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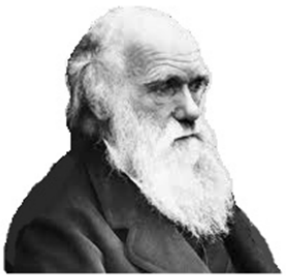

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Pharmacological, Biochemical and Therapeutic Potential of Milk Thistle (Silymarin): A Review

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ABSTRACT

Silymarin, a flavonolignan from the seeds of 'milk thistle' (*Silybum marianum* (L.) Gaertn.)), has been widely used from ancient times because of its excellent hepato-protective action. It is a mixture of mainly three flavonolignans, which are, silybin, silidianin, and silychristin, 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.

***Keywords*:** Bioavailability, hepato-protection, milk thistle, silymarin, *Silybum marianum*

PRACTICAL APPLICATION

Silymarin is known to be a complex mixture of compounds which is derived from plants. These compounds are known to be antioxidants with several biological properties, some of

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which include anticancer activity, anti-inflammatory, antifibrotic and antioxidant properties. Although, there are several side effects of silymarin in people, like allergies, insomnia, itching, bloating and diarrhea but of several benefits. Hence, this review elucidates, the biochemical and pharmacological effect of silymarin, in order to expound its therapeutic role.

1. INTRODUCTION

Silymarin is a standardized extract from the plant named *Silybum marianum* (L.) Gaertn., also known as Milk Thistle. *Silybum marianum* belongs to the Asteraceae/Compositacea family. This plant has been used over the years for treatment of liver diseases [1].

Silybum marianum can also be classified as a member of *Carduus marianum* family, is a plant which has been used for the past two decades for treatment of different diseases such as liver and gallbladder disorders, also protecting the liver against snake bite and insect stings, mushroom poisoning and alcohol abuse [1].

Milk Thistle can be found in countries such as Kashmir, North America, Canada and Mexico. It is usually seen with large leaves and a reddish-purple flower that are all thorny. The medicinal part in Milk Thistle is either the seeds or fruits [2].

Figure 1. Common Names: Milk thistle, Mary thistle, holy thistle Latin Names: *Silybum marianum* (L.) Gaertn., synonym *Carduus marianus* L.

Apart from the liver disease, silymarin effects have also been indicated in various illnesses of different organ such as prostate, lungs, Central Nervous System, kidneys, breast, pancreas, and skin [3].

Silymarin can also be produced in callus and cells suspensions of *Silybum marianum* [4].

Silymarin is a complex mixture of compounds that can be derived from plants. This compounds include flavonolignans, flavonoids (taxifolin, quercetin) and polyphenolic

molecules [5]. These compounds are known to be antioxidants and also they have other biologic properties [6]. Silymarin has the ability to antagonize the toxin of *Amanita phalloides* [7].

Apart from the hepato-protective action against several liver diseases, silymarin also exhibits anticancer activity, anti-inflammatory, antifibrotic and antioxidant properties [8]. This means silymarin can be used in the treatment of cancer, inflammation and fibrosis.

The long term effect of the use of milk thistle brings about the following side effects in people: allergies, insomnia, itching, bloating and diarrhea. Because of the lack of conclusive findings on the safety of milk thistle, it is not advised to be used by children and pregnant women and if it is to be used it must be recommended by the doctor.

The general benefits of Milk Thistle are:

- It provides safety for the liver against invading diseases.
- It manages and slows down the deterioration of the brain processes due to advancing age.
- It helps in retaining the best conditions for your bones.
- It contributes to the treatment of cancer.
- It aids in increasing the breast milk production in lactating mothers.
- It could help in treating pimples and other facial and skin problems.
- It helps in reducing the high sugar blood level of diabetic people.

2. BIOCHEMICAL CONSTITUENTS, PHYTOCHEMICAL AND PROXIMAL CONSTITUENTS OF SILYMARIN

Silymarin is made up of different biochemical constituents which include 10% of silydianin, 5% of isosilybin and within the range of 10 to 30% of an unidentified organic polymer fraction.

Also, there is a minor fraction of other flavanols which include 2,3-dehydrosilybin (DHSB), quercetin, taxifolin, kaempferol and others is present [9,10]. Silymarin is a flavonoid complex that can be extracted from the seeds of milk thistle and it is composed of three isomers [11]. In a milk thistle that is standardly extracted, 70% of silymarin, a mixture of the flavonolignans (silydianin, silychristin), and silibinin are the major constituents that will be found in it [12]. Other constituents present in Milk Thistle include dehydrosilybin, desoxysilydianin, and silybinomer.

The structure of the constituents of silymarin is shown below. The structure was clarified in the 1960s (Figure 2).

Silibinin, also known as silybin, is the major active constituent of silymarin, a standardized extract of the milk thistle seeds, containing a mixture of flavonolignans consisting of silibinin, isosilibinin, silichristin, silidianin, and others. [13]. The four main flavonolignan isomers in silymarin are silibinin, isosilibinin, silichristin and silidianin, but the most active biological constituent is silibinin (also called silybin). This shows that about 50–60% of the silymarin component is silibinin, with the other flavonolignan isomers which comprise about 35%: silichristin (20%), silidianin (10%) and isosilibinin (5%) [14, 9].

Silibinin is a polyphenolic flavonoid antioxidant with the molecular formula of $C_{25}H_{25}O_{10}$ and with a molecular weight of 482.44 g/mol [15]. It is mixture of two diastereomers which include silibinin A and silibinin B, in an approximately equimolar ratio (Figure 3).

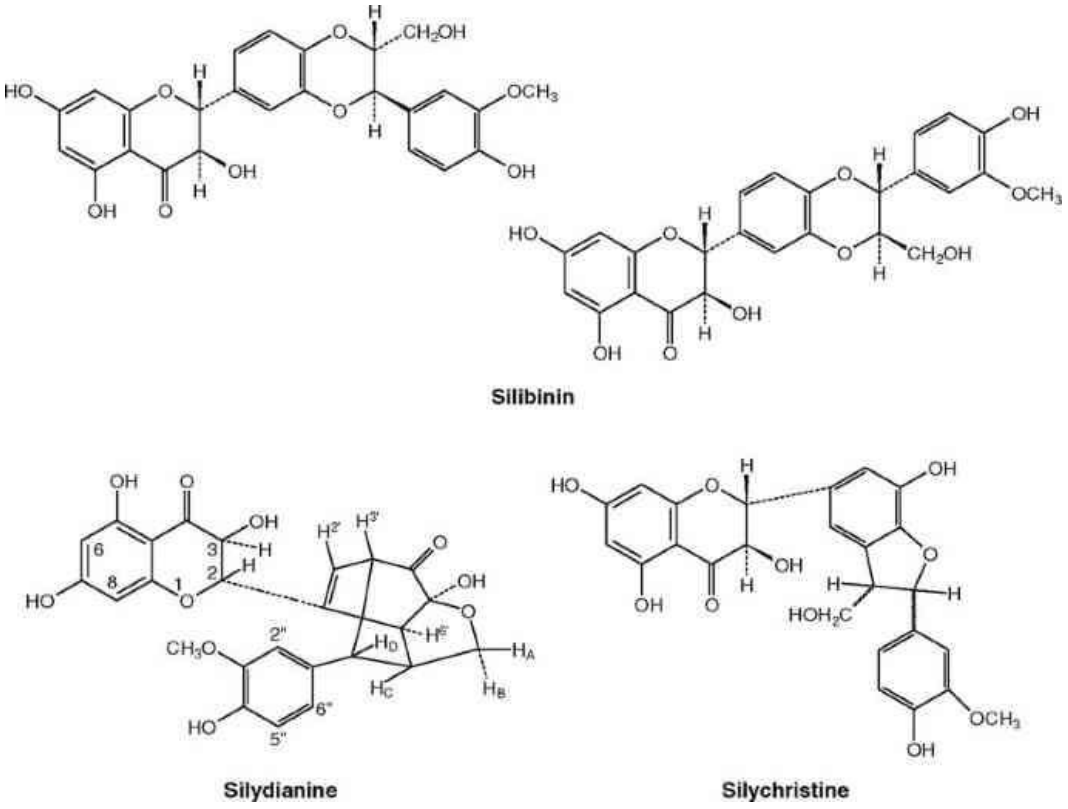

Figure 2. The three structural components of the constituents of silymarin: silibinin, silydianin, and silychristin [10].

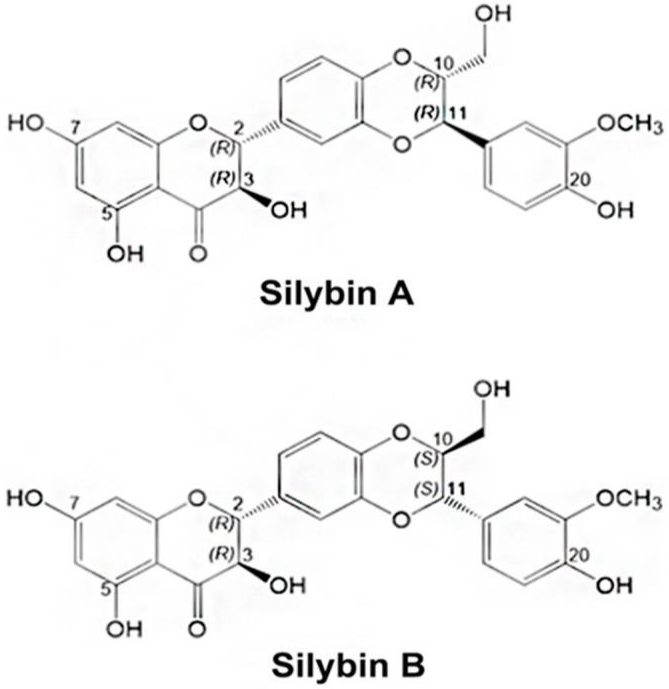

Figure 3. Chemical structures of silybin diastereomers [1].

3. PHARMACOLOGY OF SILYMARIN

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

3. 1. Carbon tetrachloride

Carbon tetrachloride is known for its hepatotoxic properties and many hepato-protective 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 to phospholipids and sphingomyelin to 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 tetrachloride-induced chronic liver damage [17, 20, 21].

3. 2. Hepatectomy

Rats with partial hepatectomy, (the removal of 70% of the liver), when subjected to silymarin pretreatment, showed increased synthesis of DNA, RNA, protein and cholesterol suggesting the regeneration of liver [22]. Interestingly, the increased protein synthesis was found in damaged livers with partial hepatectomy, but not in the respective control organism (rats) [23]. The mechanism of increased protein synthesis is not known, but probably silymarin initiates a physiologic regulator, so the silybin fits in to a specific binding site on the polymerase, thus stimulating ribosome formation [24]. Probably silymarin is able to enter the nucleus and specifically stimulate RNA polymerase I, owing to its structural similarity to steroids.

3. 3. Acetaminophen

Acetaminophen, (N-acetyl-p-aminophenol, paracetamol, APAP) is a well-known antipyretic and analgesic, effective at therapeutic doses. At high doses, acetaminophen induces toxicity to the liver, which is usually characterized by chest pain, vomiting diarrhea, and sometimes shock. Moreover, hepatic failure, myocardial and kidney dysfunctions have been attributed to excessive ingestion of acetaminophen [25]. Silymarin has been studied for its protective action against acetaminophen induced toxicity in animal models. Ramellini & Meldolesi in their 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 [26].

3. 4. Ethanol

Acute and chronic administration of ethanol produces a drastic decrease in the hepatic content of reduced glutathione (GSH); an important biomolecule against chemically induced cytotoxicity [27]. The hepato-protective activity of silymarin against ethanol-induced damage has been tested in different animals. The administration of ethanol has reduced a marked

increase in serum alanine transaminase ALT), aspartate transaminase (AST) and gamma glutamyl transferase (γ`-GT) levels, with a disturbance in reduced and oxidized glutathione ratio. The group of organisms into which silymarin was induced did not show any significant changes in these parameters, showing its protective role against ethanol [28].

3. 5. Iron

Iron overload is associated with liver damage, characterized by massive iron deposition in hepatic parenchymal cells, leading to fibrosis and eventually to hepatic cirrhosis [29]. The oxidative stress due to increased hepatic lipid peroxidation is the major mechanism of iron induced hepatotoxicity. Pre-treatment in rats with silymarin educed iron induced increase in lipid peroxidation and levels of serum enzymes, indicating their hepato-protective action [30].

3. 6. Manganese Toxicity

It is well known that manganese toxicity in animals is associated with increased oxidative stress, apoptosis and inflammation [31]. In fact, animals exposed to manganese chloride were characterized by a significant increase in TBARS levels associated with a decrease of enzymatic (SOD, CAT, GSH-Px) and non-enzymatic (GSH, Vitamin C) antioxidant [32, 33], increased lipid and protein oxidation [34], DNA fragmentation and urinary hydrogen peroxide [35]. Co-administration of SM (100 mg/kg/BW) to Mn-treated rats significantly restored antioxidant defenses and attenuated oxidative damages observed in the liver, kidney and brain [32-35].

3. 7. Cisplatin

Cisplatin (CDDP) is a chemotherapeutic drug widely used against a variety of cancers and its nephrotoxicity is mainly due to ROS production and oxidative stress [36]. It was shown that CDDP caused decreased activities of AO enzymes (SOD and GSH-Px) and GSH, increased MDA in rat liver [37, 38] and significantly elevated serum activities of lactate dehydrogenase (LDH) and creatine kinase (CK) [38]. SM (100 mg/kg/BW) significantly prevented the cisplatin-evoked disturbances in the above-mentioned antioxidant indexes [37, 38].

Furthermore, *in vitro* pre-treatment with 25–200 μM of SM significantly protected against cisplatin-induced cell death in a dose-dependent manner [39], inhibited apoptotic cascade and increased cell viability in the HEI-OC1 cells [40].

4. ABSORPTION AND METABOLISM OF SILYMARIN

Silymarin and its main constituent silibinin sources, metabolism and bioavailability have already been reviewed extensively [41-43]. It has been shown that after oral consumption silibinin is characterized by comparatively low availability, for example in rats, it is only about 0.95% [44]. In fact, after the oral administration of the standardized milk thistle extract Legalon, flavonolignans were rapidly absorbed and eliminated [43] with a half-life for silibinin of 6 h [45-47]. The main biotransformation route of silybin and derivatives was identified to be conjugation [41]. It is interesting to note that silibinin, similar to other flavonoids is recognized by the body as a foreign matter and quickly metabolized via phase II enzymes. Indeed, oral consumption of silibinin was associated with a significant increase in both glutathione *S*-

transferase (GST) and quinone reductase (QR) activities in liver, lung, stomach, skin and small bowel in a dose- and time-dependent manner [48].

Silibinin present in the systemic circulation was found mainly in conjugated form [49, 50]. In fact, after oral SM administration to healthy volunteers, only 10% [51] to 17% [52] of the total silibinin in the plasma was found in the free unconjugated form. Indeed, mono-, di-, and sulpho-glucuronides are shown to be formed, and 31 metabolites have been identified [53]. Indeed, silibinin in humans and rats shows fast elimination of both the free and conjugated forms with the mean elimination half-life being 6.32h [45]. Therefore, similar to other flavonoids, after oral consumption silibinin, the main constituent of Silymarin, is characterized by comparatively low availability, fast metabolism and its concentration in plasma is mainly in nano-molar range and only in some cases reaching micro-molar concentrations.

4. 1. Absorption

Silymarin has oral absorption only about 23-47% and quick phase II conjugation, leading to low bioavailability [5]. It is commonly agreed that silymarin suffers from low bioavailability due to poor solubility in water [54]. However, efflux transporters on the apical side of the intestinal epithelium further throttled the absorption of silybin as seen in Figure 4.

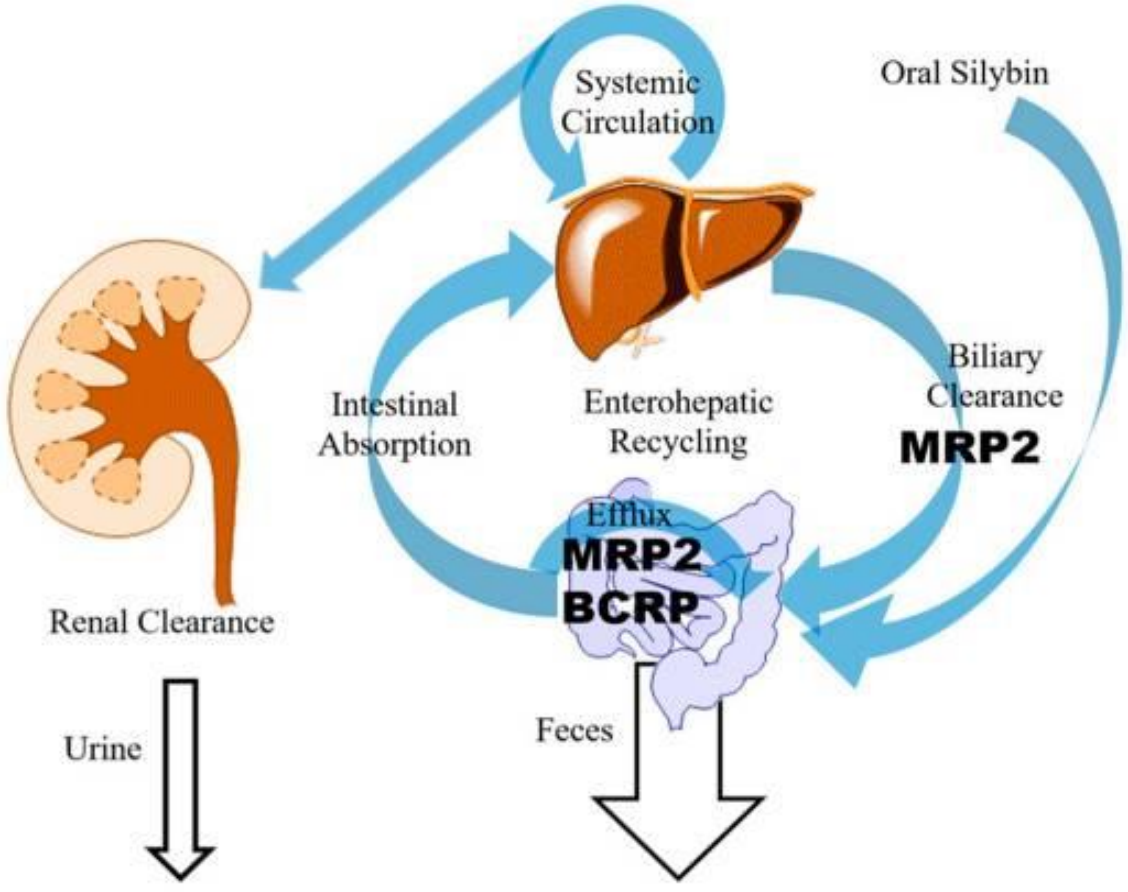

Figure 4. Transporters related to the disposition and elimination of silybin. BCRP: breast cancer resistance protein; MRP2: multidrug resistance-associated protein [82, 55].

The Caco-2 cell monolayer model was used to investigate the intestinal absorption of silybin [55]. The measured mean efflux ratios of silybin A and silybin B were 5.05 and 4.61, respectively, indicating an active transport mechanism. Moreover, MK571 (a multidrug resistance-associated protein (MRP2)-specific inhibitor) significantly decreased the efflux ratio of silybin, while Ko143 (a breast cancer resistance protein (BCRP)-specific inhibitor) and cyclosporin A (both a BCRP and P-glycoprotein (P-gp) inhibitor) were less potent, suggesting that intestinal efflux of silybin is mediated by MRPs and possibly BCRP. Studies carried out with Madin-Darby canine kidney cells II (MDCKII) lines overexpressing transporters (MRP2, BCRP, or MDR1) and a sandwich-cultured hepatocyte model confirmed that the transporters involved in the absorption and excretion of silybin are MRP2 and BCRP, but not P-gp [55].

4. 2. Metabolism

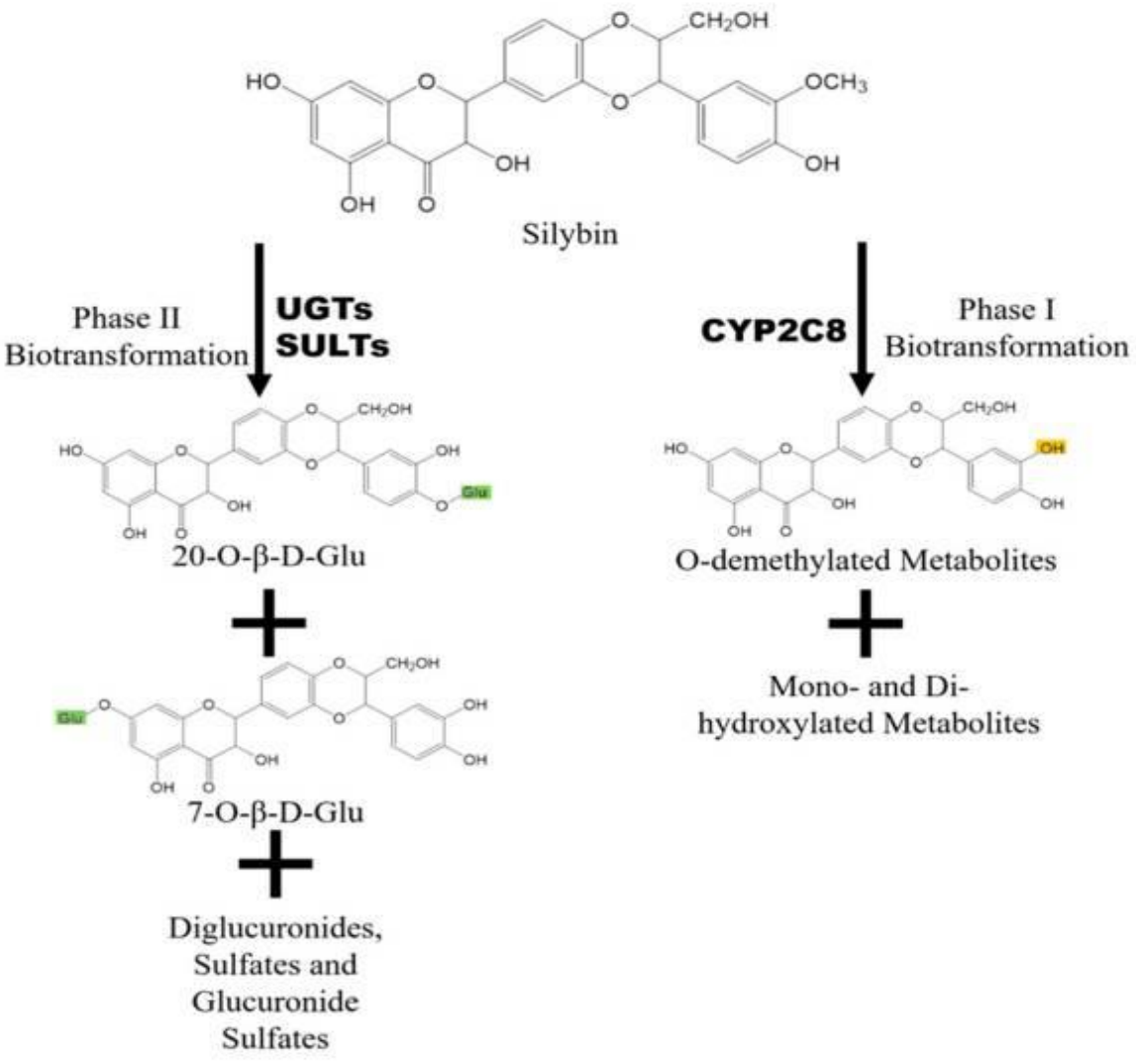

Figure 5. Metabolism of silybin and its major metabolites. UGT: UDP-glucuronosyltransferase; SULTs: sulfotransferases; CYP2C8: Cytochrome P450 2C8 [11, 41, 56-58].

After oral administration, silybin/silymarin undergoes both phase I and phase II biotransformation, especially phase II [41]. Figure 5 shows that phase I metabolites of silybin mainly include O-demethylated ones mediated by the CYP2C8 (Cytochrome P450 Family 2 Subfamily C Member 8) isoenzyme [56]. In addition, four minor metabolites including three monohydroxy ones and one dihydroxy one are also observed, though their structures are not confirmed [11]. Silybin and its phase I metabolites undergo extensive phase II biotransformation, as most of the silybin in the system exists as conjugates including 55% glucuronidated conjugates and about 28% sulfated ones [57, 58].

Glucuronidation reactions of silybin are mediated by UDP-glucuronosyltransferase (UGT) 1A1, 1A6, 1A7, 1A9, 2B7, and 2B15, while sulfidation reactions are mediated by sulfotransferases (SULTs) [59]. Four monoglucuronides, 1 diglucuronide, 3 monosulfates, 2 glucuronide sulfates, and O-demethylated glucuronide have been detected in plasma after silymarin administration [60, 61]. There are two major glucuronidation sites, C-7 and C-20. Silybin is glucuronidated in a stereoselective manner, with silybin B more efficiently glucuronidated at the C-20 position, while silybin A is glucuronidated similarly on both sites [49] due to the stereoselective activity of certain UGT isoforms [59].

The rapid and extensive phase II metabolism of silybin has been considered as the major reason contributing to its low bioavailability.

5. ANTIOXIDANT PROPERTIES OF SILYMARIN

It should be noted that Silymarin can contribute to the antioxidant defenses in different ways. Firstly, by direct free radical scavenging. Secondly, by preventing free radical formation by inhibiting specific enzymes responsible for free radical production, or by maintaining the integrity of electron-transport chain of mitochondria in stress conditions. In most studies pure silybin, as the main component of Silymarin, was used, however, in some cases Silymarin also showed antioxidant action *in vivo*.

5. 1. Direct free radical scavenging

The effects of silibinin on the formation of ROS and eicosanoids by human platelets, white blood and endothelial cells were studied [53]. Silibinin showed to be a strong scavenger of HOCI (IC_{50} 7 μM), but not of O_2^- (IC_{50} > 200 μM) produced by human granulocytes. Similarly, production of O_2^- and NO in isolated rat Kupffer cells were inhibited by silibinin in a dose-dependent manner, with IC_{50} 80 μM [62]. Indeed, silybin has no superoxide anion scavenging capability but was able to significantly decrease (at 20 μM) hem-mediated low density lipoprotein (LDL) oxidation and showed slight inhibition of hydroxyl radical formation [63]. The rate constants of silybin with OH radical (1.8×10^{10} dm/mol/s) is diffusion controlled, suggesting that silybin is a potent free radical scavenger [64]. Indeed, silibinin (2.5 μM) significantly decreased the concentration of H_2O_2 in Ab_{1-42}-stressed neurons and prevented oxidative-related injuries [64]. Furthermore, silybin (10 μM and higher) is shown to have protective activity in ameliorating DNA damage in a model system [64]. Treatment *in vitro* with silibinin significantly inhibited spontaneous O_2^- and H_2O_2 release and TNF-α production by monocytes from pre-eclamptic women. The main effect of silibinin was obtained at 50 μM concentration [66].

5. 2. Protective effects of silybin on mitochondria, a main source of free radical production in the cell

Mitochondria are the primary cellular consumers of oxygen and contain numerous redox enzymes capable of transferring single electrons to oxygen, generating the ROS superoxide (O_2^-). It is well documented that mitochondrial enzymes known to generate ROS include the tricarboxylic acid (TCA) cycle enzymes aconitase and α-ketoglutarate dehydrogenase; the electron-transport chain (ETC) complexes I, II and III; pyruvate dehydrogenase and glycerol-3-phosphate dehydrogenase; dihydroorotate dehydrogenase; the monoamine oxidases (MAO) A and B; and cytochrome b5 reductase (Lin et al., 2006). Furthermore, mitochondrial insults, including oxidative damage itself, can cause an imbalance between ROS production and removal, resulting in net ROS production. For example, ROS can induce protein modifications, lipid peroxidation and mitochondrial DNA damage, which ultimately results in mitochondrial dysfunction [67].

One of the mechanisms responsible for the decrease in oxidative stress is the protective effect of SM/silibinin on mitochondrial structure and function. Indeed SM protects mitochondria from pathological events by triggering pro-survival cell signaling. For example, silibinin supplementation is shown to optimize electron-transport chain, decreasing electron leakage and ROS formation and directly reducing activities of ROS-producing enzymes in the mitochondria. In rats subjected to ischemia/reperfusion (I/R), compared with the control group, a severe impairment of mitochondrial bioenergetics was observed. SM prevented the most significant changes that occurred in mitochondria during I/R (decreased ATP levels, membrane potential and state 3 respiration), and the associated cell dysfunction [68].

Silibinin (100-500 μM), was evaluated for its protective effect against beta-adrenergic agonist isoproterenol-induced injury in cultured rat neonatal cardiac myocytes [69]. It was shown that silibinin addition was associated with increased SOD activity and upregulated mitochondrial membrane potential and with a prevention of mitochondrial dysfunction and cell injury [68]. Silibinin, at a concentration as low as 10 μM, fully mitigated the rise in metabolic flow-driven ROS formation in perfused rat hepatocytes. In addition, studies on isolated liver mitochondria revealed that this low dose of silibinin depressed ROS production linked to the electron transfer chain activity [70]. It has been shown that cold preservation and warm reperfusion of the rat liver were associated with increased lipid peroxidation and superoxide anion generation, as well as with decreased GSH, mitochondrial ATP content and respiratory control ratio (RCR). However, preservation conducted in presence of silibinin (100 μM) improved parameters affected by preservation and reperfusion. Indeed, silibinin promoted an increase of ATP and RCR by 39 and 16% respectively and decreased oxidative stress to values observed in livers never preserved nor perfused [71].

It has been suggested that the uncoupler-like activity of dehydrosilybin could be the basis of its ROS modulation effect in various experimental systems. In fact, dehydrosilybin uncoupled the respiration of isolated rat heart mitochondria with a very high potency in suppressing ROS formation in isolated rat heart mitochondria with IC(50) = 0.15 μM [72]. It is interesting to note that silibinin in mitochondria was far more effective than its effect in a purely chemical system generating superoxide or in cells capable of oxidative burst, where the IC(50) for dehydrosilybin exceeds 50 μM. Changes in mitochondrial respiratory complexes in fatty hepatocytes were also attenuated by silibinin-vitamin E complex (15 mg vitamin E and 47 mg silybin) fed to rats with a major protective effect on Complex II subunit CII-30 [73]. Similarly, silybin (0.4 g/kg) in complex with phospholipid (SILIPHOS) was effective in decreasing severe

oxidative stress and preserving hepatic mitochondrial bioenergetics and mitochondrial proton leak and ATP reduction in nonalcoholic steatohepatitis induced by the methionine- and choline-deficient (MCD) diet [74]. Silymarin oil (10 mL/kg/BW) significantly increased levels of membrane fluidity and membrane potential of liver mitochondria [76].

It has been suggested that protective mechanism of action of silibinin (50–200 μM) in intrastriatal MPP+-injected rats may be linked to maintenance of mitochondrial bioenergetics and integrity [77]. An *in vitro* study demonstrated that silibinin inhibits the activity of ROS-generating monoamine oxidase (MAO) that catalyzes the oxidative deamination of monoamines [75]. Similarly, silymarin oil (10 mL/kg/BW) decreased MAO activity in mice livers [76]. The formation of leukotrienes via the 5-lipoxygenase pathway was indicated to be strongly inhibited by silibinin. In particular, in human granulocytes IC50-values of 15 μM and 14.5 μM silibinin were detected for LTB4 and LTC4/D4/E4/F4 formation respectively.

However, much higher silibinin concentrations (45–69 μM) were necessary to inhibit the cyclooxygenase pathway [78]. Rats exposed to a carcinogen (1,2-dimethylhydrazine DMH) showed increased activities of phase I enzymes (cytochrome b5, cytochrome b5 reductase, cytochromeP450, cytochromeP450 reductase, cytochromP4502E1) in the liver and colonic mucosa as compared to control rats. Silibinin supplementation (50 mg/kg/BW) modulates the xenobiotic metabolizing enzymes including decreasing activity of ROS-producing cytochrome b5 reductase [79]. Although energy generation in the mitochondrion is an essential and extremely important process for cell survival, excessive mitochondrial ROS production also has detrimental consequences for the cell and the whole body.

The different behaviour of silymarin/silibinin in normal and cancerous cells should be mentioned. In particular, SM is shown to have a protective effect against diabetes-induced cardiomyocyte apoptosis [80] as well as apoptosis caused by various toxicants, while it causes apoptosis in cancerous cells. For example, SM effectively suppressed cell growth in a dose- and time-dependent manner, and arrested cell cycle progression at G1/S phase in human ovarian cancer line A2780s and PA-1 cells via up-regulation of p53, p21, and p27 protein expression, and down-regulation of CDK2 protein expression [81, 82].

6. CONCLUSION

The excellent hepato-protective activity of silymarin, besides its 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 has established efficacy in the restoration of liver function and regeneration of liver cells. 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. Silymarin may make a breakthrough as a new approach to protect other organs in addition to liver.

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