

MILK THISTLE

111 ABSTRACTS

PubMed search of:

Milk Thistle, therapeutic = 52 hits

Milk Thistle, deficiency = 1 hit

Milk Thistle, human = 58 hits

Milk Thistle, animal = 38 hits

Milk Thistle, benefit = 7 hits

MESH search of:

Milk Thistle, therapeutic = 7 hits

Milk Thistle, deficiency = 0 hits

Milk Thistle Overview

The milk thistle plant grows from 4-10 feet in height and produces thick thistle, reddish-purple flowers, large prickly leaves, and tube-like pointed flowers. Milk thistle grows around the world and is typically found along roadsides and upon cultivated ground. Milk thistle is an edible plant, and its leaves can be eaten like artichokes. The seeds can be roasted and brewed like coffee. *Silybum marianum*, a constituent of milk thistle, has been used for over 2,000 years as a traditional medicine specifically for liver ailments. Milk thistle protects liver tissue; aids in the regeneration of damaged liver tissue; decreases liver and bile cholesterol; alleviates inflammation; and limits liver damage resulting from a disrupted oxygen supply.

Scientifically, researchers have found that silymarin, as well as an isolated form of flavonolignan called silybin, can prevent or counteract damage to the liver caused by toxins such as alcohol, acetaminophen (Tylenol) and other drugs, as well as environmental (heavy metals) and bacterial toxins, and even poisons such as those found in the lethal Deathcap mushroom. Silymarin combats lipid peroxidation in the liver of rats; may hasten the restoration of liver cells in damaged liver tissue. The mechanism of liver damage may be depletion of glutathione and silymarin and silybin actually elevated glutathione levels in rats given alcohol. Human subjects with liver damage caused by chronic alcoholism, cirrhosis, hepatitis, or other toxicities were significantly benefited by treatment with silymarin.

Research on milk thistle as a liver cholesterol-lowering agent shows that rats given silybin had significantly lower cholesterol levels in their bile relative to rats given placebo. Even topical silymarin, applied to the ears of mice with dermatitis, caused a decrease in inflammation. Silymarin has also decreases histamine release from cells. And, giving silybin to rats following oxygen supply depletion to the liver decreased the severity of cell death.

Dosage: Milk thistle, standardized extract of the milk thistle seed contains 80% silymarin flavonoids (silybin, silydianin, and silychristin). Typical dosage recommendations are for 175 mg of 80% silymarin extract, taken 1-3 times per day.

Side Effects: Milk thistle is quite safe as a supplement. Neither toxicity nor drug interactions have been reported following high doses of milk thistle or its components.

(Source: www.supplementwatch.com)

Research Overview

Milk Thistle research shows the following:

1. Has hepaprotective qualities
2. May be used safely in chronic liver disease
3. Acts as a powerful antioxidant in liver tissues
4. In synergy with vitamin E, act as liver free radical scavenger
5. Is valuable in post-operative liver repair
6. Is an effective treatment in alcoholic cirrhosis
7. Inhibits prostate tumor growth
8. Regenerates liver cells
9. Is a cancer preventative
10. Is anti-carcinogenic
11. Inhibits colon cancer cell proliferation
12. Inhibits tongue cancer cell proliferation
13. Has immunostimulating properties
14. May be of benefit to the nervous system
15. May protect against skin cancer
16. May protect against atherosclerosis
17. May protect pancreas against alcohol damage
18. Improves milk metabolism in cows
19. Prevents LDL oxidation
20. Inhibits Nitric Oxide production
21. Has antiinflammatory properties
22. May protect against UVB rays

MILK THISTLE: 111 RESEARCH ABSTRACTS FILED ACCORDING TO CONDITION OR FUNCTION

Liver

1. Biosci Biotechnol Biochem. 2003;67(9):1857-1863. **(Animal Study)**

Suppression of Ethanol and Lipopolysaccharide-induced Liver Injury by Extracts of Hydrangeae Dulcis Folium in Rats.

Hashizume E, Nakagiri R, Shirai A, Kayahashi S, Yasushi S, Kamiya T.

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In female SD rats that were injected with 4 g/kg BW ethanol p.o. followed by a 5 mg/kg BW lipopolysaccharide (LPS) i.v. injection, serum glutamic pyruvic transaminases (GPT) activity increased to about eight times that of normal rats. In this model, rats that had been fed a diet containing 1% Hydrangeae Dulcis Folium (HDF) extracts for fifteen days showed significantly lower serum GPT activity (380.0+/-58.2 IU/l) than the control group (3527.0+/-774.1 IU/l). HDF's efficacy was far superior to **milk thistle** in this model (2950.0+/-915.9 IU/l). When mouse macrophages were treated with HDF extracts at 50 microg/ml, TNF-alpha production induced by LPS was suppressed to about 10% of the control. Rat serum TNF-alpha levels induced by LPS was decreased to 58.7% of the control by administering 1000 mg/kg BW HDF extract p.o. These results indicate that HDF prevents alcohol-induced liver injury through the inhibition of TNF-alpha production.

2. *Toxicon*. 2003 Sep;42(4):339-49.

Cytotoxic fungi-an overview.

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Among fungal toxins causing organ damage in the human body, amatoxins and orellanine remain exceptional. Amatoxins, a group of bicyclic octapeptides occurring in some *Amanita*, *Galerina* and *Lepiota* species, induce deficient protein synthesis resulting in cell death, but might also exert toxicity through inducing apoptosis. Target organs are intestinal mucosa, liver and kidneys. Poisoning will result in dehydration and electrolyte derangement, liver necrosis and possibly kidney damage. In established poisoning the mainstay of treatment is optimum symptomatic and supportive care. No specific treatment is available, but some pharmaceuticals, like **silibinin**, benzylpenicillin and acetylcysteine, might have a role in limiting the extent of hepatic damage. Orellanine is a nephrotoxic bipyridine N-oxide found in some *Cortinarius* species. Its mechanism of action is not fully understood, but it has been shown to inhibit protein synthesis and to generate free oxygen radicals. As early symptoms often are lacking or vague, poisoning may initially be overlooked or misinterpreted and the patients usually present with established renal damage. Supportive care is the only therapeutic option. *Tricholoma equestre* might contain a myotoxin and repeated ingestion may cause significant rhabdomyolysis. Ingestion of *Amanita*

smithiana and *A. proxima* has been reported to result in kidney damage. Gyromitrin, a toxic compound that is converted to hydrazines in the stomach, occurs in some *Gyromitra* species. It is mainly neurotoxic, but may also induce moderate hepatic damage and haemolysis.

3. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2003 Sep 5;794(2):303-10.

Application of liquid chromatography-electrospray ionization-ion trap mass spectrometry to investigate the metabolism of **silibinin** in human liver microsomes.

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Silibinin is the main isomer of a group of flavanoids extracted from the seeds of the **milk thistle** weed, a common herb that is widely used to maintain liver health and for the treatment of liver disorders. **Silibinin** when incubated with human liver microsomes produced one major metabolite and at least two minor metabolites. Tandem mass spectrometry (MS) was used to identify the metabolite structures partially. MS studies confirmed that the major metabolite is demethylated **silibinin** and the two minor metabolites are mono-hydroxy and di-hydroxy **silibinin**. The $K(m)$ value for the demethylation shows that **silibinin** has a strong affinity for the cytochrome P450 enzymes.

4. *J Hepatol.* 2003 Sep;39(3):333-40. (**Animal Study**)

Silibinin protects mice from T cell-dependent liver injury(small star, filled).

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BACKGROUND/AIMS: **Silibinin** is the major pharmacologically active compound of the

Silybum marianum fruit extract **silymarin**. Its well-known hepatoprotective activities are mostly explained by antioxidative properties, inhibition of phosphatidylcholine synthesis or stimulation of hepatic RNA and protein synthesis. Here, we characterized the hepatoprotective potential of **silibinin** as an immune-response modifier in T cell-dependent hepatitis in vivo. METHODS: **Silibinin** was tested in the mouse model of concanavalin A (ConA)-induced, T

cell-dependent hepatitis. Liver injury was assessed by quantification of plasma transaminase activities and intrahepatic DNA fragmentation. Plasma cytokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA), intrahepatic cytokine and inducible NO synthase (iNOS) mRNA levels by reverse transcriptase polymerase chain reaction, intrahepatic iNOS expression by immunofluorescent staining, and intrahepatic nuclear factor kappa B (NF-kappaB) activation by electrophoretic mobility shift assay. RESULTS: **Silibinin** significantly inhibited ConA-induced liver disease. **Silibinin** proved to be an immune-response modifier in vivo, inhibiting intrahepatic expression of tumor necrosis factor, interferon-gamma, interleukin (IL)-4, IL-2, and iNOS, and augmenting synthesis of IL-10. In addition, **silibinin** inhibited intrahepatic activation of NF-kappaB. CONCLUSIONS: **Silibinin**, suppressing T cell-dependent liver injury as an immune-response modifier, might be a valuable drug in therapeutic situations in which intrahepatic immunosuppression is required.

5. Alcohol Alcohol. 2003 Sep-Oct;38(5):411-4.

Primary human hepatocytes are protected against prolonged and repeated exposure to ethanol by **silibinin**-dihemisuccinate.

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AIMS AND METHODS: We investigated the effect of **silibinin**-C-2',3'-dihydrogensuccinate (SDH) on primary human hepatocytes when exposed to ethanol for 14 days. At regular intervals, the medium was refreshed and liver enzymes and secreted protein in the medium were determined. RESULTS: The ethanol-induced release of lactate dehydrogenase (at 34 mM ethanol) was completely blocked by 20 microM SDH. SDH itself stimulated fibrinogen release and had no toxic effect. CONCLUSIONS: We can conclude that SDH has a beneficial effect on human hepatocytes when exposed to ethanol in vitro.

6. Arzneimittelforschung. 2003;53(6):420-7.

Preparation and pharmacological evaluation of **silibinin** liposomes.

Maheshwari H, Agarwal R, Patil C, Katare OP.

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

The aim of the present study was to encage a drug into liposomal structures to make them more effective, safe and targeted to liver cells. The investigation deals with critical parameters controlling the formulation and evaluation of **silibinin (silymarin, CAS22888-70-6)** liposomes. Small unilamellar liposomal vesicles were prepared using the ethanol injection method. The various formulation and process variables were optimized to improve the drug entrapment efficiency. The study includes the selection of lipid composition, impact of charge imparting agent and the nature of hydration medium. The stability and size parameters were critically monitored. The liposomal systems were also studied for hepatoprotective activity in mice against carbon tetrachloride induced hepatotoxicity and gastroprotective activity using the pyloric ligation method. The results indicate a significant effect of cholesterol on drug-entrapment and drug-leakage characteristics. The size distribution range was from 0.056-1.270 microns with the most frequent size ranging from 0.266-0.466 micron. The amount of drug loaded in these vesicles was approx. 90%. Lipid cholesterol mass ratio of 10:2 has a maximum entrapment of 87.2% (+/- 1.77). The results obtained from the in vivo studies indicate the improved performance of **silymarin** in liposomes at a level of 55.6% hepatoprotection in comparison to 33.08% of plain drug. Plain liposomes showed hepatoprotection though to a lower degree of 24.2%. Liposomal **silymarin** and plain liposomes also showed significant antiulcer activity as compared with plain **silymarin** and control groups.

7. J Pharm Belg. 2003;58(1):28-31.

[St. Mary's Thistle: an overview]

[Article in French]

Laekeman G, De Coster S, De Meyer K.

K.U.Leuven.

St. Marys Thistle has been approved for registration as a regular medicine in Belgium. The hepatotropic properties of this plant are rather difficult to evaluate objectively. Mortality rate in case of life-threatening hepatic diseases is the most objective parameter. Legalon is the only drug registered in Belgium. It has a prescription only status. The plant *Silybum marianum* is a thistle and as a consequence belongs to the Compositae. There is a limited production of St.-Marys Thistle in Pajottenland, west of Brussels. The seeds are exported to Italy in order to extract **silymarine**, a mixture of flavonolignanes with antioxidant properties. **Silymarine** has been tested in living animals deliberately intoxicated with mushroom toxins, medicines, heavy metals or toxic organic solvents. Preventive as well as curative activity has been confirmed.

Silymarine accumulates in the liver, which is also the target organ in therapy. **Silymarine** improves the prognosis after accidental ingestion of the toxic *Amanita phalloides*. Patients infected with hepatitis B and C might benefit from **Silymarine**, but more data have to be generated. **Silymarine** given to patients with liver damages by alcohol lowers the death toll. The drug has a general safety pattern comparable to placebo.

8. *Phytother Res.* 2002 Nov;16(7):632-8.

Effect of silybin and its congeners on human liver microsomal cytochrome P450 activities.

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Silybin and related flavonolignans form a major part of the *Silybum marianum* extract, **silymarin**, which has been used to treat liver diseases for hundreds of years. Although regarded as safe, many of the extract constituents remain thus far untested for their possible effects on liver biotransformation enzymes. Cytochromes P450 (CYP) are very important in this regard. We tested the effect of four flavonolignans: silybin, its hemisynthetic derivative dehydrosilybin, silydianin, and silycristin on three specific CYP activities: bufuralol 1'-hydroxylation (CYP2D6), p-nitrophenol hydroxylation (CYP2E1), and nifedipine oxidation (CYP3A4). All flavonolignans displayed dose-dependent inhibition of these activities with IC(50) values in the micromolar range. The inhibition was competitive or mixed as revealed by double reciprocal plots of kinetic experiments. However, the inhibition is not considered to be relevant for therapy because physiological concentrations of the individual flavonolignans do not exceed 0.5 microM. The data support the use of the extract as a dietary supplement. Copyright 2002 John Wiley & Sons, Ltd.

9. *Planta Med.* 2002 Aug;68(8):676-9. **(Animal Study)**

Physiological responses to a natural antioxidant flavonoid mixture, **silymarin**, in BALB/c mice: I induction of transforming growth factor beta1 and c-myc in liver with marginal effects on other genes.

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Silymarin, a mixture of flavonolignans isolated from *Silybum marianum*, is known for its hepatoprotective properties. We investigated the expression of cytokines in mouse liver following treatment with 0, 10, 50, and 250 mg/kg of **silymarin** once daily for 5 days. A dose-related but insignificant decrease of circulating alanine aminotransferase and aspartate aminotransferase after **silymarin** treatment was observed, suggesting that **silymarin** treatment did not induce hepatic damage. **Silymarin treatment caused significant increases in the expressions of transforming growth factor (TGF) beta1 and c-myc in liver.** No significant difference was detected among these treatments in the expression of hepatocyte growth factor, interferon gamma, tumor necrosis factor alpha, and class II major histocompatibility complex. **These results suggest that alterations of TGFbeta1 and c-myc expression in the liver may be involved in the hepatoprotective effects of silymarin observed in other studies.**

10. Tidsskr Nor Laegeforen. 2002 Mar 20;122(8):777-80.

[Serious mushroom poisoning by *Cortinarius* and *Amanita virosa*]

[Article in Norwegian]

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BACKGROUND: Following a characteristic long latent period (3-17 days), the nephrotoxins of *Cortinarius rubellus* and *Cortinarius orellanus* can cause the orellanus syndrome, due to severe damage of the proximal tubular epithelium. *Amanita virosa* is known to produce serious toxic effects in the liver and the kidneys after an initial asymptomatic latent phase. **MATERIAL AND METHODS:** We discuss the toxicity, clinical features and treatment of the orellanus and the phalloides syndromes and present six case histories. **RESULTS AND INTERPRETATION:** Ingestion of *Cortinarius rubellus* and *Cortinarius orellanus* resulted in permanent renal failure in four out of five patients, following a latent period of about ten days. One patient who ingested *Amanita virosa*, developed hepatotoxicity. **He was given silibinin and symptomatic treatment and recovered.** After ingestion of *Cortinarius rubellus* and *Cortinarius orellanus*, no specific treatment is available. The therapy is directed toward the renal failure, including dialysis and possible transplantation. **Poisoning by *Amanita virosa* is treated with the nonspecific antidote silibinin.**

11. Drugs. 2001;61(14):2035-63.

The use of **silymarin** in the treatment of liver diseases.

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The high prevalence of liver diseases such as chronic hepatitis and cirrhosis underscores the need for efficient and cost-effective treatments. The potential benefit of **silymarin** (extracted from the seeds of *Silybum marianum* or milk thistle) in the treatment of liver diseases remains a controversial issue. Therefore, the objective of this review is to assess the clinical efficacy and safety of **silymarin** by application of systematic approach. 525 references were found in the databases, of which 84 papers were retained for closer examination and 36 were deemed suitable for detailed analysis. **Silymarin** has metabolic and cell-regulating effects at concentrations found in clinical conditions, namely carrier-mediated regulation of cell membrane permeability, inhibition of the 5-lipoxygenase pathway, scavenging of reactive oxygen species (ROS) of the R-OH type and action on DNA-expression, for example, via suppression of nuclear factor (NF)-kappaB. Pooled data from case record studies involving 452 patients with *Amanita phalloides* poisoning show a highly significant difference in mortality in favour of **silibinin** [the main isomer contained in **silymarin**] (mortality 9.8% vs 18.3% with standard treatment; $p < 0.01$). The available trials in patients with toxic (e.g. solvents) or iatrogenic (e.g. antipsychotic or tacrine) liver diseases, which are mostly outdated and underpowered, do not enable any valid conclusions to be drawn on the value of **silymarin**. The exception is an improved clinical tolerance of tacrine. In spite of some positive results in patients with acute viral hepatitis, no formally valid conclusion can be drawn regarding the value of **silymarin** in the treatment of these infections. Although there were no clinical end-points in the four trials considered in patients with alcoholic liver disease, histological findings were reported as improved in two out of two trials, improvement of prothrombin time was significant (two trials pooled) and liver transaminase levels were consistently lower in the **silymarin**-treated groups. Therefore, **silymarin** may be of use as an adjuvant in the therapy of alcoholic liver disease. Analysis was performed on five trials with a total of 602 patients with liver cirrhosis. The evidence shows that, compared with placebo, **silymarin** produces a nonsignificant reduction of total mortality by -4.2% [odds ratio (OR) 0.75 (0.5 - 1.1)]; but that, on the other hand, the use of **silymarin** leads to a significant reduction in liver-related mortality of -7% [OR: 0.54 (0.3 - 0.9); $p < 0.01$]. An individual trial reported a reduction in the number of patients with encephalopathy of -8.7% ($p = 0.06$). In one study of patients with cirrhosis-related diabetes mellitus, the insulin requirement was reduced by -25% ($p < 0.01$). We conclude that available evidence suggests that **silymarin** may play a role in the therapy

of (alcoholic) liver cirrhosis. **Silymarin** is has a good safety record and only rare case reports of gastrointestinal disturbances and allergic skin rashes have been published. This review does not aim to replace future prospective trials aiming to provide the 'final' evidence of the efficacy of **silymarin**.

12. Am Fam Physician. 2001 Nov 1;64(9):1555-60.

Erratum in:

Am Fam Physician 2002 Jun 15;65(12):2438.

Comment in:

Am Fam Physician. 2001 Nov 1;64(9):1515-6.

Preventive strategies in chronic liver disease: part I. Alcohol, vaccines, toxic medications and supplements, diet and exercise.

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Chronic liver disease is the 10th leading cause of death in the United States. Hepatitis C virus infection is the most frequent cause of chronic liver disease and the most common indication for liver transplantation. Preventive care can significantly reduce the progression of liver disease. Alcohol and hepatitis C virus are synergistic in hastening the development of cirrhosis; therefore, patients with hepatitis C infection should abstain from alcohol use. Because superinfection with hepatitis A or B virus can lead to liver failure, vaccination is recommended. Potentially hepatotoxic medications should be used with caution in patients with chronic liver disease. In general, nonsteroidal anti-inflammatory drugs should be avoided; acetaminophen in a dosage below 2 g per day is the safest choice. **Many herbal remedies are potentially hepatotoxic, and only milk thistle can be used safely in patients who have chronic liver disease.** Weight reduction and exercise can improve liver function in patients with fatty liver.

13. Am J Gastroenterol. 2001 Nov;96(11):3195-8.

Comment in:

Am J Gastroenterol. 2002 May;97(5):1272-3.

Mushroom poisoning--from diarrhea to liver transplantation.

Broussard CN, Aggarwal A, Lacey SR, Post AB, Gramlich T, Henderson JM, Younossi

ZM.

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Mushroom poisoning from the genus *Amanita* is a medical emergency, with *Amanita phalloides* being the most common species. The typical symptoms of nausea, vomiting, abdominal pain, and diarrhea are nonspecific and can be mistaken for gastroenteritis. If not adequately treated, hepatic and renal failure may ensue within several days of ingestion. In this case series, patients poisoned with *Amanita virosa* are described with a spectrum of clinical presentations and outcomes ranging from complete recovery to fulminant hepatic failure. Although there are no controlled clinical trials, a few anecdotal studies provide the basis for regimens recommended to treat *Amanita* poisoning. Use of i.v. penicillin G is supported by most reports. **Silibinin, although preferred over penicillin**, is not easily available in the United States. In those with acute liver failure, liver transplantation can be life saving.

14. Eksp Klin Farmakol. 2001 Jul-Aug;64(4):53-5.

[Effect of *Silybum marianum* oil and legalon on lipid peroxidation and liver antioxidant systems in rats intoxicated with carbon tetrachloride]

[Article in Russian]

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An oil obtained from the seeds of Saint-Mary thistle (*Silybum marianum*) and the drug legalon (silybinin) prepared from this plant produce an antioxidant effect on liver tissues of rats poisoned with carbon tetrachloride. Legalon (25 mg/kg) and the oil (2000 mg) reduced the level of lipid peroxidation, increased the catalase activity, but did not reduce the concentration of selenium in liver (which decreased as a result of CCl₄ intoxication). Legalon significantly increased the activity of superoxide dismutase in liver tissues, while the Saint-Mary thistle oil did not produce such effect.

J Ethnopharmacol. 2001 Oct;77(2-3):227-32. **(Animal Study)**

15. Effect of **silibinin** and vitamin E on restoration of cellular immune response after partial hepatectomy.

Horvath ME, Gonzalez-Cabello R, Blazovics A, van der Looij M, Barta I, Muzes G, Gergely P, Feher J.

Second Department of Medicine, Semmelweis University, Szentkiralyi str. 46, H-1088 Budapest, Hungary.

Our aim was to study the antioxidant and immunomodulatory effect of **silibinin** and vitamin E on the early postoperative course in rats that had undergone a partial hepatectomy (PHX). Male Wistar rats that were treated with **silibinin** (50 mg/b.w.kg i.p.) and/or vitamin E (500 mg/b.w.kg p.o.) were randomised to undergo 70% PHX. At 72 h after operation, Concanavalin A (Con-A) induced lymphocyte proliferation, and lipopolysaccharide (LPS) induced interleukin-1 (IL-1) mitogenicity and tumour necrosis factor-alpha (TNF-alpha) cytotoxicity were measured in the spleen. In addition, total free radical scavenger capacity of the liver was analysed. In PHX animals, Con-A induced lymphocyte proliferation was significantly decreased, and both LPS induced IL-1 and TNF-alpha activity were significantly increased as compared to Sham treated animals. **Treatment with silibinin and vitamin E synergistically restored both lymphocyte proliferation (P<0.01) and cytokine activity (P<0.001) in PHX animals. In addition, silibinin and vitamin E synergistically (P<0.001) restored total hepatic free radical scavenger capacity as well as serum levels of AST and gammaGT, that were all markedly decreased in PHX animals. Our results suggest that preoperative treatment with silibinin and/or vitamin E modulates the cellular immunoresponse and restores impaired liver function following PHX, presumably through their antioxidant capacity. This may explain their beneficial effects on the postoperative course of liver repair.**

16. Hepatology. 2001 Aug;34(2):329-39. **(Animal Study)**

Beneficial effects of **silymarin** on estrogen-induced cholestasis in the rat: a study in vivo and in isolated hepatocyte couplets.

Crocenzi FA, Sanchez Pozzi EJ, Pellegrino JM, Favre CO, Rodriguez Garay EA, Mottino AD, Coleman R, Roma MG.

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The effect of **silymarin** (SIL) on 17alpha-ethynylestradiol (EE)-induced cholestasis was evaluated in rats. EE (5 mg/kg, subcutaneously, daily, for 5 days) decreased both the bile-salt-dependent and the bile-salt-independent fractions of the bile flow. The decrease in the former was associated to a reduction in the bile salt pool size (-58%), and this effect was completely prevented by SIL. This compound also counteracted the inhibitory effect induced by EE on HCO₃⁻ but not glutathione output, 2 major determinants of the bile-salt-independent bile flow. EE decreased the secretory rate maximum (SRM) of tauroursodeoxycholate, (-71%) and bromosulfophthalein (BSP; -60%), as well as the expression of the BSP canalicular carrier, mrp2; SIL failed to increase mrp2

expression, and had only a marginal beneficial effect on both taurooursodeoxycholate and BSP SRM values. However, the two-compartment model-based kinetic constant for BSP canalicular transfer was significantly improved by SIL (+262%). SIL decreased rather than increased CYP3A4, the cytochrome P450 isoenzyme involved in the oxidative metabolism of EE, and had no inhibitory effect on the UDP-glucuronosyltransferase isoforms involved in the formation of its 17beta-glucuronidated, more cholestatic metabolite.

Pretreatment of isolated rat hepatocyte couplets with **silibinin**, the major, active component of SIL, counteracted the estradiol 17beta-glucuronide-induced decrease in the percentage of couplets secreting apically the fluorescent bile acid analogue, cholyl-lysyl-fluorescein. These results show that SIL protects against EE-induced cholestasis by normalizing mainly the decrease in the bile salt pool size and HCO₃⁻ output, and probably by counteracting the cholestatic effect of its cholestatic, glucuronidated metabolite.

17. Vopr Pitan. 2000;69(5):20-3.

[Effects of bioflavonoids on the toxicity of T-toxin in rats. A biochemical study]

[Article in Russian]

Kravchenko LV, Avren'eva LI, Tutel'ian VA.

The enrichment of a diet of rats by flavonoids of **milk thistle**, *Silybum marianum*, reduced toxicity of T-2 toxin and was accompanied by reduction of a degree of change of total and nonsedimentable activity of lysosomal enzymes and microsomal xenobiotic metabolizing enzymes.

18. Drug Metab Dispos. 2000 Nov;28(11):1270-3.

Milk thistle, a herbal supplement, decreases the activity of CYP3A4 and uridine diphosphoglucuronosyl transferase in human hepatocyte cultures.

Venkataramanan R, Ramachandran V, Komoroski BJ, Zhang S, Schiff PL, Strom SC.

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Milk thistle extract is one of the most commonly used nontraditional therapies, particularly in Germany. **Milk thistle** is known to contain a number of flavonolignans. We evaluated the effect of **silymarin**, on the activity of various hepatic drug-metabolizing enzymes in human hepatocyte cultures. Treatment with **silymarin** (0.1 and 0.25 mM) significantly reduced the activity of CYP3A4 enzyme

(by 50 and 100%, respectively) as determined by the formation of 6-beta-hydroxy testosterone and the activity of uridine diphosphoglucuronosyl transferase (UGT1A6/9) (by 65 and 100%, respectively) as measured by the formation of 4-methylumbelliferone glucuronide. **Silymarin** (0.5 mM) also significantly decreased mitochondrial respiration as determined by MTT reduction in human hepatocytes. [These observations point to the potential of silymarin to impair hepatic metabolism of certain coadministered drugs in humans.](#) Indiscriminate use of herbal products may lead to altered pharmacokinetics of certain drugs and may result in increased toxicity of certain drugs.

19. Ther Apher. 2000 Aug;4(4):303-7.

Treatment of Amanita phalloides poisoning: I. Retrospective evaluation of plasmapheresis in 21 patients.

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Amanita phalloides poisoning is the most common cause of lethal mushroom poisoning (lethality >20% in adults). A specific antidote against the amanitin toxins is not available. This retrospective study reports results in 21 patients (12 males, 9 females; ages 9-59 years) treated for amanita phalloides poisoning between 1984 and 1993. Plasmapheresis was carried out using a commercial plasma protein solution (Biseko, Biotest, Dreieich, Germany) in 17 patients, fresh plasma in 2 patients, and human albumin/Ringer's solution in 2 patients. Ancillary drugs, including penicillin and **silibinin**, also were used for detoxification, correction of blood-clotting deficiencies, and hepatic protection. One patient died of acute hepatic failure. The results, assessed using mortality (4.8% overall) and frequency of complications, indicate that plasmapheresis is a safe and effective treatment for amanita phalloides poisoning but that further investigations are needed, especially involving measurements of efficacy and the efficiency of toxin removal.

20. Pharmacol Toxicol. 2000 Jun;86(6):250-6.

Inhibitory effects of **silibinin** on cytochrome P-450 enzymes in human liver microsomes.

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Silibinin, the main constituent of **silymarin**, a flavonoid drug from *Silybum marianum* used in liver disease, was tested for inhibition of human cytochrome P-450 enzymes. Metabolic activities were determined in liver microsomes from two donors using selective substrates. With each substrate, incubations were carried out with and without **silibinin** (concentrations 3.7-300 μM) at 37 degrees in 0.1 M KH_2PO_4 buffer containing up to 3% DMSO. Metabolite concentrations were determined by HPLC or direct spectroscopy. First, **silibinin** IC_{50} values were determined for each substrate at respective $K(M)$ concentrations. **Silibinin** had little effect ($\text{IC}_{50} > 200 \mu\text{M}$) on the metabolism of erythromycin (CYP3A4), chlorzoxazone (CYP2E1), S(+)-mephenytoin (CYP2C19), caffeine (CYP1A2) or coumarin (CYP2A6). A moderate effect was observed for high affinity dextromethorphan metabolism (CYP2D6) in one of the microsomes samples tested only ($\text{IC}_{50} = 173 \mu\text{M}$). Clear inhibition was found for denitronifedipine oxidation (CYP3A4; $\text{IC}_{50} = 29 \mu\text{M}$ and $46 \mu\text{M}$) and S(-)-warfarin 7-hydroxylation (CYP2C9; $\text{IC}_{50} = 43 \mu\text{M}$ and $45 \mu\text{M}$). When additional substrate concentrations were tested to assess enzyme kinetics, **silibinin** was a potent competitive inhibitor of dextromethorphan metabolism at the low affinity site, which is not CYP2D6 ($K_{i,c} = 2.3 \mu\text{M}$ and $2.4 \mu\text{M}$). Inhibition was competitive for S(-)-warfarin 7-hydroxylation ($K_{i,c} = 18 \mu\text{M}$ and $19 \mu\text{M}$) and mainly non-competitive for denitronifedipine oxidation ($K_{i,n} = 9 \mu\text{M}$ and $12 \mu\text{M}$). With therapeutic **silibinin** peak plasma concentrations of $0.6 \mu\text{M}$ and biliary concentrations up to $200 \mu\text{M}$, metabolic interactions with xenobiotics metabolised by CYP3A4 or CYP2C9 cannot be excluded.

21. J Pharmacol Exp Ther. 2000 Jul;294(1):160-7.

Hepatotoxicity of tacrine: occurrence of membrane fluidity alterations without involvement of lipid peroxidation.

Galisteo M, Rissel M, Sergent O, Chevanne M, Cillard J, Guillouzo A, Lagadic-Gossmann D.

Institut National de la Sant inverted question marke et de la Recherche M inverted question markedicale U456, D inverted question marketoxication et R inverted question markeparation Tissulaire, Facult inverted question marke des Sciences Pharmaceutiques et Biologiques, Universit inverted question marke de Rennes I, Rennes, France.

Tacrine (THA), used in the treatment of Alzheimer's disease, is known to induce hepatotoxicity, the mechanisms of which remain to be fully established. We have previously shown that THA reduced intracellular glutathione concentration in rat hepatocytes in primary culture, thus pointing to a possible role for oxidative stress in THA toxicity. To test this, the effects of antioxidant molecules, namely, the flavonoids **silibinin**, **silibinin** dihydrogensuccinate, and **silymarin**, were evaluated on the toxicity of THA in cultured rat hepatocytes. This toxicity

was investigated after a 24-h treatment over a concentration range from 0 to 1 mM, in the presence or absence of antioxidant (1 and 10 microM). We found that simultaneous treatment of hepatocytes with any of the antioxidants and THA remained ineffective on the lactate dehydrogenase release induced by THA. Then, the production of lipid-derived radicals (to estimate lipid peroxidation) was measured in THA (0.05-0.50 mM)-treated cells using a spin-trapping technique coupled to electron paramagnetic resonance (EPR) spectroscopy. No increase of the EPR signal was observed over the period of 30 min to 24 h. In contrast, treatment of cells with the spin label 12-doxyyl stearic acid followed by EPR spectroscopy showed that THA (0.05 and 0.25 mM) rapidly increased hepatocyte membrane fluidity. Extracellular application of GM1 ganglioside (60 microM) both reversed this increase in fluidity and partially reduced lactate dehydrogenase release on THA exposure. In conclusion, this work indicates that early alterations of membrane fluidity, not resulting from lipid peroxidation, are likely to play an important role in the development of THA toxicity.

22. Eksp Klin Farmakol. 1999 Mar-Apr;62(2):36-8.

[The effect of aqueous extracts of hepatotropic medicinal plants on free-radical oxidation processes]

[Article in Russian]

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The authors studied the effect of decoctions and infusions of medicinal plants (common barberry, sandy immortelle, common maize, spotted **milk thistle**) on free-radical oxidation (FRO) in model systems in vitro and in experiments in vivo on nonbred albino mice. In various model systems (in which active forms of oxygen are generated and lipid peroxidation takes place) the plants under study suppressed as well as intensified the processes of lipid peroxidation, depending on the concentration of the phytopreparation and the type of the model systems. [In in vivo experiments the drugs of plant origin suppressed lipid peroxidation, reducing the parameters induced by iron and chemoluminescence and the malonic dialdehyde level in the liver.](#)

23. Free Radic Biol Med. 1998 May;24(7-8):1316-23. **(Animal Study)**

Cold-induced release of reactive oxygen species as a decisive mediator of hypothermia injury to cultured liver cells.

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The mechanisms of hypothermia-induced cell injury are still unclear. The present study provides experimental evidence for the involvement of reactive oxygen species in hypothermia injury: cultured rat hepatocytes incubated in cold (4 degrees C) Krebs-Henseleit buffer or cell culture medium were injured under normoxic conditions and even more so under hyperoxic conditions, whereas the hepatocytes were protected under hypoxic conditions. During warm (37 degrees C) incubation in cell culture medium, on the other hand, cell injury was minimal under normoxic conditions, only slightly increased under hyperoxic conditions, but substantially increased under hypoxic conditions. **The injury occurring during cold normoxic incubation was also largely decreased by the addition of the spin-trap 5,5-dimethyl-1-pyrroline N-oxide, the hydroxyl radical scavenger dimethyl sulfoxide, the flavonoid silibinin, or the transition metal chelator 2,2'-dipyridyl to the medium, or by preincubating the cells with the iron chelator deferoxamine or the lipophilic antioxidant alpha-tocopherol before the hypothermic incubation.** In addition, marked lipid peroxidation was observed during cold incubations without inhibitors, but not during warm incubations. Similar results were obtained with cultured rat liver endothelial cells. These results suggest that in hepatocytes and in liver endothelial cells, cold-induced release of reactive oxygen species, most likely of hydroxyl radicals, is the main injurious factor under hypothermic conditions.

24. Ren Fail. 1998 May;20(3):471-9.

The effect of **silibinin** on experimental cyclosporine nephrotoxicity.

Zima T, Kamenikova L, Janebova M, Buchar E, Crkowska J, Tesar V.

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The immunosuppressive drug cyclosporine A (CsA), is metabolized by cytochrome P-450 IIIA. It causes acute reversible as well as chronic largely irreversible nephrotoxic effects. This effect is based on vasoconstriction of the afferent and efferent glomerular arterioles which leads to a reduction in glomerular plasma flow and glomerular filtration rate. The mechanisms of the vasoconstriction are unclear with a number of different pathways under discussion. **Silibinin** is the main constituent of **silymarin**. **Silibinin** inhibits lipid peroxidation on hepatic microsomes and mitochondria of rats and is also able to reduce the activity of various monooxygenases. Cyclosporin-induced lipid peroxidation and affected cytochrome P-450 may even contribute to cyclosporine nephrotoxicity. We examined the possibility that **silibinin** had a protective

effect as a result of its radical scavenging properties. **Silibinin**, 5 mg/kg BW i.p., was administered 30 min before cyclosporine application at dose of 30 mg/kg BW daily i.p. The biochemical parameters, total malondialdehyde (MDA) in whole blood and kidney homogenates and specific content of cytochrome P-450 in microsomal liver suspension were estimated. Three groups were studied: controls (con), cyclosporine alone (CsA), and cyclosporine plus **silibinin** (CsA + Sili). Creatinine was significantly increased after 2 weeks in both cyclosporine treated groups compared to controls (CsA 60.2 +/- 10.6 versus 45.8 +/- 10.4 $\mu\text{mol/L}$, $p < 0.05$; and CsA + Sili 72.0 +/- 8.3 versus 45.8 +/- 10.4 $\mu\text{mol/L}$, $p < 0.001$) and glomerular filtration rate (GFR) was significantly decreased ($p < 0.0001$) in the same groups. Total MDA was elevated only in CsA rats (2.26 +/- 0.35 $\mu\text{mol/L}$, $p < 0.05$) in comparison with controls (1.60 +/- 0.44 $\mu\text{mol/L}$, $p < 0.05$) and with rats treated by CsA + Sili (1.65 +/- 0.27 $\mu\text{mol/L}$, $p < 0.05$). The specific content of cytochrome P-450 in microsomal liver suspension was increased in group CsA + Sili (1.179 +/- 0.115 nmol/mg prot) compared to control group (0.775 +/- 0.086 nmol/mg prot., $p < 0.05$) and also CsA group (0.806 +/- 0.098 nmol/mg prot., $p < 0.05$). **In conclusion, silibinin decreased cyclosporine-induced lipid peroxidation without a protective effect on GFR.** These data indicate that this pathway is not be important in cyclosporine-induced nephrotoxicity. Administration of both drugs (CsA + sili) increased the specific content of cytochrome P-450 in liver microsomes. This suggests that the effect of **silibinin** on cyclosporine biotransformation in the liver is via cytochrome P-450.

25. Vnitr Lek. 1997 Oct;43(10):686-90.

[Amanita poisoning and the importance of sorption hemoperfusion in its therapy]

[Article in Czech]

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Intoxications with poisonous mushrooms, in particular toadstools, are still a serious medical problem. The author presents contemporary views on the etiopathogenesis of intoxications with *Amanita phalloides*, the clinical picture of the phalloid syndrome and its prognosis. He emphasizes the importance of a comprehensive therapeutic approach, incl. the administration of antidotes (penicillin G and **silibinin**) and extracorporeal haemoelimination treatment. Early sorption haemoperfusion, either alone or combined with haemodialysis or plasmapheresis, prevent the development of hepatic and renal failure and significantly reduce the mortality from mushroom poisoning. The results of amanitine sorption in in vitro experiments and in the treatment of human intoxications justify the use of biocompatible synthetic resin sorbents

(Amberlite XAD-2) in the treatment of mushroom poisoning rather than active charcoal.

26. C R Seances Soc Biol Fil. 1997;191(5-6):821-35.

[Effect of **silibinin** on oxidative damage of blood constituents]

[Article in French]

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Silibinin (SDH) is a flavonoid with ascertained hepatoprotective effects, which have been partially attributed to its antioxidant properties. Oxidation of blood constituents could have a role in atherogenesis and interfere with the rheologic properties of the blood. In this study we investigated, whether SDH could protect some blood constituents against oxidative modification. [In human plasma we measured TBARS and fluorescence generation as indicators of copper or azobis amidinopropane hydrochloride \(AAPH\) at 760 mm Hg PO₂-induced lipid peroxidation. SDH at 50 microM inhibited copper-induced TBARS formation by 25% and fluorescence by 47%. SDH also inhibited AAPH-induced lipid peroxidation, but at 175 microM concentration only. Oxidative modification of albumine was evaluated by fluorescence generation. SDH at 50 microM inhibited copper/hydrogen peroxide fluorescence generation by 54% and at 2.5 microM it inhibited EDTA-Fe \(II\)/hydrogen peroxide fluorescence generation by 31%. The protection of albumin by SDH was confirmed by SDS-PAGE electrophoresis. Copper-induced red-cell lipid peroxidation was evaluated by TBARS formation. SDH at 250 microM inhibited copper-induced lipid peroxidation and hemolysis by 45% and 94%, respectively. SDH also inhibited hemolysis in red-cell suspensions exposed to hydrogen peroxide, but not lipid peroxidation. Our results show that SDH may protect blood constituents from oxidative damage.](#)

27. Am J Gastroenterol. 1998 Feb;93(2):139-43.

Comment in:

Am J Gastroenterol. 1999 Feb;94(2):545-6.

Milk thistle (*Silybum marianum*) for the therapy of liver disease.

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Silymarin, derived from the **milk thistle** plant, *Silybum marianum*, has been used for centuries as a natural remedy for diseases of the liver and biliary tract. As interest in alternative therapy has emerged in the United States, gastroenterologists have encountered increasing numbers of patients taking **silymarin** with little understanding of its purported properties. **Silymarin** and its active constituent, silybin, have been reported to work as antioxidants scavenging free radicals and inhibiting lipid peroxidation. Studies also suggest that they protect against genomic injury, increase hepatocyte protein synthesis, decrease the activity of tumor promoters, stabilize mast cells, chelate iron, and slow calcium metabolism. In this article we review **silymarin's** history, pharmacology, and properties, and the clinical trials pertaining to patients with acute and chronic liver disease.

28. *Arzneimittelforschung*. 1997 Dec;47(12):1383-7. (**Animal Study**)

Effects of **silibinin** and of a synthetic analogue on isolated rat hepatic stellate cells and myofibroblasts.

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Hepatic stellate cells and the derived myofibroblasts play a central pathogenic role in liver fibrogenesis. In order to identify the still unknown hepatoprotective properties of the flavonoid **silibinin** and the related pyridylchromone NH₄ x HCl (2-(3-pyridyl)-4-H-1-benzopyran-4-one hydrochloride), their effects on isolated rat hepatic stellate cells and derived myofibroblasts were determined. Concentrations of 10⁽⁻⁴⁾ mol/l **silibinin** reduced the proliferation of freshly isolated rat hepatic stellate cells by about 75%, but had no detectable effect on their viability, morphology and their cytoskeletal architecture. It reduced the transformation towards myofibroblasts and down-regulated the gene expression of extracellular matrix components and the profibrogenic transforming growth beta. Whereas **silibinin** concentrations higher than 10⁽⁻⁴⁾ mol/l were toxic, lower concentrations had no effects on the proliferation and transformation behavior. Although 10⁽⁻⁴⁾ mol/l NH₄ x HCl reduced the proliferation rate by about 50%, this substance had no significant effect on the transformation process. The results indicate that one important aspect of the potential antifibrotic properties of **silibinin** might be the inhibition of hepatic stellate cell proliferation and transformation.

29. *Hepatology*. 1997 Sep;26(3):643-9. (**Animal Study**)

Comment in:

Hepatology. 2001 Feb;33(2):483-4.

Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats.

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Silymarin (SIL), a standardized plant extract containing about 60% polyphenole **silibinin**, is used as a hepatoprotective agent. Its antifibrotic potential in chronic liver diseases has not been explored. Therefore, we applied SIL to adult Wistar rats that were subjected to complete bile duct occlusion (BDO) by injection of sodium amidotrizoate (Ethibloc). This treatment induces progressive portal fibrosis without significant inflammation. Rats with sham-operation that received SIL at 50 mg/kg/d (n = 10) and rats with BDO alone (n = 20) served as controls, whereas groups of 20 animals were fed SIL at a dose of 25 and 50 mg/kg/d during weeks 1 through 6 or doses of 50 mg/kg/d during weeks 4 through 6 of BDO. Animals were sacrificed after 6 weeks for determination of blood chemistries, total and relative liver collagen (as hydroxyproline [HYP]), and the serum aminoterminal propeptide of procollagen type III (PIIINP). BDO in untreated rats caused an almost ninefold increase in total liver collagen (16.1 +/- 3.1 vs. 1.8 +/- 0.4 mg HYP, P < .001). SIL at 50 mg/kg/d reduced total HYP by 30% to 35%, either when given from week 1 through 6 or from week 4 through 6 after BDO (10.6 +/- 2.7 and 10.2 +/- 3.9 mg HYP, both P < .01 vs. BDO alone), whereas 25 mg/kg/d were ineffective. **Because SIL at 50 mg/kg/d also reduced the collagen content per gram of liver tissue, it acted as a true antifibrotic agent.** The single value of PIIINP at killing paralleled the antifibrotic activity of SIL with 11.6 +/- 3.8 and 9.9 +/- 3.7 vs. 15.3 +/- 5.2 microg/L in both high-dose groups (P < .05 and P < .01, respectively, vs. rats with BDO alone). Except for a decreased alkaline phosphatase and a lower histological fibrosis score in the groups that received SIL, clinical-chemical parameters were not different among all groups with BDO. We therefore conclude that 1) BDO with Ethibloc is a suitable model to test for pure antifibrotic drugs because it induces progressive rat secondary biliary fibrosis without major inflammation; 2) oral SIL can ameliorate hepatic collagen accumulation even in advanced (biliary) fibrosis; and 3) PIIINP appears to be a suitable serum marker to monitor the inhibition of hepatic fibrogenesis in this model of biliary fibrosis.

30. Am Fam Physician. 1997 Apr;55(5):1797-800, 1805-9, 1811-2.

Mushroom poisoning.

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The majority of cases of mushroom poisoning occur in children and involve benign gastrointestinal irritants. Critical poisonings most frequently occur in adults who ingest *Amanita phalloides* or other mushrooms containing amanitin. Critical versus noncritical poisonings can be diagnosed with a high degree of confidence by the patient's history and initial symptoms. The most promising new medical treatment for *Amanita* mushroom poisoning is **silibinin**. In suspected cases of mushroom poisoning, it is important to obtain specimens of the ingested mushrooms, if possible, since treatment is specific to the species.

31. *Minerva Anestesiol.* 1996 May;62(5):187-93.

[**Silibinin** and acute poisoning with *Amanita phalloides*]

[Article in Italian]

Carducci R, Armellino MF, Volpe C, Basile G, Caso N, Apicella A, Basile V.

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The aim of the present study was to show the therapeutic effect of **silibinin** dihemisuccinate in a case of intoxication by mushrooms of *Amanita* gender. We report a clinical case of a 4-person family intoxicated by ingestion of mushrooms *Amanita phalloides* and admitted to the center for poisoning treatment of the Hospital "A. Cardarelli" in Naples. Although all were treated with standard therapy, there was a worsening of the clinical picture till the third day, when it was decided to add **silibinin** dihemisuccinate by the intravenous route to the therapy. After the beginning of **silibinin** administration the patients showed a favourable course with a rapid resolution of the clinical picture, although the prognosis appeared severe on the basis of hematochemical examination results. On day 9 **silibinin** dihemisuccinate was replaced with **silibinin** betacyclodextrine per os. All patients were discharged on day 10-13. After two months all hematological parameters are in the normal range also a hepatobiliopancreatic echography does not show any morphological alteration. As in the case of polytherapies and because of the lack of comparative studies, it seems difficult to establish which therapeutic component had the major role in the resolution of the clinical picture. However, on the basis of our experience, and of the literature data, [we think that silibinin may play a significant role in protecting hepatic tissue not yet injured. However we believe that other studies are necessary to confirm our hypothesis.](#)

32. Hepatology. 1996 Apr;23(4):749-54. **(Animal Study)**

Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of **silibinin**.

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The flavonoid **silibinin**, the main compound extracted from the **milk thistle** *Silybum marianum*, displays hepatoprotective properties in acute and chronic liver injury. To further elucidate the mechanisms by which it acts, we studied the effects of **silibinin** on different functions of isolated rat Kupffer cells, namely the formation of superoxide anion radical (O₂⁻), nitric oxide (NO), tumor necrosis factor alpha (TNF-alpha), prostaglandin E(2) (PGE(2)), and leukotriene B(4) (LTB(4)). **Production of O₂⁻ and NO were inhibited in a dose-dependent manner, with an 50 percent inhibitory concentration (IC(50)) value around 80 micro mol/L. No effect on TNF-alpha formation was detected.** Opposite effects were found on the cyclooxygenase and 5-lipoxygenase pathway of arachidonic acid metabolism. **Whereas no influence on PGE(2) formation was observed with silibinin concentrations up to 100 micro mol/L, a strong inhibitory effect on LTB(4) formation became evident.** The IC(50)-value for inhibiting the formation of this eicosanoid was determined to be 15 micro mol/L **silibinin**. The strong inhibition of LTB(4) formation by **silibinin** was confirmed in experiments with phagocytic cells isolated from human liver. Hence, while rather high concentrations of **silibinin** are necessary to diminish free radical formation by activated Kupffer cells, significant inhibition of the 5-lipoxygenase pathway already occurs at **silibinin** concentrations which are achieved in vivo. Selective inhibition of leukotriene formation by Kupffer cells can at least partly account for the hepatoprotective properties of **silibinin**.

33. Life Sci. 1996;58(18):1591-600.

Scavenging of reactive oxygen species and inhibition of arachidonic acid metabolism by **silibinin** in human cells.

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The effects of the flavonoid **silibinin**, which is used for the treatment of liver diseases, on the formation of reactive oxygen species and eicosanoids by human platelets, white blood and endothelial cells were studied. **Silibinin** proved to be a strong scavenger of HOCl (IC₅₀ 7 microM), but not of O₂⁻ (IC₅₀ > 200

microM) produced by human granulocytes. The formation of leukotrienes via the 5-lipoxygenase pathway was strongly inhibited. In human granulocytes IC₅₀-values of 15 microM and 14.5 microM **silibinin** were detected for LTB₄ and LTC₄/D₄/E₄/F₄ formation, respectively. In contrast to this, three- to fourfold **silibinin** concentrations were necessary to half maximally inhibit the cyclooxygenase pathway. For PGE₂ formation by human monocytes an IC₅₀-value of 45 microM **silibinin** was found. IC₅₀-values of 69 microM and 52 microM **silibinin** were determined for the inhibition of TXB₂ formation by human thrombocytes and of 6-K-PGF₁ alpha formation by human omentum endothelial cells, respectively. Thus, the deleterious effects of HOCl that can lead to cell death, and those of leukotrienes that are especially important in inflammatory reactions, can be inhibited by **silibinin** in concentrations that are reached in vivo after the usual clinical dose. **Silibinin is thought not only to display hepatoprotective properties but might also be cytoprotective in other organs and tissues.**

34. Clin Investig. 1994 Oct;72(10):794-8.

Amanita poisoning during the second trimester of pregnancy. A case report and a review of the literature.

Nagy I, Pogatsa-Murray G, Zalanyi S Jr, Komlosi P, Laszlo F, Ungi I.

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Amanita phalloides-type mushroom poisoning is well recognized as causing acute liver injury and often death. Less is known, however, of whether maternal Amanita poisoning is associated with fetal damage or not. In August 1991 four members of a family were hospitalized with food intoxication caused by Amanita phalloides and Amanita verna. One of them died from hepatic and renal failure. The survivors included a 26-year-old woman in the 23rd week of pregnancy. Her clinical symptoms and blood chemistry data (lowest prothrombin activity 23%) indicated intoxication of medium severity. The management consisted of i.v. hydration, forced diuresis, and administration of **silibinin**, high-dose penicillin, thioctic acid, hydrocortisone, vitamin K, and fresh frozen plasma. Sonographic and obstetric controls failed to show any fetal abnormalities in the acute phase of poisoning. In the 38th week of pregnancy she gave birth to a healthy baby, who has subsequently undergone an undisturbed development. This observation indicated that severe fetal damage did not occur in maternal Amanita poisoning in the second trimester of pregnancy. Thus, at least from the second trimester on, maternal Amanita poisoning is not necessarily an indication for induced abortion.

35. Biol Res. 1994;27(2):105-12.

Biochemical bases of the pharmacological action of the flavonoid **silymarin** and of its structural isomer **silibinin**.

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The flavonoid **silymarin** and one of its structural components, **silibinin**, have been well characterized as hepato-protective substances. However, little is known about the biochemical mechanisms of action of these substances. This review deals with recent investigations to elucidate the molecular action of the flavonoid. Three levels of action have been proposed for **silymarin** in experimental animals: a) as an antioxidant, by scavenging prooxidant free radicals and by increasing the intracellular concentration of the tripeptide glutathione; b) regulatory action of the cellular membrane permeability and increase of its stability against xenobiotic injury; c) at the nuclear expression, by increasing the synthesis of ribosomal RNA by stimulating DNA polymerase I and by exerting a steroid-like regulatory action on DNA transcription. The specific hepatoprotective action of **silibinin** against the toxicity of ethanol, phenylhydrazine and acetaminophen is also discussed. It is suggested that the biochemical effects observed for the flavonoid in experimental models may settle the basis for understanding the pharmacological action of **silymarin** and **silibinin**.

36. Orv Hetil. 1993 Apr 25;134(17):907-10.

[Amanita phalloides poisoning in a 15-year case load of a pediatric intensive care unit]

[Article in Hungarian]

Mikos B, Biro E.

Borsod-A. -Z. Megyei Korhaz, Gyermekegeszsegugyi Kozpont-OTE II. Gyermekgyogyaszati Tanszek, Miskolc.

The clinical course of eight patients with Amanita phalloides poisoning is reviewed. Early diagnosis was based on the history, characteristic clinical features and non-specific laboratory data. A complex supportive therapy with gastric lavage, bowel irrigation, correction of volume and electrolyte abnormalities, and penicillin-G (Penicillin, Biogal), **silibinin** (Legalon SIL, Madaus), thioctacid (Thioctacid, Asta), corticosteroid (Di-Adreson-F aquosum, Organon) administration was commenced in every case before identification of

the mushroom. Haemoperfusion was performed in six cases, and in one patient plasmapheresis was applied as well. Seven children recovered completely. Unfortunately, a girl of 12 years died. According to the authors' experience, the use of non-invasive and invasive methods of the non-specific detoxication is proposed in case of severe *Amanita phalloides* poisoning.

37. J Cardiovasc Electrophysiol. 1993 Apr;4(2):161-77. **(Animal Study)**

The role of free radicals in the pathogenesis of amiodarone toxicity.

Vereckei A, Blazovics A, Gyorgy I, Feher E, Toth M, Szenasi G, Zsinka A, Foldiak G, Feher J.

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INTRODUCTION: In vitro and in vivo studies were performed to elucidate the pathogenesis of amiodarone toxicity. METHODS AND RESULTS: Rats were treated with amiodarone alone (500 mg/kg body weight per day) or together with antioxidants (**silibinin** or MTDQ-DA: 50 mg/kg body weight per day) or with either antioxidant alone. They received amiodarone for 30 days and antioxidant for 33 days (3 days pretreatment). In vitro, amiodarone induced a dose-dependent chemiluminescence signal, which was inhibited by the two dihydroquinolin-type antioxidants (MTDQ-DA, CH 402). Chemiluminometric results from liver homogenate demonstrated that simultaneous **treatment with silibinin partially prevented the liver homogenate superoxide anion radical scavenger capacity decreasing effect of amiodarone. Amiodarone treatment caused a significant increase of NADPH and Fe³⁺ induced lipid peroxidation in the liver microsomal fraction, which antioxidants (silibinin, MTDQ-DA) were unable to prevent.** Light microscopy of the lung tissue in amiodarone-treated rats showed accumulation of foamy macrophages with thickening of the interalveolar septa, pneumonitis, and variable interstitial fibrosis. Antioxidant treatment did not prevent these changes. Electron micrographs of lung from amiodarone-treated rats showed lysosomal phospholipoidosis, intralysosomal electron dense deposits, and increased lysosome number and size. In contrast to rats treated with amiodarone alone, those treated with both amiodarone and **silibinin** had significantly fewer lysosomes ($P < 0.01$); the lysosome size, shape, and internal characteristics remained the same. Simultaneous treatment with **silibinin** and amiodarone decreased lysosomal phospholipoidosis compared to amiodarone treatment alone. Simultaneous treatment with MTDQ-DA and amiodarone did not show any beneficial effect. Pulse radiolysis and cobalt 60-gamma (⁶⁰Co-gamma) radiolysis studies showed that the main free radical product in a reducing environment was a very reactive aryl radical formed after the partial deiodination of the amiodarone molecule. The radiosensitizing effect of amiodarone was also verified in rat liver microsomal preparations using in vivo amiodarone with or without MTDQ-DA

pretreatment and ^{60}Co -gamma irradiation with or without the in vitro addition of antioxidants (CH 402, MTDQ-DA). In vivo, the MTDQ-DA treatment also had a radiosensitizing effect; however, the in vitro addition of both antioxidants resulted in a radioprotective effect. The aryl radical also may emerge in vivo during the metabolism of amiodarone. CONCLUSION: These observations suggest that amiodarone in vitro and in vivo generates free radicals that may play a role in the pathogenesis of amiodarone toxicity beside other well-established mechanisms, and antioxidants may have a partial protective effect against amiodarone toxicity.

38. Acta Physiol Hung. 1992;80(1-4):375-80.

The effect of **silibinin** (Legalon) on the the free radical scavenger mechanisms of human erythrocytes in vitro.

Altorjay I, Dalmi L, Sari B, Imre S, Balla G.

2nd Department of Medicine, University Medical School, Debrecen, Hungary.

The effect of Legalon was investigated parallel with that of Adriblastina (doxorubicin) and paracetamol on some parameters characterizing the free radical scavenger mechanisms of human erythrocytes in vitro and on the time of acid hemolysis performed in aggregometer. Observations suggest that Adriblastina enhances the lipid peroxidation of the membrane of red blood cells, while paracetamol causes significant depletion of intracellular glutathione level, thus decreasing the free radical eliminating capacity of the glutathione peroxidase system. Legalon on the other hand, is able to increase the activity of both superoxide dismutase and glutathione peroxidase, which may explain the protective effect of the drug against free radicals and also the stabilizing effect on the red blood cell membrane, shown by the increase of the time of full haemolysis.

39. Orv Hetil. 1991 Jun 9;132(23):1265-8.

[Possibilities of antioxidant therapy in the prevention of side effects of amiodarone]

[Article in Hungarian]

Vereckei A, Feher E, Gyorgy I, Foldiak G, Toth M, Toncsev H, Feher J.

Semmelweis OTE II. Belklinika, Budapest.

The authors demonstrated the generation of a very reactive phenyl radical from amiodarone in a reducing molecular environment by pulse radiolysis study. The

various antioxidants are probably not capable of preventing the generation of phenyl radical, as well as to protect against its damaging effects on the neighboring molecules. Electron microscopic studies from lung tissue of in vivo treated rats showed that the simultaneous **Silibinin** (a flavonoid type antioxidant) treatment with amiodarone decreased significantly the lysosomal phospholipoidosis induced by amiodarone compared with the amiodarone treated group, but it didn't prevent entirely the accumulation of lysosomal phospholipids. The in vitro lysosomal beta-glucuronidase enzyme release measured from the liver tissue of in vivo treated rats increased significantly on amiodarone treatment, the antioxidants used (**Silibinin**, and the dihydroquinoline type MTDQ-DA) didn't exert any favorable effect. The authors discuss in details the possible relationships between free radical reactions and lysosomal phospholipoidosis.

40. Lijec Vjesn. 1991 Jan-Feb;113(1-2):16-20.

[Mushroom poisoning with a long period of development]

[Article in Croatian]

Gasparovic V, Puljevic D, Radonic R, Gjurasin M, Ivanovic D, De Wollf F, Pisl Z.

Klinika za unutarnje bolesti, KBC, Medicinski fakultet Sveucilista Zagrebu.

A group of 87 patients with the signs of poisoning with mushrooms with along period of incubation ($t = 12.4 \pm 6.2$ h) has been reported. Nausea, vomiting and diarrhea dominate in the clinical picture in the first phase and hepatic and/or renal insufficiency in the second phase. Forty-one patients (47.1%) had "only" clinical symptoms without severe parenchymatous impairments. Forty-six (54.9%) had evidence of a hepatic lesion and 8 patients (10.8%) had renal function impaired, 6 of which needed hemodialysis. There was a significant correlation between elevation of serum transaminases and prolongation of prothrombin complex, resulting from the decreased synthetic liver function (SGPT1/PV1r = -0.424, $p = 0.00$; SGOT1/PV1r = -0.448, $p = 0.000$) during the first days after poisoning. Hepatic and renal damage was not identical in all the cases, and there was no correlation between the elevation of serum transaminases and retention of nitrogen substances. When analysing the effect of therapy on elevation of serum transaminases and prolongation of prothrombin complex, a significant difference between elevation of serum transaminases and prolongation of prothrombin time was found in patients on competitive inhibition with penicillins or **silibinin**, as compared to the patients only on plasmapheresis ($p = 0.004$ for SGOT, $p = 0.000$ for SGPT). These data unquestionably suggest the efficacy of competitive inhibition in the treatment of poisoning with mushrooms of a long period of incubation. In favour of this therapy also speaks the group of seriously ill patients who were simultaneously on plasmapheresis and

competitive inhibition and who had better improvement than those "only" on plasmapheresis ($p = 0.004$ for SGOT).(ABSTRACT TRUNCATED AT 250 WORDS)

41. Schweiz Rundsch Med Prax. 1990 Sep 4;79(36):1031-3.

[Severe vomiting, diarrhea]

[Article in German]

Richter M, Simmen R.

Medizinische Klinik, Stadtspital Triemli, Zurich.

Because of heavy vomiting and a watery diarrhoea after consumption of two *Amanita phalloides* mushroom taken in suicidal attempt this 31-year old female patient came to our emergency ward. The diagnosis being clear a therapy with stomach irrigation, substitution of fluid and electrolytes as well as application of penicillin and **Silibinin** was begun. The toxin concentration in the urine was not too high and the Quick value did not fall to low. The course was benign and the patient could leave the hospital only a few days later.

42. Biochem Pharmacol. 1990 Jun 15;39(12):2027-34. (**Animal Study**)

Mechanism for the protective effects of **silymarin** against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice. Evidence that **silymarin** acts both as an inhibitor of metabolic activation and as a chain-breaking antioxidant.

Letteron P, Labbe G, Degott C, Berson A, Fromenty B, Delaforge M, Larrey D, Pessayre D.

Unite de Recherches de Physiopathologie Hepatique (INSERM U-24), Hopital Beaujon, Clichy, France.

Administration of **silymarin** (800 mg/kg i.p.) 30 min before carbon tetrachloride (18 microL/kg i.p.) did not modify total hepatic levels of CCl₄ and metabolites in mice, but decreased by 40% the in vivo covalent binding of CCl₄ metabolites to hepatic lipids at 2 hr. This pretreatment decreased by 60% the exhalation of ethane during the first hour after CCl₄, and decreased by 50% the incidence of liver cell necrosis. In vitro, **silymarin** (800 micrograms/mL) decreased by 50 to 70% various monooxygenase activities, and decreased by 20% the covalent binding of CCl₄ metabolites to microsomal proteins. **Silymarin** (800 micrograms/mL) decreased by 70% in vitro lipid peroxidation mediated by CCl₄ metabolites, and decreased by 90% peroxidation mediated by NADPH alone. **Silibinin**, one of the

three isomers composing **silymarin**, also decreased carbon tetrachloride-induced lipid peroxidation; this effect, however, was less than that of **silymarin** in vitro, and was more transient in vivo. Pretreatment with **silibinin** (800 mg/kg i.p.) 30 min before CCl₄ (18 microL/kg i.p.) did not improve SGPT activity or liver histology at 24 hr. We conclude that **silymarin** prevents carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice, firstly, by decreasing the metabolic activation of CCl₄, and, secondly, by acting as a chain-breaking antioxidant.

43. *Planta Med.* 1989 Oct;55(5):420-2. **(Animal Study)**

Selectivity of **silymarin** on the increase of the glutathione content in different tissues of the rat.

Valenzuela A, Aspillaga M, Vial S, Guerra R.

Silymarin, a flavonoid extracted from the seeds of the **milk thistle**, *Silybum marianum*, increases the redox state and the total glutathione content of the liver, intestine, and stomach of the rat. The same treatment does not affect the levels of the tripeptides in the kidney, lung, and spleen. This selective effect of the flavonoid on the digestive organs is ascribed to its pharmacokinetics on the digestive track, where the biliary concentration of **silymarin** is increased and maintained via the entero-hepatic circulation.

44. *J Hepatol.* 1989 Jul;9(1):105-13.

Randomized controlled trial of **silymarin** treatment in patients with cirrhosis of the liver.

Ferenci P, Dragosics B, Dittrich H, Frank H, Benda L, Lochs H, Meryn S, Base W, Schneider B.

1st Department of Gastroenterology and Hepatology, University of Vienna, Austria.

Silymarin, the active principle of the **milk thistle** *Silybum marianum*, protects experimental animals against various hepatotoxic substances. To determine the effect of **silymarin** on the outcome of patients with cirrhosis, a double blind, prospective, randomized study was performed in 170 patients with cirrhosis. 87 patients (alcoholic 46, non-alcoholic 41; 61 male, 26 female; Child A, 47; B, 37; C, 3; mean age 57) received 140 mg **silymarin** three times daily. 83 patients (alcoholic 45, non-alcoholic 38; 62 male, 21 female; Child A, 42; B, 32; C, 9; mean age 58) received a placebo. Non-compliant patients and patients who failed to come to a control were considered as 'drop outs' and were withdrawn from the

study. All patients received the same treatment until the last patient entered had finished 2-years of treatment. The mean observation period was 41 months. There were 10 drop outs in the placebo group and 14 in the treatment group. In the placebo group, 37 (+2 drop outs) patients had died, and in 31 of these, death was related to liver disease. In the treatment group, 24 (+4 drop outs) had died, and in 18 of these, death was related to liver disease. The 4-year survival rate was 58 +/- 9% (S.E.) in **silymarin**-treated patients and 39 +/- 9% in the placebo group (P = 0.036). **Analysis of subgroups indicated that treatment was effective in patients with alcoholic cirrhosis (P = 0.01) and in patients initially rated 'Child A' (P = 0.03).** No side effects of drug treatment were observed.(ABSTRACT TRUNCATED AT 250 WORDS)

45. Acta Physiol Hung. 1989;73(2-3):285-91.

Liver cell protection in toxic liver lesion.

Feher J, Cornides A, Pal J, Lang I, Csomos G.

2nd Department of Medicine, Semmelweis University, Budapest, Hungary.

Most of the hepatoprotective drugs belong to the group of free radical scavengers. The mechanism of their action involves membrane stabilisation, neutralisation of free radicals and immunomodulation. The authors demonstrate the effect of different-drugs used in therapy of liver diseases (**silymarin**, **silibinin**, Aica-P) in human clinico-pharmacological study and in animal experiments. A wide number of methods was used. Both the **silymarin** preperates and the Aica-P corrected the altered immunreaction and the decreased superoxid-dismutase (SOD) activity of erythrocytes and lymphocytes in patients with alcoholic liver cirrhoses. The scavenger effect of these drugs was demonstrated in the subcellular fractions of liver cells in animal experiments. The data support the therapeutic effect of these drugs in liver diseases.

46. Burns Incl Therm Inj. 1988 Feb;14(1):25-30. **(Animal Study)**

Changes in the pattern of microsomal fatty acids in rat liver after thermal injury and therapeutic intervention.

Schmidt KH, Muller U, Horer W, Braatz R.

Department of Surgery, University of Tuebingen, FR Germany.

Despite substantial progress in handling the acute phase after thermal injury, severely burned patients still succumb to systemic sepsis as a consequence of a compromised defence system. It is likely that autotoxic mechanisms play an important role in the aetiology of the impaired host defence. One of the primary

target systems of autotoxic cell damage is the liver. In the present work oxidative alterations in the microsomal compartment of liver cells have been investigated. [It was found that thermal burns are associated with extensive oxidation of polyunsaturated fatty acids which can be antagonized by antioxidants such as silibinin.](#)

47. Toxicol Appl Pharmacol. 1984 May;73(3):355-62. **(Animal Study)**

Protection by **silibinin** against Amanita phalloides intoxication in beagles.

Vogel G, Tuchweber B, Trost W, Mengs U.

A single oral dose of the lyophilized deathcap fungus Amanita phalloides (85 mg/kg body wt) caused gastrointestinal signs of diarrhea, retching, and vomiting in beagles after a latent period of 16 hr. The pathologic lesions; the increases in serum transaminase (GOT, GPT), alkaline phosphatase, and bilirubin, as well as the fall in prothrombin time all indicated that liver damage was maximal at about 48 hr after poisoning. Four of twelve dogs given A. phalloides died with signs of hepatic coma within 35 to 54 hr with the biochemical values in the survivors reverting to normal by the ninth day. **Silibinin** administration (50 mg/kg) 5 and 24 hr after intoxication suppressed the serum changes and the fall in prothrombin time. [The degree of hemorrhagic necrosis in the liver was markedly reduced, and none of the silibinin-treated dogs died.](#)

48. Hoppe Seylers Z Physiol Chem. 1984 May;365(5):555-66.

[Mechanism of action of **silibinin**. V. Effect of **silibinin** on the synthesis of ribosomal RNA, mRNA and tRNA in rat liver in vivo]

[Article in German]

Sonnenbichler J, Zetl I.

The influence of the flavonolignane **Silibinin** on the rate of RNA synthesis in rat livers was studied in detail and the time course of the stimulatory effect was determined: 8 h after i.p. application a maximal increase of about 60% in nuclear RNA synthesis can be observed. The analysis of the RNA by electrophoresis on agarose and by sucrose gradient centrifugation demonstrated that in particular the ribosomal RNA (28S, 18S, 5.8S) synthesis is accelerated followed by enhanced incorporation of rRNA into mature ribosomes. During stimulation also changes in the pattern of 45S RNA can be observed. The synthesis of mRNAs, 5S RNA and tRNAs is not influenced by **Silibinin**, which was shown after separation of these moieties on oligo(dT)-cellulose, and by polyacrylamid electrophoresis, respectively. [The clinically observed enhancement](#)

of liver cell regeneration during **Silibinin** treatment thus can be explained by an increase of the protein synthetic apparatus.

49. Hum Toxicol. 1983 Apr;2(2):183-95.

Chemotherapy of *Amanita phalloides* poisoning with intravenous **silibinin**.

Hruby K, Csomos G, Fuhrmann M, Thaler H.

1 A total of 18 cases of *Amanita phalloides* intoxication was treated by combined chemotherapy during 1980 and 1981. After attempted primary elimination of the toxin all patients received **silibinin** as basic therapy mainly by infusion and in two instances orally. 2 In order to evaluate the effect of **silibinin** therapy a retrospective study of the followed-up case records was made. The cases were arbitrarily classified into three groups according to the severity of liver damage (light, medium and severe). 3 [There was found a close relationship between the severity of liver injury and the delay between mushroom ingestion and the onset of **silibinin** therapy.](#) With the exception of one fatality in a particularly high dosage suicidal intoxication, all patients survived. 4 Administration of silibinin even up to 48 h after mushroom ingestion appears to be an effective measure to prevent severe liver damage in *Amanita phalloides* poisoning. Contrarily, the onset of general supportive treatment together with penicillin therapy which was throughout several hours before **silibinin** administration did not correlate with the severity of liver damage.

Components

50. J Nat Prod. 2003 Sep;66(9):1171-4.

Molecular Structure and Stereochemistry of Silybin A, Silybin B, Isosilybin A, and Isosilybin B, Isolated from *Silybum marianum* (**Milk thistle**).

Lee DY, Liu Y.

Bio-Organic and Natural Product Laboratory, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, Massachusetts 02478.

Two pairs of diastereoisomeric flavonolignans, silybin A, silybin B, isosilybin A, and isosilybin B, were successfully separated from *Silybum marianum* by sequential silica gel column chromatography, preparative reversed-phase HPLC, and recrystallization. Complete stereochemical assignments at C-2, C-3, C-7', and C-8' of these flavonolignans have been achieved. On the basis of X-ray crystallographic analysis and optical rotation data, coupled with comprehensive (1)H and (13)C NMR spectral data interpretation including COSY, HMQC, and HMBC,

the stereochemistry of these diastereoisomers was determined unambiguously as silybin A (4), 2R, 3R, 7'R, 8'R; silybin B (5), 2R, 3R, 7'S, 8'S; isosilybin A (6), 2R, 3R, 7'R, 8'R; and isosilybin B (7), 2R, 3R, 7'S, 8'S.

51, *Org Biomol Chem.* 2003 May 21;1(10):1684-9.

Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*).

Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME.

Natural Product Laboratory, Chemistry and Life Sciences, Research Triangle Institute, 3040 Cornwallis Road, Research Triangle Park, North Carolina 27709, USA. nck@rti.org, mcw@rti.org

Complete separation, isolation, and structural characterization of four diastereoisomeric flavonolignans, silybins A (1) and B (2), and isosilybins A (3) and B (4) from the seeds of **milk thistle** (*Silybum marianum*) were achieved for the first time using a preparative reversed-phase HPLC method. In addition, three other flavonolignans, silychristin (5) isosilychristin (6) and silydianin (7), and a flavonoid, taxifolin (8) were isolated. Structures, including absolute stereochemistries of 1-4, were confirmed using 2D NMR and CD spectroscopy.

Prostate Cancer

52. *Cancer Epidemiol Biomarkers Prev.* 2003 Sep;12(9):933-9. (**Animal Study**)

Suppression of advanced human prostate tumor growth in athymic mice by **silibinin** feeding is associated with reduced cell proliferation, increased apoptosis, and inhibition of angiogenesis.

Singh RP, Sharma G, Dhanalakshmi S, Agarwal C, Agarwal R.

Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA.

Recently, we observed that dietary feeding of **silibinin** strongly prevents and inhibits the growth of advanced human prostate tumor xenografts in athymic nude mice without any apparent signs of toxicity together with increased secretion of insulin-like growth factor-binding protein 3 from the tumor in to mouse plasma (R. P. Singh et al., *Cancer Res.*, 62:3063-3069, 2002). In the present study, we investigated the effect of **silibinin** feeding [0.05% and 0.1% (w/w) in diet for 60 days] on the prognostic biomarkers (namely, proliferation, apoptosis, and angiogenesis) in the prostate tumor xenografts of the above-reported study.

Immunohistochemical analysis of the tumors for proliferating cell nuclear antigen and Ki-67 showed that **silibinin** decreases proliferation index by 28-60% and 30-60% ($P < 0.001$) as compared with their controls, respectively. In situ detection of apoptosis by terminal deoxynucleotidyl transferase dUTP-mediated nick end labeling staining of tumors showed a 7.4-8.1-fold ($P < 0.001$) increase in apoptotic cells in **silibinin**-fed groups over that of control group. **Silibinin** also increased activated caspase 3-positive cells by 2.3-3.6-fold ($P < 0.001$). CD31 staining for tumor vasculature showed a significant decrease (21-38%; $P < 0.001$) in tumor microvessel density in **silibinin**-fed groups of tumors as compared with control group of tumors. Tumor sections were also analyzed for vascular endothelial growth factor and insulin-like growth factor-binding protein 3 protein expression, and a slightly decreased and a moderately increased cytoplasmic immunostaining in **silibinin**-fed groups were observed as compared with the control group, respectively. **Together, these results suggest that inhibition of advanced human prostate tumor xenograft growth in athymic nude mice by silibinin is associated with its in vivo antiproliferative, proapoptotic, and antiangiogenic efficacy in prostate tumor.**

53. Int J Cancer. 2003 Sep 20;106(5):699-705.

Silibinin sensitizes human prostate carcinoma DU145 cells to cisplatin- and carboplatin-induced growth inhibition and apoptotic death.

Dhanalakshmi S, Agarwal P, Glode LM, Agarwal R.

Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262, USA.

In several recent studies, we have shown that **silibinin** inhibits the growth of human prostate cancer cells (PCA) both in vitro and in vivo. Here, we investigated the effect of **silibinin** in combination with cisplatin and carboplatin on human PCA DU145 cell growth and apoptosis. Cisplatin alone at 2 microg/ml dose produced 48% cell growth inhibition, whereas a combination with 50-100 microM **silibinin** resulted in 63-80% ($p < 0.05-0.001$) growth inhibition. Similarly, compared to 68% growth inhibition at 20 microg/ml carboplatin, addition of 50-100 microM doses of **silibinin** caused 80-90% inhibition ($p < 0.005-0.001$). In the studies assessing the effect of these combinations on cell cycle progression, a combination of cisplatin or carboplatin with **silibinin** resulted in a stronger G2-M arrest, compared to these agents alone showing a moderate G2-M and G1 arrests in case of cisplatin and **silibinin**, and a complete S phase arrest with carboplatin, respectively. A stronger G2-M arrest by these combinations was accompanied by a substantial decrease in the levels of cdc2, cyclin B1 and cdc25C. **Silibinin**/platinum compound combinations were also effective in inducing apoptosis where cisplatin and carboplatin when combined with **silibinin** enhanced apoptosis from 8 to 15% and from 20 to 40%,

respectively. Apoptosis induction was further confirmed by PARP and caspases 3, 9 and 7 whose cleaved levels were also enhanced by combination treatment. In addition, there was a significant increase in cytochrome c release in the cytosol following treatment of DU145 cells with these combinations. Together, these results show a substantial increase in the efficacy of platinum compounds on human PCA cells, when combined with **silibinin**, which provide a rationale for further investigations with these combinations. Copyright 2003 Wiley-Liss, Inc.

54. Mol Cancer Ther. 2002 May;1(7):525-32.

Inhibition of retinoblastoma protein (Rb) phosphorylation at serine sites and an increase in Rb-E2F complex formation by **silibinin** in androgen-dependent human prostate carcinoma LNCaP cells: role in prostate cancer prevention.

Tyagi A, Agarwal C, Agarwal R.

Several studies have identified **silibinin** as an anticarcinogenic agent. Recently, we showed that **silibinin** inhibits cell growth via G1 arrest, leading to differentiation of androgen-dependent human prostate carcinoma LNCaP cells (X. Zi and R. Agarwal, Proc. Natl. Acad. Sci. USA, 96: 7490-7495, 1999). Here, we extend this study to assess the effect of **silibinin** on total retinoblastoma protein (Rb) levels and its phosphorylation status, levels of E2F family members, and Rb-E2F binding in LNCaP cells. Compared with controls, **silibinin** resulted in an increase in total Rb levels that was largely attributable to an increase in unphosphorylated Rb (up to 4.1-fold). This effect of **silibinin** was mainly attributable to a large decrease (70-97%) in the amount of Rb phosphorylated at specific serine sites. In other studies, **silibinin** showed a moderate effect on E2F1 but up to 98 and 90% decreases in E2F2 and E2F3 protein levels, respectively. **Silibinin** treatments also resulted in an increase in the amount of Rb binding to E2F1 (3.8-fold), E2F2 (2.2-fold), and E2F3 (2.2-fold). Cyclin-dependent kinases (CDKs), together with their catalytic subunit cyclins, phosphorylate Rb, which makes transcription factor E2Fs free from Rb-E2F complexes, resulting in cell growth and proliferation. Conversely, CDK inhibitors inhibit this phosphorylation, maintaining E2Fs bound to Rb, which causes growth inhibition. On the basis of our data showing that **silibinin** induces both unphosphorylated Rb levels and Rb-E2F binding, we also assessed its effect on upstream cell cycle regulators. **Silibinin**-treated cells showed up to 2.4- and 3.6-fold increases in Cip1/p21 and Kip1/p27 levels, respectively, and a decrease in CDK2 (80%), CDK4 (98%), and cyclin D1 (60%). Consistent with these results, **silibinin** showed both G1 arrest and growth inhibition. Together, these findings identify modulation of Rb levels and its phosphorylation status as a molecular mechanism of **silibinin**-induced neuroendocrine differentiation of human prostate carcinoma LNCaP cells and suggest that this could be a novel approach for prostate cancer prevention by **silibinin**.

55. Cell Cycle. 2002 Mar-Apr;1(2):137-42.

The cancer preventive flavonoid **silibinin** causes hypophosphorylation of Rb/p107 and Rb2/p130 via modulation of cell cycle regulators in human prostate carcinoma DU145 cells.

Tyagi A, Agarwal C, Agarwal R.

Department of Pharmaceutical Sciences; School of Pharmacy; University of Colorado Health Sciences Center; Denver Colorado 80262, USA.

Phosphorylation status of retinoblastoma (Rb) and related proteins is important to drive cell cycle progression. In hyperphosphorylated state, they are growth stimulatory, but their hypophosphorylation is growth inhibitory. Here we assessed whether **silibinin** causes hypophosphorylation of Rb-related proteins as its growth inhibitory response in human prostate cancer (PCA) DU145 cells. **Silibinin** treatment of cells resulted in a strong increase (up to 2.3- and 5.4-fold) in the levels of hypophosphorylated Rb/p107 and Rb2/p130, respectively, but a strong decrease (91, 78 and 45%) in protein levels of transcription factors E2F3, E2F4 and E2F5, respectively. In the studies analyzing whether this effect of **silibinin** is via modulation of cell cycle regulators, **silibinin**-treated cells showed a strong increase (up to 13- and 6-fold) in Cip1/p21 and Kip1/p27 levels, respectively. **Silibinin** treatment also resulted in 90 and 70% decrease in CDK4 and CDK2 levels, respectively, but did not alter the protein levels of cyclin D1 and cyclin E. Consistent with its effect on G1 cell cycle regulators, **silibinin** treated cells exhibited a strong G1 arrest, almost complete growth inhibition, and morphological changes suggestive of differentiation. Together, these results suggest that **silibinin** caused hypophosphorylation of Rb-related proteins may in part be responsible for its cancer preventive and anti-carcinogenic efficacy in different cancer models including PCA.

56. Clin Cancer Res. 2002 Nov;8(11):3512-9.

Comment in:

Clin Cancer Res. 2002 Nov;8(11):3311-4.

Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth Inhibition, G2-M arrest, and apoptosis.

Tyagi AK, Singh RP, Agarwal C, Chan DC, Agarwal R.

Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA.

PURPOSE: We recently demonstrated the strong anticancer efficacy of **silibinin**, an active constituent of a widely consumed dietary supplement **milk thistle** extract, against human prostate cancer cells in culture and nude mice xenografts. We also observed that pharmacologically achievable concentrations of **silibinin** in animal studies were in the range of 25-100 microM, depending on the dose regimen, which did not show any apparent toxicity to the animals. In this study, we assessed whether **silibinin** synergizes the therapeutic potential of the chemotherapeutic drug doxorubicin against prostate cancer, the effectiveness of which is limited because of high systemic toxicity. **EXPERIMENTAL DESIGN:** Prostate cancer cells were treated with **silibinin** and doxorubicin, either alone or in combination, and cell growth was determined by manual cell counting. Cell cycle progression was assessed by saponin/propidium iodide staining and fluorescence-activated cell sorter analysis. Protein levels of cell cycle regulators were determined by Western blotting, and cdc2/p34 kinase activity was analyzed by in-beads kinase assay. Apoptosis was quantified by annexin V/propidium iodide staining and fluorescence-activated cell sorter analysis. **RESULTS:** **Silibinin** strongly synergized the growth-inhibitory effect of doxorubicin in prostate carcinoma DU145 cells (combination index, 0.235-0.587), which was associated with a strong G(2)-M arrest in cell cycle progression, showing 88% cells in G2-M phase by this combination compared with 19 and 41% of cells in **silibinin** and doxorubicin treatment alone, respectively. The underlying mechanism of G2-M arrest showed a strong inhibitory effect of combination on cdc25C, cdc2/p34, and cyclin B1 protein expression and cdc2/p34 kinase activity. **More importantly, this combination caused 41% apoptotic cell death compared with 15% by either agent alone.** **Silibinin** and doxorubicin alone as well as in combination were also effective in inhibiting the growth of androgen-dependent prostate carcinoma LNCaP cells. **CONCLUSION:** **These findings suggest a need for in vivo studies with this combination in preclinical prostate cancer models.** Positive outcomes might be relevant for a clinical application in prostate cancer patients.

57. Prostate. 2002 Nov 1;53(3):211-7. **(Animal Study)**

Antiproliferative and apoptotic effects of **silibinin** in rat prostate cancer cells.

Tyagi A, Bhatia N, Condon MS, Bosland MC, Agarwal C, Agarwal R.

Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA.

BACKGROUND: The tremendous impact of prostate cancer (PCA) on the US male population has led to an increased attention on its prevention and on therapeutic intervention. Short-term models are needed to quickly screen the efficacy of promising agents against PCA. We have established recently several

rat PCA cell lines from primary PCA in rats induced by a MNU-testosterone protocol, but their usefulness as a model for screening PCA preventive and therapeutic agents remains to be established. With the rationale that agents found effective in these cells could be promising for efficacy testing in long-term in vivo experiments, e.g., with MNU-testosterone-induced PCA in rats, the major goal of our study was to assess the antiproliferative and apoptotic efficacy in rat PCA cell lines of **silibinin**, a major active flavonoid component of **silymarin**, which consists of a group of flavonoid antioxidants occurring in **milk thistle** (*Silybum marianum*). METHODS: Three rat PCA cell lines, namely H-7, I-8, and I-26, were treated with **silibinin** or **silymarin**, a crude **silibinin**-containing preparation, at various doses for varying lengths of time. Cell growth and viability studies were carried out by using hemocytometer and Trypan blue dye exclusion methods. Cell cycle distribution studies were conducted by using PI staining and flow cytometry analysis, and DNA synthesis was assessed by bromodeoxyuridine incorporation. Apoptotic cell death was assessed as DNA damage by using an enzyme-linked immunosorbent assay method and by annexin V and PI staining followed by flow cytometry analysis. RESULTS: **Silibinin** resulted in a significant growth inhibition and reduction in cell viability in each cell line studied in both a dose- and a time-dependent manner. **Silibinin** treatment of H-7 and I-8 cells at 100 microM dose for 12 and 24 hr resulted in a G1 arrest but caused S phase arrest after a 48-hr treatment period in each cell line studied. Similar **silibinin** treatment of I-26 cells resulted in a slight S phase arrest at all time points studied. Consistent with these findings, **silibinin** showed a strong inhibition of DNA synthesis. **Silibinin** also induced a substantial apoptotic death in each cell line studied. Similar to **silibinin**, **silymarin** induced growth inhibition and reduced viability in a dose- and time-dependent manner. CONCLUSION: This study demonstrates that **silibinin** as well as **silymarin** induce growth inhibition and apoptosis in rat PCA cells. These results form a strong rationale for PCA prevention and therapeutic intervention studies with **silibinin** and **silymarin** in animal models, such as the MNU-testosterone rat PCA model, to establish their efficacy and to further define their mechanisms of action under in vivo conditions. Copyright 2002 Wiley-Liss, Inc.

58. Cancer Res. 2002 Jun 1;62(11):3063-9. (Animal Study)

Dietary feeding of **silibinin** inhibits advanced human prostate carcinoma growth in athymic nude mice and increases plasma insulin-like growth factor-binding protein-3 levels.

Singh RP, Dhanalakshmi S, Tyagi AK, Chan DC, Agarwal C, Agarwal R.

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We have reported recently the anticancer effect of flavonoid antioxidant **silymarin**, the major part of **milk thistle** extract, against advanced human prostate carcinoma DU145 cells (X. Zi et al., *Cancer Res.*, 58: 1920-1929, 1998) and later identified that **silibinin** is the main active component in **silymarin** responsible for its effect in cell culture studies. On the basis of these observations, here we assessed in vivo growth inhibitory potential of **silibinin** against advanced human prostate cancer (PCA). Dietary feeding of **silibinin** at 0.05 and 0.1% doses (w/w) for 60 days, 24 h after s.c. DU145 tumor xenograft implantation in athymic male nude mice, significantly inhibited tumor volume by 35 and 58% ($P < 0.05$), and wet weight of tumor by 29 and 40% ($P < 0.05$), respectively. In a second experiment where mice were fed with these test diets for 3 weeks before tumor xenograft implantation and continued on these diets for a total of 63 days, tumor volume and wet weight of tumor were reduced by 53-64% ($P < 0.001-0.05$) and 31-52% ($P < 0.05$), respectively. In both studies, animals did not show weight loss or reduced food consumption. These in vivo anticancer effects of **silibinin** were associated with an increased accumulation (up to 5.8 fold; $P < 0.05$) of human insulin-like growth factor-binding protein-3 in mouse plasma. In additional studies assessing biological availability of **silibinin** in nude mice and its antiproliferative activity at such doses in DU145 cells in culture, **silibinin** levels in plasma and prostate were found to be in the range of 7-13 microg/ml and 3.7-4.6 microg/g, respectively. At these biologically achievable **silibinin** concentrations, increased IGFBP-3 level in DU145 cell culture medium and a strong DU145 cell growth inhibition were observed that were irreversible in the absence of **silibinin** in culture medium. These findings extend and translate our observations on in vitro anticancer effect of **silibinin/silymarin** to an in vivo preclinical PCA model, which may form the basis for a Phase I clinical trial in PCA patients.

59. *Oncogene*. 2002 Mar 7;21(11):1759-67.

Silibinin inhibits constitutive and TNFalpha-induced activation of NF-kappaB and sensitizes human prostate carcinoma DU145 cells to TNFalpha-induced apoptosis.

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Prostate cancer (PCA) is one of the most common invasive malignancies of men in the US, however, there have been limited successes so far in its therapy. Even most potent agents (e.g. TNFalpha) are ineffective in killing human PCA cells possibly due to constitutive activation of NF-kappaB that subsequently activates a large number of anti-apoptotic genes. In such a scenario, strong apoptotic agent TNFalpha, further induces NF-kappaB activation rather than inducing apoptosis. In several recent studies, we have demonstrated both cancer

preventive and anti-cancer efficacy of **silymarin** and its constituent **silibinin** in a variety of experimental tumor models and cell culture systems. Here we examined whether **silibinin** is effective in inhibiting constitutive NF-kappaB activation in human PCA cells, which would help in overcoming TNFalpha-insensitivity. Our studies reveal that **silibinin** effectively inhibits constitutive activation of NF-kappaB in advanced human prostate carcinoma DU145 cells. Consistent with this, nuclear levels of p65 and p50 sub-units of NF-kappaB were also reduced. In the studies assessing molecular mechanism of this effect, **silibinin** treatment resulted in a significant increase in the level of IkappaBalpha with a concomitant decrease in phospho-IkappaBalpha. Kinase assays revealed that **silibinin** dose-dependently decreases IKKalpha kinase activity. **The effect of silibinin on IKKalpha seemed to be direct as evidenced by the in vitro kinase assay, where immunoprecipitated IKKalpha was incubated with silibinin.** This shows that **silibinin** does not necessarily need an upstream event to bring about its inhibitory effect on IKKalpha and downstream effectors. **Additional studies showed that silibinin also inhibits TNFalpha-induced activation of NF-kappaB via IkappaBalpha pathway and subsequently sensitizes DU145 cells to TNFalpha-induced apoptosis.** These results indicate that **silibinin** could be used to enhance the effectiveness of TNFalpha-based chemotherapy in advanced PCA.

60. Carcinogenesis. 2001 Sep;22(9):1399-403.

Silymarin inhibits function of the androgen receptor by reducing nuclear localization of the receptor in the human prostate cancer cell line LNCaP.

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A number of reports have shown that the polyphenolic flavonoid **silymarin** (SM) is an effective anticancer agent. Agents with novel mechanisms of blocking androgen receptor (AR) function may be useful for prostate cancer prevention and therapy. Previous studies showed that **silibinin** (SB), the major active component of SM, could inhibit cell proliferation of a human prostate cancer cell line, LNCaP, by arresting the cell cycle at the G(1) phase without causing cell death. This study further delineates the potential molecular mechanism by which SM and SB exhibit antiproliferative effects on androgen-responsive prostate cancer cells by inhibiting function of the AR. **We observed that SM and SB inhibited androgen-stimulated cell proliferation as well as androgen-stimulated secretion of both prostate-specific antigen (PSA) and human glandular kallikrein (hK2).** Additionally, for the first time, we show that an immunophilin, FKBP51, is androgen regulated and that this up-regulation is suppressed by SM and SB. **We further demonstrate that transactivation activity of the AR was diminished by SM**

and SB using gene transfer of PSA promoter and hK2 androgen-responsive element constructs. However, expression and steroid-binding ability of total AR were not affected by SM in western blotting and ligand-binding assays. Intriguingly, we found that nuclear AR levels are significantly reduced by SM and SB in the presence of androgens using western blotting assay and immunocytochemical staining. This study provides a new insight into how SM and SB negatively modulate androgen action in prostate cancer cells.

61. Mol Carcinog. 2001 Apr;30(4):224-36.

Inhibitory effect of **silibinin** on ligand binding to erbB1 and associated mitogenic signaling, growth, and DNA synthesis in advanced human prostate carcinoma cells.

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We recently showed the inhibitory effect of a flavonoid antioxidant, **silymarin**, on erbB1-Shc activation in prostate cancer (PCA) DU145 cells. In the present study, we performed more detailed mechanistic and molecular modeling studies with pure **silibinin** to assess and define its effect on membrane signaling related to erbB1 activation in human PCA LNCaP and DU145 cells. Studies also were performed to establish the biologic responses toward extracellular signal-regulated protein kinase 1/2 (ERK1/2) activation, cell growth, and DNA synthesis. Treatment of serum-starved cells with various doses of **silibinin** for 2 h followed by (125)I-epidermal growth factor (EGF) showed 30-75% inhibition in ligand binding and 55-95% inhibition in its internalization in LNCaP cells and 20-64% and 12-27% inhibition in these two events in DU145 cells. Time-response studies showed similar effects. In further studies, treatment of serum-starved cultures with **silibinin** followed by EGF showed strong inhibitory effects on membrane and cytoplasmic signaling molecules. In the case of erbB1 activation, **silibinin** showed a 58-75% decrease in LNCaP and a 40-100% decrease in DU145 cells at 50, 75, and 100-microg/mL doses. Inhibitory effects of **silibinin** also were evident on ERK1/2 activation (20-80% inhibition) in both cell lines. Treatment of serum-starved cultures with **silibinin** resulted in 20-40% and 30-55% inhibition of LNCaP and DU145 cell growth, respectively, at similar doses after 1-3 d of treatment, and 10-50% cell death in both cell lines. Under 10% serum conditions, identical **silibinin** treatments resulted in 20-65% inhibition of cell growth in LNCaP and DU145 cells but did not cause any cell death. Similar doses of **silibinin** treatments for 24 h also resulted in 25-60%, 35-40%, and 36-50% inhibition of DNA synthesis when cells were cultured in 10% serum, totally serum starved, and serum starved plus stimulated with EGF, respectively. Molecular modeling of **silibinin** showed that it is a highly lipophilic compound, suggesting

that it interacts with lipid-rich plasma membrane, including binding with erbB1, thereby competing with the EGF-erbB1 interaction. Because the ligand-erbB1 autocrine-loop is causally involved in advanced and androgen-independent PCA, the observed effects of **silibinin** and its strong lipophilic nature could be useful in developing this agent for the prevention and therapy of PCA. Copyright 2001 Wiley-Liss, Inc.

62. Cancer Res. 2000 Oct 15;60(20):5617-20.

Silibinin up-regulates insulin-like growth factor-binding protein 3 expression and inhibits proliferation of androgen-independent prostate cancer cells.

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Silibinin, a naturally occurring flavonoid antioxidant found in the milk thistle, has recently been shown to have potent antiproliferative effects against various malignant cell lines, but the underlying mechanism of action remains to be elucidated. We investigated the effect of **silibinin** on androgen-independent prostate cancer PC-3 cells. At pharmacologically achievable **silibinin** concentrations (0.02-20 microM), we observed increased insulin-like growth factor-binding protein 3 (IGFBP-3) accumulation in PC-3 cell conditioned medium and a dose-dependent increase of IGFBP-3 mRNA abundance with a 9-fold increase over baseline at 20 microM **silibinin**. An IGFBP-3 antisense oligodeoxynucleotide that attenuated **silibinin**-induced IGFBP-3 gene expression and protein accumulation reduced the antiproliferative action of **silibinin**. We also observed that **silibinin** reduced insulin receptor substrate 1 tyrosine phosphorylation, indicating an inhibitory effect on the insulin-like growth factor I receptor-mediated signaling pathway. These results suggest a novel mechanism by which **silibinin** acts as an antiproliferative agent and justify further work to investigate potential use of this compound or its derivatives in prostate cancer treatment and prevention.

63. Biochem Pharmacol. 2000 Oct 15;60(8):1051-9.

Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents.

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Prostate cancer (PCA) is the most common invasive malignancy and leading cause (after lung) of cancer deaths in males. Since PCA is initially androgen-dependent, strategies are targeted toward androgen depletion for its control. However, tumor re-growth mostly occurs following this modality, and is androgen-independent. A loss of functional androgen receptor and an enhanced expression of growth factor receptors (e.g. erbB family members) and associated ligands have been shown to be the causal genetic events in PCA progression. These genetic alterations lead to an epigenetic mechanism where a feed-back autocrine loop between membrane receptor (e.g. epidermal growth factor receptor [erbB1] and associated ligand (e.g. transforming growth factor- α) results in an enhanced activation of extracellular signal-regulated protein kinase 1/2 (ERK1/2) as an essential component of the uncontrolled growth of PCA at an advanced and androgen-independent stage. Together, we rationalized that inhibiting these epigenetic events would be useful in controlling advanced PCA growth. Dietary polyphenolic flavonoids and isoflavones are being studied extensively as cancer-preventive and interventive agents. Therefore, we focused our attention on **silymarin**, genistein, and epigallocatechin 3-gallate (EGCG), present in **milk thistle**, soy beans, and green tea, respectively. The effect of these agents was assessed on the erbB1-Shc-ERK1/2 signal transduction pathway, cell cycle regulatory molecules, and cell growth and death. In androgen-independent human prostate carcinoma DU145 cells, **silymarin**, genistein, and EGCG resulted in a significant to complete inhibition of transforming growth factor- α -caused activation of membrane receptor erbB1 followed by inhibition of downstream cytoplasmic signaling target Shc activation and a decrease in its binding with erbB1, without an alteration in their protein expression. **Silymarin** and genistein also inhibited ERK1/2 activation, suggesting that these agents impair the activation of erbB1-Shc-ERK1/2 signaling in DU145 cells. In the case of EGCG, a further increase in ERK1/2 activation was observed that was related to its pro-oxidant and apoptotic activities. **Silymarin**, genistein, and EGCG also resulted in a significant induction of Cip1/p21 and Kip1/p27 and a decrease in cyclin-dependent kinase (CDK) 4, but a moderate inhibition of CDK2, cyclin D1, and cyclin E was observed. An enhanced level of Cip1/p21 and Kip1/27 also led to an increase in their binding to CDK4 and CDK2. Treatment of cells with **silymarin**, genistein, and EGCG also resulted in strong cell growth inhibition at lower doses, and complete inhibition at higher doses. In contrast to **silymarin**, higher doses of genistein also showed cell death. A more profound cytotoxic effect was observed in the case of EGCG, with strong cell death at lower doses and complete loss of viability at higher doses. Together, these results suggest that cell signaling and regulators of cell cycle are potential epigenetic molecular targets for prostate cancer prevention by dietary agents. More studies, therefore, are needed with these agents to explore their anticarcinogenic potential against human prostate cancer.

Silibinin decreases prostate-specific antigen with cell growth inhibition via G1 arrest, leading to differentiation of prostate carcinoma cells: implications for prostate cancer intervention.

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Reduction in serum prostate-specific antigen (PSA) levels has been proposed as an endpoint biomarker for hormone-refractory human prostate cancer intervention. We examined whether a flavonoid antioxidant **silibinin** (an active constituent of **milk thistle**) decreases PSA levels in hormone-refractory human prostate carcinoma LNCaP cells and whether this effect has biological relevance. **Silibinin** treatment of cells grown in serum resulted in a significant decrease in both intracellular and secreted forms of PSA concomitant with a highly significant to complete inhibition of cell growth via a G1 arrest in cell cycle progression. Treatment of cells grown in charcoal-stripped serum and 5alpha-dihydrotestosterone showed that the observed effects of **silibinin** are those involving androgen-stimulated PSA expression and cell growth. **Silibinin-induced G1 arrest was associated with a marked decrease in the kinase activity of cyclin-dependent kinases (CDKs) and associated cyclins because of a highly significant decrease in cyclin D1, CDK4, and CDK6 levels and an induction of Cip1/p21 and Kip1/p27 followed by their increased binding with CDK2.** **Silibinin treatment of cells did not result in apoptosis and changes in p53 and bcl2, suggesting that the observed increase in Cip1/p21 is a p53-independent effect that does not lead to an apoptotic cell death pathway. Conversely, silibinin treatment resulted in a significant neuroendocrine differentiation of LNCaP cells as an alternative pathway after Cip1/p21 induction and G1 arrest.** Together, these results suggest that **silibinin** could be a useful agent for the intervention of hormone-refractory human prostate cancer.

65. Cancer Res. 1998 May 1;58(9):1920-9.

A flavonoid antioxidant, **silymarin**, inhibits activation of erbB1 signaling and induces cyclin-dependent kinase inhibitors, G1 arrest, and anticarcinogenic effects in human prostate carcinoma DU145 cells.

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Prostate cancer (PCA) is the most common nonskin malignancy and the second

leading cause of cancer deaths in United States males. One practical and translational approach to control PCA is to define a mechanism-based anticarcinogenic agent(s). Recently, we showed that **silymarin**, a flavonoid antioxidant isolated from **milk thistle**, possesses exceptionally high to complete protective effects against experimentally induced tumorigenesis. Because the epidermal growth factor receptor (erbB1) and other members of the erbB family have been shown to play important roles in human PCA, efforts should be directed to identify inhibitors of this pathway for PCA intervention. In this study, we assessed whether **silymarin** inhibits erbB1 activation and associated downstream events and modulates cell cycle regulatory proteins and progression, leading to growth inhibition of human prostate carcinoma DU145 cells. **Treatment of serum-starved cells with silymarin resulted in a significant inhibition of transforming growth factor alpha-mediated activation of erbB1 but no change in its protein levels. Silymarin treatment of cells also resulted in a significant decrease in tyrosine phosphorylation of an immediate downstream target of erbB1, the adapter protein SHC, together with a decrease in its binding to erbB1. In the studies analyzing cell cycle regulatory molecules, silymarin treatment of cells also resulted in a significant induction of cyclin-dependent kinase inhibitors (CDKIs) Cip1/p21 and Kip1/p27, concomitant with a significant decrease in CDK4 expression, but no change in the levels of CDK2 and CDK6 and their associated cyclins E and D1, respectively. Cells treated with silymarin also showed an increased binding of CDKIs with CDKs, together with a marked decrease in the kinase activity of CDKs and associated cyclins. In additional studies, treatment of cells grown in 10% serum with anti-epidermal growth factor receptor monoclonal antibody clone 225 or different doses of silymarin also resulted in significant inhibition of constitutive tyrosine phosphorylation of both erbB1 and SHC but no change in their protein levels. Furthermore, whereas silymarin treatment resulted in a significant increase in the protein levels of both Cip1/p21 and Kip1/p27, monoclonal antibody 225 showed an increase only in Kip1/p27. These findings suggest that silymarin also inhibits constitutive activation of erbB1 and that the observed effect of silymarin on an increase in CDKI protein levels is mediated via inhibition of erbB1 activation only in the case of Kip1/p27; however, additional pathways independent of inhibition of erbB1 activation are possibly responsible for the silymarin-caused increase in Cip1/p21 in DU145 cells. In other studies, silymarin treatment also induced a G1 arrest in the cell cycle progression of DU145 cells and resulted in a highly significant to complete inhibition of both anchorage-dependent and anchorage-independent growth of DU145 cells in a dose- and time-dependent manner. Taken together, these results suggest that silymarin may exert a strong anticarcinogenic effect against PCA and that this effect is likely to involve impairment of erbB1-SHC-mediated signaling pathway, induction of CDKIs, and a resultant G1 arrest.**

Colon Cancer

66. J Surg Res. 2003 Jul;113(1):133-8.

Anti-angiogenic effect of **silymarin** on colon cancer LoVo cell line.

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OBJECTIVE: This study was designed to evaluate the anti-angiogenic effect of **silymarin** (SM) and its major pure component **silibinin** (SB), and also thalidomide (TH). **MATERIALS AND METHODS:** A modified in vitro system using a coculture of endothelial (EA.hy 926) and colon cancer (LoVo) cell lines was adopted in this study. **RESULTS:** In cytotoxicity assay, SM/SB/TH concentrations causing 20% (IC(20)) inhibition of cellular growth were 41.8 microg/ml/0.22 mM/0.088 mM for EA.hy 926 cells, and 16.1 microg/ml/0.12 mM/0.099 mM for LoVo cells, respectively. All 3 drugs showed concentration dependent inhibition of migration and differentiation assay. The IC(50) inhibiting chemotaxis migration of EA.hy 926 towards LoVo by SM/SB/TH was 1.15 microg/ml/0.66 microM/1.98 microM, respectively. In differentiation assay, SM/SB/TH inhibited in vitro capillary tube formation by 50% at 1.25 microg/ml/2.6 micro/6.3 microM, respectively. In an analysis of vascular endothelial growth factor secreted by LoVo cells, SM/SB/TH decreased 50% secretion at 6.52 microg/ml/6.6 microM/131.7 microM, respectively. **CONCLUSION:** **SM/SB has a strong anti-angiogenesis effect on the colon cancer cell line, and this might provide an alternative treatment option for anti-cancer treatment.**

67. Int J Cancer. 2002 Oct 10;101(5):461-8. **(Animal Study)**

Silymarin, a naturally occurring polyphenolic antioxidant flavonoid, inhibits azoxymethane-induced colon carcinogenesis in male F344 rats.

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The modifying effect of dietary administration of the polyphenolic antioxidant flavonoid **silymarin**, isolated from **milk thistle** [*Silybum marianum* (L.) Gaertneri], on AOM-induced colon carcinogenesis was investigated in male F344 rats. In the short-term study, the effects of **silymarin** on the development of AOM-induced colonic ACF, being putative precursor lesions for colonic adenocarcinoma, were assayed to predict the modifying effects of dietary **silymarin** on colon tumorigenesis. Also, the activity of detoxifying enzymes (GST and QR) in liver and colonic mucosa was determined in rats gavaged with **silymarin**. Subsequently, the possible inhibitory effects of dietary feeding of

silymarin on AOM-induced colon carcinogenesis were evaluated using a long-term animal experiment. In the short-term study, dietary administration of **silymarin** (100, 500 and 1,000 ppm in diet), either during or after carcinogen exposure, for 4 weeks caused significant reduction in the frequency of colonic ACF in a dose-dependent manner. **Silymarin** given by gavage elevated the activity of detoxifying enzymes in both organs. In the long-term experiment, dietary feeding of **silymarin** (100 and 500 ppm) during the initiation or postinitiation phase of AOM-induced colon carcinogenesis reduced the incidence and multiplicity of colonic adenocarcinoma. The inhibition by feeding with 500 ppm **silymarin** was significant ($p < 0.05$ by initiation feeding and $p < 0.01$ by postinitiation feeding). Also, **silymarin** administration in the diet lowered the PCNA labeling index and increased the number of apoptotic cells in adenocarcinoma. beta-Glucuronidase activity, PGE(2) level and polyamine content were decreased in colonic mucosa. These results clearly indicate a chemopreventive ability of dietary **silymarin** against chemically induced colon tumorigenesis and will provide a scientific basis for progression to clinical trials of the chemoprevention of human colon cancer. Copyright 2002 Wiley-Liss, Inc.

Lung Cancer

68. Anticancer Res. 2003 May-Jun;23(3B):2649-55.

Silibinin induces growth inhibition and apoptotic cell death in human lung carcinoma cells.

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BACKGROUND: The high systemic toxicity of chemotherapeutic agents limits their use to treat clinical lung cancer. These limitations could be minimized/overcome by using non-toxic phytochemicals, like, **silibinin**. **MATERIALS AND METHODS:** We used small cell lung carcinoma cells (SCLC) SHP-77 and non-small cell lung carcinoma cells (NSCLC) A-549, analyzing cell growth inhibition and death with Trypan blue exclusion, indices of the cell cycle progression with flow cytometry and apoptosis with propidium iodide and Hoechst 33342. **RESULTS:** **Silibinin** (25, 50 and 100 microM) treatment of SHP-77 and A-549 cells resulted in their growth inhibition and cell death. Cell cycle studies showed a small increase in G0-G1 population at all the time intervals in SHP-77 cells, however, in A-549 cells, a slight increase in G0-G1 but strong increase in S-phases was observed at lower treatment times, and a strong increase in G0-G1 population at 72 hours. Quantitative apoptotic studies showed that **silibinin** causes apoptotic cell death in both a dose- and a time-dependent manner with SHP-77 cells showing more

apoptotic effect than A-549 cells. CONCLUSION: **Silibinin** significantly induces growth inhibition, a moderate cell cycle arrest and a strong apoptotic death in both small cell and non-small cell human lung carcinoma cells, which warrants further studies to assess the efficacy of this non-toxic agent in animal lung tumor models.

Alcoholic liver disease

69. Best Pract Res Clin Gastroenterol. 2003 Aug;17(4):625-47.

Advances in alcoholic liver disease.

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Alcoholic liver disease (ALD) remains a major cause of morbidity and mortality worldwide. For example, the Veterans Administration Cooperative Studies reported that patients with cirrhosis and superimposed alcoholic hepatitis had a 4-year mortality of >60%. Interactions between acetaldehyde, reactive oxygen and nitrogen species, inflammatory mediators and genetic factors appear to play prominent roles in the development of ALD. The cornerstone of therapy for ALD is lifestyle modification, including drinking and smoking cessation and losing weight, if appropriate. Nutrition intervention has been shown to play a positive role on both an inpatient and outpatient basis. Corticosteroids are effective in selected patients with alcoholic hepatitis and pentoxifylline appears to be a promising anti-inflammatory therapy. Some complementary and alternative medicine agents, such as **milk thistle** and S-adenosylmethionine, may be effective in alcoholic cirrhosis. Treatment of the complications of ALD can improve quality of life and, in some cases, decrease short-term mortality.

70. Blutalkohol. 1991 Nov;28(6):405-8.

Ethanol elimination in man under influence of hepatoprotective **silibinin**.

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The effect of a single dose of hepatoprotective **silibinin** on blood alcohol elimination was investigated. Neither influence on the blood alcohol curve, nor detectable increase in the beta 60 value was found, although biochemical considerations suggest such an effect. Though **silibinin** has a protective effect

on chronic alcohol liver injury, **it does not influence acute alcohol elimination, and is therefore not suitable for use as a "sobering-up" agent.**

71. Free Radic Res Commun. 1987;3(6):373-7.

Effect of **silibinin** on the activity and expression of superoxide dismutase in lymphocytes from patients with chronic alcoholic liver disease.

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The in vitro and in vivo effects of the naturally occurring flavolignan hepatoprotective agent **silibinin** on the expression and activity of superoxide dismutase (SOD) enzyme were studied in lymphocytes from patients with chronic alcoholic liver disease. In vitro incubation with **silibinin** in a concentration corresponding to the usual therapeutic dosage markedly increased the SOD--expression of lymphocytes as measured by flow-cytofluorimetry following staining with monoclonal anti-Cu, Zn-SOD--antibody and FITC-conjugated anti-mouse Ig. In vivo treatment with the drug restored the originally low SOD activity of the patients' lymphocytes. **These data indirectly suggest that antioxidant activity might be one of the important factors in the hepatoprotective action of silibinin.**

Cancer

72. J Altern Complement Med. 2003 Jun;9(3):411-6.

Milk thistle: is there a role for its use as an adjunct therapy in patients with cancer?

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The use of complementary and alternative medicine (CAM) is common among patients with cancer. Many of these patients use CAM therapies to decrease the risk of late effects that are sometimes associated with cancer therapy. Certain classes of effective anticancer agents can induce short- and long-term toxicity to the liver. Currently, there are no safer alternatives to these medications. Milk thistle (*Silybum marianum*) is a botanical that may be useful in the prevention or treatment of liver dysfunction in patients undergoing anticancer therapy.

73. Carcinogenesis. 2002 May;23(5):787-94. (**Animal Study**)

Dietary **silymarin** suppresses 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in male F344 rats.

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The modifying effect of dietary administration of a polyphenolic antioxidant flavonoid **silymarin** isolated **milk thistle** [*Silybum marianum* (L.) Gaertneri] on 4-nitroquinoline 1-oxide (4-NQO)-induced tongue tumorigenesis was investigated in male F344 rats. Based on the results in pilot studies showing that **silymarin** treatment together with 4-NQO significantly reduced the occurrence of tongue dysplasia and gavaged with **silymarin** significantly elevated the phase II detoxifying enzymes' activities in the liver and tongue, the effects of dietary feeding of **silymarin** on tongue carcinogenesis were investigated in a long-term experiment, where rats were initiated with 4-NQO and fed **silymarin** containing diets during or after 4-NQO exposure. At 5 weeks of age, all animals except those treated with **silymarin** alone and untreated rats were given 20 p.p.m. 4-NQO in drinking water for 8 weeks to induce tongue neoplasms. Starting 1 week before 4-NQO administration, animals were fed the experimental diets containing **silymarin** (100 and 500 p.p.m.) for 10 weeks, and then maintained on a basal diet for 24 weeks. Starting 1 week after the cessation of 4-NQO exposure, the experimental groups given 4-NQO and a basal diet were fed the experimental diets containing 100 or 500 p.p.m. **silymarin** for 24 weeks. At week 34, feeding of 500 p.p.m. **silymarin** during the promotion phase significantly inhibited the incidence of tongue carcinoma, when compared with 4-NQO alone group (20% versus 64%, $P = 0.019$). Dietary **silymarin** decreased the cell proliferating activity and increased apoptotic index of tongue carcinoma. The treatment with **silymarin** decreased the polyamine content and prostaglandin (PG) E(2) level in the tongue mucosa. Thus, the results indicate that feeding of **silymarin** (500 p.p.m.) during the promotion phase of 4-NQO-induced rat tumorigenesis exerts chemopreventive ability against tongue squamous cell carcinoma through modification of phase II enzymes activity, cell proliferation, and/or PGE(2) content.

74. Cancer Lett. 1999 Dec 1;147(1-2):77-84.

Inhibition of human carcinoma cell growth and DNA synthesis by **silibinin**, an active constituent of **milk thistle**: comparison with **silymarin**.

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Several studies from our laboratory have shown the cancer chemopreventive and anti-carcinogenic effects of **silymarin**, a flavonoid antioxidant isolated from **milk thistle**, in long-term tumorigenesis models and in human prostate, breast and cervical carcinoma cells. Since **silymarin** is composed mainly of **silibinin** with small amounts of other stereoisomers of **silibinin**, in the present communication, studies were performed to assess whether the cancer preventive and anti-carcinogenic effects of **silymarin** are due to its major component **silibinin**. Treatment of different prostate, breast, and cervical human carcinoma cells with **silibinin** resulted in a highly significant inhibition of both cell growth and DNA synthesis in a time-dependent manner with large loss of cell viability only in case of cervical carcinoma cells. When compared with **silymarin**, these effects of **silibinin** were consistent and comparable in terms of cell growth and DNA synthesis inhibition, and loss of cell viability. Based on the comparable results of **silibinin** and **silymarin**, we suggest that the cancer chemopreventive and anti-carcinogenic effects of **silymarin** reported earlier are due to the main constituent **silibinin**.

75. J Immunol. 1999 Dec 15;163(12):6800-9.

Silymarin suppresses TNF-induced activation of NF-kappa B, c-Jun N-terminal kinase, and apoptosis.

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Silymarin is a polyphenolic flavonoid derived from **milk thistle** (*Silybum marianum*) that has anti-inflammatory, cytoprotective, and anticarcinogenic effects. How **silymarin** produces these effects is not understood, but it may involve suppression of NF-kappa B, a nuclear transcription factor, which regulates the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis. In this report, we investigated the effect of **silymarin** on NF-kappa B activation induced by various inflammatory agents. **Silymarin** blocked TNF-induced activation of NF-kappa B in a dose- and time-dependent manner. This effect was mediated through inhibition of phosphorylation and degradation of Iota kappa B alpha, an inhibitor of NF-kappa B. **Silymarin** blocked the translocation of p65 to the nucleus without affecting its ability to bind to the DNA. NF-kappa B-dependent reporter gene transcription was also suppressed by **silymarin**. **Silymarin** also blocked NF-kappa B activation induced by phorbol ester, LPS, okadaic acid, and ceramide, whereas H₂O₂-induced NF-kappa B activation was not significantly affected. The effects of **silymarin**

on NF-kappa B activation were specific, as AP-1 activation was unaffected. **Silymarin** also inhibited the TNF-induced activation of mitogen-activated protein kinase kinase and c-Jun N-terminal kinase and abrogated TNF-induced cytotoxicity and caspase activation. **Silymarin** suppressed the TNF-induced production of reactive oxygen intermediates and lipid peroxidation. Overall, the inhibition of activation of NF-kappa B and the kinases may provide in part the molecular basis for the anticarcinogenic and anti-inflammatory effects of **silymarin**, and its effects on caspases may explain its role in cytoprotection.

76. Carcinogenesis. 1999 Nov;20(11):2101-8. (**Animal Study**)

Tissue distribution of **silibinin**, the major active constituent of **silymarin**, in mice and its association with enhancement of phase II enzymes: implications in cancer chemoprevention.

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Polyphenolic antioxidants are being identified as cancer preventive agents. Recent studies in our laboratory have identified and defined the cancer preventive and anticarcinogenic potential of a polyphenolic flavonoid antioxidant, **silymarin** (isolated from **milk thistle**). More recent studies by us found that these effects of **silymarin** are due to the major active constituent, **silibinin**, present therein. Here, studies are done in mice to determine the distribution and conjugate formation of systemically administered **silibinin** in liver, lung, stomach, skin, prostate and pancreas. Additional studies were then performed to assess the effect of orally administered **silibinin** on phase II enzyme activity in liver, lung, stomach, skin and small bowel. For tissue distribution studies, SENCAR mice were starved for 24 h, orally fed with **silibinin** (50 mg/kg dose) and killed after 0.5, 1, 2, 3, 4 and 8 h. The desired tissues were collected, homogenized and parts of the homogenates were extracted with butanol:methanol followed by HPLC analysis. The column eluates were detected by UV followed by electrochemical detection. The remaining homogenates were digested with sulfatase and beta-glucuronidase followed by analysis and quantification. Peak levels of free **silibinin** were observed at 0.5 h after administration in liver, lung, stomach and pancreas, accounting for 8.8 +/- 1.6, 4.3 +/- 0.8, 123 +/- 21 and 5.8 +/- 1.1 (mean +/- SD) microg **silibinin**/g tissue, respectively. In the case of skin and prostate, the peak levels of **silibinin** were 1.4 +/- 0.5 and 2.5 +/- 0.4, respectively, and were achieved 1 h after administration. With regard to sulfate and beta-glucuronidate conjugates of **silibinin**, other than lung and stomach showing peak levels at 0.5 h, all other tissues showed peak levels at 1 h after **silibinin** administration. The

levels of both free and conjugated **silibinin** declined after 0.5 or 1 h in an exponential fashion with an elimination half-life ($t_{(1/2)}$) of 57-127 min for free and 45-94 min for conjugated **silibinin** in different tissues. In the studies examining the effect of **silibinin** on phase II enzymes, oral feeding of **silibinin** at doses of 100 and 200 mg/kg/day showed a moderate to highly significant ($P < 0.1-0.001$, Student's t-test) increase in both glutathione S-transferase and quinone reductase activities in liver, lung, stomach, skin and small bowel in a dose- and time-dependent manner. **Taken together, the results of the present study clearly demonstrate the bioavailability of and phase II enzyme induction by systemically administered silibinin in different tissues, including skin, where silymarin has been shown to be a strong cancer chemopreventive agent, and suggest further studies to assess the cancer preventive and anticarcinogenic effects of silibinin in different cancer models.**

Immune

77. *Planta Med.* 2003 Jan;69(1):44-9. **(Animal Study)**

Physiological responses of a natural antioxidant flavonoid mixture, **silymarin**, in BALB/c mice: III. **Silymarin** inhibits T-lymphocyte function at low doses but stimulates inflammatory processes at high doses.

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Silymarin is a mixture of bioactive flavonoids isolated from **Milk thistle** (*Silybum marianum*). Crude extracts from this plant have been used for centuries as a natural remedy and **silymarin** is now effectively used in the treatment of inflammatory liver toxicity and disease in humans. In vitro studies show that **silymarin** can inhibit the production and damage caused by tumor necrosis factor alpha (TNFalpha) and is a potent antioxidant both in vitro and in vivo. Such findings suggest **silymarin** may impact the immune system but little information exists following in vivo exposure. Therefore, we tested the hypothesis that exposure to **silymarin** will modulate the inflammatory immune response. Male BALB/c mice (6/group) were treated intraperitoneally once daily for five days with 0, 10, 50 or 250 mg/kg of **silymarin**. **Silymarin exposure did not produce any signs of overt toxicity or any changes in relative organ weights.** Flow cytometric examination of splenic lymphocyte populations showed that the absolute number of CD3+ T-lymphocytes was reduced in the 10 and 50 mg/kg groups although significance was evident only in the 10 mg/kg group. Concomitant decreases in CD4+ and CD8+ T-cell populations were observed but only the CD4+ population in mice treated with 10 mg/kg of **silymarin** was significantly different from control. Functional examination of secondary lymphoid cells

revealed that phytohemagglutinin-induced T-lymphocyte proliferation was increased in the lowest dose group only. B-lymphocyte blastogenesis induced by lipopolysaccharide was increased following exposure to 10 and 50 mg/kg of **silymarin**. Similarly, expression of TNFalpha, inducible nitric oxide synthase, IL-1beta and IL-6 mRNA were increased dose-dependently. The expression of IL-2 and IL-4 were reduced in mice treated with 10 and 50 mg/kg of **silymarin** although only the 10 mg/kg group was significantly different from control. **The results indicate that in vivo parenteral exposure to silymarin results in suppression of T-lymphocyte function at low doses and stimulation of inflammatory processes at higher doses. Further studies investigating the effects of silymarin on the immune system are warranted.**

78. *Planta Med.* 2002 Nov;68(11):961-5.

Physiological responses to a natural antioxidant flavonoid mixture, **silymarin**, in BALB/c mice: II. alterations in thymic differentiation correlate with changes in c-myc gene expression.

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Silymarin is a mixture of bioactive flavonoids isolated from the seeds and fruits of **Milk thistle** [*Silybum marianum* (L.) Gaertner]. We tested the hypothesis that exposure to **silymarin** will modulate differentiation and cell selection in the thymus via alterations in gene expression. Male BALB/c mice were treated intraperitoneally once daily for five days with 0, 10, 50 or 250 mg/kg of **silymarin**. Flow cytometric examination of thymic lymphocyte populations showed that the absolute numbers of CD4 + and CD8 + positive T-lymphocytes were increased by **silymarin**. The c- myc proto-oncogene is important in controlling differentiation and functions of thymocytes. Treatment with **silymarin** resulted in increased c- myc expression in the thymus. In contrast, the expressions of IL-2 and IL-4 were decreased by **silymarin**, while MHC II expression did not change. These results indicate that in vivo exposure to **silymarin** influences phenotypic selection processes in the thymus at doses that may be encountered in natural medicinal use. Further studies investigating the effects of **silymarin** on the immune system are warranted.

79. *Med Sci Monit.* 2002 Nov;8(11):BR439-43.

Immunostimulatory effect of *Silybum Marianum* (**milk thistle**) extract.

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BACKGROUND: Herbal products are increasingly used for their effects on the immune system. **Milk thistle**, a commonly used herbal product is known to inhibit growth of certain tumors, although the mechanism of this effect remains unknown. Previously we have shown that **Milk thistle** extracts stimulate neurons in culture. Since other drugs that affect the neuronal; system also affect the immune system, we investigated the effects of **Milk thistle** on the immune system. **MATERIAL/METHODS:** Standardized **Milk thistle** extract was studied in murine lymphocyte proliferation tests using Concanavalin A (ConA) as mitogen for non-specific stimulation and mixed lymphocyte culture (MLC) as allospecific stimulation. Th1 and Th2 cytokine levels in MLC were assayed by two antibody capture ELISA technique. All tests were performed in triplicate and repeated twice. **RESULTS:** We found that **Milk thistle** is immunostimulatory in vitro. It increased lymphocyte proliferation in both mitogen and MLC assays. These effects of **Milk thistle** were associated with an increase in interferon gamma, interleukin (IL)-4 and IL-10 cytokines in the MLC (table). This immunostimulatory effect increased in response to increasing doses of Milk Thistle. **CONCLUSIONS:** Our study has uncovered a novel effect of **milk thistle** on the immune system. This immunostimulatory effect may be of benefit in increasing the immunity to infectious diseases.

80. Am Surg. 2002 Oct;68(10):860-4.

In vitro immunomodulatory effects of herbal products.

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Immunosuppressive drugs have been developed from natural products such as soil and fungi, which are also the sources of some commonly used herbal products. However, the effect of herbal products on immune response has not been investigated. Because these products can affect the host immune system they can induce either rejection or tolerance after a transplant procedure. To investigate the effects of ten commonly used herbal products on transplant-related immune function we performed in vitro lymphocyte proliferation tests using phytohemagglutinin, mixed lymphocyte culture (MLC) assay, and interleukin (IL)-2 and IL-10 production from MLC. Dong quai, ginseng, and **milk thistle** had nonspecific immunostimulatory effects on lymphocyte proliferation, whereas ginger and green tea had immunosuppressive effects. Dong quai and **milk thistle** increased alloresponsiveness in MLC, whereas ginger and

tea decreased these responses. **The immunostimulatory effects of dong quai and milk thistle were consistently seen in both cell-mediated immune response and nonspecific lymphoproliferation**, whereas that of ginseng was not. The immunosuppressive effect of green tea and ginger were mediated through a **decrease in IL-2 production, but the immunostimulatory effects of dong quai and milk thistle were not**. We conclude that green tea, dong quai, ginseng, milk thistle, and ginger have effects on in vitro immune assays that may be relevant in transplantation in humans.

81. J Altern Complement Med. 2002 Aug;8(4):467-75.

In vitro immunomodulatory effects of ten commonly used herbs on murine lymphocytes.

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OBJECTIVES: Physicians are increasingly encountering patients who use herbal products. Some of these products are known to modulate the immune system but their scientific basis is not well established. Because these products can affect the host immune system, they could be beneficial in the treatment of immune-related diseases, or alternatively, they could cause inadvertent side-effects. The purpose of this study was to determine which of these common herbal products modulate lymphocyte proliferation in vitro. **METHODS:** Lymphocyte proliferation assays using concanavalin A (mitogen stimulation) and mixed lymphocyte culture (alloantigen stimulation) were used as in vitro tests to investigate the immunomodulatory effects of 10 commonly used herbal products. **RESULTS:** Ginger and tea were consistently immunosuppressive while dong quai, **milk thistle, and St. John's wort were consistently immunostimulatory in vitro**. Ginseng enhanced lymphocyte proliferation only in the mitogen-stimulation assay. The magnitude of the enhancement or suppression of the individual herbal products was different in the two assays. **CONCLUSION:** Our study provides a uniform survey of the immunomodulatory properties of 10 commonly used herbal products and paves the way for testing these effects in vivo and in clinical setting.

82. Free Radic Res. 2001 Feb;34(2):137-51.

Restoration of the cellular thiol status of peritoneal macrophages from CAPD patients by the flavonoids **silibinin** and **silymarin**.

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During continuous ambulatory peritoneal dialysis (CAPD) the peritoneal immune cells, mainly macrophages, are highly compromised by multiple factors including oxidative stress, resulting in a loss of functional activity. One reason for the increase of inflammatory reactions could be an imbalance in the thiol-disulfide status. Here, the possible protective effects of the antioxidant flavonoid complex **silymarin** and its major component **silibinin** on the cellular thiol status were investigated. Peritoneal macrophages from dialysis fluid of 30 CAPD patients were treated with **silymarin** or **silibinin** up to 35 days. A time-dependent increase of intracellular thiols was observed with a nearly linear increment up to 2.5-fold after 96 hours, reaching a maximum of 3.5-fold after 20 days of culture. Surface-located thiols were also elevated. The stabilization of the cellular thiol status was followed by an improvement of phagocytosis and the degree of maturation as well as significant changes in the synthesis of IL-6 and IL-1ra. Furthermore, the treatment of peritoneal macrophages with flavonoids in combination with cysteine donors resulted in a shortened and more efficient time course of thiol normalization as well as in a further increased phagocytosis. In addition, GSH-depletion in thiol-deficient media simulating CAPD procedures led to intracellular thiol deficiency similar to the *in vivo* situation. It is concluded that treatment with **milk thistle** extracts **silymarin** and **silibinin** alone or, more effectively in combination with cysteine donors, provide a benefit for peritoneal macrophages of CAPD-patients due to a normalization and activation of the cellular thiol status followed by a restoration of specific functional capabilities.

83. Schweiz Med Wochenschr. 1990 Mar 10;120(10):345-8.

Agents Actions. 1990 Mar;29(3-4):239-46.

Silibinin (Legalon-70) enhances the motility of human neutrophils immobilized by formyl-tripeptide, calcium ionophore, lymphokine and by normal human serum.

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Experiments reported here were designed to investigate the effect of **silibinin** (extracted from *Silybum marianum*) on human polymorphonuclear leukocyte (PMN) motility and on leukocyte immobilizing activity of lymphokine (leukocyte inhibitory factor, LIF), formyl-Met-Leu-Phe (fMLP), calcium ionophore A-23187 and human sera inactivated by heat (HI-S). **In the *in vitro* experiments, silibinin (1-10 micrograms/ml) failed to influence the random motility of unstimulated PMNS in agarose droplet assay, but enhanced the motility of the**

PMNs immobilized by fMLP, calcium ionophore, LIF or by autologous human sera. In the in vivo study, **silibinin** (Legalon-70) two hours after the administration was effective in enhancing spontaneous motility of leukocytes obtained from health volunteers which action could be regarded as a consequence of the decrease of leukocyte immobilizing activity being present in normal human plasma.

Neurodegenerative diseases

84. Eur J Neurosci. 2002 Dec;16(11):2103-12.

Silymarin protects dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation.

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An inflammatory response in the central nervous system mediated by activation of microglia is a key event in the early stages of the development of neurodegenerative diseases. **Silymarin** is a polyphenolic flavanoid derived from **milk thistle** that has anti-inflammatory, cytoprotective and anticarcinogenic effects. In this study, we first investigated the neuroprotective effect of **silymarin** against lipopolysaccharide (LPS)-induced neurotoxicity in mesencephalic mixed neuron-glia cultures. **The results showed that silymarin significantly inhibited the LPS-induced activation of microglia and the production of inflammatory mediators, such as tumour necrosis factor-alpha and nitric oxide (NO), and reduced the damage to dopaminergic neurons.** Therefore, the inhibitory mechanisms of **silymarin** on microglia activation were studied further. The production of inducible nitric oxide synthase (iNOS) was studied in LPS-stimulated BV-2 cells as a model of microglia activation. **Silymarin** significantly reduced the LPS-induced nitrite, iNOS mRNA and protein levels in a dose-dependent manner. Moreover, LPS could induce the activation of p38 mitogen-activated protein kinase (MAPK) and c-jun N-terminal kinase but not extracellular signal-regulated kinase. The LPS-induced production of NO was inhibited by the selective p38 MAPK inhibitor SB203580. These results indicated that the p38 MAPK signalling pathway was involved in the LPS-induced NO production. **However, the activation of p38 MAPK was not inhibited by silymarin.** Nevertheless, **silymarin** could effectively reduce LPS-induced superoxide generation and nuclear factor kappaB (NF-kappaB) activation. It suggests that the inhibitory effect of **silymarin** on microglia activation is mediated through the inhibition of NF-kappaB activation.

85. J Mol Neurosci. 2002 Jun;18(3):265-9. **(Animal Study)**

Neurotrophic and neuroprotective effects of **milk thistle** (*Silybum marianum*) on neurons in culture.

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Herbal products are being increasingly used as dietary supplements and therapeutic agents. However, much more research must be performed in order to determine the biological basis for their putative clinical effects. We tested the effects of **milk thistle** (*Silybum marianum*) extract on the differentiation and survival of cultured neural cells. **Milk thistle** enhanced nerve growth factor (NGF)-induced neurite outgrowth in PC-12 neural cells and prolonged their survival in culture. **Milk thistle** extract also protected cultured rat hippocampal neurons against oxidative stress-induced cell death. [Our data demonstrate that **milk thistle** extract can promote neuronal differentiation and survival, suggesting potential benefits of chemicals in this plant on the nervous system.](#)

Skin Cancer

86. Int J Oncol. 2002 Dec;21(6):1213-22. (**Animal Study**)

Treatment of **silymarin**, a plant flavonoid, prevents ultraviolet light-induced immune suppression and oxidative stress in mouse skin.

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It is well documented that ultraviolet (UV) light-induced immune suppression and oxidative stress play an important role in the induction of skin cancers. Earlier, we have shown that topical treatment of **silymarin**, a plant flavonoid from **milk thistle** (*Silybum marianum* L. Gaertn.), to mouse skin prevents photocarcinogenesis, but the preventive mechanism of photocarcinogenesis in vivo animal system by **silymarin** is not well defined and understood. To define the mechanism of prevention, we employed immunostaining, analytical assays and ELISA which revealed that topical treatment of **silymarin** (1 mg/cm² skin area) to C3H/HeN mice inhibits UVB (90 mJ/cm²)-induced suppression of contact hypersensitivity (CHS) response to contact sensitizer dinitrofluorobenzene. Prevention of UVB-induced suppression of CHS by **silymarin** was found to be

associated with the inhibition of infiltrating leukocytes, particularly CD11b+ cell type, and myeloperoxidase activity (50-71%). **Silymarin** treatment also resulted in significant reduction of UVB-induced immunosuppressive cytokine interleukin-10 producing cells and its production (58-72%, $p < 0.001$). Topical treatment of **silymarin** also resulted in significant reduction of the number of UVB-induced H₂O₂ producing cells and inducible nitric oxide synthase expressing cells concomitant with decrease in H₂O₂ (58-65%, $p < 0.001$) and nitric oxide (65-68%, $p < 0.001$) production. Together, these data suggest that prevention of UVB-induced immuno-suppression and oxidative stress by **silymarin** may be associated with the prevention of photocarcinogenesis in mice. The data obtained from this study also suggest: i) phase-I clinical trial of **silymarin** in high skin cancer risk human population and ii) development of sunscreen containing **silymarin** as an antioxidant (chemopreventive agent) or **silymarin** can be supplemented in skin care products.

87. Carcinogenesis. 2002 Mar;23(3):499-510. **(Animal Study)**

Silymarin inhibits growth and causes regression of established skin tumors in SENCAR mice via modulation of mitogen-activated protein kinases and induction of apoptosis.

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This study reports in vivo therapeutic efficacy of **silymarin** against skin tumors with mechanistic rationale.

7,12-Dimethylbenz[a]anthracene-12-O-tetradecanoyl-phorbol-13-acetate (DMBA-TPA)-induced established skin papilloma (tumor)-bearing SENCAR mice were fed with 0.5% **silymarin** in AIN-93M-purified diet (w/w), and both tumor growth and regression were monitored during 5 weeks of feeding regimen. **Silymarin** feeding significantly inhibited (74%, $P < 0.01$) tumor growth and also caused regression (43%, $P < 0.01$) of established tumors. Proliferating cell nuclear antigen and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling immunohistochemical staining of tumors showed that **silymarin** decreases proliferation index by 48% ($P < 0.001$) and increases apoptotic index by 2.5-fold ($P < 0.001$), respectively. Skin tumor growth inhibition and regression by **silymarin** were also accompanied by a strong decrease ($P < 0.001$) in phospho-ERK1/2 levels in tumors from **silymarin**-fed mice compared with controls. In the studies evaluating bioavailability and physiologically achievable level of **silymarin** (as **silibinin**) in plasma, skin tumor, skin, liver, lung, mammary gland and spleen, we found 10, 6.5, 3.1, 13.7, 7.7, 5.9 and 4.4 microg **silibinin**/ml plasma or per gram tissue, respectively. In an attempt to translate these findings to human skin cancer and to establish biological significance of

physiologically achievable level, effect of plasma concentration of **silibinin** was next examined in human epidermoid carcinoma A431 cells. **Silibinin** treatment of cells in culture at 12.5, 25 (plasma level) and 50 microM doses resulted in 30-74% ($P < 0.01-0.001$) growth inhibition and 7-42% death of A431 cells in a dose- and time-dependent manner; apoptosis was identified as a cell death response by **silibinin**. Similar **silibinin** treatments also resulted in a significant decrease in phospho-mitogen-activated protein kinase/extracellular signal-regulated protein kinase 1/2 (MAPK/ERK1/2) levels, but an up-regulation of stress-activated protein kinase/jun NH(2)-terminal kinase (SAPK/JNK1/2) and p38 mitogen-activated protein kinase (p38 MAPK) activation in A431 cells. The use of MEK1 inhibitor, PD98059, showed that inhibition of ERK1/2 signaling, in part, contributes to **silibinin**-caused cell growth inhibition. Together, the data suggest that an inhibition of ERK1/2 activation and an increased activation of JNK1/2 and p38 by **silibinin** could be possible underlying molecular events involved in inhibition of proliferation and induction of apoptosis in A431 cells. These data suggest that **silymarin** and/or its major active constituent **silibinin** could be an effective agent for both prevention and intervention of human skin cancer.

88. Nutr Cancer. 2001;39(2):292-9.

Differential responses of skin cancer-chemopreventive agents **silibinin**, quercetin, and epigallocatechin 3-gallate on mitogenic signaling and cell cycle regulators in human epidermoid carcinoma A431 cells.

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Silibinin, quercetin, and epigallocatechin 3-gallate (EGCG) have been shown to be skin cancer-preventive agents, albeit by several different mechanisms. Here, we assessed whether these agents show their cancer-preventive potential by a differential effect on mitogenic signaling molecules and cell cycle regulators. Treatment of human epidermoid carcinoma A431 cells with these agents inhibited the activation of the epidermal growth factor receptor and the downstream adapter protein Shc, but only **silibinin** showed a marked inhibition of mitogen-activated protein kinase-extracellular signal-regulated kinase-1 and -2 activation. In terms of cell cycle regulators, **silibinin** treatment showed an induction of Cip1/p21 and Kip1/p27 together with a significant decrease in cyclin-dependent kinase (CDK)-4, CDK2, and cyclin D1. Quercetin treatment, however, resulted in a moderate increase in Cip1/p21 with no change in Kip1/p27 and a decrease in CDK4 and cyclin D1. EGCG treatment also led to an induction of Cip1/p21 but no change in Kip1/27, CDK2, and cyclin D1 and a decrease in CDK4 only at low doses. Treatment of cells with these agents resulted in a strong

dose- and time-dependent cell growth inhibition. A high dose of **silibinin** and low and high doses of quercetin and EGCG also led to cell death by apoptosis, suggesting that a lack of their inhibitory effect on mitogen-activated protein kinase-extracellular signal-regulated kinase-1 and -2 activation possibly "turns on" an apoptotic cell death response associated with their cancer-preventive and anticarcinogenic effects. Together, these results suggest that **silibinin**, quercetin, and EGCG exert their cancer-preventive effects by differential responses on mitogenic signaling and cell cycle regulators.

89. Cancer Res. 1999 Feb 1;59(3):622-32. **(Animal Study)**

A flavonoid antioxidant, **silymarin**, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model.

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In cancer chemoprevention studies, the identification of better antitumor-promoting agents is highly desired because they may have a wider applicability against the development of clinical cancers. Both epidemiological and animal studies have suggested that microchemicals present in the diet and several herbs and plants with diversified pharmacological properties are useful agents for the prevention of a wide variety of human cancers. **Silymarin**, a flavonoid isolated from **milk thistle**, is used clinically in Europe and Asia as an antihepatotoxic agent, largely due to its strong antioxidant activity. Because most antioxidants afford protection against tumor promotion, in this study, we assessed the protective effect of **silymarin** on tumor promotion in the SENCAR mouse skin tumorigenesis model. [Application of **silymarin** prior to each 12-O-tetradecanoylphorbol 13-acetate \(TPA\) application resulted in a highly significant protection against tumor promotion in 7,12-dimethylbenz\(a\)anthracene-initiated mouse skin.](#) The protective effect of **silymarin** was evident in terms of reduction in tumor incidence (25, 40, and 75% protection, $P < 0.001$, X2 test), tumor multiplicity (76, 84, and 97% protection, $P < 0.001$, Wilcoxon rank sum test), and tumor volume (76, 94, and 96% protection, $P < 0.001$, Student's t test) at the doses of 3, 6, and 12 mg per application, respectively. To dissect out the stage specificity of **silymarin** against tumor promotion, we next assessed its effect against both stage I and stage II of tumor promotion. [Application of **silymarin** prior to that of TPA in stage I or mezerein in stage II tumor promotion in dimethylbenz\(a\)anthracene-initiated SENCAR mouse skin resulted in an exceptionally high protective effect during stage I tumor promotion, showing 74% protection against tumor incidence \(\$P < 0.001\$, X2 test\), 92% protection against tumor multiplicity \(\$P < 0.001\$, Wilcoxon rank sum test\), and 96% protection](#)

against tumor volume ($P < 0.001$, Student's t test). With regard to stage II tumor promotion, **silymarin** showed 26, 63, and 54% protection in tumor incidence, multiplicity, and volume, respectively. Similar effect of **silymarin** to that in anti-stage I studies, were also observed when applied during both stage I and stage II protocols. In other studies, **silymarin** significantly inhibited: (a) TPA-induced skin edema, epidermal hyperplasia, and proliferating cell nuclear antigen-positive cells; (b) DNA synthesis; and (c) epidermal lipid peroxidation, the early markers of TPA-caused changes that are associated with tumor promotion. Taken together, these results suggest that **silymarin** possesses exceptionally high protective effects against tumor promotion, primarily targeted against stage I tumors, and that the mechanism of such effects may involve inhibition of promoter-induced edema, hyperplasia, proliferation index, and oxidant state.

90. Biochem Biophys Res Commun. 1997 Oct 9;239(1):334-9. **(Animal Study)**

Novel cancer chemopreventive effects of a flavonoid antioxidant **silymarin**: inhibition of mRNA expression of an endogenous tumor promoter TNF alpha.

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In this study we describe exceptionally high protective effects of **silymarin**, a flavonoid antioxidant isolated from **milk thistle**, against 12-O-tetradecanoylphorbol 13-acetate (TPA)- and okadaic acid (OA)-caused tumor promotion in SENCAR mouse skin. Pre-application of **silymarin** to that of TPA in 7, 12-dimethylbenz(a)anthracene (DMBA)-initiated mouse skin resulted in almost complete protection in terms of tumor incidence (85%) as well as multiplicity (94%). In OA-caused tumor promotion studies, [application of silymarin prior to that of OA in DMBA-initiated mouse skin resulted in a complete protection against tumorigenicity](#). We next assessed the effect of **silymarin** on TPA- and OA-caused induction of mRNA expression of tumor necrosis factor alpha (TNF alpha) which is an endogenous tumor promoter and a central mediator of tumor promotion in vivo in the case of both TPA and OA tumor promotion. [Topical application of silymarin on mouse skin prior to that of TPA or OA resulted in a highly significant to complete inhibition in a dose-dependent manner against both TPA- and OA-caused induction of TNF alpha mRNA expression in mouse epidermis](#). These results indicate that **silymarin** exerts novel chemopreventive effects against tumorigenicity by inhibiting endogenous tumor promoter TNF alpha. Additional studies are warranted in other tumor models to further evaluate the cancer chemopreventive effect of **silymarin** and to define the involvement of TNF alpha as a molecular target for such an effect.

91. J Natl Cancer Inst. 1997 Apr 16;89(8):556-66. **(Animal Study)**

Protective effects of **silymarin** against photocarcinogenesis in a mouse skin model.

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BACKGROUND: Nonmelanoma skin cancer is the most common cancer among humans; solar UV is its major cause. Therefore, it is important to identify agents that can offer protection against this cancer. **PURPOSE:** We evaluated the protective effects of **silymarin**, a flavonoid compound isolated from the **milk thistle** plant, against UVB radiation-induced nonmelanoma skin cancer in mice and delineated the mechanism(s) of its action. **METHODS:** For long-term studies, three different protocols of treatment were employed, each evaluating protection by **silymarin** at a different stage of carcinogenesis. Female SKH-1 hairless mice were subjected to 1) UVB-induced tumor initiation followed by phorbol ester-mediated tumor promotion, 2) 7,12-dimethylbenz[a]anthracene-induced tumor initiation followed by UVB-mediated tumor promotion, and 3) UVB-induced complete carcinogenesis. Forty mice were used in each protocol and were divided into control and treatment groups. **Silymarin** was applied topically at a dose of 9 mg per application before UVB exposure, and its effects on tumor incidence (% of mice with tumors), tumor multiplicity (number of tumors per mouse), and average tumor volume per mouse were evaluated. In short-term studies, the following parameters were measured: formation of sunburn and apoptotic cells, skin edema, epidermal catalase and cyclooxygenase (COX) activities, and enzymatic activity and messenger RNA (mRNA) expression for ornithine decarboxylase (ODC), a frequently observed marker at tumor promotion stage. Fisher's exact test was used to evaluate differences in tumor incidence, two-sample Wilcoxon rank sum test was used for tumor multiplicity and tumor volume, and Student's t test was used for all other measurements. All statistical tests were two-sided. **RESULTS:** In the protocol with UVB-induced tumor initiation, **silymarin** treatment reduced tumor incidence from 40% to 20% ($P = .30$), tumor multiplicity by 67% ($P = .10$), and tumor volume per mouse by 66% ($P = .14$). In the protocol with UVB-induced tumor promotion, **silymarin** treatment reduced tumor incidence from 100% to 60% ($P < .003$), tumor multiplicity by 78% ($P < .0001$), and tumor volume per mouse by 90% ($P < .003$). The effect of **silymarin** was much more profound in the protocol with UVB-induced complete carcinogenesis, where tumor incidence was reduced from 100% to 25% ($P < .0001$), tumor multiplicity by 92% ($P < .0001$), and tumor volume per mouse by 97% ($P < .0001$). **In short-term experiments, silymarin application resulted in statistically significant inhibition in UVB-caused sunburn and apoptotic cell formation, skin edema, depletion of catalase activity, and**

induction of COX and ODC activities and ODC mRNA expression. CONCLUSIONS AND

IMPLICATION: **Silymarin** can provide substantial protection against different stages of UVB-induced carcinogenesis, possibly via its strong antioxidant properties. Clinical testing of its usefulness is warranted.

Diabetes

92. Diabetes Nutr Metab. 2002 Aug;15(4):222-31.

Silybin-beta-cyclodextrin in the treatment of patients with diabetes mellitus and alcoholic liver disease. Efficacy study of a new preparation of an anti-oxidant agent.

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BACKGROUND AND AIMS: In patients with non-insulin dependent diabetes mellitus (T2DM) and associated chronic liver disease, plasma levels of glucose, insulin and triglycerides are high, lipid peroxidation is increased and natural antioxidant reserves are reduced. Thus, we hypothesised that the re-balancing of cell redox levels and amelioration of liver function could result in a better glucose and lipid metabolism. To study this, we assessed the effect of a new oral formulation of an antioxidant agent - silybin-beta-cyclodextrin (named IBI/S) - in patients with chronic alcoholic liver disease and concomitant T2DM. **METHODS:** Sixty outpatients were enrolled in a three-centre, double blind, randomised, IBI/S vs placebo study. Forty-two (21 in the group IBI/S - 135 mg/d silybin per os - and 21 in the placebo group) concluded the 6-month treatment period. The efficacy parameters included fasting and mean daily plasma glucose levels, glycosylated hemoglobin (HbA1c), basal, stimulated C-peptide and insulin levels, total-, HDL-cholesterol and triglycerides levels in addition to conventional liver function tests. Insulin sensitivity was estimated by HOMA-IR. Malondialdehyde (MDA) was also measured before and after treatment as an index of oxidative stress. **RESULTS:** Fasting blood glucose levels, which were similar at baseline in IBI/S group and in the placebo group (173.9 mg/dl and 177.1 mg/dl, respectively), decreased to 148.4 mg/dl (-14.7% vs baseline; $p = 0.03$) in the IBI/S group while they were virtually unchanged in the placebo group. The comparison between the groups at mo 6 (T6) also showed a significant reduction of glucose levels in the IBI/S group ($p = 0.03$). The same trend was observed in mean daily blood glucose levels, HbA1c and HOMA-IR, although differences were not significant. Basal and stimulated C-peptide values showed that only a few changes had occurred in both groups. Such results indicate that insulin secretion

was virtually unaffected, as confirmed also by the insulinemia data. Plasma triglycerides concentrations dropped from a baseline value of 186 mg/dl to 111 mg/dl (T6) in the IBI/S group, with significant differences at all instances with respect to baseline values. By contrast, triglycerides increased from 159 mg/dl at entry to 185 mg/dl (T6) in the placebo group. The difference between the groups at T6 was highly significant ($p < 0.01$). Total and HDL cholesterol as well as liver function tests did not change significantly during the study in both groups. MDA decreased significantly only in the group receiving IBI/S. No clinically relevant side effects were observed in either group. CONCLUSIONS: Oral administration silybin-beta-cyclodextrin in patients with T2DM and compensated chronic alcoholic liver disease causes a significant decrease in both glucose and triglyceride plasma levels. These effects may be due to the recovery of energy substrates, consistent with a reduced lipid peroxidation and an improved insulin activity.

Hepatitis

93. Gastroenterol Nurs. 2001 Mar-Apr;24(2):95-7.

Milk thistle and the treatment of hepatitis.

Giese LA.

Gastroenterology nurses and associates will find it helpful to be informed about **milk thistle** (*silybum marianum*), a popular, safe and promising herb used by patients with liver disease. **Silymarin** is a derivative from the **milk thistle** plant with few side effects that has been safely used for centuries to treat liver ailments. Since the 1970s, there has been a reemergence of the marketing and use of **silymarin**. Research results of some small studies suggest **silymarin** has hepatoprotective, antiinflammatory, and regenerative properties producing a beneficial effect for some types of hepatitis. It is unclear, however, whether **silymarin** might interfere with the effect of interferon or ribavirin. A well-designed, placebo-controlled study of a larger population is needed. It is certainly encouraging that a large collaborative study is currently underway for **milk thistle** therapy in hepatitis C. This study is funded by NCCAM, the National Institute of Allergy and Infectious Diseases (NIAID), and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Research updates are available online at www.nccam.nih.gov and through the NCCAM Clearinghouse at 1-888-644-6226.

Bladder Cancer

94. Jpn J Cancer Res. 2002 Jan;93(1):42-9. **(Animal Study)**

Chemopreventive effects of a flavonoid antioxidant **silymarin** on N-butyl-N-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in male ICR mice.

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The modifying effects of dietary administration of a flavonoid antioxidant, **silymarin**, a mixture of three flavonoids isolated from **milk thistle** seeds, on N-butyl-N-(4-hydroxybutyl)nitrosamine (OH-BBN)-induced urinary bladder carcinogenesis were examined in male ICR mice. Animals were divided into 5 groups, and groups 1 to 3 were given OH-BBN (500 ppm) in drinking water for 6 weeks. Mice in group 2 were fed a diet containing 1000 ppm **silymarin** for 8 weeks during the initiation phase starting 1 week before OH-BBN exposure, and mice in group 3 were fed the diet for 24 weeks during the postinitiation phase. Animals in group 4 were given only the test compound, and those in group 5 were given the basal diet alone throughout the experiment. Animals were sacrificed at the end of week 32. The frequency of bladder lesions, cell proliferation and cell cycle progression activity estimated in terms of the 5-bromodeoxyuridine (BrdU) labeling index or cyclin D1-positive cell ratio were compared among the groups. [Administration of **silymarin** in the initiation or postinitiation phase significantly decreased the incidences of bladder neoplasms and preneoplastic lesions. Dietary exposure to this agent significantly reduced the labeling index for BrdU and the cyclin D1-positive cell ratio in various bladder lesions.](#) These findings suggest that **silymarin** is effective in preventing OH-BBN-induced bladder carcinogenesis in mice.

Hydrogen Peroxide

95. *Phytother Res.* 2001 Nov;15(7):608-12.

Inhibition of the superoxide anion release and hydrogen peroxide formation in PMNLs by flavonolignans.

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The mixture of flavonolignans [Legalon: silybin (2a), isosilybin (3), silydianin (4) and silychristin (5)] and derivatives of silybin (2b-d) were assessed for their inhibitory activity on the oxidative burst of PMA-stimulated human PMNLs. The inhibitory effect of flavonolignans on O₂(⁻) release were compared with

that of vitamin E (1). The flavonolignans tested exhibited the following order in inhibition of O_2^- release by PMA-stimulated PMNLs: 5,7,4"-trimethylsilybin (2c) approximately vitamin E (1) > Legalon >or= peracetylsilybin (2b) > silybin (2a) > peracetyl-5,7,4"-trimethylsilybin (2d). The flavonolignans inhibited not only the O_2^- release, but also the H_2O_2 formation in PMA-stimulated PMNLs. [The inhibitory capacity of flavonolignans on \$H_2O_2\$ formation was similar to their inhibitory capacity on \$O_2^-\$ release.](#) These data suggest that the flavonolignans have antioxidant properties on the PMNL oxidative burst. The fact that the trimethyl derivative of silybin (2c) has a greater inhibitory effect than silybin itself suggests that the efficacy of the antioxidant properties is dependent on the lipophilicity of the molecules. This is underlined by the fact that peracetylation of all of the hydroxyl groups in silybin resulted in a total loss of the antioxidant activity of the molecule. In summary, flavonolignans inhibit the oxidative burst of PMNLs, and this inhibitory effect depends on the chemical structure of the flavonolignans. Copyright 2001 John Wiley & Sons, Ltd.

96. *Biochem Pharmacol.* 1994 Aug 17;48(4):753-9. **(Animal Study)**

Scavenging of reactive oxygen species by **silibinin** dihemisuccinate.

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Silibinin dihemisuccinate (SDH) is a flavonoid of plant origin with hepatoprotective effects which have been partially attributed to its ability to scavenge oxygen free radicals. In the present paper the antioxidant properties of SDH were evaluated by studying the ability of this drug to react with relevant biological oxidants such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO\cdot$) and hypochlorous acid ($HOCl$). In addition, its effect on lipid peroxidation was investigated. SDH is not a good scavenger of O_2^- and no reaction with H_2O_2 was detected within the sensitivity limit of our assay. However, it reacts rapidly with $HO\cdot$ radicals in free solution at approximately diffusion-controlled rate ($K = (1.0-1.2) \times 10^{10} M^{-1} sec^{-1}$) and appears to be a weak iron ion chelator. SDH at concentrations in the micromolar range protected alpha 1-antiproteinase against inactivation by $HOCl$, showing that it is a potent scavenger of this oxidizing species. Luminol-dependent chemiluminescence induced by $HOCl$ was also inhibited by SDH. The reaction of SDH with $HOCl$ was monitored by the modification of the UV-visible spectrum of SDH. The studies on rat liver microsome lipid peroxidation induced by $Fe(III)$ /ascorbate showed that [SDH has an inhibitory effect, which is dependent on its concentration and the magnitude of lipid peroxidation.](#) This work supports the reactive oxygen species scavenger action ascribed to SDH.

Leukemia

97. *Biochem Pharmacol.* 2001 Jun 15;61(12):1487-95.

Induction of human promyelocytic leukemia HL-60 cell differentiation into monocytes by **silibinin**: involvement of protein kinase C.

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The effect of **silibinin**, an active component of *Silybum marianum*, on cellular differentiation was investigated in the human promyelocytic leukemia HL-60 cell culture system. **Treatment of HL-60 cells with silibinin inhibited cellular proliferation and induced cellular differentiation in a dose-dependent manner.** Cytofluorometric analysis and morphologic studies indicated that **silibinin** induced differentiation of HL-60 cells predominantly into monocytes. **Importantly, strongly synergistic induction of differentiation into monocytes was observed when silibinin was combined with 5 nM 1alpha,25-dihydroxyvitamin D(3) [1,25-(OH)(2)D(3)], a well-known differentiation inducer of HL-60 cells into the monocytic lineage. Silibinin enhanced protein kinase C (PKC) activity and increased protein levels of both PKCalpha and PKCbeta in 1,25-(OH)(2)D(3)-treated HL-60 cells. PKC and extracellular signal-regulated kinase (ERK) inhibitors significantly inhibited HL-60 cell differentiation induced by silibinin alone or in combination with 1,25-(OH)(2)D(3), indicating that PKC and ERK may be involved in silibinin-induced HL-60 cell differentiation.**

Sleeping sickness

98. *J Mol Med.* 2001 Apr;79(2-3):121-7.

Identification and characterization of trypanocides by functional expression of an adenosine transporter from *Trypanosoma brucei* in yeast.

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The causative agents of sleeping sickness, *Trypanosoma brucei rhodesiense* and *T. brucei gambiense*, do not synthesize purines de novo but salvage purine bases and nucleosides from their hosts. We used yeast as an expression system for

functional characterization of the trypanosomal adenosine transporter TbAT1. A selection of purine analogs and flavonoids were tested for their ability to interfere with adenosine transport, with the aims of identifying (a) trypanocidal TbAT1 substrates, and (b) inhibitors of trypanosomal purine transport. Cordycepin (3'-deoxyadenosine) was a TbAT1 substrate of high activity against *T. brucei rhodesiense* (IC₅₀ 0.2 nM). Inhibitors of mammalian nucleoside transport were not active, while the flavonol **silibinin** was a potent, noncompetitive inhibitor of TbAT1-mediated adenosine transport in yeast. **Silibinin** also inhibited melarsen-induced lysis of bloodstream form trypanosomes. IC₅₀ values to *T. brucei rhodesiense* and to human carcinoma cells were 0.6 and 140 µM, respectively, indicating a good selectivity towards the parasites. Further studies are necessary to elucidate the effects of flavonoids on trypanosomal purine transport and their potential as trypanocides.

Kidney

99. *J Pharmacol Exp Ther.* 1999 Sep;290(3):1375-83.

Stimulatory effects of **silibinin** and silicristin from the **milk thistle** *Silybum marianum* on kidney cells.

Sonnenbichler J, Scalera F, Sonnenbichler I, Weyhenmeyer R.

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The biochemical influence of flavonolignans from the **milk thistle** *Silybum marianum* has been tested on kidney cells of African green monkeys. Two nonmalignant cell lines were selected, with the focus of the work on the fibroblast-like Vero line. Proliferation rate, biosynthesis of protein and DNA, and the activity of the enzyme lactate dehydrogenase (as a measure of the cellular metabolic activity) were chosen as parameters for the effect of the flavonolignans. **Silibinin** and silicristin show remarkable stimulatory effects on these parameters, mainly in Vero cells; however, **isosilibinin** and silidianin proved to be inactive. [In vitro experiments with kidney cells damaged by paracetamol, cisplatin, and vincristin demonstrated that administration of silibinin before or after the chemical-induced injury can lessen or avoid the nephrotoxic effects.](#) The results warrant in vivo evaluations of the flavonolignan derivatives.

100. *Br J Cancer.* 1996 Dec;74(12):2036-41. **(Animal Study)**

Silibinin protects against cisplatin-induced nephrotoxicity without compromising cisplatin or ifosfamide anti-tumour activity.

Bokemeyer C, Fels LM, Dunn T, Voigt W, Gaedeke J, Schmoll HJ, Stolte H, Lentzen H.

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Cisplatin is one of the most active cytotoxic agents in the treatment of testicular cancer, but its clinical use is associated with side-effects such as ototoxicity, neurotoxicity and nephrotoxicity. Long-term kidney damage from cisplatin particularly affects the proximal tubular apparatus and can be detected by increased urinary excretion of brush-border enzymes, such as L-alanine-aminopeptidase (AAP), and magnesium. In the current study, the flavonoid **silibinin** was used as a nephroprotectant for cisplatin-induced nephropathy in a rat animal model. **Infusion of silibinin before cisplatin results in a significant decrease in glomerular (indicated by creatinine clearance and serum urea level) and tubular kidney toxicity (excretion of brush-border enzymes and magnesium). Silibinin given alone had no effect on renal function.** In order to exclude an inhibition of the anti-tumour activity of cisplatin and 4-hydroperoxy-ifosfamide by co-administration of **silibinin**, in vitro studies were performed in three established human testicular cancer cell lines. Dose-response curves for cisplatin (3-30 000 nmol) combined with non-toxic **silibinin** doses (7.25×10^{-6} or 7.25×10^{-5} mol l⁻¹) did not deviate significantly from those of cisplatin alone as measured by relative cell survival during a 5 day assay using the sulphorhodamine-B staining technique. **Also silibinin did not influence the cytotoxic activity of 4-hydroperoxy-ifosfamide (30-10 000 nmol) in vitro.** In summary, these in vitro data rule out a significant inhibition of the anti-tumour activity of the major nephrotoxic components, cisplatin and 4-hydroperoxy-ifosfamide, by co-administration of **silibinin** in a human germ cell tumour cell line model. Together with these demonstrated cytoprotection effects in the rat animal model, these data form the basis for a randomised clinical trial of **silibinin** for the protection of cisplatin-associated nephrotoxicity in patients with testicular cancer.

101. J Pharmacol Exp Ther. 1996 Dec;279(3):1520-6.

Effects of **silibinin** and antioxidants on high glucose-induced alterations of fibronectin turnover in human mesangial cell cultures.

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To elucidate the primary mechanism of high glucose cytotoxicity, the cytoprotective properties of antioxidants against metabolic disorders were assessed in human mesangial cell (HMC) cultures. An 8-day incubation of HMC with

high glucose concentration (30 mM) resulted in an extracellular accumulation of the matrixprotein fibronectin (FN), owing to both an expansion of the matrix-associated pericellular FN and a 60% increase of the soluble molecule in the culture medium. The high glucose-induced FN alterations were not due to osmotical effects, as assessed by an iso-osmotic mannitol control. Rather, they are mediated by oxygen-free radicals because the combined treatment of HMC with high glucose and either the antioxidative flavonoid **silibinin** (given as the water soluble derivative **silibinin-C-2,3-dihydrogensuccinate disodium salt**) or a radical scavenger cocktail totally prevented the extracellular FN accumulation. This is corroborated further by the determination of malondialdehyde, a product of lipid peroxidation. **Incubation of HMC with high glucose resulted in an increase of malondialdehyde in cell homogenates which was completely counteracted by either silibinin or a radical scavenger cocktail. Silibinin alone had no effects on protein synthesis and culture growth.** The data presented are compatible with oxidative stress induced by high glucose concentration in HMC cultures. The study further substantiates the proposed role of **silibinin** in the amelioration of glucose cytotoxicity in renal cells.

Nitric Oxide

102. Proc Soc Exp Biol Med. 1998 Sep;218(4):390-7.

In vitro attenuation of nitric oxide production in C6 astrocyte cell culture by various dietary compounds.

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Excessive nitric oxide (NO) production in the brain has been correlated with neurotoxicity and the pathogenesis of several neurodegenerative diseases. NO production from neuroglial cells surrounding neurons contributes significantly to the pathogenesis of these diseases. The suppression of NO production in these cells may be beneficial in retarding many of these disorders. The present study was designed to evaluate the capacity of dietary-derived polyphenolic compounds, flavonoids, crude extracts, oils, and other food constituents in suppressing the release of NO from lipopolysaccharide (LPS)/gamma-interferon (IFN-gamma) stimulated C6 astrocyte cells. In this experiment, 61 compounds were tested, and 36 showed significant suppressive effects of NO production. The results indicate that the following compounds exhibited a dose-dependent suppressive effect of NO production with an IC₅₀ less than 10(-3) M: quercetin, (-)-epigallocatechin gallate, morin, curcumin, apigenin, sesamol, chlorogenic acid, fisetin, (+)-taxifolin, (+)-catechin, ellagic acid, and caffeic acid. **Compounds, which reduce NO production at less than 300 ppm, include milk thistle, silymarin,**

grapenol, and green tea. These results demonstrate a possible value for dietary compounds to inhibit the excessive production of NO.

Breast Cancer

103. Clin Cancer Res. 1998 Apr;4(4):1055-64.

Anticarcinogenic effect of a flavonoid antioxidant, **silymarin**, in human breast cancer cells MDA-MB 468: induction of G1 arrest through an increase in Cip1/p21 concomitant with a decrease in kinase activity of cyclin-dependent kinases and associated cyclins.

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There is an increasing interest in identifying potent cancer preventive and therapeutic agents against breast cancer. **Silymarin**, a flavonoid antioxidant isolated from **milk thistle**, exerts exceptionally high to complete anticarcinogenic effects in tumorigenesis models of epithelial origin. In this study, we investigated the anticarcinogenic effect of **silymarin** and associated molecular mechanisms, using human breast carcinoma cells MDA-MB 468. **Silymarin** treatment resulted in a significantly high to complete inhibition of both anchorage-dependent and anchorage-independent cell growth in a dose- and time-dependent manner. The inhibitory effects of **silymarin** on cell growth and proliferation were associated with a G1 arrest in cell cycle progression concomitant with an induction of up to 19-fold in the protein expression of cyclin-dependent kinase (CDK) inhibitor Cip1/p21. Following **silymarin** treatment of cells, an incremental binding of Cip1/p21 with CDK2 and CDK6 paralleled a significant decrease in CDK2-, CDK6-, cyclin D1-, and cyclin E-associated kinase activity with no change in CDK2 and CDK6 expression but a decrease in G1 cyclins D1 and E. Taken together, these results suggest that **silymarin** may exert a strong anticarcinogenic effect against breast cancer and that this effect possibly involves an induction of Cip1/p21 by **silymarin**, which inhibits the threshold kinase activities of CDKs and associated cyclins, leading to a G1 arrest in cell cycle progression.

Atherosclerosis

104. Arzneimittelforschung. 1998 Mar;48(3):236-9.

Inhibitory action of **silibinin** on low density lipoprotein oxidation.

Locher R, Suter PM, Weyhenmeyer R, Vetter W.

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Low density lipoprotein (LDL) oxidation and smooth muscle cell growth represent key events in atherogenesis. Any mean to reduce these two phenomena may decrease the risk of coronary artery disease and atherosclerosis in general. The effects of **silibinin** (CAS 22888-70-6) on LDL oxidation and proliferation of vascular smooth muscle cells were evaluated in vitro. **Silibinin (50-200 $\mu\text{mol/l}$) prolonged the lag times of both LDL autooxidation and oxidation by copper by > 50%, as assessed by recordings of diene formation. However, **silibinin** (up to 500 $\mu\text{mol/l}$) did not interfere with LDL-stimulated radiolabeled thymidine incorporation. These findings indicate that **silibinin**, apart from its hepatoprotective effects, has inhibitory properties on LDL oxidation in vitro. Therefore **silibinin** might represent a novel tool in the prevention and therapy of atherosclerosis.**

105. Ann Acad Med Stetin. 1997;43:41-56. **(Animal Study)**

[The effect of bioflavonoids and lecithin on the course of experimental atherosclerosis in rabbits]

[Article in Polish]

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Atherosclerosis and its clinical manifestations are still one of the most important civilization problems. New questions arise: is it really an inevitable process? Are there any rational methods to prevent the development of atherosclerotic changes or to facilitate its regression? The aim of the work was to evaluate the influence of bioflavonoids extracted from **milk thistle** (*Silybum marianum* L), troxerutin (O-(beta-hydroxy-ethyl)-ruozid and lecithin, administered together and as a single therapy, on the experimental atherosclerosis development in rabbits. Sixty male mixed-breed rabbits were randomly assigned to 6 equal groups: I--control, II--fed on fat-rich diet (FR/DB), III--fed on FR-diet and sylimaryn concentrate (S), IV--animals fed on FR-diet and troxerutin (T), V--rabbits fed on FR-diet and soya bean lecithin (L), VI--animals fed on FR-diet and sylimaryn-phospholipid complex (SF). The whole experiment lasted 12 weeks. Following tests have been performed: electrocardiographic, biochemical, pathomorphological (including macroscopic and microscopic evaluations of aorta). Biochemical analysis included: cholesterol concentration (total, low density lipoprotein fraction cholesterol and high

density fraction cholesterol), triglycerides, b-lipoproteins, phospholipids, fibrinogen, trace elements (calcium, magnesium, zinc and copper) and dimalonialdehyde concentration. Concentrations of ascorbyl free radical, total cholesterol, triglycerides, P-450 cytochrome and phospholipids in liver have been estimated. Evident normalization of lipid metabolism and inhibition of atherosclerotic changes have been observed in the group of animals fed on SF complex. Concentrations of total cholesterol, LDL-cholesterol fraction, phospholipids and triglycerides decreased in serum. Decrease of serum dimalonialdehyde was followed by increase of ascorbyl free radicals concentration in liver. Significant increase of serum zinc has been also noted, which exceeded values observed in control group. Concentration of P-450 cytochrome increased in liver microsomes. Sylimaryn and lecithin showed less anti-atherosclerotic activity, and troxerutin displayed the least anti-atherosclerotic activity (Tab. 1-2, Fig. 1-2). On the basis of the achieved results the following conclusions were drawn: 1) **Sylimaryn and lecithin have anti-atherosclerotic activity in rabbits.** 2) **Sylimaryn-phospholipid complex shows the strongest anti-atherosclerotic activity.** 3) The achieved results allow us to undertake clinical trials using SF-complex in prevention and treatment of atherosclerosis.

106. Morphol Igazsagugyi Orv Sz. 1990 Jul;30(3):202-7. **(Animal Study)**

[Morphologic and morphometric analysis of atheromatous changes in the aorta in **silibinin**-treated cholesterol-fed rabbits]

[Article in Hungarian]

Schneider F, Hidvegi J, Bartfai Z, Somogyi A, Blazovics A.

SOTE II. Pathologiai Intezet.

Authors studied the effect of **Silibinin** of antioxidative effect on cholesterol sclerosis of rabbits. Qualitative analysis of aorta sections, macroscopic and microscopic morphometric examination of aorta were the applied methods. Their results seem to show that **Silibinin** has a favourable influence on cholesterol sclerosis. According to their opinion only joint use of qualitative and quantitative macroscopic and microscopic methods can provide basis for really accurate judgment of any atheromatose change.

Pancreas

107. Cell Mol Life Sci. 1997 Dec;53(11-12):917-20. **(Animal Study)**

Silibinin, a plant extract with antioxidant and membrane stabilizing properties, protects exocrine pancreas from cyclosporin A toxicity.

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Silymarin can be extracted from the **milk thistle**, and **silibinin** is the main component of the plant extract. Possibly due to their antioxidant and membrane-stabilizing properties, the compounds have been shown to protect different organs and cells against a number of insults. Thus liver, kidney, erythrocytes and platelets have been protected from the toxic effects of ethanol, carbon tetrachloride, cold ischemia and drugs, respectively. The effect of **silibinin** on endocrine and exocrine pancreas, however, has not been studied. We therefore investigated whether **silibinin** treatment attenuates cyclosporin A (CiA) toxicity on rat endocrine and exocrine pancreas. Groups of 15 male Wistar rats were treated for 8 days with CiA and/or **silibinin**. On day 9, endocrine and exocrine pancreatic functions were tested in vitro. At the end of the treatment period, blood glucose levels in vivo were significantly higher in rats treated with CiA while **silibinin** did not affect glucose levels. In vitro, insulin secretion was inhibited after treatment with **silibinin**, but amylase secretion was not affected. After treatment with CiA both insulin and amylase secretion were reduced. **Silibinin** and CiA had an additive inhibitory effect on insulin secretion, but **silibinin** attenuated CiA-induced inhibition of amylase secretion. Despite CiA treatment, amylase secretion was in fact restored to normal with the highest dose of **silibinin**. Thus **silibinin** inhibits glucose-stimulated insulin release in vitro, while not affecting blood glucose concentration in vivo. This combination of effects could be useful in the treatment of non-insulin-dependent diabetes mellitus. Furthermore, **silibinin** protects the exocrine pancreas from CiA toxicity. As this inhibitory effect is probably unspecific, **silibinin** may also protect the exocrine pancreas against other insult principles, such as alcohol.

Prostaglandin endoperoxide synthase

108. Pharmacology. 1992;44(1):1-12. **(Animal Study)**

In vitro inhibition and stimulation of purified prostaglandin endoperoxide synthase by flavonoids: structure-activity relationship.

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We studied the effects of 37 flavonoids on prostaglandin endoperoxide synthase (EC 1.14.99.1) purified from sheep vesicular glands. Nonplanar flavans were more potent inhibitors than planar flavones and flavonols (IC₅₀ values were, e.g., 40 μmol/l for catechin and epicatechin, 110 μmol/l for galangin, 490 μmol/l for

quercetin and 450 $\mu\text{mol/l}$ for kaempferol). Different inhibition mechanisms were observed, i.e. uncompetitive inhibition for nonplanar flavonoids and competitive or noncompetitive inhibition for planar flavonoids. Potent inhibitors in the group of flavones were substances with an o-dihydroxy structure in the B ring and in the group of flavonol substances with two hydroxyl groups in position 5 and 7 of the A ring. **None of the flavanones studied caused significant inhibition, except for the flavanone-3-ol, silibinin (silybin), which caused potent inhibition with an IC_{50} of 120 $\mu\text{mol/l}$.** Several flavonoids, which were able to inhibit the prostaglandin endoperoxide synthase at higher concentrations, were also able to stimulate the enzyme at lower concentrations. These results indicate that the flavonoids should be divided into two groups according to their capacity to inhibit the prostaglandin endoperoxide synthase, represented by planar and nonplanar substances as in each group a close correlation between structure and inhibitory activity was observed.

109. Vet Med (Praha). 1991 Jun;36(6):321-30. **(Animal Study)**

[**Milk thistle** (*Silybum marianum*, L., Gaertn.) in the feed of ketotic cows]

[Article in Czech]

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Two comparative trials were performed, each with 16 cows which in the period of 2-6 weeks after parturition had 7.9 mg and more acetone in 1 litre of milk. The cows, crossbreeds of the Czech Red-Pied cattle with the Holstein cattle, were divided into control and test groups, eight in each using the system of pairs. The cows of test groups were given for a fortnight feed rations containing a meal of **milk thistle** (*Silybum marianum*, L., Gaert.) seeds, at a rate of 0.3 kg per head/day with the contents of 2.34% silybin and silydianin (substances of the so called **silymarin** complex of the flavonolignane group). In comparison with the control cows, in the blood and milk of the former ones a decrease was demonstrated in the sum of acetone + acetoacetic acid (up to P less than 0.01) and beta-hydroxybutyric acid in the blood (up to P less than 0.05). The ketonuria degree dropped remarkably. Although there were not observed any differences in the parameters of acid-base metabolism in the blood (pH, PCO_2 , BE, SB, BB), the pH values and net acid-base output in urine were higher in these cows. Milk production in the cows of control groups was decreasing during the trial (up to P 0.01), but in the test cows it was higher by 7.7% (trial 1) and by 3.4% (trial 2), in comparison with the milk yield at the beginning of the trials. **Differences in metabolism parameters and milk production in favour of the cows which were given milk thistle in their feed rations were observed even**

in a fortnight after the diet stopped to contain this ingredient.

Cholesterol

110. J Hepatol. 1991 May;12(3):290-5.

Effect of **Silibinin** on biliary lipid composition. Experimental and clinical study.

Nassuato G, Iemmolo RM, Strazzabosco M, Lirussi F, Deana R, Francesconi MA, Muraca M, Passera D, Fragasso A, Orlando R, et al.

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The effect of **Silymarin**, a natural flavonoid, on biliary lipid composition, was studied in rats and humans. Bile flow, biliary cholesterol, phospholipid and total bile salt concentrations were measured in 23 control rats and in 27 rats treated with **Silibinin**, the active component of **Silymarin**, at the dose of 100 mg/kg body weight i.p. (n = 21) or 50 mg/kg body weight i.p. (n = 6) for 7 days. Biliary cholesterol and phospholipid concentrations were significantly reduced after the higher **Silibinin** dose (60.9 and 72.9% of the control values), whereas bile flow and biliary total bile salt concentration were unchanged. After the lower **Silibinin** dose all parameters remained unchanged. Total liver cholesterol content was not affected by **Silibinin**. On the other hand, in vitro determination of rat liver microsomal 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity showed a significant dose-dependent inhibition by **Silibinin** (0.5-8 mg/kg). Biliary lipid composition was also assayed in four gallstone and in 15 cholecystectomized patients before and after **Silymarin** (420 mg per day for 30 days) or placebo administration. In both groups, biliary cholesterol concentrations were reduced after **Silymarin** treatment and the bile saturation index significantly decreased accordingly. [These data suggest that **Silibinin**-induced reduction of biliary cholesterol concentration both in humans and in rats might be, at least in part, due to a decreased synthesis of liver cholesterol.](#)

Corticosteroid secretion

111. J Endocrinol. 1990 Feb;124(2):341-5.

An antioxidant drug, **silibinin**, modulates steroid secretion in human pathological adrenocortical cells.

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Because human adrenocortical cells from different adrenal disorders exhibit pathologically altered corticosteroid synthesis, and free radical mechanisms may induce pathological changes in the activities of corticosteroid biosynthetic enzymes (cytochrome P-450), we examined the effect of an antioxidant, **silibinin**, on basal and ACTH-stimulated secretion of several corticosteroids in isolated adrenal cells from an aldosterone-producing adenoma, atrophied adrenal tissues surrounding the adenoma, and hyperplastic adrenals from Cushing's syndrome. In the presence of a high concentration (100 $\mu\text{mol/l}$) of **silibinin**, variably diminished secretion of basal aldosterone, corticosterone, cortisol, 18-OH-corticosterone and 11-deoxycorticosterone was found. In contrast, the addition of 0.01 μmol **silibinin/l**, which failed to produce a clear effect on basal corticosteroid secretion, resulted in a potentiation of ACTH-stimulated secretion of several corticosteroids in the adenomatous and hyperplastic adrenocortical cells. These results suggest that the dose-dependent dual effect of **silibinin** on corticosteroid secretion may be attributed to corresponding changes in the activities of cytochrome P-450 enzymes, and that stimulation of ACTH-induced corticosteroidogenesis by **silibinin** is presumably due to the antioxidant property of the drug.