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Progress in Research and Application of Silymarin

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ABSTRACT

Silymarin (SL), a mixture of flavonolignans mainly including silybin A and B, isosilybin A and B, silidianin, and silychristine from the seeds of *Silybum marianum* L., has been widely used to treat acute and chronic viral hepatitis, toxin/drug-induced hepatitis, cirrhosis, alcoholic fatty liver diseases and other ailments because of its excellent hepatoprotective effect. Silybin is the most active component of SL, which is administered orally in most cases of clinical use. However, its bioavailability is low due to poor water solubility. The present paper reviews the research results on isolation methods, pharmacological activities, action mechanisms, preparation techniques, quality control and market state of SL in the hope that it would be helpful to better understand and use this traditional Chinese medicine.

Keywords: action mechanism, isolation methods, pharmacological activity, preparation techniques, quality control

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INTRODUCTION

Silybum marianum L. was originally planted in India and the Kashmir area of Pakistan, then introduced and cultivated in Europe, America and Australia. The plant was introduced into China from Germany in 1927, mainly grown in Jiangsu, Shaanxi and Beijing. Silymarin (SL) is a mixture of flavonolignans isolated from the seeds of *S. marianum*, including many isomers with poor water solubility. The main chemical constituents of SL are silybin A and B, isosilybin A and B, silidianin, and silychristin, among which, silybins are the most active components with the highest content. SL has an excellent clinical effect to treat acute and chronic viral hepatitis, cirrhosis and metabolic toxin-induced liver injury by improving the hepatic function, and enhancing activity of the cell membrane. This article reviews research advances in the isolation methods, pharmacological activities, clinical uses, action mechanisms, preparation techniques, quality control and market state of SL.

EXTRACTION AND SEPARATION

Early in 1974, Wagner *et al.* (1974) extracted silybin, isosilybin, silychristine and silidianin from seeds of *S. marianum*. In 2003, Lee *et al.* (2003) extracted two isomers of silybin and isosilybin, silybin A and B, isosilybin A and B for the first time. Chinese scientists (Chang and Wu 1985)

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extracted silybin and silidianin from the seeds of *S. marianum* for the first time in 1985.

Regarding the isolation method of SL, many studies were carried out. Chen *et al*. (1997) compared several solvents to extract total flavones from *S. marianum*, and screened ethyl acetate as the best, meanwhile a technique improved with continuous circumfluence and repeated recovery was put forward for extraction of total flavones (recovery rate = 5.01%), silybin (with purity of 98.9%) could be obtained after repeated crystallization with methanol. Ding *et al.* (1999) noted that diacolation with organic solvent and thermal reflux were popular for the extraction of SL, and extraction by ethyl acetate, ethanol and pot groups forced-cycle counter-current extraction were the main methods. Based on a square orthogonal regression design with five variables, Zhao *et al.* (2000) put forward the optimal parameters to extract SL: temperature 0°C, solvent volume 330 mL, ultrasound time 1.13 h, seeds of *S. marianum* 332.8 g, immersion time 27.8 h. In recent years, one study proved that ethyl acetate extraction assisted by ultrasound yielded over 7.0% SL, and shortened the extraction time to less than 10 h (Shi *et al.* 2006). Scientists also used acetonitrile-ultrasound in the extraction of SL; the best extraction condition was obtained by means of an orthogonal test: ultrasonic temperature 60°C, time 60 min, power 100%, the proportion of materials was 1: 15 ($g \cdot ml^{-1}$), and the rate of extraction for SL in the repeated test was on average 10.09 $mg \cdot g^{-1}$ (Li *et al.* 2006). At the same time, two methods, column chromatography and conventional re-crystallization were compared, and the latter was improved with the use of ultrasonic waves, and optimal conditions of re-crystallization were: ultrasonic time 45 min, 60°C, power 100%, and ethanol as solvent (Li *et al.* 2006). Zhang *et al.* (2007) investigated the extracting technique of SL by high pressure extraction (HPE); the best technical conditions were selected through an orthogonal test: 75% ethanol, pressure 400 Mpa, ratio of solvent to sample = 50: 1 ($mL \cdot g^{-1}$), extraction time 3 min. Under these conditions, the purity and yield of SL extract reached 28.8 and 2.424%, respectively.

PHARMACOLOGICAL ACTIVITIES

Hepatoprotective effect

Treatent of liver cirrhosis

In a prospectively double-blind randomized trial (Cheng and Xiao 2007), 170 liver cirrhosis patients including 91 alcohol-induced and 79 non-alcohol-induced patients were randomly grouped to be treated with SL for 2 years. 37 patients of the placebo group died, of which 31 died of liver disease; 24 patients of the treatment group died, 18 of liver disease. The 4-year actual survival rate of the treatment group was 49~67%, while that of the control group was 30~48%. Further analysis discovered that treatment with SL was useful to patients with alcohol-induced liver cirrhosis. In another study (Angulo *et al.* 2000), 27 patients of primary biliary cirrhosis (PBC) whose treatment was ineffective with ursodeoxycholic acid (UDCA, 13-15 mg/kg/day) were treated with UDCA and SL (140 mg 3 times daily for 1 year) together for one year. There was no significant change in serum alkaline phosphatase (AKP), alanine transaminase (ALT) and aspartate transaminase (AST), albumin, and bilirubin (BIL). So the efficacy of SL to treat PBC should be further verified through a randomized controlled clinical trial. Saller *et al.* (2001) found that SL could lower serum glutamyl transferase, improve the prothrombin time and histological grade, and suggested SL as an adjuvant drug to treat alcohol-induced liver cirrhosis (AILC). Further research on the mechanism of SL to treat AILC found that SL treatment could recover serum ALT, AST and BIL to normal levels, decrease γ-glutamyl transpeptidase (γ-GT), significantly increase lymphocyte transformation rate, and obviously decrease the number of CD_8^+ cells, while the placebo group did not show these effects. This revealed that the mechanism of SL to treat AILC was due to immunomodulating activity. In addition, a clinical trial showed that SL did not have any significant effect on the clinical course and survival rate of AILC patients, which may be related to abstention from wine or not, or the poor compliance of patients to drugs.

Treatment of chronic hepatitis C virus (HCV)

Core protein and non-structure protein of hepatitis C virus (HCV) can cause lipid metabolism disorder, induce active oxygen free radicals, damage hepatocytes, activate hepatic stellate cells further and start liver fibrosis. So, finding an effective and safe antioxidant may change the outcome of chronic HCV infection. Melhem *et al.* (2005) administered 50 patients with a combination of 7 antioxidants (glycyrrhizin, schisandrin, SL, vitamin C, lipoic acid, vitamin E) to treat chronic HCV for 20 weeks. The ALT of 44% of patients recovered to normal, the viral load of 25% patients decreased, the histology of 36% patients improved, and the life quality of 58% patients improved, so it was concluded that the combination of antiviral agents and antioxidants could increase general response rate of the chronic HCV patients. SL was also shown to be a good antioxidant.

Treatment of liver cirrhosis complicating diabetes

Cheng and Xiao (2007) used SL to treat diabetic patients with liver cirrhosis; the treatment group was given SL and insulin together while the control group insulin only. The levels of fasting blood glucose, urine glucose, and glycosylated hemoglobin of the treatment group were significantly lower than those of the control group. After treatment for 4 months, the fasting insulin and the average dosage of exogenous insulin of the treatment group were significant lower than that of the control group. This reveals that SL can reduce lipid peroxidation of the cell membrane and insulin resistance, promote secretion of endogenous insulin and decrease the dosage of exogenous insulin.

Treatment of nonalcoholic fatty liver

Xing and He (2007) investigated the effect of SL on nonalcoholic fatty liver of rat and its mechanisms. Thirty SD rats were randomly divided into a control, model and SL groups. The nonalcoholic fatty liver was induced by feeding fat-enriched foods and intraperitoneally injecting oxytetracycline together, and the rats in SL group were treated with 100 $mg \cdot kg^{-1} \cdot d^{-1}$ of SL for 8 weeks. Serum ALT, triglyeride (TG), total cholesterol (TC), malondialdehyde (MDA) and hepatic TG, TC, MDA in SL group rats were markedly lower, while the serum and hepatic superoxide dismutase (SOD) were significantly higher, while the mRNA expression of peroxisome proliferator activated receptor α (PPARα) in liver tissue increased ($P<0.01$, compared with the model group).

Anti-inflammatory effect

SL has a protective effect against acute lung injury induced by lipopolysaccharide (LPS) in rats. The concentration of TNF-α, IL-1β and MCP-1 in serum and lung tissue, and the level of lipid peroxidation in lung tissue in LPS rats were significantly ($P < 0.05$) decreased after the administration of SL (50 mg/kg). The inferred mechanism is to decrease inflammatory reaction and oxidative stress (Wang *et al.* 2007).

Antitumor effect

Protection against carcinogen-induced tumors

Agarwal *et al.* (1994) found that SL, on skin application, inhibited the activity of epidermal ornithine decarboxylase (ODC) induced by 12-*O*-tetradecanoylphorbol-13-acetate

(TPA), and implied that SL could be an effective anti-tumor agent. In recent years, some animal tests have also proved that SL, especially silibin, the main active component, provides strong protection against tumor models in mice and rats induced by many kinds of tumor promoters.

Oral administration of SL at a dose of 6 mg per mouse resulted in significant protection against benzoyl peroxide (BPO)-induced tumor promotion in SENCAR mouse skin, and a 70% reduction ($P < 0.001$) in tumor incidence, a 67% reduction ($P<0.001$) in tumor multiplicity and a 44% decrease ($P < 0.001$) in tumor volume (Zhao *et al.* 2000). SL also inhibited SENCAR mouse from skin edema, hyperplasia, DNA synthesis, and epidermis lipid peroxidation induced by TPA, thereby resisting the generation of skin tumors (Lahiri-Chatterjee *et al.* 1999). In addition, SL can also resist against the generation and development of tumors induced by other chemical carcinogens, such as 4-nitroquinoline-1 -oxide (4-NQO) (Yanaida *et al.* 2002) and *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) (Vinh *et al.* 2002).

Protection against UV-induced skin cancer

Ultraviolet (UV) is the main cause of skin cancer. Long-term irradiation of UV may cause DNA injury, the release of tumor necrosis factor α (TNF-α), abnormality of oncogenes and anti-oncogenes, injury to the immune system, oxidative stress, etc. then produce cancer (Nong *et al*. 2002). Silibin provides strong protection against skin cancer induced by UVB in nude mouse, and administration before or after radiation both can inhibit the generation of tumors and delay the incubation period (Malikarjuna *et al.* 2004). Silibin simultaneously inhibited the apoptosis of HaCaT cells induced by a low dose of UVB irradiation (15, 30 $mJ \cdot cm^{-2}$), and enhanced the apoptosis of HaCaT cells induced by a higher dose of UVB irradiation (120 $mJ \cdot cm^{-2}$), suggesting that silibinin possibly works as a UVB damage sensor to exert its biological action (Dhanalakshmi *et al.* 2004).

Synergistic interaction with antitumor preparations

TNF-α is used to treat tumors clinically because of its direct inhibition of multiplication and cellular necrosis. Silibin can regulate the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis (Manna *et al.* 1999), it can also conquer the insensitivity of some tumors to TNF-α. A study by Dhanalakshmi *et al.* (2002) showed that SL can inhibit the activity of NF-κB induced by TNF-α, thereby increasing the sensitivity of prostate cancer DU145 cells on apoptosis of tumor cells induced by TNF-α, which can increase the efficacy of TNF-α antitumor preparation.

Doxorubicin, a cytochalasin preparation, is widely used to treat prostate cancer. There is strong synergism between silybin and doxorubicin to treat DU145 cells together (Tyagi *et al.* 2002). This implies that silybin has the potential value for clinical therapy of tumors.

Ding *et al.* (2006) divided patients with renal damage induced by multiple myeloma into treatment and control groups; patients in the former group were given protective renal treatment, and patients in the control group were given SL tablets at a dose of 77 $mg \cdot d^{-1}$ for 4 weeks. The thrombopoietin (TPD) receptor mRNA of the treatment group was higher than that of the control group ($P < 0.05$), and the medical index of the control group decreased significantly ($P < 0.05$), suggesting that SL might realize its antitumor effects by inhibiting TPD and the receptor mRNA.

The combination of SL and baicalein eradicates tumor cells efficiently (6.75 $\mu g \cdot ml^{-1}$ baicalein and 100 $\mu g \cdot ml^{-1}$ SL for 48 h), which inhibited the growth of HepG2 and increased the percentages of cells in G0/G1 phase and decreased those in S-phase (Chen *et al*. 2009).

Anti-diabetes effect

Bai *et al.* (2001) found that SL can prevent diabetic neuropathy by regulating tissue metabolism disorder, ameliorate hemorheology and relieve endoneurium ischemia when observing the changes of ultrastructure in the superior cervical ganglionic cell of diabetic rats and the effect of the administration of SL. Moreover, SL plays a significant role in the prevention of diabetic autonomic neuropathy (Bai *et al*. 2001).

MECHANISMS FOR THE PROTECTIVE EFFECT AGAINST LIVER DISEASE

Antioxidation

SL has a protective effect on liver injury induced by CCl_4, galactosamine, alcohols and other hepatotoxins (such as *Amanita phalloides*). It can also resist against the activation of hepatic stellate cells (HSCs) and liverish Kupffer cells, and inhibit synthesis of the extracellular matrix (ECM). The anti-lipid peroxidation of SL has been proved by many tests. In 1990, a report by Lotteron *et al.* (1990) showed that SL could prevent lipid peroxidation induced by CCl_4 and coenzyme (NADPH) in liver microsomes of mice, and decrease hepatotoxicity. In another study (Tasduq 2005), the biochemical manifestations of liver toxicity caused by co-administration of anti-TB drugs, rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA), in a sub-chronic mode for 12 weeks, were investigated. The levels of ALT, AST, alkaline phosphatase (ALP) and BIL in serum increased significantly, the activity of lipid peroxidation, intracellular calcium [Ca^{2+}] and cytopigment P4502EI in the liver were enhanced, while the content of glutathione (GSH) and the activity of glutathione peroxidase (GPx) and catalase in the liver decreased. SL reversed these abnormal alterations. SL showed a significant protective effect on hepatotoxicity and hepatic injury induced by different modes of radiation (3 or 6 Gy; 1, 3 and 7 days) due to its antioxidant and free radical scavenging properties (Ramadan *et al*. 2002). Masin *et al.* (2000) found that SL obviously inhibits oxidative stress induced by iron. Deng *et al.* (2005) found that SL can not only improve liver injury of nonalcoholic fatty hepatitis (NASH), but also decrease blood-lipid level and plasma MDA content, increase the activity of SOD and GPx, improve the state of lipid peroxidation of NASH patients, and decrease or prevent the development of NASH. These data indicate that SL is a kind of chain-breaking antioxidant or free-radical scavenger, whose antioxidation plays an important role in the protection of the liver. The molecular mechanism of the antioxidant activity can be inferred from the structure of the dimeric products obtained from radical-mediated reactions of selectively methylated derivatives of silybin. The radical oxidation of silybin methylated at 7-OH yields C-C dimmers, which enable the molecular mechanism of their E-ring interaction with radicals to be elucidated and shows the importance of the 20-OH group in this respect (Gazak *et al.* 2009).

Inhibit the production of NO

Nitric oxide (NO), also termed vascular endothelium derivative relaxation factor, is induced by mononuclear macrophages, endothelial cells, etc. In the abnormal status of a fatty liver, overproduction of NO is the main medium to induce hyperdynamic circulation and hypoxemia and increase the toxicity of active mononuclear macrophage. SL can inhibit active liver Kupffer cells from producing NO, then decrease the interaction with O_2^- (Dehmlow *et al.* 1996).

Decrease the activity of phospholipase

Phospholipid in the membrane is metabolized to arachidonic acid (AA) by phospholipase, and the latter is transformed into leukotriene (LTs) by 5′-lipoxygenase, or trans-

formed into prostaglandin E (PGE) and thromboxane A (TXA). LTs, which are mainly produced by active mononuclear macrophages, are strong chemokines that aggregate leukocytes, mediate injury of endotheliocytes in hepatic sinusoid and formation of microthrombosis, etc. PGE can dilate blood vessels and inhibit platelet aggregation. SL can decrease the activity of phospholipase and protect the liver membrane by means of resisting against TXA, contracting the blood vessels strongly, improving platelet aggregation, preventing the toxic reaction of T cells and the formation of TNF-α, and increasing the intracellular cAMP level (Yang *et al*. 2006).

Protection of the cell membrane

Through the anti-lipid oxidation reaction, SL can maintain the fluidity of the membrane, protect the hepatocyte membrane, and interrupt the combination of phalloidine and α-amanitine with specific receptors in the hepatocyte membrane to inhibit these toxins from attacking the hepatocyte membrane, transmembrane transport and enterohepatic circulation. In a study by Wu *et al.* (2003), SL regulated the fluidity of liver microsomes and mitochondrial membrane, and kept membrane fluidity in a perfect state of movement. SL's mechanism to protect the cell membrane is to recover the shallow layer fluidity of membrane lipids and to alleviate enlargement of the cell membrane brittleness induced by lipid peroxidation. On the other hand, it also could recover the deep layer fluidity of membrane lipids, increase barrier function of the membrane, and prevent toxic substances such as lipid peroxidation products from entering the membrane to damage cells.

Steady cell membrane and facilitate energy metabolism

In an animal test (Masini *et al.* 2000), gerbils were dosed with weekly injections of iron-dextran alone or in combination with sylibin, by gavage for 8 weeks. A strict correlation was found between lipid peroxidation and the level of desferrioxamine chelatable iron pool. A consequent derangement in the mitochondrial energy-transducing capability, resulting from a reduction in the respiratory chain enzyme activities, occurred. These irreversible oxidative anomalies brought about a dramatic drop in tissue ATP level. The mitochondrial oxidative derangement was associated with the development of fibrosis in the hepatic tissue. SL administration significantly reduced both functional anomalies and the fibrotic process by chelating desferrioxamine chelatable iron.

Promote hepatic cytothesis

SL could combine the estradiol receptor and activate it in liver cells, and the activated receptor could enhance the activity of liver endonuclear RNA polymerase I, promote the transcription of ribosome RNA, increase the number of ribosomes in intracytoplasm, promote the synthesis of enzyme and structure protein, and indirectly promote the synthesis of cellular DNA, which are beneficial to hepatic cytothesis (Yang *et al.* 2006).

Cytokine and immunomodulatory function

Transforming growth factor β (TGF-β) is the most important factor in the occurrence of liver fibrosis. Jeong *et al.* (2005) examined the mechanism of SL by using rats with hepatic cirrhosis induced by CCl_4. At an early stage of hepatic cirrhosis, the number of hepatic stellate cells and the content of TGF-β in the SL-treated group rats were lower than in the control group. Based on the results, it was presumed that anti-fibrotic and anti-inflammatory effects of SL were associated with activation of hepatic stellate cells through the expression of TGF-β and stabilization of mast cells. Jia *et al.* (2001) found that SL could reduce the mRNA level of TGF-β, precollagen type I and tissue inhibitor of matrix metalloproteinase (TIMP)-1 in the hepatic fibrosis model induced by biliary obstruction. Interkeukin-10 (IL-10) is an important negative-regulatory factor in the process of liver fibrosis. SL could protect hepatocytes and prevent hepatic fibrosis by raising the IL-10 level (Yen *et al.* 2005). IL-10 further up-regulates IL-1 receptor expression from LPS-stimulated neutrophils, which suggested IL-10 played a role as an anti-inflamatory cytokines in immunomodulated pathway (Cassatella *et al*. 1994). A study on alcoholic fatty liver showed that acetaldehyde adducts produced in the alcoholic metabolic process could stimulate the immune system to increase the number and activity of cytotoxic lymphocytes (CTLs) and natural killer cells (NKCs) and aggravate the immune injury of hepacytes as exogenous antigens. SL treatment could decrease the number and activity of CTLs and NKCs in blood, which indicates that SL has an immunoregulatory function (Schroeter *et al.* 1995).

Inhibition of the synthesis of extracellular matrix (ECM)

Muriel *et al.* (2004) investigated the effects of a mixture, including SL, phosphate lecithin and vitamins E, on liver fibrosis induced by dimethylnitrosamine in rats. The expression of procollagen type a1, TGF-β, TIMP1 and TIMP2 significantly decreased after the treatment. In the model of prolonged biliary liver fibrosis induced by biliary occlusion, the content of collagen in the model group was 9-fold higher than that in the SL treatment group while the content of hydroxyproline in the SL high-dosage group (50 $mg \cdot kg^{-1} \cdot d^{-1}$) decreased by 30% to 35%, ALP decreased significantly and histological grade improved.

ACHIEVEMENTS OF RESEARCH IN SILYMARIN PREPARATION

SL has very poor bioavailability due to its poor water solubility with its flavonolignan structure, which limits its clinical application and efficacy. So how to increase its oral bioavailability and enrich the drug in liver in order to improve its clinical application is an important task of preparation researchers. New SL preparations are being developed and manufactured around the world to increase its solubility or change its properties of digestion and absorption in order to increase its bioavailability. So far, the main preparations of SL are capsules, compound preparation, tablets, suspension, injection of SL complex salt, solid dispersion made up of polyethlene glycol (PEG), polyvinylpyrrolidone (PVP) and other supplementary agents, cyclodextrin inclusion compounds, complexes of soybean phospholipid, liposomes, and/or phosphatidylcholine, among others.

Tablets

The tablets reported or clinically used are Yiganling tablets (SL tablets), Complex Prescription Yiganling tablets, and Dan-ning tablets.

The procedure to prepare Yiganling tablets is to blend SL, dextrin, starch and sucrose according to a certain proportion (unspecified) and then make it into granules. After drying and adding the magnesium stearate, ingredients are mixed well and squashed into a tablet, finally coating sugar on it (Chinese State Drug Administration 2002).

The prescription of Complex Prescription Yiganling tablet is 30 g SL and 80 g schizandrol extract. The preparative method is same as the Yiganling tablet (Chinese Ministry of Health 1998).

The Dan-ning tablet is made of artificial *calculus bovis*, SL and Yanhusuo (*Corydalis yahusuo*), and is used to treat chronic cholecystitis, biliary tract infection and cholelithiasis (Xu *et al.* 2004).

Capsules

The capsules reported and clinically used are Yiganling capsules (SL capsules), Complex Prescription Yiganling capsules, Baoganling capsules and SL Soft Gelatin capsules (Chinese State Drug Administration 2002).

According to the National Drug Standards, the method to prepare Yiganling capsules is to smash SL and sieve it into a particle size of 60 mesh. Starch is sieved into a particle size of 100 mesh. Both components are weighed as prescribed, and fully blended in a mixer (for ~15 min). After drying for 4 h at 80°C, the content is examined and the loading capacity is calculated. Finally, the qualifications are tested, the charge is split and packed.

Complex Prescription Yiganling capsules are modified from Complex Prescription Yiganling tablets, and the concentration of SL can be determined by HPLC (Ling *et al.* 2004).

Baoganling capsules (Ling *et al.* 1998) are a natural drug preparation made by TYLER Encapsulation Co. in America, whose main components are 15 natural drugs, such as black radish, beet leaves, North American fringe tree, greater celandine, dandelion, SL, choline, methionine, *myo*-inositol, magnesium aminosuccinate, ox gall extract and vitamin B_6, of which, SL is the main composition (2 mg per capsule).

Liang *et al.* (2004) prepared SL soft capsule to improve oral bioavailability of silybin. The crude drug was micronized into micropowder with a size of less than 10 μm, followed by grinding and emulsification to make soft capsules. The content of silybin in the capsules was determined by HPLC. The soft capsules reached the standard of Pharmacopoeia of the People's Republic of China. The content of silybin was 62.7 $mg \cdot g^{-1}$, the average recovery was 97.5% with an RSD of 0.80% (n=6).

Granules

Hepatitis B Xintai granules (Sun *et al.* 2003) are a Chinese patent medicine with local standard originating from Guizhou Province whose prescription includes five traditional Chinese medicines: gardenia, lightyellow sophora root, amoorcorn tree bark, danshen root and SL. This granular drug, with heat-clearing, detoxicating and dampness-eliminating functions, is used to treat acute and chronic hepatitis, or persistent hepatitis with wetness-heat.

Injections

Meglumine salt, a product from the reaction between silibin and organic amine, is one of the earliest organic amine salts of SL to change silybin into a water-soluble preparation. Lu *et al.* (1999) injected multiple low-doses of *cis*-platin to rats to observe the ongoing change of nitrogen content in blood urine and NO content in the renal cortex; the results showed that NO might play an important role in the renal injury induced by *cis*-platin. After oral administration with SL-meglumine salt, the nitrogen content in blood urine and NO content in renal cortex was reduced to the same level as the control group. This implies that SL can effectively prevent renal injury induced by *cis*-platin with the possible mechanism to decrease the NO content in the renal cortex. The clinical test manifested that this drug could produce an effect quickly and strongly, and the total effective rate was 74.6%, the significant effective rate was 52.0%, and the curative effect was better than that of Yiganling tablets.

Di-meta-succinate sodium salt is also one of the early-researched water-soluble preparations of SL, with a strong function against liver injury. The complex salt of 0.4 $mg \cdot ml^{-1}$ could almost completely prevent the reperfused mice from amanita toxin, and prevent dogs and humans from absorbing the toxin in hepatoenteral circulation, which was very significant in the treatment of amanita poisoning (Mira *et al*. 1994). The complex salt not only has an effect of protecting the liver, but can also decrease the content of free fatty acids and triglycerides in serum, and inhibit the synthesis of cellular cholesterol. In recent research, this complex salt was found to react quickly with free hydroxyl radicals and clear active oxygen (Valenzuela *et al*. 1989).

Other preparations

The β-cyclodextrin (β-CD) inclusion complex of SL

Li *et al.* (1996) wrapped SL with β-CD to increase the apparent solubility so as to improve the absorption speed of SL, quicken the drug action and shorten the treatment course. The result of an orthogonal test showed that the optimal condition to wrap SL with β-CD by saturated a water solution method is as follows: ratio of SL to β-CD = 0.75:4; temperature = 40°C; stirring time = 1 h; intensity = 600 $r \cdot min^{-1}$.

The solid dispersant of SL

Solid dispersion technology is a common pharmaceutical method to increase solubility, and the combined use of surfactant and polymer can increase the dissolution of difficult soluble drugs (Tang *et al.* 2001). At present, in order to improve water solubility and the dissolution rate of SL, PEG and PVP are commonly used as supplementary agents to prepare solid dispersant, and meglumine salt is used to prepare water-soluble compound as well (Zhu *et al.* 2001). Deng *et al.* (2000) compared the solubility of some solid dispersants made of PVP, urea and poloxamer as supporter. The results showed that Poloxamer-188 was better to improve the solution and dissolution rate of the drug, and it had good effect as a solubilizing and wetting agent. Poloxamer is a good supporter with better potential to be used to make solid dispersant of SL, due to its property of increasing solution and dissolution rate. Tang *et al.* (2001) prepared dispersion using PVP-K30, PEG-6000, PEC-6000 and Tween-80 together as carrier, and prepared an inclusion complex using β-CD and HP-β-CD as wrapper, to optimize the technology to increase the solution and dissolution rate of SL. The result showed that PVP solid dispersion has higher solubility in water than the inclusion complex. When the carriers were at the same concentration, PVP dispersion has the highest dissolution rate. The conclusion is that PVP-K30 has a high drug loading and solubilization efficiency as a carrier. In another study (Yang *et al.* 2005), solid dispersant using Poloxamer-188 was prepared as carrier by hot melt extrudation technology (HME); the results showed that the dissolution rate of SL was higher than that by the melting method. This is because HME technology has strong mixing and shearing effects, which make the drug well dispersed in carriers.

Liposome

Liposome, a new carrier of target drugs, could congregate in the liver and release slowly after intravenous injection. This passive targeting induced by phagocytosis of liver Kupffer cells is good for the treatment of hepatic lesions, especially hepatitis. The method to prepare SL liposome is: place silybin and fabaceous lecithin together in a flask, add CH_3OH-$CHCl_3$; the mixed solvent and other supplementary agents are dissolved in vacuum; after drying for several hours, 0.9% NaCl is added, then rotated to hydrate for 2 h to get a silybin liposome suspension; finally, this is hydrated fully under 25°C for several hours (Yu *et al.* 2003). The silybin liposome with a 0.02 to 0.06 ratio of drug to lipid was determined by first-order derivative ultraviolet spectrophotometry; the result showed that the enveloping efficiency was 65.1-83%, with the average content of silybin being 760 $mg \cdot L^{-1}$ (n=4).

Phospholipid complex

SL-phosphatidylcholine complex, as also termed IdB 1016, has high lipophilicity, rapid absorption and high efficacy, and can not only increase the bioavailability of SL, but also remove free radicals and resist against lipid peroxidation (Zhong *et al.* 2003). Barzaghi *et al.* (1990) determined the silybin levels in plasma after oral administration of IdB 1016 and SL (equivalent to 360 mg silybin) to 9 healthy volunteers. The peak concentration of the former was 298 $ng \cdot ml^{-1}$, and the area under curve (AUC) was 881 $ng \cdot ml^{-1} \cdot h^{-1}$, while the peak concentration of the latter was 102 $ng \cdot ml^{-1} \cdot h^{-1}$, and AUC was 257 $ng \cdot ml^{-1} \cdot h^{-1}$. The relative bioavailability of IdB1016 was 4.6, which indicated that bioavailability was improved when SL was included as a phospholipid complex. The study also indicated that IdB1016 could also increase the bioactivity of SL, such as liver protection. The SL-phospholipid complex could decrease the content of hepatic TC and plasma MAD of high-fat feeding rabbits, and increase the content of some metal ions (such as Zn^{2+} and Ca^{2+}) in serum and cytochrome P450 in microsomes, thereby resisting against lipid peroxidation, eliminating free radicals and stabilizing membranes (Du *et al.* 1998). Jiang *et al.* (2002) carried out a test to evaluate the bioavailability of the SL-phospholipid complex in rabbits. Their results showed that the content of plasma free-silybin was lower than that of bound silybin, and the trend was similar to the report of Barzaghi *et al.* (1990). Compared with SL, the time for the SL-phospholipid complex to reach a peak was 0.5 h (down from 1.4 h), and the peak concentration was 2 times higher than SL. The AUC was 11.95 $ug \cdot ml^{-1} \cdot h^{-1}$ (up from 5.55 $\mu g \cdot ml^{-1} \cdot h^{-1}$), but the half-life of silybin almost did not change. These results proved that complex formation increases the rate and degree of absorption, but it does not affect the processes of metabolism and elimination.

Nano-particles

The solution enhanced dispersion by supercritical fluid (SEDS) technique was used to precipitate SL nanoparticles from an organic solution of acetone (Yu *et al.* 2005). The effects of process parameters, such as temperature, pressure, and solution concentration on morphology, particle size (PS), and particle size distribution (PSD) of the particles gained were investigated by using scanning electron microscope (SEM), and the results showed that all particles were solid microspheres, and the temperature and solution concentration were the main factors to influence PS. Through optimization of process parameters, SL particles of 100~300 nm in diameter can be obtained at 9.5 MPa, 40°C and a solution concentration of 60 $mg \cdot mL^{-1}$. He *et al.* (2005) prepared solid lipid nanoparticles (SLN) of various sizes (150, 500 and 1000 nm) using Compritol 888ATO as the material and SL as a model drug to investigate the effect of particle size on oral absorption of SL-loaded solid lipid nanoparticles; the results showed that the AUC of 150 nm SLN was 2.08 times higher that of 500 nm SLN, and 2.54 times higher that of 1 000 nm SLN when treated orally to rats ($P < 0.05$), which implies that the oral bioavailability of 150 nm SLN was remarkably higher than the other two sizes of SLN. At the same time, the parameters of the lyophilization process were optimized for shape, color and redispersibility. A mixture of 2% lactose and 2% glucose was better to prevent nanoparticles from aggregating. So the following optimal lyophilization process was applied: pre-cooling at -45°C for 10 h, then maintaining at -25°C for 5 h, at -5°C for 2 h, at 0°C for 2 h, at 10°C for 3 h, an finally dried at 30°C for 6 h.

QUALITY CONTROL

To improve the controllability for SL preparations, HPLC, Spectrophotometry and TLC methods were used to build the method of quality control. Some major studies are described as below (**Tables 1-3**).

MARKET TRADE

China is the main producer of SL, and countries in Europe and America mostly import SL from China. The required amount of SL is more than 1.2 Mt every year in the world with 300 t every year in China, but the annual output in

Table 1 HPLC method

Aim	Column	Mobile phase	Detection wavelength	Reference
investigate the content of silybin	Prodigy 5 μm C_{18}-A (250 mm × 4.6 mm)	methanol-water (45:55)	288 nm	Lin *et al.* 2004
establish the quality evaluation method for fruits, pericarp and their extracts of *S. marianum*.	5 μm Hypersil C_{18} column (250 mm × 4.6 mm)	methanol-acetonitrile-ammonium dihydrogen phosphate buffer (24:24:50, pH=5)	287 nm	Yuan *et al.* 2003
determine the content of silybin in SL capsules	C_{18} column	gradient elution (0-13 min, methanol 30-70%, 13-20 min, methanol 70%)	288 nm	Liang *et al.* 2004
separate seven components in SL	YWG-C_{18} (100 nm × 4.6 mm)	gradient elution (solution A: methanol-water-0.2 $mol \cdot L^{-1}$ phosphate-0.5 $mol \cdot L^{-1}$ potassium dihydrogen phosphate 80:120:1:8, solution B: ethanol; 0-14 min, solution A; 14-19 min, solution A: solution B 80:20; 19-20 min, solution A: solution B 90:10; 20-22 min, solution B	280 nm	Wang *et al.* 1998
separate and determine content of SL in Legalon, Yiganling and Complex Prescription Yiganling tablets	5 μm C_{18} (10 mm × 4.6 mm)	methanol and solvent mixture of water-dioxane (9:1)	288 nm	Ding *et al.* 1999
determine the content of silybin in Yiganling tablet	C_{18} column	methanol-water-0.5 $mol \cdot L^{-1}$ potassium dihydrogen phosphate (10:10:1, pH=4.0)	288 nm	Chinese National Drug Standard

Table 2 Spectrophotometry method.

Aim	Method	Absorbance wavelength	Reference
detect the content of SL in Yiganling capsule	spectrophotometry	288 ± 1 nm	Chinese National Drug Standard
detect the content of SL in Complex Prescription Yiganling tablet	spectrophotometry	288 nm	Chinese National Drug Standard
detect the content of SL in the fruits, pericarp and their extracts of *S. marianum*	spectrophotometry	287 nm	Yuan *et al.* 2003
detect the content of flavonoids in SL solid dispersion	first-order derivative ultraviolet spectrophotometry	338 nm	Li *et al.* 2002
detect the content of SL in β-CD inclusion complex	spectrophotometry	288 nm	Li *et al.* 1996

Table 3 TLC method.

Aim	Chromatographic condition	Scanning method	Scanning wavelength	References
determine the content of SL	silica gel G thin layer plate, $CHCl_3$-$CH_3CH_2COOC_2H_5$-methanol-water (30:40:20:10, kept overnight at 10°C) as developer	TLC scanning	323 nm	Lin *et al.* 1998
determine the content of SL in Yiganling capsules	silica gel G thin layer plate, $CHCl_3$-CH_3OH-H_2O (15:2:0.1) as developer	Two-wavelength TLC scanning	288~360 nm	Liu *et al.* 1999
determine the content of silybin in the natural drug preparation Legalon from Germany	GF_{254} thin layer plate, $CHCl_3$-CH_3COCH_3-CH_2O_2 (9:2:1) as developer	Dual wavelength reflection sawtooth scannning	288~360 nm	Chen *et al.* 1999

China is less than 1 Mt.

The forms of SL products are single-component silybin separated from *S. marianum*. The annual export amount of SL and silybin in China is more than 300 t; the purity of SL is about 70%, and the purity of silybin is > 99%.

Organic solvents are often used in producing SL in China, which limits the usage of SL. Meanwhile, the benefit of production decreases as the price of organic solvents increases. The confusion of product quality standard and retail price, antiquated detection methods and high import thresholds severely test production enterprises of SL in China. Thus technology should be improved, quality should be increased, new products should be developed and management improved to decrease production costs, thereby increasing the competitive ability to export SL (Zhao *et al.* 2006).

PROSPECTS

S. marianum L. is an excellent plant to protect the liver, and has been recorded in the classical works of Greece and Rome early in the first century AD. Use of the plant to treat liver diseases has thousands of years of history in Europe. SL is the only natural product extracted from plants to protect the liver and to cure liver diseases due to its outstanding efficacy since the 1970s. It is reported that there are 2 billion people infected by hepatitis B virus and 6 million people have already died from liver-related cancer (WHO 2008). China is a high incidence area with 10 million the new hepatitis B patients every year. So SL has great potential as a raw medicine to protect the liver and treat liver diseases. Besides, SL also shows significant activity to resist against tumors and to treat diabetes in some pharmacological tests in recent years.

Thus far, the liposoluble SL has been the chief product (70-80%) of *S. marianum* extract in the market (Zhao *et al.* 2006). The remaining solvent is harmful to humans; furthermore, water-insolubility leads to low bioavailability. So it is urgent to develop water-soluble SL for target preparations to realize a new formulation breakthrough for injections, granules, beverages and health food.

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