to 2 meters high. It has large, bright leaves with characteristic white stains along the nerves and with waved and spiny margins. At the bottom of the stem and branches appears the flower capitulum between 4 and 8 cm diameter, composed by tubular purple flowers wrapped in several rows of coriaceous bracts. The most external ones ending in a strong spine. The fruit is achenium type, brown-black colour with grey stains, bright and non-striated. Its size is about 7 mm length coronated by a white down of simple and deciduous hair. Its fruits may be confounded with the seeds, due to its size and shape.[2] The leaves are characterized by milky veins, from which the plant derives its name(3). The extracts of milk thistle is being used as a general medicinal herb from as early as 4$^{th}$ century B.C. and first reported by Theophrastus.[1, 4] This plant is used as emetic in 1$^{st}$ century A.D. by Dioskurides and also became favored medicine for hepatobiliary diseases in 16$^{th}$ century and the drug was revived again in 1960 in central euripi.[3,4] The active constituents of the plant are obtained from the dried seeds and consist of four flavonolignans which are collectively known as silymarin. Wagner *et a* [5] characterized these active compounds and Flora *et al* [6] reviewed its history, properties and the clinical effects. Silymarin is widely prescribed by herbalists and has almost no side effects. The plant is native to the Mediterranean and grows throughout Europe and north America. It also grows in India, china, South America, Africa and Australia. [7]

## CHEMISTRY

The seeds of milk thistle contain approximately 70-80% silymarin flavonolignans and approximately 20-30% of chemically undefined fraction, composed of mostly polymeric and oxidized polyphenolic compounds.[8] Silymarin is a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin as shown

in figure 1 with an empirical formula $C_{25}H_{22}O_{10}$. Among the isomers silybin is the major and most active component and represents about 60-70 per cent, followed by silychristin (20%), silydianin (10%), and isosilybin (5%).[9] The seeds also contain betaine, trimethylglycine and essential fatty acids that may contribute to silymarins hepatoprotective and anti-inflammatory activities.[3,7,9]

## PHARMACOKINETICS

Due to poor solubility of silymarin it is administered in the form of encapsulation, sugar coated tablets, self-microemulsifying drug delivery system (SMEDDS) or beta cyclodextrin inclusion complex etc. The absorption by oral route is as low as 2-3 per cent of the silybin recovered from rat bile in 24 h. About 20-40 per cent of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates in human beings. [9] The peak plasma concentration is achieved in 6 to 8 hrs and its elimination half life is approximately 6 hrs.[1,8] 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. [10] Pharmacokinetic studies with silybin phosphatidylcholine complex have shown an increase in the oral bioavailability of silybin in healthy human subjects, probably by a facilitatory role of drug complex on the passage of the drug across the gastrointestinal tract.[11] Silybin dihemisuccinate is given in emergency cases with the poisoning of *Amanita phalloides*.[12]

## TOXICITY

Acute toxicity studies of silymarin after intra venous infusion have been carried out on mice, rat, rabbit and dog. The $LD_{50}$ values were 400mg/kg for mice, 385mg/kg for rats and 140mg/kg for rabbits and dogs though these values may vary depending on infusion rate. With slow infusion over 2 to 3 hours the LD50 was 2gm/kg in rats and after oral administration it was 10g/Kg. [13]

Silybin

Silydianin

Isosilybin

Silycristin

**Figure 1 Structures of flavonolignan isomers of silymarin.**

**PHARMACODYNAMICS**.

*Antioxidant Properties*

Silymarin is an antioxidant and free radical scavenger. It can also interact directly with the cell membrane components to prevent any abnormalities in the content of lipid fraction responsible for maintaining normal fluidity. [14] 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. [15]

*Activity against Lipid Peroxidation*

Lipid peroxidation is caused by interaction of free radicals with unsaturated fatty acids in lipids resulting in broad spectrum of alterations and the consequent degeneration of cell membranes. Silymarin appears to act as an antioxidant not only because it acts as a scavenger of the free radicals that induce lipid peroxidation, [16,17] but also because it influences enzyme systems associated with glutathione and superoxide dismutase.[18]

*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.[19] Interestingly, the increase in protein synthesis was induced by silibinin only in injured livers, not in healthy controls.[20] 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.[3]

*Anti-Inflammatory and Anticarcinogenic Properties*

The molecular bases of the anti-inflammatory and anticarcinogenic effects of silymarin are not yet known; they might be related to the inhibition of the transcription factor NF-kB, which regulates the expression of various genes involved in the inflammatory process, in cytoprotection and carcinogenesis.[21,22] 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.[3,10] The inhibitory effect on 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis is a pivotal pharmacological property of silymarin.[9] 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 non melanoma 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 cyclooxygenase and ornithine decarboxylase activity. This effect provides protection against photocarcinogenesis. [10, 23]

*Antifibrotic Effects*

On prolong exposure to ethanol or carbon tetrachloride hepatic stellate cells proliferate and transform into myofibroblast responsible for deposition of collagen fibres in liver. Recently, the effects of silibinin on the transformation of stellate cells into myofibroblasts have been investigated. The results have shown that silibinin, at a concentration of 100µmol/L reduces the proliferation of stellate cells isolated from fresh liver of rats by about 75%, reduces the conversion of such cells into myofibroblasts, and down regulates gene expression of extracellular matrix components indispensable for fibrosis.[24]

*Amanita phalloides toxin*:
Prevention of liver toxicity is possible if silymarin is given as pretreatment or up to 10 min after amanita toxin poisoning.[1] 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.[10] Silymarin has also been found to protect liver cells from injury caused by viral hepatitis.[3]

**Overview of Mechanisms of Action**

Silymarin's hepatoprotective action is due to following mechanisms. These includes

- activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of GSH
- inhibition of the transformation of stellate hepatocytes into myofibroblasts, which are responsible for the deposition of collagen fibres leading to cirrhosis.
- 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.
- Stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration.
- Enhanced glucoronidation and protection from glutathione depletion.[25]
- Immunomodulatory effects on diseased liver.

## THERAPEUTIC ACTIVITY

*Viral Hepatitis*

Studies show that silymarin is effective in both acute and chronic hepatitis. Studies showed that administration of silymarin shortens treatment time and lowers serum bilurubin, AST and ALT. In acute hepatitis silymarin 140mg dose three times daily for three weeks shows lower levels of AST then control groups. In patients with chronic hepatitis 420 mg of silymarin per day for six months resulted in a significant improvement in serum liver enzyme levels. [27]

*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.[3] 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.[6]

**Figure 2 Mechanism of action of silymarin as proposed by Valenzuela and Garrido [26]**

*Alcohol liver diseases and cirrhosis*

Silymarin given to patients has showed lower levels of bilurubin and liver enzymes with alcoholic liver diseases. In patients with cirrhosis, a long term administration of silymarin at 420 mg per day resulted in significant increase in survival compared to a placebo group [27]

*Psoriasis*

Silymarin has shown to improve livers activity in removal of endotoxin, inhibit cAMP phosphodiesterase and inhibit leukotriene synthesis. This causes improvent in condition of psoriasis as increases levels of cAMP and leukotrieins are characteristics of psoriasis.[28]

*Neuroprotective and neurotropic activity*

Silymarin may be useful in the treatment and prevention of some neurodegenerative and neurotoxic process partly due to antioxidant activity and may be due to some unknown mechanism. Silymarin inhibits TNF-_ and reduced production of inducible nitric oxide synthase which cause microglia activation78. Further silymarin may be of use in protecting primary hippocampal neurone against oxidative stress induced apoptosis and neuromodulatory action against persistent viral infections leading to encephalitis.[1]

## ADVERSE EVENTS

Silymarin is reported to have a very good safety profile 5. Both animal and human studies showed that silymarin is non toxic even when given at high doses (>1500 mg/day). However, a laxative effect is noted at these doses may be due to increased bile secretion and bile flow.[29] Most commonly noted adverse effects were related to gastrointestinal tract like bloating, dyspepsia, nausea, irregular stool and diarrhoea. It was observed in 2 to 10 per cent of patients in clinical trials, which were similar to placebo.[30] It also produced pruritus, headache, exanthema, malaise, asthenia, and vertigo.[9]

## BIOAVAILABILITY ENHANCEMENT OF SILYMARIN

Oral absorption of silymarin is of about 23-47%, leading to low bioavailability of the drug. This is due to the poor water solubility and hence different approaches by the researchers are been used to improve the solubility in turn improving the bioavailability of the drug.

In order to improve the solubility and dissolution of silymarin Soo Woo j. et al. formulated silymarin in the form of self-micro emulsifying drug delivery system (SMEDDS). The optimum formulation of SMEDDS containing silymarin was obtained based on the study of pseudo-ternary phase diagram. The SMEDDS consisted of 15% silymarin, 10% glyceryl monooleate as the oil phase, a mixture of polysorbate 20 and HCO-50 (1:1) as the surfactant, Transcutol as the co-surfactant with a surfactant/co-surfactant ratio of 1. The mean droplet size of the oil phase in the micro-emulsion formed from the SMEDDS was 67 nm. The percentage release of silybin from the SMEDDS after 6 hours was 2.5 times higher than that from the reference capsule (Legalon$^{R}$). After its oral administration to rats, the bioavailability of the drug from the SMEDDS was 3.6 times higher than the reference capsule.[31]

Garg R. et al. formulated floating effervescent tablets of silymarin using various materials like hydroxypropyl methylcellulose (HPMC) K 4M, K 15M, psyllium husk, swelling agent such as crospovidone, microcrystalline cellulose gas generating agent like sodium bicarbonate, citric acid and evaluated the floating properties, swelling characteristics and *in vitro* drug release studies of the formulations. Floating non effervescent tablets were prepared by polypropylene foam powder and different matrix forming polymers like HPMC K 4M, Carbopol 934P, xanthan gum and sodium alginate. *In vitro* drug release studies were performed and drug release kinetics evaluated using the linear regression method was found to follow both the Higuchi and the Korsemeyer and Peppas equation. The drug release mechanism was found fickian type in most of the formulations. The developed floating tablets of Silymarin may be used in clinic for prolonged drug release for at least 24 h, thereby improving the bioavailability and patient compliance.[32]

Nakhat P D et al. in order to improve the bioavailability of silymarin formulated tablets

of silymarin HP-β-CD solid dispersion. Tablets were prepared by direct compression using superdisintegrants such as crosscarmellose sodium, sodium starch glycolate and polyplasdone XL in different concentration. Developed formulations were evaluated for hardness, friability, weight variation, drug content disintegration time and invitro dissolution profiles. Among different batches, formulation containing crosscarmellose sodium showed improved dissolution over marketed formulation reflecting vital role of HP-β-CD dispersion in promoting oral bioavailability of silymarin. More over optimized formulation showed stability at varying temperature and relative humidity.[33]

Abrol S et al. studied the synergistic hepatoprotective effect of silymarin with phospholipids when it is encaged in microspheres so as to passively target it to liver and to compare these silymarin formulations with silymarin solution. Various silymarin loaded lipid emulsions were formulated which include formulation A prepared with soyabean oil as an internal oily phase, soya lecithin as surfactant and tween 80 as co-surfactant; formulation B which was same as formulation A but was filtered through 0.45µ membrane filter and finally steam sterilized for intravenous administration; formulation C containing soyabean oil as an internal oily phase, soya lecithin as surfactant, tween 80 and propylene glycol as co-surfactant/ cosolvent. These formulations were compared for their release profile with silymarin solution in propylene glycol, i.e. formulation D. I*n vivo* evaluation was carried out using three models i.e. phenobarbitone induced sleep time in mice, biochemical estimation of SGOT and SGPT enzyme levels and histopathological examination of rat livers. Results revealed that there was significant reduction in sleep time in the mice treated with silymarin loaded lipid microspheres (both p.o. as well as i.v.) when compared with control and even with plain lipid microspheres and silymarin solution and significant reduction in enzyme levels in silymarin lipid microspheres treated group when compared with control, plain lipid microspheres as well as silymarin solution treated group. Histopathological studies also supported the results obtained from the other two models. A positive outcome of these studies gave an insight that if silymarin is coupled with phospholipid in such microparticulate delivery systems, hepatoprotective effect of drug molecules can be pronounced further by self targeting nature and synergistic action.[34]

In another study conducted by Qiu Ming-feng et al. solid dispersions in the form of "dripping pills" were designed to enhance solubility. Dripping pills of silymarin were prepared at a 1:4 ratio by the traditional fusion method with the use of a mixture of silymarin and polyethylene glycol 6000 (PEG 6000). The prepared dripping pills were spherical and 3 to 4 mm in diameter, with an average weight of 30 mg per pill and with each pill containing 5 mg of silymarin. The dissolution rates of silymarin in dripping pill and of 3 other silymarin preparations, including Yiganling Film-Coating Tablet, Yiganling Sugar-Coating Tablet, and Legalon Capsule, were determined in pH 1.2 medium. The dissolution rate ($T_{50}$) of the silymarin dripping pill was found to be significantly higher (by a factor of 7.5–11) than those of the other 3 preparations.[35]

Yanyu et al. formulated proliposomes of silymarin to increase the bioavailability of silymarin in beagle dogs. The proliposomes were prepared by film- deposition of silymarin and phospholipid mixture on a mannitol carrier in a round bottle flask. Dissolution of proliposomes at pH 1.2 and 6.8 showed that dissolution was completed after 20 min irrespective of the ph of the media. The dissolution of silymarin fro the proliposomes was more than that of the control(prepared by same method but without phospholipids). The encapsulation efficiency of the formulation was more than 90%, mean particle size of 196.4 nm, and was stable for 3 months at 40$^{o}$C. The pharmacokinetics data of 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 697ng/ml.

respectively. This data shows enhanced GI absorption of silymarin from proliposomes.[36] Arcari et al. formulated silybin β- cyclodextrin molecular inclusion complex to overcome the low bioavailability of silymarin. The complex prepared was subjected to invitro dissolution study and in vivo test (rat bile elimination) with silybin, silymarin and one traditional formulation based on silybin. The results showed an manifold increase in dissolution of complex as compared with silybin. The in vivo results also agrees with the dissolution results; after oral administration of silybin complex, silybin concentration in rat bile was 20% more than after administration of silybin as such or in traditional formulation. In last two cases, the silybin concentration was up to six times less then after administration of same amount of silymarin. These data revealed that inclusion complex could increase the bioavailability of silybin.[37]

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