Fructus Silybi Mariae

Definition

Fructus Silybi Mariae consists of the dried ripe fruits, freed from the pappus, of *Silybum marianum* (L.) Gaertn., Asteraceae (*1*, *2*).

Synonyms

Carduus marianus L., *Carthamus maculatum* Lam., *Cirsium maculatum* Scop., *Mariana mariana* (L) Hill., *Silybum maculatum* Moench. (*3*, *4*). Asteraceae are also known as Compositae.

Selected vernacular names

Akùb, Artichnuat sauvage, blessed thistle, bull thistle, cardo blanco, cardo de burro, cardo mariano, carduo mariano, chardon argente, chardon-marie, épine blanche, Frauendistelfrüchte, fructus cardui mariae, fruit de chardon marie, holy thistle, kharshat barri, khorfeish, kocakavkas, kuub, Lady's milk, Lady's thistle, lait de Notre Dame, marian thistle, máriatövis-termés, mariazami, Mariendistel, Mariendistelfrüchte, Marienkörner, maritighal, mild marian thistle, milk thistle, pternix, shawkeddiman, Silberdistil, silybe, silybon, silybum, St Mary's thistle, thistle, thistle of the Blessed Virgin, true thistle, variegated marian thistle (*3–7*).

Geographical distribution

Indigenous to North Africa, Asia Minor, southern Europe and southern Russian Federation; naturalized in North and South America, Australia, China and Central Europe (*3*, *4*).

Description

An annual or biennial herb, stem 20–150 cm high, green, glabrous or slightly arachnoid-pubescent. Leaves alternate, large, glossy green, white-veined or variegated, glabrous with strongly spiny margins, basal leaves (25–50 cm long, 12–25 cm wide) cauline, pinnatifid. Inflorescence large, composed of red-purple, hermaphrodite, tubular florets gathered into a capitulum (2.5–4.0 cm in diameter), tucked in an involucre with thorny external bracts. Fruits 6–7 mm long, composed of 6–8 hard-skinned achenes with a white, silky pappus (15–20 mm in diameter) at apex. $2n = 34$ (*3*, *7–12*).

Plant material of interest: dried ripe fruits, freed from the pappus

General appearance

Obliquely obovoid with remainder of a flower crown on its top; 6–7 mm long, up to 3 mm wide, 1.5 mm thick. Testa shiny brownish-black or matt greyish-brown, with dark or greyish-white dots. At the tip, there is a projecting yellowish cartilaginous, swollen ring, and at the bottom at the side, a canaliculate hilum. Silvery pappus absent from the drug. Varieties are white, grey and black (*2*, *4*).

Organoleptic properties

Odour: scarcely perceptible; taste: oily, bitter (*2–4*).

Microscopic characteristics

Pericarp epidermis a colourless palisade layer of cells (about 75 µm long and 8 µm wide) with a strongly thickened outside wall, which reduces the lumen in that part of the cell to a slit; subepidermal layer composed of colourless, thin-walled, parenchyma cells or groups of parenchyma cells alternating with a variable number of pigmented cells; innermost layer mostly collapsed and containing cigar-shaped or monoclinic prismatic crystals of calcium oxalate. Testa epidermis consists of large, lemon-yellow, palisade-like, elongated cells (about 150 µm long) with striated walls and narrow lumen widening slightly at the ends; subepidermal layers have lignified and pitted cells (*2*, *4*).

Powdered plant material

Brownish-yellow. Fragments of colourless palisade-like epidermal cells from the fruit wall with attached pigment layer; epidermal cells about 75 µm long and 8 µm wide; cigar-shaped or monoclinic prismatic crystals of calcium oxalate; fragments of lemon-yellow, palisade-like testa cells about 150 µm long; fragments of embryo with thin-walled cells, small druses and lipophilic substances (*2*).

General identity tests

Macroscopic and microscopic examinations (*2*, *4*), and thin-layer chromatography for the presence of marker compounds (taxifolin, silybin, silydianin and silychristin) (*2*, *13*).

Purity tests

Microbiological

Tests for specific microorganisms and microbial contamination limits are as described in the WHO guidelines on quality control methods for medicinal plants (*14*).

Foreign organic matter

Not more than 2% (*1*, *2*).

Total ash

Not more than 8% (*1*, *2*).

Acid-insoluble ash

Not more than 1% (*1*).

Water-soluble extractive

Not less than 10% (*1*).

Loss on drying

Not more than 8% (*2*).

Pesticide residues

The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (*15*). For other pesticides, see the *European pharmacopoeia* (*15*), and the WHO guidelines on quality control methods for medicinal plants (*14*) and pesticide residues (*16*).

Heavy metals

For maximum limits and analysis of heavy metals, consult the WHO guidelines on quality control methods for medicinal plants (*14*).

Radioactive residues

Where applicable, consult the WHO guidelines on quality control methods for medicinal plants (*14*) for the analysis of radioactive isotopes.

Other purity tests

Chemical, sulfated ash and alcohol-soluble extractive tests to be established in accordance with national requirements.

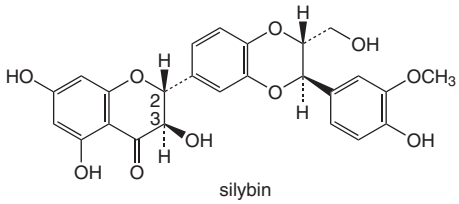
Chemical assays

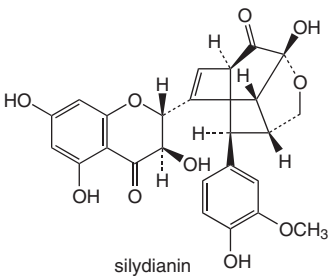
Contains not less than 1.5% silymarin, calculated as silybin, as analysed by high-performance liquid chromatography (*2*). Other high-performance liquid chromatography methods are also available (*3*, *17*, *18*).

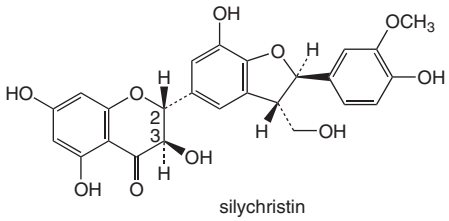
Major chemical constituents

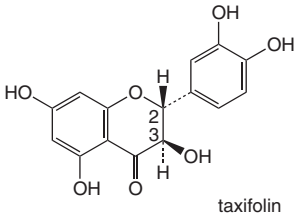
The major active constituents are flavonolignans (1.5–3.0%), collectively known as silymarin. The major components of the silymarin complex are the four

isomers silybin and isosilybin (a 1 : 1 mixture of diastereoisomers), silychristin and silydianin. Other flavonolignans identified include 2,3-dehydrosilybin and 2,3-dehydrosilychristin. Taxifolin, a 2,3-dihydroflavonol, which may be regarded as the parent flavonol of the silymarin compounds, is another major marker for Fructus Silybi Mariae (*3*, *4*, *6–8*, *19*, *20*). The structures of the major silymarin components and taxifolin are presented below.









Medicinal uses

Uses supported by clinical data

Supportive treatment of acute or chronic hepatitis and cirrhosis induced by alcohol, drugs or toxins (*21–34*).

Uses described in pharmacopoeias and in traditional systems of medicine

Treatment of dyspeptic complaints and gallstones (*7*, *35*).

Uses described in folk medicine, not supported by experimental or clinical data

Treatment of amenorrhoea, constipation, diabetes, hay fever, uterine haemorrhages and varicose veins (*6*).

Pharmacology

Most of the biochemical and pharmacological studies have been performed using a standardized silymarin preparation, or its major constituent, silybin.

Experimental pharmacology

Antioxidant activity

Silymarin and silybin have antioxidant activity in vitro: both react with oxygen-free radicals such as hydroxyl anions, phenoxy radicals and hypochlorous acid in various model systems such as human platelets, human fibroblasts, rat liver microsomes and mitochondria, and using enzymatically and non-enzymatically generated free inorganic radicals (*36–42*). The production of superoxide anion radicals and nitric oxide was inhibited after treatment of isolated rat Kupffer cells with silybin (IC_{50} 80 µmol/l) (*43*). Both silymarin and silybin inhibited free radical-induced lipid peroxidation in microsomal and mitochondrial preparations of human red blood cells, thereby stabilizing the structure of the cell membrane (*36*, *44–52*). Inhibition of cyclic AMP-dependent phosphodiesterase by silybin, silydianin and silychristin has been demonstrated in vitro. Since cyclic AMP is known to stabilize lysosomal membranes, an increase in the concentration of this nucleoside has been proposed to be the mechanism of membrane stabilization and thus the anti-inflammatory activity of silymarin (*53*). Silybin also inhibits phospholipid synthesis and breakdown in rat liver membranes in vitro, and corrects the alteration in phospholipid metabolism in ethanol-treated rats (*54*). Both silymarin and silybin are incorporated into the hydrophobic–hydrophilic interface of the rat microsomal membrane bilayer and alter the structure by influencing the packing of the acyl chains (*47*).

Antihepatotoxic activity

Silymarin and silybin inhibited hepatotoxicity induced by paracetamol (acetaminophen), amitriptyline, carbon tetrachloride, ethanol, erythromycin estolate, galactosamine, nortriptyline and *tert*-butyl hydroperoxide in rat hepatocytes in vitro (*55–58*). Silybin reduced ischaemic damage to nonparenchymal hepatic cells and improved post-ischaemic function in pig livers (*59*). Allyl alcohol-induced toxicity, and associated lipid peroxidation and glutathione depletion were suppressed after treatment of isolated rat hepatocytes with silymarin and silybin at concentrations of 0.1 and 1.0 mmol/l, respectively (*60*).

Silybin stimulated macromolecular biosynthesis in vitro and in vivo (*61–64*). Silybin increased the rate of ribosomal RNA synthesis by 20% in rat liver, cultured hepatocytes and isolated liver nuclei, via activation of DNA-dependent RNA polymerase I (*63*). Silybin binds to the regulatory subunit of DNA-dependent RNA polymerase I at the estrogen binding site, thereby acting as a natural steroid effector, and thus activating the enzyme and increasing the rate of ribosomal RNA synthesis (*64*). Silybin had no effect on the transcription of RNA polymerase II or III. The increase of ribosomal RNA synthesis in the liver stimulates the formation of mature ribosomes, and hence protein biosynthesis (*63*). Furthermore, an increase in DNA synthesis was observed in livers from hepatectomized rats treated with silybin (27 mg/kg body weight) (*65*).

Intraperitoneal or intragastric administration of silymarin (15–800 mg/kg body weight) to dogs, mice and rats prevented carbon tetrachloride-induced liver damage (*46*, *66–68*). This effect of silymarin was attributed to its antioxidant activity, a decrease in the metabolic activation of carbon tetrachloride, and stabilization of hepatocyte membranes (*46*, *66*, *67*, *69*). Intragastric administration of silymarin (50 mg/kg body weight) improved the metabolism and tissue distribution of aspirin in rats with carbon tetrachloride-induced liver toxicity (*70*). Intraperitoneal administration of either silymarin or silybin markedly inhibited liver damage induced by paracetamol (acetaminophen), *Amanita phalloides* toxins (e.g. phalloidin and α-amanitin), ethanol, galactosamine, halothane, polycyclic aromatic hydrocarbons, rare earth metals (e.g. cerium, praseodymium and lanthanum) and thallium in various rodent models (*50*, *71–81*). Furthermore, intravenous administration of silybin hemisuccinate sodium salt (50 mg/kg body weight) to dogs given sublethal doses of *Amanita phalloides* (85 mg/kg body weight) prevented the increase in concentration of liver enzymes in the blood and the decrease in clotting factors (*82*). The uptake of [^{3}H]dimethyl phalloidin in isolated rat hepatocytes was inhibited by 79% in cells treated with silybin ester (100 µg/ml) (*73*). However, intravenous administration of silybin (50 mg/kg body weight) to rats inhibited the protective effect of ethanol on paracetamol-induced hepatotoxicity. The combination of ethanol and silybin appeared to lead to inhibition of paracetamol metabolism by microsomes (*83*). Intravenous administration of silybin hemisuccinate sodium salt (50 mg/kg body weight) to mice preinfected with sublethal doses of frog virus 3 attenuated histological changes in hepatocyte nuclei; animals treated with a lethal dose of frog virus 3 showed increased survival times (*84–86*).

Intragastric administration of silymarin (50 mg/kg body weight) to rats inhibited collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct occlusion induced by sodium amidotrizoate (*87*). Silymarin increased the redox state and the total glutathione content in the liver, intestine and stomach of rats after intraperitoneal administration (200 mg/kg body weight) (*42*, *88*).

In a transplantation experiment, explanted pig liver was subjected to cold-induced ischaemia by storage of the liver at 4°C for 24 hours, followed by extracorporeal reperfusion for 4 hours. Intravenous administration of 500 mg silybin ester prior to removal of the liver, followed by 400 mg/l during cold storage and 100 mg/h during reperfusion, reduced histological damage to the liver cells (measured by bile production) and improved liver function during reperfusion by 24–66% (measured by bile acid excretion) (*59*).

Anti-inflammatory and anti-allergic activity

Silybin inhibited neutrophil-mediated histamine release induced by *f*-met peptide and anti-IgE from human basophil leukocytes. The inhibitory effect was significantly attenuated ($P < 0.05$) by elevating the extracellular calcium concentration. However, no effect was observed on histamine release induced by the calcium ionophore A23187 (*89*). Silymarin inhibited neutrophil-

mediated histamine release activated by *N*-formylmethionyl-leucyl-phenylalanine from rat peritoneal mast cells at a concentration of 25 µg/ml (*90*). Silybin inhibited the synthesis of leukotriene B_4 (IC_{50} 15 µmol/l) in isolated rat Kupffer cells, but had no effect on prostaglandin E_2 formation at concentrations up to 100 µmol/l (*43*). Silymarin, silybin, silydianin and silychristin inhibited the activity of lipoxygenase and prostaglandin synthetase in vitro (*91–93*). The anti-inflammatory activity of silybin was assessed in human polymorphonuclear leukocytes in vitro. The chemotactic and phagocytic activities of the polymorphonuclear leukocytes were not modified by silybin at concentrations of 0.5–25.0 µg/ml. However, the compound did inhibit luminol-enhanced chemiluminescence, suggesting that the mechanism of anti-inflammatory activity involved the inhibition of hydrogen peroxide formation (*94*). Intragastric administration of silymarin reduced carrageenan-induced footpad oedema in rats (ED_{50} 62.42 mg/kg body weight). Topical application of silymarin inhibited xylene-induced ear inflammation in mice, and its activity was similar to that of indometacin (25 mg/kg body weight). In addition, silymarin inhibited leukocyte accumulation in inflammatory exudates following intraperitoneal administration of carrageenan to mice (*95*).

Intragastric administration (25–1000 mg/kg body weight) of an acetone extract of the fruit containing silybin increased the volume and dry mass of excreted bile in rats (*96*). Intragastric administration of silymarin (100 mg/kg body weight) prevented gastric ulceration in rats induced by cold-restraint and pyloric ligation, but was not effective against ethanol-induced ulcers (*97*). Intragastric administration of silymarin (100 mg/kg body weight) to rats prevented gastric injury induced by ischaemia-reperfusion (*98*).

Clinical pharmacology

Alcohol-induced hepatitis

The efficacy of a standardized silymarin preparation for the treatment of alcohol-induced cirrhosis was assessed in six placebo-controlled clinical trials (*24–27, 31, 33, 99*). The majority of these studies involved between 50 and 100 patients, with one study including 170 patients (*26*). Patients generally received an oral dose of 280–420 mg (140 mg two or three times daily) of a standardized silymarin preparation or placebo. One of the studies had a treatment period of up to 4 years, and used survival rates as their outcome parameter. The results of this study showed a significant decrease in the mortality of patients treated with silymarin as compared with placebo ($P < 0.05$) (*26*). After treatment with the silymarin preparation (140 mg twice daily), a decrease in total bilirubin, liver enzymes and serum N-terminal propeptide of collagen type III levels was observed (*25*). A 6-month trial that was also double-blind assessed the efficacy of silymarin in patients who had histological documentation of chronic alcoholic hepatitis. Silymarin treatment improved histology, and lymphocyte proliferation and lipid peroxidation (*24*). In two studies that were also randomized and double-blind, treatment of 163 patients with the

silymarin preparation decreased serum levels of liver enzymes, improved liver function, and returned sulfobromophthalein levels to normal, as compared with placebo (*27*, *31*). Another trial that was also randomized and double-blind analysed the effects of silymarin in 116 patients with alcohol-induced hepatitis, 58 of whom had liver cirrhosis. Patients received 420 mg silymarin or placebo daily for 3 months. A significant improvement was noted in both groups ($P < 0.05$); however, silymarin was not more effective than placebo (*99*).

Five double-blind clinical trials assessed the efficacy of silymarin in the treatment of various chronic liver diseases induced by alcohol (*22*, *23*, *25*, *29*, *30*). In four of these trials, treatment of patients with 420 mg of the silymarin preparation daily for 6 months decreased the serum levels of bilirubin, procollagen III peptide and liver enzymes, and increased serum glutathione peroxidase activity and lectin-induced lymphoblast transformation (*23*, *25*, *29*, *30*). In the fifth study, which was also placebo-controlled, the efficacy of silymarin was assessed in 20 patients with various chronic liver diseases. After 13 months of treatment (420 mg daily), histopathological findings showed improvements in the treated group as compared with the group that received placebo (*22*).

In a randomized trial of 60 patients with diabetes caused by alcohol-induced cirrhosis, patients received either 600 mg silymarin daily or no treatment for 6 months (*100*). The blood glucose and malondialdehyde levels, daily insulin need and fasting insulinaemia levels were all significantly lower in treated patients than in those that were untreated ($P < 0.05$), and lower than initial baseline values (*100*, *101*). A study without controls assessed the efficacy of a standardized silymarin preparation (420 mg daily) in inhibiting fibrotic activity in 277 patients with various chronic liver diseases. In liver fibrosis, the serum level of the procollagen III peptide increases. The elevated levels of this peptide decreased over the 4-week treatment period (*102*). In a drug monitoring study without controls, 108 patients with alcohol-induced hepatotoxicity and liver inflammation were treated with silymarin (200–400 mg/kg body weight, in a single dose) daily for 5 weeks. After treatment, the serum procollagen III peptide and liver enzyme levels were lower in comparison to the initial baseline values. The preparation was generally well tolerated in 98% of patients (*103*). The safety and efficacy of silymarin were evaluated in over 3500 patients in two drug-monitoring studies. In one study, 2637 patients with various liver disorders were treated with a standardized silymarin preparation (560 mg, given in four divided doses) daily for 8 weeks. Subjective symptoms decreased by 63%, clinical findings improved and elevated serum levels of liver enzymes were reduced in the treated group. Treatment was rated as very good, good or satisfactory by 88% of the physicians (*21*). Minor gastrointestinal side-effects were reported in 1% of patients (*21*, *28*).

Acute and chronic viral hepatitis

Three controlled trials assessed the efficacy of silymarin in the treatment of acute viral hepatitis (*104–106*). In a randomized, double-blind study of 57

patients with acute viral hepatitis A or B, patients received 420 mg of a standardized silymarin preparation or placebo daily for 3 weeks. In the treatment group, 40% of patients had a normalized blood bilirubin level, as compared with 11% of the placebo group; 82% of the treated patients had a normalized blood level of aspartamine transaminase, as compared with 52% of the placebo group. There was no difference between the two groups in the number of patients who developed immunity (*105*). In another trial, the duration of inpatient care was shown to be shorter for patients treated with silymarin, compared to those who received supportive care (23.3 and 30.4 days, respectively). In patients with viral hepatitis B, treatment with silymarin led to a shorter interval to the development of immunity (30.4 days), compared to supportive therapy only (41.2 days) (*104*). A double-blind study in patients with acute viral hepatitis indicated that daily treatment with 420 mg silymarin (three doses of 140 mg) decreased the complications associated with the infection (*106*).

A 12-month study combining two double-blind, placebo-controlled trials assessed the efficacy of silymarin in the treatment of chronic hepatitis, with or without cirrhosis, in 36 patients. Patients were treated with 420 mg of a standardized silymarin preparation or placebo daily for 3–12 months. Assessment of serum levels of bilirubin and liver enzymes did not reveal any significant differences in liver function between the treatment and placebo groups. However, histological improvements were noted in patients who received silymarin (*107*).

Organic compound-induced hepatitis

A controlled clinical study of patients with a 5–20-year history of occupational exposure to toluene and/or xylene vapours was performed to assess the efficacy of a standardized silymarin preparation on liver function. Thirty patients were treated orally with 140 mg of the preparation three times daily for 30 days, and the results were compared with those from 19 untreated matched controls. Both liver function and platelet counts markedly improved in the treated patients (the elevated serum levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were reduced, and the low platelet numbers increased) as compared with the controls (*32*). In another study, the effects of a silymarin preparation (420 mg/day) on liver function in 14 patients chronically exposed to the organophosphate malathion were assessed. After treatment, patients showed no improvement in liver function tests when compared with the controls (10 healthy volunteers) (*108*).

Drug-induced hepatitis

A double-blind, placebo-controlled study assessed the efficacy of silymarin in the prevention of hepatic damage induced by psychotropic drugs. Sixty patients receiving chronic therapy with psychotropic drugs (butyrophenones or phenothiazines) were treated orally with 800 mg silymarin or placebo daily for 90 days. Silymarin treatment improved liver function and reduced lipoperoxida-

tive hepatic damage as determined by serum malondialdehyde levels (the end-product of the oxidation of polyunsaturated fatty acids) (*109*). A small clinical study found improvements in biochemical parameters in 19 patients using psychotropic drugs after 6 months of treatment with silymarin (*110*).

Toxin-induced hepatitis

Numerous case reports have indicated that silymarin and silybin are effective in the treatment of poisoning due to ingestion of the deathcap mushroom *Amanita phalloides* (*34*, *111–114*). *Amanita* toxins inhibit the activity of RNA polymerase in hepatocytes, causing cell death after 12–24 hours. In a clinical trial without controls, 60 patients were treated intravenously with silybin (20 mg/kg body weight, daily for 1–2 days), 24–36 hours after ingestion of *Amanita phalloides*. The survival rate was 100% (*34*). Results of a multicentre study of 252 cases of poisoning due to ingestion of *Amanita phalloides* indicated that intravenous infusion of silybin (20 mg/kg body weight, daily for 1–2 days), in combination with the standard management techniques, dramatically reduced mortality, without producing side-effects (*111–113*).

Assessment of the clinical trials of silymarin for the treatment of hepatitis induced by alcohol, drugs or toxins, and acute and chronic viral hepatitis should be interpreted with caution because of the small number of patients involved, the heterogeneity of diagnoses and outcome parameters, and the inconsistent reporting of alcohol intake by patients during the studies (*115*).

Pharmacokinetics

In a randomized, four-way crossover study without controls, a single dose of 102, 153, 203 or 254 mg silybin was administered orally to six healthy males. Silybin and isosilybin concentrations in plasma were measured as unconjugated compounds as well as total isomers after hydrolysis using high-performance liquid chromatography. Areas under the curve were linear with the dose, and only 10% of total silybin in the plasma was in the conjugated form. The elimination half-life of unconjugated silybin was less than 1 hour; that of total silybin was estimated to be 6 hours. Approximately 5% of the dose was excreted into the urine as total silybin, corresponding to a renal clearance rate of 30 ml/min (*116*).

After oral administration of a single dose of 560 mg silymarin (equivalent to 240 mg silybin) to six healthy volunteers, maximum serum concentrations of silybin were low, ranging from 0.18 to 0.62 µg/ml. Only 1–2% of the dose was excreted in the urine during the 24 hours following administration. After oral administration of a single dose of 140 mg silymarin (equivalent to 60 mg silybin) to 14 patients who had undergone cholecystectomy, bile collected from the T-tube drains contained 11–47 µg/ml silybin, equivalent to 7–15% of the dose, after 24 hours (*117*).

Following oral administration of a single dose of a standardized silymarin preparation (140 mg) to nine patients who had undergone cholecystectomy, the urinary and biliary excretion of silybin, silydianin and silychristin were mea-

sured. The urinary excretion of silybin and silychristin was insignificant. Both silybin and silychristin were excreted in the bile in the form of sulfate and glucuronide conjugates. The total elimination of silybin was estimated to be 20–40% and that of silychristin was 4–10%. Urinary excretion of silymarin occurred over a 24-hour period, with maximum excretion occurring between 2 and 9 hours after administration (*118*).

The bioavailability of silymarin varies considerably and is dependent on the formulation of the product (*119*).

Contraindications

Fructus Silybi Mariae is contraindicated in cases of known allergy to plants of the Asteraceae family (*120*).

Warnings

No information available.

Precautions

No information available on general precautions or precautions concerning drug interactions; drug and laboratory test interactions; carcinogenesis, mutagenesis, impairment of fertility; teratogenic and non-teratogenic effects in pregnancy; nursing mothers; or paediatric use. Therefore, Fructus Silybi Mariae should not be administered during pregnancy or lactation or to children without medical supervision.

Adverse reactions

Crude drug: one case of anaphylactic shock was reported in a patient ingesting a tea prepared from Fructus Silybi Mariae (*120*). Standardized preparation: a mild laxative effect has been reported (*35*).

Dosage forms

Usually standardized extracts for phytomedicine; crude drug for decoction (*4*). Store in a well-closed container, protected from light and humidity (*2*).

Posology

(Unless otherwise indicated)

Daily dosage: 12–15 g crude drug (*35*); 200–400 mg silymarin, calculated as silybin, in standardized preparations (*35*).

A parenteral preparation, silybin hemisuccinate sodium salt, is available in Germany for treatment of poisoning due to ingestion of *Amanita phalloides* (*111–114, 121*). The total dosage is 20 mg/kg body weight, given as four infusions over a 24-hour period, with each dose administered over a 2-hour period (*121*).

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