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Silymarin: an old remedy with a challenging future?

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ABSTRACT

Silymarin, a flavanolignan-containing standardized extract obtained from the fruits of *Silybum marianum*, is a traditional phytotherapic product widely used for the treatment of liver diseases including hepatitis , cirrhosis and bile secretion dysfunction. Recent preclinical data and some clinical investigations also support its use in the management of non-alcoholic fatty liver disease (NAFLD). Despite this evidence, silymarin efficacy has not yet been supported by clear-cut dose-related pharmacokinetic data, although several attempts have been made to enhance the oral absorption of flavanolignans, its putative active principles. However, positive results have been obtained with lecithin-based formulations (Phytosome®) of silybin, the main flavanolignans in silymarin, which showed improved oral absorption in preclinical and clinical trials. The Phytosome® delivery system has also been applied to silymarin with promising preclinical results consistent with the oral bioavailability of most of the flavanolignans present in the extract. These data may help optimize the dose for clinical efficacy in liver protection and also pave the way for the use of silymarin in new therapeutic areas where recent findings indicate a major role for the entire phytocomplex.

Keywords
Silymarin
Flavanolignan
Pharmacokinetics
Phytosome®

Introduction

Silybum marianum (L.) Gaertn. (syn. *Carduus marianus* L.) is a herbaceous annual or biennial plant belonging to the *Asteraceae* family (*Compositae*) and a native of the Mediterranean region. The plant has been used for medicinal purposes since the time of Pliny and Dioscorides (1st century A.D.) and is thought to have been first used for liver protection in the 16th and 17th centuries. In the 18th, 19th and first half of the 20th century, it was employed to treat liver disease and disorders of the bile duct [1]. In the second half of the 20th century, several components were identified, including a mixture of flavanolignans (1.5–3.0%) called silymarin. Purified components of silymarin were then isolated and their structures elucidated, mainly by German phytochemists [2, 3]. Clinically certified silymarin (Legalon®, originally developed by Madaus Pharma, Germany) contains 65–80% of a mixture of seven flavanolignans having molecular weight 482 and formula $C_{25}H_{22}O_{10}$ (i.e., silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) resulting from the chemo-, regio- and stereoisomeric oxidative coupling of the flavonol taxifolin and the lignin coniferyl alcohol. Silymarin also contains small amounts (1.6–2.2%) of taxifolin (Fig. 1), fatty acids and polyphenolics. Silybin is the major constituent of silymarin (40–65%), with silychristin and silydianin accounting for 20–45% and isosilybin for 10–20%.

Following isolation of the active ingredients of silymarin, in 1975 pharmacological evaluation of the extract and its flavanolignans (i.e., silybin, silydianin and silychristin) revealed its efficacy in several experimental models of liver intoxication induced by carbon tetrachloride, thioacetamide, α-amanitin and phalloidin [4]. Subsequently, silymarin and particularly silybin (the most active flavanolignan) have been studied for their anti-hepatotoxic effects (Table 1) as well as their capacity to modulate several parameters associated with liver damage in animal models of NAFLD (Table 2) [5]. Biochemical assays have also been conducted to elucidate the potential mechanisms of action (Table 3).

Most results support the use of silymarin (daily dosage 210–800 mg) for the treatment of liver diseases (hepatitis, cirrhosis and bile secretion dysfunction) caused by alcohol, other toxins, or viral infection. In addition, preclinical evidence derived from NAFLD models has been promising and studies are ongoing [6–12].

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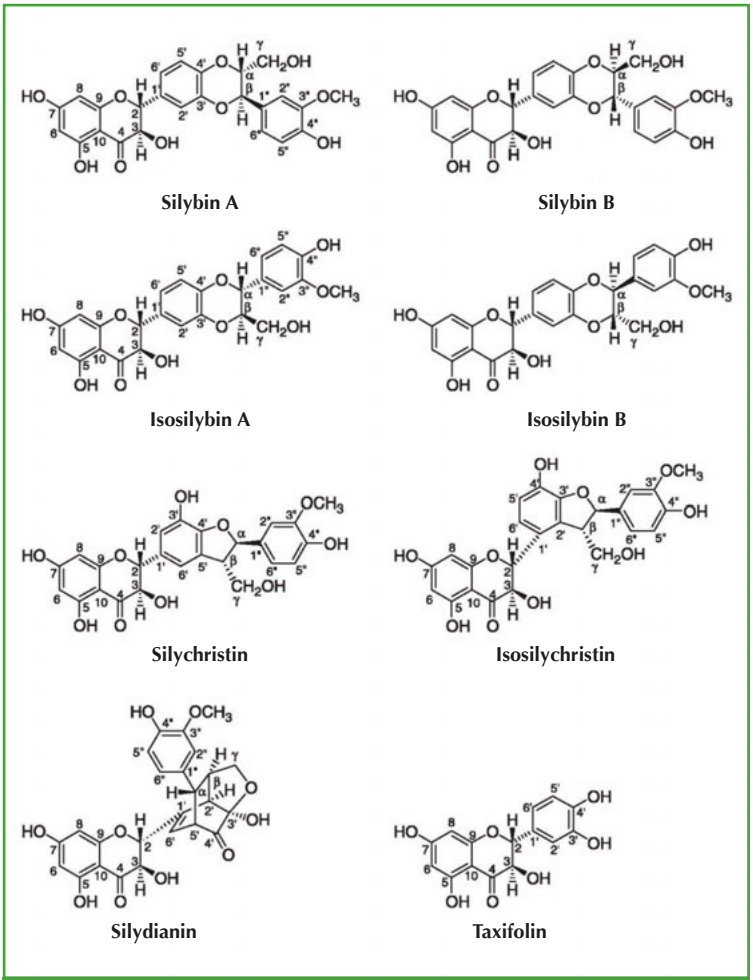

Figure 1 - Structure of silymarin flavanolignans and taxifolin

Intoxicant	Animal	Silymarin/silybin
Carbon tetrachloride (CCl_4)	Rat	Active
Heavy metals (lead, cadmium)	Rat	Active
Drugs		
Azathioprine	Rat	Active
Indomethacin		
Isoniazide		
Lorazepam	Rat	Active
Tolbutamide		
Clofibrate		
Estradiol	Chicken	Active
Halothane	Mouse	Active
Thioacetamide	Rat	Active
Endotoxin of *Escherichia coli*	Rat	Active

Table 1 - Experimental models of liver intoxication effects of silymarin and silybin (from [1] updated 2018)

Parameter	Number of studies (animal)	Treatment duration (weeks)
Body weight	6 (rat, mouse, gerbil)	3–12
Liver histology	7 (rat, mouse, gerbil)	3–12
AST/ALT	5 (rat, mouse, gerbil)	4–12
Insulin sensitivity	5 (rat, mouse, gerbil)	3–12
Liver oxidative stress	5 (rat, mouse, gerbil)	3–8

Table 2 - Experimental models of the effects of silymarin and silybin on non-alcoholic fatty liver disease (adapted from [5])

Activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of glutathione (GSH)
Ability to regulate membrane permeability and to increase membrane stability in the presence of xenobiotic damage
Capacity to regulate nuclear expression by means of a steroid-like effect (attributed to a structural similarity of silymarin to steroid hormones) followed by tissue regeneration
Inhibition of the transformation of quiescent hepatic stellate cells into activated myofibroblasts which are responsible for the deposition of collagen fibres leading to cirrhosis
Anti-inflammatory effect resulting in a decrease in hepatic inflammation and inflammatory cytokines, possibly as a result of reduced tissue damage

Table 3 - Main reported properties of silymarin flavanolignans (from [6])

Pharmacokinetics

Preclinical and clinical studies of silymarin have shown mixed results. The relationship between clinical efficacy and dose–exposure is still unclear due to lack of information on levels of the active silymarin flavanolignans after treatment with standard dosage regimens. In addition, inter-subject variability in the kinetics of silymarin flavanolignans has complicated investigations. Silymarin flavanolignans are characterized by low water solubility, low gastrointestinal absorption and low and erratic oral bioavailability of 0.73–0.95% in rats. This finding is confirmed by pharmacokinetic parameters measured after oral administration of clinically effective doses in healthy human volunteers (Table 4). After a single oral dose of 600 mg silymarin, plasma concentrations of total flavanolignans are about 300 ng/ml (C_{max} values), which is far below the expected therapeutic values of 12–60 µg/ml [13]. However, the type of liver condition strongly affects the kinetics of silymarin flavanolignans [14–16]: an oral dose of 600 mg of silymarin in patients resulted in C_{max} val-

Plasma concentration	SC	SD	SB_A	SB_B	ISB_A	ISB_B	Silymarin
	ng/ml (% total)						
Free	7.52 (14.0)	ND	21.7 (60.0)	16.9 (15.2)	3.72 (5.73)	6.30 (18.5)	53.1 (17.4)
Sulfated	19.8 (36.8)	ND	5.83 (15.8)	12.8 (14.1)	38.6 (59.5)	7.26 (21.4)	84.3 (27.6)
Glucuronidated	26.5 (49.2)	24.4 (100)	9.26 (25.2)	64.4 (70.7)	22.6 (34.8)	20.50 (60.1)	168.0 (55.0)
Total	53.8	24.4	36.8	91.1	64.9	34.1	305

ND not detected; *SC*: silychristin; *SD*: silydianin; SB_A: silybin A; SB_B: silybin B; ISB_A: isosilybin A; ISB_B: isosilybin B

Table 4 - Summary of pharmacokinetics parameters of silymarin flavanolignans in healthy volunteers after oral dosing (from [13])

ues of total flavanolignans which were 2.4 to 4.7-fold higher than in healthy volunteers. The increases in plasma AUCs in patients was dependent on the type of pathology, with four-fold higher concentrations observed in those with HCV and cirrhosis and three-fold higher concentrations seen in those with NAFLD. Patients with NAFLD showed clear evidence of enterohepatic cycling of flavanolignans, which could partially explain the efficacy of silymarin in these subjects.

Optimizing flavanolignan oral bioavailability: a multi-faceted approach

Silybin

Only a few attempts to improve/optimize flavanolignan bioavailability have concentrated on silybin, which is the major component (50–60%) of silymarin and contains an approximately equimolar mixture of the two diasteroisomers silybin A and silybin B. Similarly to other flavanolignans with highly hydrophobic and non-ionizable structures, silybin has very low solubility in water, which accounts for its extremely poor oral bioavailability. The oral bioavailability of silybin in rats is estimated to be 0.95% or less of the administered dose. Various methods have been proposed to increase silybin bioavailability, including the production of a lecithin-formulated product (Phytosome®) which, since the early 1990s, has allowed the development of new orally bioavailable products in the pharmaceutical and nutraceutical fields [17–21]. An orally bioavailable silybin combined with vitamin E (Siliphos®, Indena S.p.A., Milano, Italy) has been recently developed for the management of NAFLD [22].

Preclinical tests have also been conducted to investigate the ability of mixed micelles formed from bile salts to improve silybin oral bioavailability, with some positive results being obtained in dogs [23].

Silymarin

Several suggestions have been made to improve the oral bioavailability of silymarin flavanolignans using different formulations. This would also allow the correct dose-related response to be determined and the efficacy of the entire silymarin 'phytocomplex' to be studied and its therapeutic use expanded. Recent findings have shown that silydianin and isosilybin, which are other flavanolignans present in silymarin, have additional biological properties. Silydianin has been reported to enhance the anti-inflammatory effect of silymarin through its ability to activate caspase-3 and also to contribute to the unique antioxidant profile of silymarin [24, 25]. In addition, isosilybin can modulate key steps of cellular proliferation in prostate cancer, suggesting that silymarin might be used in the management of this slowly progressing condition [26–28]. Silymarin also recently has been reported to impact the immune system by targeting inflammatory and oncogenic translational pathways, including the interaction with T-lymphocyte function [29]. In addition, silymarin exerts antitumor effects in patients with advanced systemic disease including brain metastasis [30], which may be partially explained by the capacity of silymarin flavanolignans to specifically inhibit STAT3 with a unique bimodal mechanism [31].

Most of these data indicate that silymarin as a phytocomplex (Fig. 2) administered in a formulation with good oral bioavailability could be used in controlled clinical investigations. The Indena food-grade delivery system (Phytosome®) was applied to silymarin standardized extract to generate pre-

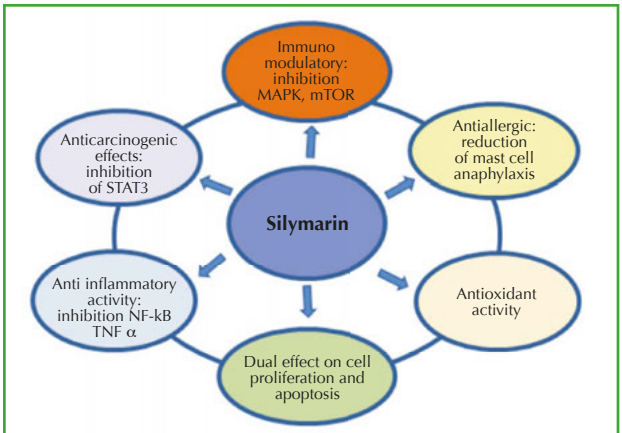

Figure 2 - Therapeutic potential of silymarin (modified from [29])

clinical pharmacokinetic data. The findings show improved oral absorption of the major flavanolignans. In this study conducted in rats, the bioavailability of the major flavanolignans (silybin, isosilybin, silydianin and silychristin) was studied after oral administration of silymarin versus silymarin-Phytosome® (19.9% silybin-like substances) at the dose of 1 g/kg (as silymarin). Two groups of fasting male rats were given an aqueous suspension of silymarin or silymarin-Phytosome® and blood samples were taken 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours after administration. Plasma levels of unmodified silybin, isosilybin, silydianin and silychristin were measured using a validated HPLC method (detection limit 150–200 ng/ml). The results demonstrate that silybin and isosilybin were detectable in each animal in a μg/ml order of magnitude; silydianin was measurable in only three out of five silymarin-Phytosome®-treated animals; silychristin was undetectable in both silymarin and silymarin-Phytosome®-treated animals. The AUC (0–24 hours) values calculated using the trapezoidal rule for total flavanolignans were 7.0±4.3 and 1.1±0.8 μg/h/ml after silymarin-Phytosome® and silymarin, respectively, showing a 6.3-fold increase in oral bioavailability (Fig. 3). In the same plasma samples, the content of total silybin, isosilybin, silydianin and silychristin was also determined using the same HPLC method after enzymatic hydrolysis with mixed β-glucuronidase/arylsulfatase. The results reveal that all flavanolignans were present in plasma in measurable amounts. The calculated AUC (0–24 hours) values for the flavanolignans (silybin+isosilybin+silydianin+silychristin) were 278.1±135.6 μg/h/ml for silymarin-Phytosome® and 106.2±8.9 μg/h/ml for silymarin, respectively, demonstrating a 2.62-fold increase in total absorption of the most relevant flavanolignans (Fig. 4).

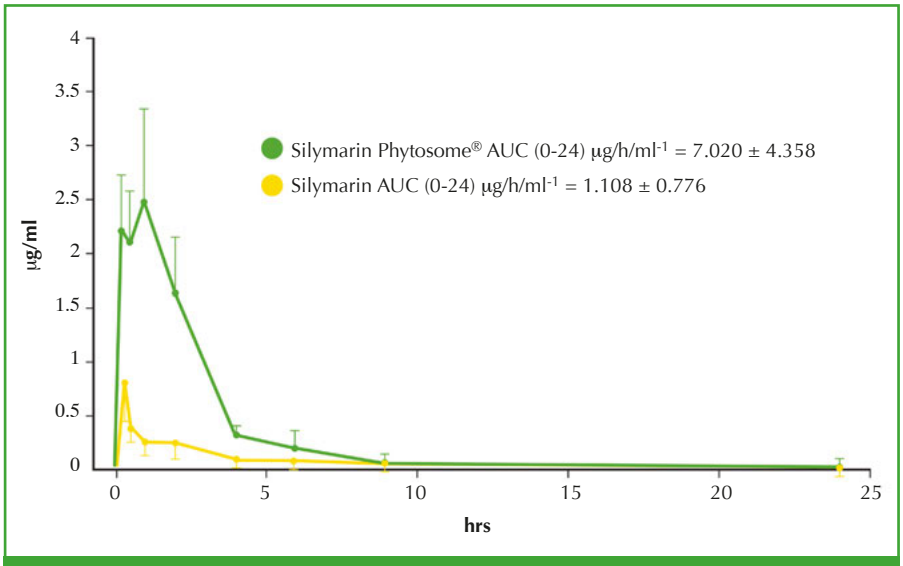

Figure 3 - Plasma profile (mean ± SE) of total immodified flavanolignans after oral administration of silymarin Phytosome® (1g/kg as silymarin; n=5) and silymarin (1g/kg; n=5) in male rats

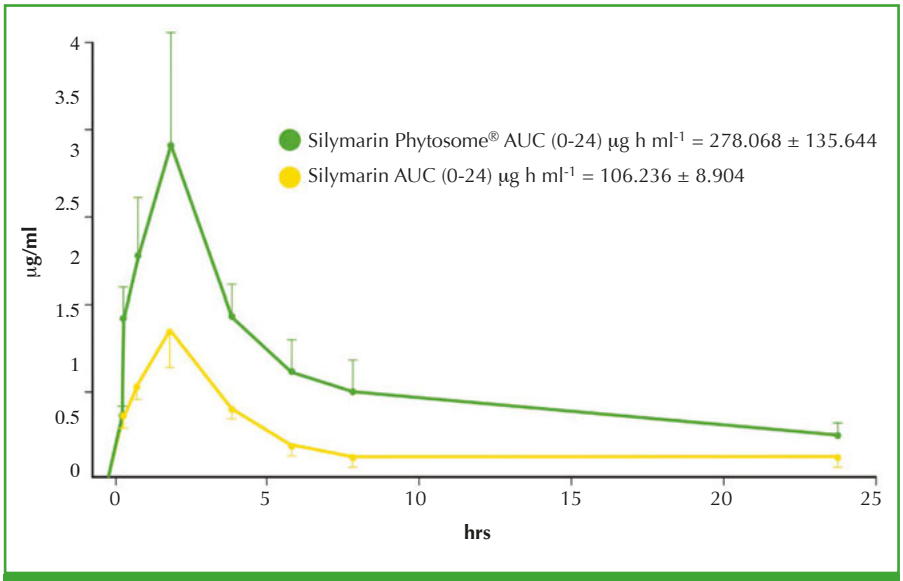

Figure 4 - Plasma profile (mean ± SE) of flavanolignans (after enzymatic hydrolysis) after oral administration of silymarin Phytosome® (1g/kg as silymarin; n=5) and silymarin (1g/kg; n=5) in male rats

Other attempts to increase silymarin flavanolignan bioavailability have used self-microemulsifying drug delivery systems (SMEDDS) which usually employ an isotropic mixture of a drug (in this case silymarin), oil, an emulsifier and a co-emulsifier. This approach has focused mainly on silybin bioavailability (which was increased several fold), but no information is available on other flavanolignans present in the administered extract [32, 33].

Another recent promising approach is related to surface-attached silymarin-loaded solid dispersion with an improved dissolution profile. When tested in rats, this formulation based on silymarin, polyvinylpyrrolidone (PVP) and Tween 80, showed better bioavailability of silybin, but again no information on additional flavanolignans was provided [34].

Conclusion

New insights into the pharmacological activities of silymarin flavanolignans suggest the entire phytocomplex should be reconsidered. In addition, the development of new validated formulations with better oral bioavailability (e.g., Phytosome®) will encourage the clinical development of this traditional phytotherapic remedy. However, defining the correct dose–effect relationships will be challenging.

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