



Role of Silymarin in Cancer Treatment: Facts, Hypotheses, and Questions

Tomas Koltai, MD, PhD¹  and Larry Fliegel, PhD²

Abstract

The flavonoid silymarin extracted from the seeds of *Silybum marianum* is a mixture of 6 flavolignan isomers. The 3 more important isomers are silybin (or silibinin), silydianin, and silychristin. Silybin is functionally the most active of these compounds. This group of flavonoids has been extensively studied and they have been used as hepato-protective substances for the mushroom *Amanita phalloides* intoxication and mainly chronic liver diseases such as alcoholic cirrhosis and nonalcoholic fatty liver. Hepatitis C progression is not, or slightly, modified by silymarin. Recently, it has also been proposed for SARS COVID-19 infection therapy. The biochemical and molecular mechanisms of action of these substances in cancer are subjects of ongoing research. Paradoxically, many of its identified actions such as antioxidant, promoter of ribosomal synthesis, and mitochondrial membrane stabilization, may seem protumoral at first sight, however, silymarin compounds have clear anticancer effects. Some of them are: decreasing migration through multiple targeting, decreasing hypoxia inducible factor-1 α expression, inducing apoptosis in some malignant cells, and inhibiting prometotic signaling among others. Interestingly, the antitumoral activity of silymarin compounds is limited to malignant cells while the nonmalignant cells seem not to be affected. Furthermore, there is a long history of silymarin use in human diseases without toxicity after prolonged administration. The ample distribution and easy accessibility to milk thistle—the source of silymarin compounds, its over the counter availability, the fact that it is a weed, some controversial issues regarding bioavailability, and being a nutraceutical rather than a drug, has somehow led medical professionals to view its anticancer effects with skepticism. This is a fundamental reason why it never achieved bedside status in cancer treatment. However, in spite of all the antitumoral effects, silymarin actually has dual effects and in some cases such as pancreatic cancer it can promote stemness. This review deals with recent investigations to elucidate the molecular actions of this flavonoid in cancer, and to consider the possibility of repurposing it. Particular attention is dedicated to silymarin's dual role in cancer and to some controversies of its real effectiveness.

Keywords

antioxidant, cancer, invasion, migration, milk thistle, silybin, silymarin

Received August 27, 2021. Received revised October 20, 2021. Accepted for publication December 6, 2021.

Introduction

Research on plants and their possible curative properties is not new. It has been occurring since ancient times. In the last 200 years this search has become more scientifically oriented and led to discoveries such as curare, strychnine, atropine, salicylate, digitalis, and more recently taxanes, artemisinin, vitamins, and many others. These naturally originated molecules “*have cellular targets similar to those of new drugs developed by pharmaceutical companies.*”¹ Many of these natural products were so strikingly important for human health that they swiftly entered clinical practice. Sometimes, they were

favorably modified by the pharmaceutical industry and then derivatives with enhanced benefits were born. While taxane compounds are one of the best examples of a success story in oncology, other compounds, not so blatantly effective as

¹ Hospital del Centro Gallego de Buenos Aires, Buenos Aires, Argentina

² University of Alberta, Edmonton, AB, Canada

Corresponding Author:

Larry Fliegel, Department of Biochemistry, Faculty of Medicine, University of Alberta, 347 Medical Science Bldg., Edmonton, AB, Canada, T6G 2H7.
Email: lfliegel@ualberta.ca



taxanes are on the waiting list. There is also a group of natural products that were, and are, used for known diseases other than cancer. In some cases, their antitumoral effects were slowly recognized and they were repurposed. Silymarin is one of this type of products, with some recognized antitumor effects however, repurposing has not yet occurred. Seeds of *Silybum marianum*,² popularly known as milk thistle, have been used since ancient times to treat diverse ailments, and more recently liver damage due to toxins, particularly *Amanita phalloides* poisoning (but including many others such as carbon tetrachloride,³ metals, allyl alcohol) and alcohol-induced damage, including hepatitis, cirrhosis, and jaundice.⁴⁻⁶ (From a technical point, what are commonly called seeds are actually fruits, but we shall call them seeds following other publication precedents). The last 15 years have witnessed a growing interest in silymarin and the plant it comes from: *Silybum marianum* (L.) Gaertn (also known as *Carduus marianus* and wild artichoke).

Although Silymarin is probably the most thoroughly studied nutraceutical, it is looked upon with skepticism by the medical profession for multiple reasons, such as:

1. ample distribution and easy accessibility to milk thistle;
2. over the counter availability;
3. the fact that it is a weed;
4. some controversial issues regarding bioavailability, and pharmacological actions;
5. its status as a nutraceutical rather than a drug according to FDA;
6. its vulgarization through many nonscientific Internet pages dedicated to silymarin compounds;
7. the enormous number of manufacturers, many of them scarcely known (Figure 1);
8. the direct consequence of this “popularization” is that it is available over the counter at the herbalist shop or through the Internet, rather than with a prescription in the pharmacy;⁷
9. the lack of striking effects on the disease;
10. the fact that it is not usually considered in university-level pharmacology courses.

Definition. Silymarin is the standardized extract obtained from the dried seeds of *Silybum marianum* (milk thistle) containing approximately 70% to 80% of the silymarin complex and an approximately 20% to 30% chemically undefined fraction, comprising mostly other polyphenolic compounds. The main component is silybin (silibinin). Silymarin and silybin are not synonyms. However, many older reports indistinctly use one or the other term, leading to some confusion. Silymarin extract and its components may frequently differ in their effects due to differences in solubility and bioavailability.

History. Silymarin has been used in Europe since the fourth century BCE by Theophrastus of Eresus, and reappears in the year 65 of current era in Pedanius Dioscorides’ *De Materia Medica*. Here he proposed milk thistle for the treatment of serpent venom bite and called it silybon.⁸ It does not seem to be part of Traditional Chinese Medicine.⁹ It was also used in

Ancient Egypt,¹⁰ however, we do not know exactly for what purpose.¹¹ During the Renaissance some of the therapeutic effects were discovered and published by herbalists and physicians such as Pietro Andrea Mattioli (1544) and Hieronymus Bock (1539), among others. In the seventeenth century, an English botanist, Nicholas Culpeper, suggested that milk thistle was useful for liver diseases.

Location and Habitat. This invasive annual plant was originally found in the Mediterranean basin, but now it is present in all the continents. It requires dry, warm soil and it is very competitive eliminating other plants.¹²

Chemistry. The standardized extract obtained from the seeds of *Silybum marianum* is known as silymarin which contains between 70% and 80% of silymarin flavolignans. *Silybum marianum* is a mixture of 8 flavolignan structurally related isomers: silybin (or silibinin), isosilibinin, silydianin, silychristin, isosilychristin, and taxifolin.^{13, 14} The main component of silymarin is Silibinin which is a compound consisting of equal amounts of silybin A and silybin B (CAS 22888-70-6).

BOX 1: Average composition of silymarin.

Silybin 60% to 70%

Silychristin 20%

Silydianin 10%

Isosilybin 5%

Taxifolin 1%

Small amounts of the flavonoids: quercetin, kaempferol, apigenin, naringin, eriodictiol.

In 1959, Möschnlin isolated silybin,¹⁵ and then in 1968 silymarin chemistry was described in detail by Wagner et al,^{16, 17} and Pelter and Hansel.¹⁸ Today, more than 50 years have elapsed since the initial hepatic antitoxic and protective function of the compound was discovered and now, its antitumor activity is under scrutiny (Figures 2 to 4). Silymarin, the active principal component of milk thistle, was originally thought to be one substance, until it was discovered that it is actually composed of a group of different flavolignans (Box 1).

Silybin is stable in acidic conditions but unstable under alkaline conditions. Alkaline media disrupt flavolignan’s skeleton. This is important because the extracellular matrix of tumors has a low pH (approximate pH = 6.8), while intracellular tumor pH is alkaline (approximate pH = 7.5), but only slightly more alkaline than normal cell intracellular pH (approximately = 7.2).¹⁹ Normal cells, on the other hand, have an alkaline extracellular milieu (approximate pH = 7.35). We presume, without evidence to sustain the presumption, that silybin can reach the malignant cell’s acidic extracellular space without degradation. This singular feature, the acidic extracellular pH of tumors,²⁰ may explain why silybin effects differ in normal versus malignant cells. Silymarin may be able to better access the malignant cell compared with normal cells. This theory needs experimental confirmation.

Production. Silymarin extract is obtained by compressing the seeds which leads to a loss of lipids. Then, the active principal component is extracted with acetone, methanol, ethanol, or



Figure 1. A glimpse of the multiple brands and presentations of silymarin compounds in the US and European markets with the resultant “vulgarization.” There are many “silymarins” developed in well accredited pharmaceutical laboratories, but there are also many produced by scarcely known sources. Most can be acquired through the Internet.

ethyl acetate. After a second lipid and impurities extraction, what is left is a mixture of flavolignans called silymarin.²¹ Silybin is obtained from silymarin through methanolic extraction.

Biological activity. In 1975, Desplaces et al²⁷ showed that silymarin had a protective effect on hepatocytes against phalloidin, the toxin of *Amanita phalloides*, when it was administered before the poison. When it was given immediately after phalloidin, it still protected hepatocytes but when given 30 min later, this protective action was negligible. Phalloidin produces acute hemorrhagic necrosis of hepatocytes. When silymarin was administered before the poison there were no morphologic (electron microscopic level) or biochemical signs of hepatic lesions.²⁸ Silymarin was adopted as an “hepato-protector” by lay persons and the medical profession based on sometimes controversial evidence.

Hepato-protection. For example in:

1. **Chronic hepatitis B and C:** silymarin was able to lower transaminases but there was no change in viral load.²⁹ However, Fried et al did not find benefits in chronic hepatitis C virus infected patients with high doses of silymarin,

- and did not find effective lowering of transaminases.³⁰ No transaminase lowering was found with silymarin in hepatitis C virus infection in another study with very high doses of silymarin.³¹ Other authors arrived to completely different results: silymarin had antiviral actions by blocking hepatitis C virus cellular entry and transmission.³² As a first conclusion we may say that there is no clear evidence of silymarin’s benefits in chronic hepatitis C.
2. **Alcoholic hepatitis:** Trinchet et al³³ found no significant favorable effects of silymarin in alcoholic hepatitis in a double blind randomized study.
3. **Nonalcoholic fatty liver disease:** In this case, silymarin has shown favorable and less controversial results.^{34–36}
4. **Reduction/inhibition of hepatic fibrosis:** silymarin showed the ability to reduce hepatic fibrosis in the early stages of liver injuries.³⁷
5. **Cirrhosis:** a large population study showed that silymarin decreased mortality in patients with hepatic cirrhosis.³⁸

In spite of the evidence favoring its benefits in chronic liver disease, “the overall efficacy of silymarin remains unclear”

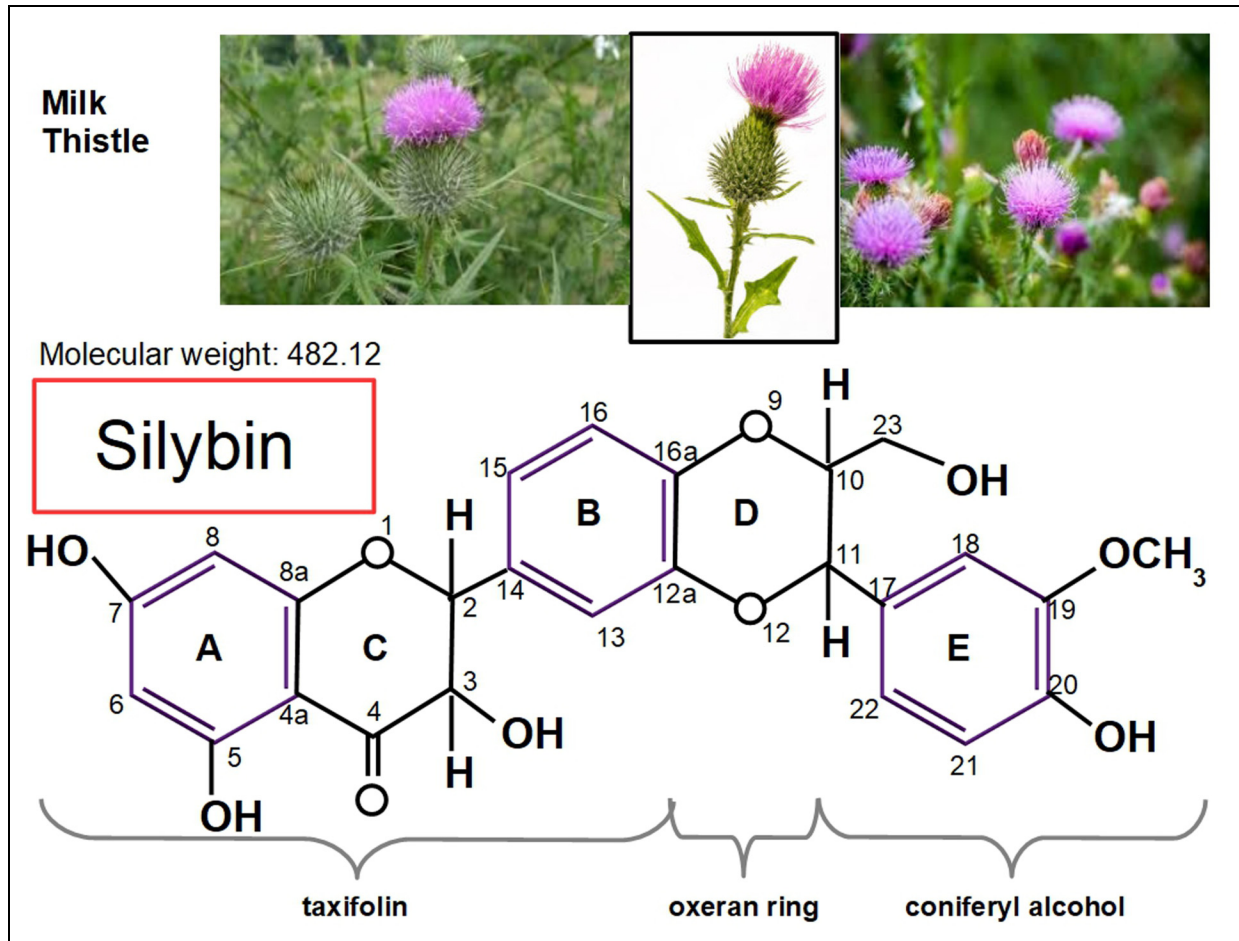


Figure 2. Milk thistle and the chemical structure of silybin ($C_{25}H_{22}O_{10}$), with its proprietary numbering. Of note, is the similarity between silybin and steroid hormones. The lower panel shows silybin's structural formula where 3 different chemical groups can be identified: a taxifolin and a coniferyl alcohol united by an oxirane ring. According to Biedermann et al,²² "the 20-OH group was established to be the most active radical scavenging moiety and also the most important group responsible for the lipoperoxidation inhibitory activity." Positions 2-3 also play a role in antioxidant activity because these positions can be oxidized to produce 2-3 dehydrosilybin (see Figure 3). Silybin has 5 hydroxyl groups in positions 3, 5, 7, 20, and 23 which are the targets to produce silybin derivatives. Positions 7 and 20 are usual sites of glucuronidation of silybin during its conjugation in human metabolism.

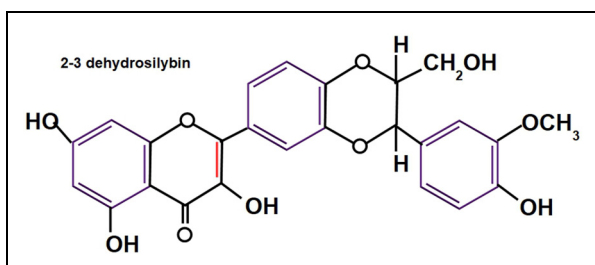


Figure 3. 2-3 dehydrosilybin has 25-fold more antioxidant activity than silybin.²³ Taxifolin and quercetin have more antioxidant activity than 2-3 dehydrosilybin. Small amounts of 2-3 dehydrosilybin are found in silymarin.

according to Tighe et al.³⁹ However, there are many, and some potentially beneficial, known biochemical effects of silymarin and silybin. For example, free radical scavenging and antioxidative properties of silybin are well known and have been

thoroughly investigated.⁴⁰ It is considered 10-fold more antioxidant than vitamin E. In 1977, Machicao and Sonnenbichler⁴¹ showed that silybin increased RNA synthesis in rat liver cells and mainly increased the production of ribosomal RNA and polymerase A. Shriever et al⁴² found that silymarin inhibited fatty acid synthesis in rat liver: fatty-acid-synthase and ATP-citrate-lyase, 2 of the main lipogenic enzymes, were diminished by about 50%. Fiebrich and Koch^{43,44} described silymarin as a blocker of prostaglandin production *in vitro* through inhibition of both prostaglandin synthetase and lipoxygenase. This reduction of lipoxygenation was confirmed on liver ribosomes and mitochondria as well and probably explains silymarin's hepatoprotective actions.⁴⁵

A few years later Sonnenbichler et al⁴⁶ presented the first evidence that silymarin acted in a different way in noncancerous hepatic tissue and malignant cells: in the first case it stimulated DNA synthesis, in the second it did not. Silymarin is also a potent blocker of cyclic AMP breakdown (*in vitro*) by a

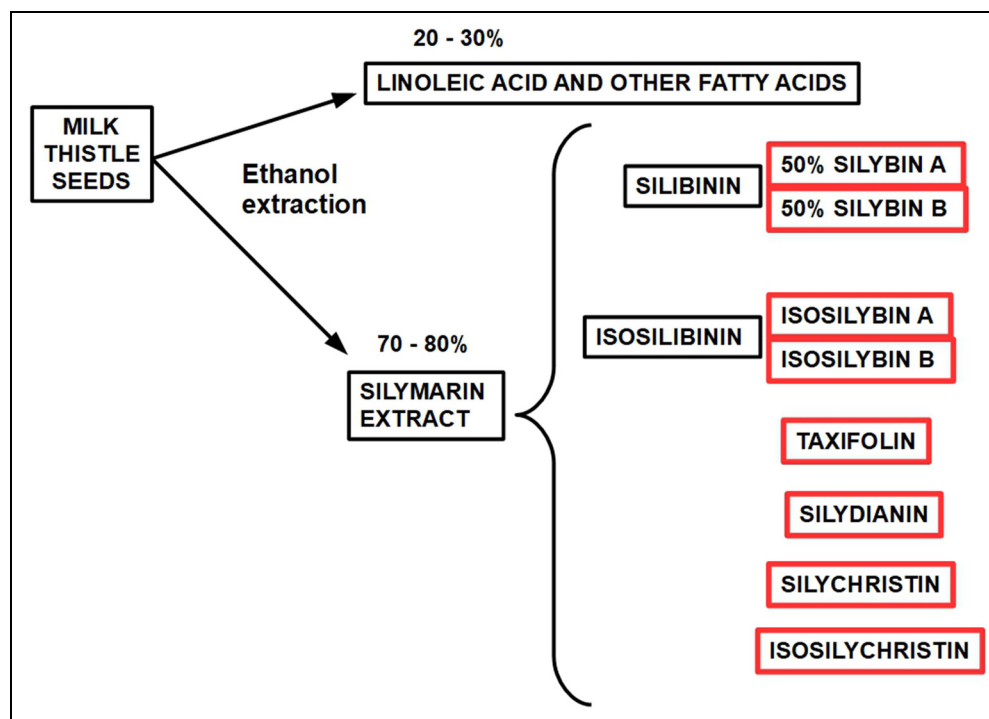


Figure 4. Nomenclature diagram of the different components of milk thistle seed and silymarin.²⁴ Isosilybin B seems to be the most powerful anticancer fraction.²⁵ Silymarin and Silibinin are different concepts, however some older publications use both terms interchangeably. Silibinin is the more active form of the silymarin extract. Only silymarin extracts are available in pharmacies while researchers usually use silibinin for their experiments. Standard silymarin extracts usually contain 33.5% silybin, 13% silychristin, 8.35% isosilybin, 3.5% silydianin²⁶ (see Figure 5).

phosphodiesterase preparation,⁴⁷ an inhibitor of histamine release from human basophil leukocytes,⁴⁸ dose-dependent downregulator of *in vitro* lymphocyte blastogenesis⁴⁹ and alters the mitochondrial electron transport chain through mitochondrial calcium release,⁵⁰ in addition to its antioxidant properties.⁵¹ Immunostimulatory effects of silymarin were also described in experimental models, but not in the context of cancer treatment.^{52,53}

Silymarin and Other Diseases (Table 1)

Silymarin has been investigated and proposed for the treatment of many different diseases, from Alzheimer dementia⁵⁴ to SARS 2 Covid-19, including diabetes,⁵⁵ diabetic complications,^{56–58} hyperlipidemia, and hypercholesterolemia,^{50–61} among others. However, in the last 15 years, the main focus has been cancer.

Silymarin and Cancer

The first observation of silymarin's possible benefits in cancer is the 1991 publication by Mehta and Moon.¹¹⁸ They showed that silymarin could act as a preventive (antipromoter) of cancer in mouse mammary glands treated with DMBA (dimethylbenzanthracene) and TPA (tetradecanoylphorbol acetate). The treatment protocol they employed made it possible to differentiate whether the chemoprevention worked at the initiation stage of carcinogenesis (DMBA phase) or during promotion (TPA phase).

A 1991 review on the advances in pharmacological studies of silymarin by Rui,¹¹⁹ did not mention anticancer activities. But in 1994, Agarwal et al¹²⁰ performed a study on skin treated with TPA confirming the protective effect of this flavonoid against tumor promotion. Silymarin protected against induction of ornithine decarboxylase by TPA. Ornithine decarboxylase inhibition protects against tumor promotion. A protective effect of silymarin was also found in colon and small intestine adenocarcinoma cells induced by 1,2-dimethylhydrazine.¹²¹ Silymarin and its components also inhibit beta-glucuronidase.¹²²

Valenzuela and Garrido¹²³ proposed 3 levels for silymarin's action in experimental animals:

- as an antioxidant, by scavenging prooxidant free radicals and by increasing the intracellular concentration of the tripeptide glutathione;
- through a regulatory action of cell membrane permeability and increase in its stability against xenobiotic injury;
- through nuclear expression, by increasing ribosomal RNA synthesis, by stimulating DNA polymerase I, and by exerting a steroid-like regulatory effect on DNA transcription.

Silymarin also inhibits rat liver cytosolic glutathione S-transferase,¹²⁴ although this function does not clearly hint towards anticancer activity. On the other hand, silymarin scavenges reactive oxygen species as noted above, and inhibits

Table I. Silymarin Research Beyond Hepatoprotection and Cancer: A Summary.

Type of disease	Specific disease	References
Neurologic diseases	General	62,63
	Parkinson's disease	64
	Alzheimer	65-67
	Multiple sclerosis	68,69
	Diabetic cognitive impairment	70
	Learning and memory deficits (in mice)	71
Diabetes	Diabetic complications	72-76
Hypercholesterolemia		77-81
Renal diseases	Cyclosporine nephrotoxicity	82
	Diabetic nephropathy	83,84
Ischemia/reperfusion	Damage prevention in general	85
	In heart muscle	86
	In the central nervous system	87,88
	In the kidney	89,90
	In intestine and bowel	91
	In the stomach	92
	In the lungs	93
	In the liver	94,95
	Multivisceral	96
	Skin	Protection against UV radiation
Melasma		100
Rosacea		101
Immune system	Inhibition of UV-induced immune suppression	102
Infections: Viral	Covid infection	103,104
	Anti-Mayaro virus	105
	Anti-Chikungunya virus	106
	Anti-Zika virus	107
Infections: bacterial	<i>Escherichia coli</i>	108
Amiodarone	Improved effects on atrial flutter	109
	Decreased Amiodarone side-effects	110
Ulcerative colitis	Prolonged remission	111
Inflammatory bowel disease		112
Irritable bowel syndrome		113
Migraine	Reduced frequency of attacks	114
Endocrine	Hyperprolactinemia	115
	Decreased hot flashes in menopause	116
	Polycystic ovarian syndrome	117

arachidonic acid metabolism in human cells,¹²⁵ has antiinflammatory effects similar to those of indomethacin,¹²⁶ protects skin against carcinogenic agents^{127,128} and ultraviolet radiation.¹²⁹⁻¹³¹ These publications strongly suggest a cancer-preventive activity and silymarin is slowly emerging as an anticancer drug. For example, Scambia et al¹³² tested the antiproliferative activity of silymarin on human ovarian and breast cancer cell lines and found a growth-inhibiting effect on both. Silymarin also showed

synergism with the commonly used anticancer compounds doxorubicin and cisplatin.

In DU145, prostate carcinoma cells, silymarin showed inhibition of Erb1 (eukaryotic ribosome biogenesis protein 1) signaling and G1 arrest.¹³³ In MDA-MB 486 breast cancer cells, G1 arrest was found due to increased p21 and decreased CDKs activity.¹³⁴ In advanced human prostate carcinoma cells, silymarin decreased ligand binding to Erb1¹³⁵ and NF-kB expression was strongly inhibited by silymarin in hepatoma cells,¹³⁶ as well as in histiocytic lymphoma, HeLa and Jurkat cells.¹³⁷

According to Zi and Agarwal, low doses of silymarin inhibited ERK1 and ERK2 Map kinases in a skin cancer cell line (human epidermoid carcinoma A431) and at higher doses activated MAPK/JNK1. This means that at lower doses the effect was antiproliferative and at higher doses proapoptotic.¹³⁸

Treating prostate carcinoma cells with silymarin the levels of PSA were significantly decreased and cell growth was inhibited through decreased CDK activity and induction of Cip1/p21 and Kip1/p27.¹³⁹

Silymarin has also been shown to have a variety of other protective effects in various cell types, such as anti-COX2 and anti-IL-1 α activity,¹⁴⁰ antiangiogenic effects through inhibition of VEGF secretion, upregulation of Insulin like Growth Factor Binding Protein 3 (IGFBP3),¹⁴¹ and inhibition of androgen receptors.¹⁴² In leukemia HL-60 cells, silymarin inhibited proliferation and induced differentiation into monocytes in a dose-dependent manner.¹⁴³ Another important effect of silymarin in cancer is the downregulation of the STAT3 pathway which was seen in many cell models. STAT3 is active in many types of cancer and is associated with poor prognosis and resistance to treatments.¹⁴⁴⁻¹⁴⁶ Telomerase activity is another important factor in promoting carcinogenesis and evading senescence, thus inducing cancer cell immortality; silymarin has the ability to decrease telomerase activity in prostate cancer cells.¹⁴⁷

Silymarin and Apoptosis

The apoptotic mechanism silymarin employs on cancer cells is generally p53 dependent, and follows the usual steps: increased proapoptotic proteins; decreased antiapoptotic proteins; mitochondrial cytochrome C release-caspase activation.¹⁴⁸ Caspase inhibitors terminate silymarin apoptotic activity. Malignant p53 negative cells show only minimal apoptosis when treated with silymarin. Therefore, one conclusion is that silymarin may be useful in tumors with conserved p53.

Silymarin and Cancer Cell Migration

Enhanced cell migration is an important part of cancer progression. The antimigratory effects of silymarin in cancer cells are the result of mechanisms that¹⁴⁹:

1. inhibit histone deacetylase activity;¹⁴⁹
2. increase histone acetyltransferase activity;¹⁴⁹
3. reduce expression of the transcription factor ZEB1;¹⁴⁹
4. increase expression of E-cadherin;¹⁴⁹

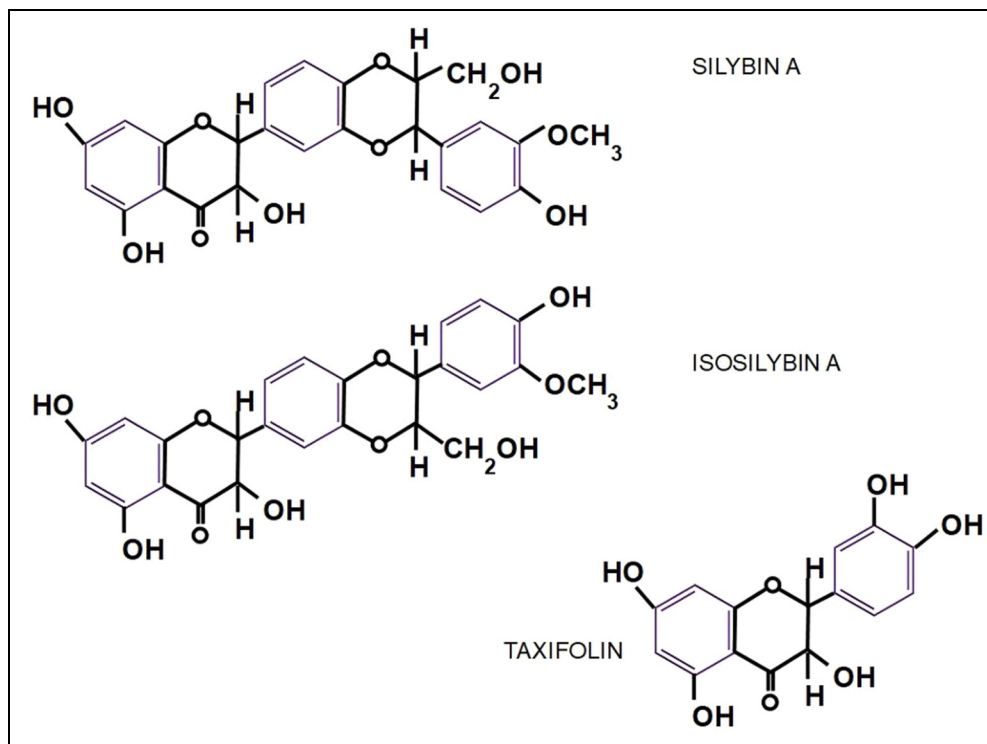


Figure 5. Similarities and differences among silymarin's components.

5. increase expression of miR-203;¹⁴⁹
6. reduce activation of sodium hydrogen isoform 1 exchanger (NHE1);¹⁵⁰
7. target β catenin and reduce the levels of MMP2 and MMP9;¹⁵¹
8. reduce activation of prostaglandin E2;¹⁵²
9. suppress vimentin expression;¹⁵³
10. inhibit Wnt signaling;¹⁵⁴
11. modulate $\beta 1$ integrin signaling.¹⁵⁵

Silymarin and Angiogenesis

Angiogenesis is important in cancer growth because solid tumors need a blood supply to grow. Silymarin inhibits angiogenesis. There are various postulated mechanisms:

1. Decreased migration of endothelial cells.¹⁵⁶
2. Flt1 (VEGFR1) upregulation.¹⁵⁷ (VEGFR1 upregulation may act as a negative regulator of VEGFA that is upheld by this receptor with low protein kinase activity and therefore VEGFA is unable to bind to KDR [VEGFR2] with much higher kinase activity).¹⁵⁸
3. VEGF downregulation.⁴⁰

Silymarin and Epithelial–Mesenchymal Transition (EMT)

EMT is involved in tumor progression and metastatic expansion. In a transcriptome study of nonsmall cell lung cancer (NSCLC) cells, Kaipa et al¹⁵⁹ found that silibinin had no

effect on EMT. However, the opposite was found in other malignant tissues^{160–162} where it showed inhibitory effects.

Silymarin and TIMP1

High expression of the tissue inactivator of metalloproteases I, or TIMP1, in cancer is a marker of poor prognosis^{163,164} because it is involved in tumor progression, metastasis, and shorter overall patient survival. TIMP1 also promotes accumulation of tumor-associated fibroblasts.¹⁶⁵ Therefore, it may be considered a target in cancer treatment. Silymarin has the capacity to decrease TIMP1 expression^{166–168} in mice.

Silymarin and LPAR1

LPAR1 and 3 (liposphatidic receptors 1 and 3) are related to cancer invasiveness.^{169–172} Silymarin has the ability to downregulate LPAR1.¹⁷³

Silymarin and TGF $\beta 2$

Silibinin reduces the expression of TGF $\beta 2$ in different tumors such as triple negative breast,¹⁷⁴ prostate, and colorectal cancers.¹⁷⁵ TGF $\beta 2$ downregulation impedes the TGF $\beta 2$ /Smad pathway reducing cellular motility and MMP2 and MMP9 (metalloproteases) reducing invasion. In the liver, TGF $\beta 2$ downregulation results in an antifibrotic effect, preventing hepatic fibrosis induced by inflammatory liver diseases.^{166,176}

Silymarin and Hypoxia Inducible Factor-1 α (HIF-1 α)

When cells are exposed to hypoxia, HIF-1 α accumulates in the nucleus activating transcription of many genes and this plays an important role in tumor progression. Silymarin was found to decrease HIF-1 α expression in rainbow trout brain¹⁷⁷ and in rat lung under hypoxic conditions.⁹³ In prostate cancer cells silibinin inhibited HIF-1 α translation.¹⁷⁸

Silymarin and CD44 and EGFR

CD44, the transmembrane receptor for hyaluronan, is increased in breast cancer and many other tumors, due to EGF (epidermal growth factor) stimulation. Silibinin decreased CD44 expression and the activation of EGFR (epidermal growth factor receptor) by EGF.¹⁷⁹ In prostate cancer, silibinin decreased/inhibited CD44 expression as well.¹⁸⁰ CD44 binding with hyaluronan triggers important protumoral signaling from its intracellular segment, inducing cancer cell survival, angiogenesis, migration, and invasion. The CD44 antigen (synonym HCAM) is a glycoprotein acting as an adhesion molecule¹⁸¹ on the cell surface. Cell adhesion molecules play an important role in cell migration. In fact, CD44 has been shown to be strongly correlated with invasion^{182,183} and metastasis.^{184–186}

Silymarin Modulation of TNF α (Tumor Necrosis Factor Alpha)

Tyagi et al¹⁸⁷ showed that silibinin pretreatment of lung cancer cells inhibited TNF α induced “phosphorylation of STAT3, STAT1, and Erk1/2, NF- κ B-DNA binding, and expression of COX2, iNOS, matrix metalloproteinases (MMP)2, and MMP9, which was mediated through impairment of STAT3 and STAT1 nuclear localization.”

Silymarin Inhibition of the Wnt/ β -Catenin Signaling

The Wnt/ β -catenin pathway is critical in cell proliferation, migration, and differentiation. It is a powerful regulator of embryonic development and tumorigenesis. Lu et al¹⁸⁸ showed that silibinin inhibited the Wnt/ β -catenin pathway in both prostate and breast cancer cells.

Silymarin Potentiation of TRAIL-Induced Apoptosis

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is part of the TNF superfamily. It is known to selectively induce apoptosis in cancer cells without having significant toxicity toward normal cells. Kauntz et al^{189,190} found silibinin potentiated TRAIL-induced apoptosis in human colon adenocarcinoma cells. Furthermore, this potentiation was also found in TRAIL-resistant cells. Silibinin upregulated Death Receptors 4 and 5, thus increasing the number of receptors for TRAIL binding. Silibinin had the ability to induce not only the extrinsic apoptotic pathway, but also the intrinsic

pathway. TRAIL sensitization by silymarin was also found in glioblastoma cells¹⁹¹ and in hepatocarcinoma.¹⁹²

Silymarin and Phospholipase A2

Secreted phospholipase A2 participates in inflammation and carcinogenesis. Silibinin downregulates secreted phospholipase A2 in cancer cells.¹⁹³

Silymarin and Platelet-Derived Growth Factor (PDGF)

PDGF and its receptor are required for fibroblast proliferation and differentiation. It was found that silibinin had the ability to downregulate PDGF in fibroblasts, thus decreasing proliferation.¹⁹⁴

Silymarin Decreases the Levels of Interleukin 8 (IL-8)

Interleukin 8 has been identified as a protumoral cytokine^{195–198} and there is evidence showing that inhibition of IL-8 reduces tumorigenesis.¹⁹⁹ Flavonoids, in general, reduce levels of IL-8. Curcumin,²⁰⁰ apigenin,²⁰¹ and silybin showed the ability to decrease IL-8 levels.^{150,202,203}

Silymarin Inhibits the Signal Transducer and Activator of Transcription 3 (STAT3) Pathway

STAT3 exists in the cytosol of cells and is a focal point of multiple oncogenic pathways. Silymarin inhibited STAT3 phosphorylation and decreased the expression of intranuclear sterol regulatory element binding protein 1 (SREBP1), decreasing lipid synthesis. The final consequences of these inhibitions were growth arrest and apoptosis.²⁰⁴

Silibinin Acts as a Mitochondrial “Poison” in Malignant Cells

Si et al,²⁰⁵ experimenting with 2 human breast cancer cell lines, MCF7 and MDA-MB-231, found that silibinin produced morphological and functional changes in mitochondria: decreased mitochondrial mass, condensed crests, reduced membrane potential and ATP content, and decreased mitochondrial biogenesis.

Silibinin and Metalloproteases

MMP2 and MMP9 play an important role in extracellular matrix remodeling and their levels correlate with progression of neuroblastoma tumors.²⁰⁶ Yousefi et al²⁰⁷ found that silibinin decreased MMP2, MMP9, and urokinase plasminogen activator receptor level (uPAR) in neuroblastoma cells. uPAR is also a marker of cell invasion.

Silymarin and COX2

COX-2 expression in cancer can stimulate angiogenesis and is associated with tumor growth, invasion, and metastasis.²⁰⁸ Silymarin decreased the expression of COX2 in a model of chemically induced hepatocarcinoma in rats.²⁰⁹

Silibinin and Programmed Death-Ligand 1 (PD-L1)

The programmed cell death protein and its ligand (PD-L1) complex play a key role in tumor progression being involved in growth regulation disturbance. This results in a defect in programmed cell death, apoptosis.²¹⁰ Silibinin inhibits PD-L1 by impeding STAT5 binding in NSCLC.²¹¹ This hints at the possible usefulness of silymarin as a complement to immune checkpoint inhibitors. A similar effect was found in nasopharyngeal carcinoma.²¹² In renal carcinoma cells, silibinin decreased PD-L1 in murine renal cancer cells *in vitro* and *in vivo*.²¹³

Silybin and Notch Signaling

The Notch signaling pathway is highly conserved, regulating development and is involved in angiogenesis and metastasis.²¹⁴ Silybin inhibited Notch signaling in hepatocellular carcinoma cells showing antitumoral effects.²¹⁵ However, dibenzazepine is much more powerful in this respect.²¹⁶ Notch was also downregulated by silibinin in breast cancer cells impeding notch-1/ERK/Akt signaling²¹⁷ and inducing apoptosis.

Silymarin and SIRT1

SIRT1 can deacetylate histones and other substrates and may act in a dual manner: as tumor suppressor or tumor promoter.^{218,219} Silymarin has the ability to increase hepatic SIRT1 expression.²²⁰ Silymarin can also increase SIRT1 expression in other tissues, such as hippocampus,²²¹ articular chondrocytes,²²² and heart muscle.²²³ Silymarin seems to act differently in tumors: in lung cancer cells SIRT1 downregulated SIRT1 and exerted multiple antitumor effects such as reduced adhesion and migration and increased apoptosis. When SIRT1 was independently downregulated with siRNA the silymarin's antitumoral effects were increased.²²⁴

Silymarin and VEGF/VEGFR

The angiogenic cytokine vascular endothelial growth factor (VEGF) and its receptor (VEGFR) play critical roles in vasculogenesis and angiogenesis. Jiang et al²²⁵ found that adding silymarin to prostate and breast cancer cells swiftly reduced the secretion of VEGF to the medium in a dose-dependent manner. Silymarin also prevented VEGF expression in myocardial cells exposed to doxorubicin toxicity²²⁶ as well as other manifestations of cardiotoxicity. A similar decrease in VEGF and VEGFR levels was found with silymarin in preeclamptic placenta, however the effect was very modest.²²⁷ There is

evidence showing that silymarin reduces VEGF expression at the transcription level.²²⁸

Silymarin and Myc

C-Myc is a multifunctional master regulator transcription factor; it is activated by oncogenic pathways, drives many functions for rapid cell division, and inhibits antiproliferative pathways.²²⁹ There is direct and indirect evidence that silymarin interacts with c-Myc,²³⁰ in some cases increasing its expression in liver cells as a response to hepatic chemical injuries²³¹ or decreasing it in malignancies.²³² Rajamanickam et al^{233,234} found that silymarin could prevent spontaneous tumorigenesis in an APC^{min/+} mouse model (prone to develop intestinal tumors) by decreasing β -catenin, cyclin D1, c-Myc, phospho-glycogen synthase kinase-3 β expression, phospho-Akt, and cyclooxygenase-2 in polyps. This report confirms silymarin's multitargeting effects in tumors and its different behavior in nonmalignant cells.

Silymarin and Carbonic Anhydrases

Carbonic anhydrases (CAs) play an important role in cancer progression, particularly those associated with the cell membrane (membrane CAs), namely isoforms CA9 (CAIX) and CA12 (CAXII). These CAs intervene in acidifying the extracellular substance and, working in tandem with sodium bicarbonate cotransporters, increase the intracellular pH. Downregulating or inhibiting membrane CAs has become a valid target for cancer therapy. Silymarin has the ability to inhibit CA isoforms CA I and CA II.^{235,236} However, we could not find any publications specifically addressing silymarin's role as a possible inhibitor of membrane CAs.

Silymarin and Mitochondria

This is a controversial relationship. On one side, silymarin showed ability to reduce oxygen consumption in mitochondria NAD-dependent substrates, while on the other hand stimulating respiration in mitochondria oxidizing succinate.⁵⁰ Silymarin increases mitochondrial release of Ca⁺⁺ and lowers mitochondrial membrane potential in cancer cells²³⁷ and increases the transmembrane potential in toxic aggressions.²³⁸ Regarding mitochondria we may presume that silymarin has context-dependent effects.

Antimetastatic Potential

Many of the features discussed above hint towards silymarin's antimetastatic potential. In a TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) model of prostate carcinoma, when mice were fed with silibinin invasion and metastasis were reduced.²³⁹ The antimetastatic effect was due to less invasion, less EMT, less collagen I-cancer cell adhesion, and less expression of CD44.²⁴⁰ In a randomized clinical study with patients harboring solid tumors, silymarin was added to standard chemotherapy. Although

Table 2. Melanoma.

Year, Ref.	Findings
2006 ²⁸⁵	Silymarin proposed for chemoprevention of melanoma.
2007 ²⁸⁶	Silymarin increased proapoptotic effects of anti-Fas agonistic antibody CH11 in melanoma cells.
2013 ²⁸⁷	Silymarin for prevention for melanoma invasion.
2013 ²⁸⁸ X	Silymarin decreased the growth of melanoma xenografts and locked MEK1/2-ERK1/2-RSK signaling that led to a reduction of NF-kB activator protein1 and STAT3, which resulted in cell cycle arrest and inhibited tumor growth <i>in vitro</i> and <i>in vivo</i>
2015 ²⁸⁹ X	Silymarin targeted cell cycle regulators, angiogenesis, and induced apoptosis <i>in vitro</i> and <i>in vivo</i> .
2016 ²⁹⁰	Silymarin as a modulator of the wnt/ β catenin pathway.

Table 3. Prostate.

Year, Ref.	Findings
2008 ²⁹¹	Silibinin had inhibitory effects on survival, motility and adhesion of highly metastatic prostate cancer cells.
2005 ²⁹²	Isosilybin B was the most potent fraction of silymarin against proliferation in different prostate cancer cell lines (LNCaP, DU145, and PC3). Silymarin used in this paper contained 5% isosilybin B. Isosilybin was a suppressor of the promoter of the topoisomerase II α gene in DU145 cells.
2001 ¹⁴²	Silymarin inhibited the androgen receptor (AR) by reducing its localization in the nucleus without modifying AR expression or binding ability. This action was probably related to silymarin's downregulation of FKBP51 that is a probable transporter of AR to the nucleus.
2020 ²⁹³	Silymarin inhibited DU 145 cell proliferation through two mechanisms: activation of the SLIT 2 protein and inhibition of CXCR4.
1999 ²⁹⁴	Silibinin inhibited growth by G1 cell cycle arrest in hormone-refractory human prostate carcinoma cells without apoptosis. It decreased CDKs and PSA and increased p21 and p27.
2006 ²⁹⁵	Silymarin and silibinin produced G1 and G2-M cell cycle arrest with a decrease in CDKs and Cdc2 kinase activity, and an increase in CDK inhibitors.
2002 ²⁹⁶	Silibinin treatment caused growth inhibition, apoptosis, and decreased viability in different prostate cancer cell lines.
2007 ²⁹⁷	Isosilybin A and Isosilybin B caused growth arrest and apoptosis in human prostate cancer cell lines.
2004 ²⁹⁸	Silibinin inhibited mitogenic signaling in prostate cancer cells.
2007 ²⁹⁹	Silibinin had a synergistic effect on Mitoxantrone inhibition of cell growth arrest and apoptosis of prostate cancer cells.
2005 ³⁰⁰ X	Dietary supplementation of silymarin in rats decreased the incidence of induced rat prostate carcinoma.
2010 ³⁰¹	Silibinin reversed epithelial mesenchymal transition, induced upregulation of cytokeratin-18, and downregulation of vimentin, MMP2, NF-kB nuclear translocation, and transcription factors ZEB1 and SLUG.
2010 ³⁰²	Isosilybin A induced apoptosis in prostate cancer cells through phosphoAkt, NF-kB, and AR downregulation.
2003 ³⁰³ X	In nude mice with xenografted prostate carcinomas, silibinin feeding increased apoptosis and reduced growth and angiogenesis.
2002 ³⁰⁴ X	In nude mice xenografted with human prostate carcinoma cells silibinin feeding decreased tumor growth by 35% to 58%.
2002 ³⁰⁵	In human androgen-dependent prostate cancer silibinin inhibited Rb phosphorylation and increased Rb-E2F.

silymarin failed to improve the results, there was a slight—not significant—trend towards reduced metastasis.²⁴¹ We think that this study had some flaws which included a small sample size (15 patients with silymarin and 15 with placebo), tumors being present in different organs, and very low dosage (420 mg/day). In spite of these flaws, the trend towards a decrease in metastasis is still interesting and further study with a larger sample population was suggested by the authors.

Silymarin: Decreasing Side Effects and Toxicity of Chemotherapeutic Drugs

Silymarin coadministered with chemotherapeutic drugs has the ability to reduce toxicity in normal organs^{242,243}.

- It protects against liver and kidney toxicities induced by methotrexate in children and adults treated for leukemia.^{244,245}

- Silibinin decreased cisplatin's nephrotoxicity without affecting its antitumoral effectiveness.^{246,247}
- There is also evidence that it protects the heart from doxorubicin toxicity, however, it is less potent than quercetin in this effect.²⁴⁸
- Silymarin reduced docetaxel central and peripheral neurotoxicity.²⁴⁹
- Silymarin was able to decrease diarrhea produced by irinotecan treatment.²⁵⁰
- Silymarin reduced hepatotoxicity in patients with nonmetastatic breast cancer receiving doxorubicin /cyclophosphamide-paclitaxel.²⁵¹

Silymarin and Resistance to Treatment

Rho et al²⁵² found that adding silymarin to epidermal growth factor receptor tyrosine kinase inhibitors could overcome

Table 4. Lung NSCLC: Nonsmall Cell Lung Cancer//SCLC: Small Cell Lung Cancer.

Year, Ref.	Findings
2004 ³⁰⁶	Silibinin impeded invasion by inhibiting expression of uPA and MMP2 and enhancing expression of TIMP2.
2013 ³⁰⁷	Silibinin meglumine impeded the epithelial–mesenchymal transition in EGFR mutant NSCLC cells.
2010 ³⁰⁸	Silibinin decreased NSCLC cell growth through cell cycle arrest and decreased cell cycle modulators.
2011 ³⁰⁹	Silymarin produced apoptosis in a highly metastatic lung cancer cell line through the mitochondrial caspase pathway.
2003 ³¹⁰	Silibinin inhibited growth and apoptosis in NSCLC and SCLC line cells in a dose-dependent manner.
2012 ³¹¹	HDAC inhibitors in combination with silibinin showed enhanced antitumor activity in NSCLC cells. There was also increased transcription of p21 through higher acetylation of its promoter. The augmented p21 was responsible for proteasomal destruction of cyclin B1.
2013 ³¹²	EGFR-mutated lung cancer cells, resistant to erlotinib and overexpressing ALDH, were resensitized by silibinin.
2009 ³¹³ X	Treating a xenograft lung cancer mouse model with silibinin resulted in a decreased tumor size through decreased angiogenesis. HIF-1 α was also decreased by silibinin.
2016 ³¹⁴	Through downregulation of STAT3, silibinin reinstated sensitivity to crizotinib therapy in ALK rearranged lung cancer.

Table 5. Bladder.

Year, Ref.	Findings
2017 ²⁵³	Silibinin reversed chemotherapeutic resistance in bladder cancer cells in a NF-kB signal-dependent and independent manner.
2017 ³¹⁵	Silibinin is an antiproliferative compound whose mechanism of action depended on p53 status (WT or mutated).
2002 ³¹⁶ X	In an induced bladder carcinogenesis mouse model, silymarin reduced the incidence of bladder lesions and cell proliferation. Silymarin acted as a preventive compound.
2017 ³¹⁷	The anticancer mechanism of silibinin in bladder cancer was through downregulation of the actin cytoskeleton, the PI3K/Akt pathway and KRAS.
2011 ³¹⁸ X	Oral silibinin prevented carcinogenesis, decreased proliferation, and increased apoptosis <i>in vivo</i> in a mouse model. Intravesical silibinin had a similar effect.
2013 ³¹⁹ X	Silibinin decreased bladder cancer metastasis and prolonged animal survival through downregulation of the GSK3 β /catenin pathway and Zeb1 expression. Silibinin also suppressed EMT and stemness.

resistance produced by the T790M mutation in NSCLC xenografts. The mechanism of action seems to be impeding EGFR dimerization. It was found that bladder cancer cell lines resistant to cisplatin could be resensitized with silymarin.²⁵³ A similar result was obtained with ovarian cancer cells resistant to paclitaxel.²⁵⁴ The mechanism involved in resensitization to chemotherapeutic drugs is not fully known, however possible factors are: inhibition of NF-kB nuclear migration,¹³⁶ inhibition of survivin protein levels, downregulation of Pgp (MDR 1),^{255–258} and multidrug resistance-associated protein 1 (MRP 1).^{258,259} Silychristin (a component of silymarin) and silychristin derivatives have shown the particular ability to inhibit Pgp activity in a concentration-dependent manner²⁶⁰ (see Tables 2 to 12).

Silymarin's Cancer Chemopreventive Actions

Table 5 summarizes the findings by Vinh et al that show that silymarin was able to significantly decrease the incidence of bladder neoplasms in male rats receiving the carcinogenic substance *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. Interestingly, these results were achieved by oral administration of silymarin and were found in those animals that received silymarin not only at the initiation of carcinogenesis, but also in those of the postcarcinogenic period (for more examples on chemoprevention, see Tables 2 to 12).

Silymarin and Hormonal Receptors

Silymarin is a selective estrogen β receptor (ER- β) agonist.²⁶¹ However, it also has some estrogenic effects through ER- α .²⁶² Silymarin has strong binding affinity to ER- β and a mild affinity for ER- α .²⁶³ Silymarin's estrogenic actions should be seriously considered as a problem in female hormone-dependent tumors. Furthermore, silymarin's estrogenic effects are confirmed by the observation that it produces benefits in menopausal women with hot flashes.¹¹⁶ Contrario sensu, it may be advantageous in benign prostate hyperplasia^{264,265} and prostate cancer. However, in an experiment carried out in albino rats, silymarin increased testosterone and LH.²⁶⁶ It also increased spermatogenesis in rats.²⁶⁷ In spite of these 2 findings, the evidence for silymarin benefits in prostate cancer is abundant (see Table 3). Our conclusion is that the possible benefits found in prostate cancer are independent of silymarin's hormonal effects.

Silymarin Inhibits Clathrin-Dependent Trafficking

Endocytosis is an important mechanism of cell intercommunication which acquires major relevance in cancer. This process is initiated by the invagination of the plasma membrane. The protein clathrin provides A coat to this invagination (Figure 5). The clathrin coated vesicle has the ability to select for the adequate cellular receptor.²⁶⁸ There is also endocytosis without clathrin coating.

Table 6. Pancreas.

Year, Ref.	Findings
2011 ³²⁰	Silibinin induced cell cycle arrest and apoptosis in certain pancreatic cancer cell lines.
2015 ³²¹	The combination of an HDAC inhibitor and silibinin had additive effects on growth inhibition and apoptosis of pancreatic cancer cells.
2015 ³²² X	In an orthotopic model of pancreatic cancer, silibinin reduced glycolytic activity of cancer cells, proliferation, and cachexia.
2013 ³²³ X	Dose-dependent cell growth inhibition was produced by silibinin concentrations between 25 and 100 μ M. In xenograft in nude mice, tumor weight was significantly decreased by dietary silibinin.
2018 ³²⁴	SW1990 pancreatic cancer cells showed G1 arrest with decreased cyclins and CDKs and apoptosis with silibinin.

Table 7. Breast.

Year, Ref.	Findings
2004 ³²⁵	Silymarin, as part of a mixture of flavonoids, downregulated the Breast Cancer Resistance Protein (BCRP). The authors propose a “flavonoid cocktail” for this purpose.
2004 ³²⁶	Silibinin synergized with conventional chemotherapeutic drugs in anticancer effects on breast cancer cells.
2009 ³²⁷	Silibinin decreased MMP9 and VEGF expression induced by TPA through downregulation of the Raf/Mek/Erk pathway.
2013 ³²⁸	Silymarin showed synergy with doxorubicin in producing MCF7 cell apoptosis
2014 ³²⁹	Silymarin showed much higher proapoptotic gene induction in a lung cancer cell line than in a breast cancer cell line.
2014 ³³⁰ X	Silibinin inhibited the accumulation of myeloid derived suppressor cells (MDSC) in murine breast cancer and increased overall survival. Silibinin decreased tumor volume.
2015 ³³¹	Silibinin induced autophagic death in breast cancer cells. Silibinin treatment decreased ATP levels and altered mitochondrial electric potential with increased ROS accumulation.
2015 ³³²	Silibinin induced apoptosis in breast cancer cells. (Comment: the concentrations used were too high and are not achievable in human use).
2015 ³³³	ER α inhibition was a key factor in silibinin-induced autophagy and apoptosis. Using ER α inhibitors with silibinin, both apoptosis and autophagia were further increased.
2016 ³³⁴	Silibinin decreased BCL2 proteins in breast cancer cells and normal breast cells and ununiformly increased PTEN in different cancer cell lines.
2017 ³³⁵	Silibinin sensitized breast cancer cells to doxorubicin treatment. (Comment: The concentrations used were excessively high and difficult to achieve in the clinical setting).
2017 ³³⁶	Silymarin-loaded iron nanoparticles produced cell cycle arrest in triple negative breast cancer cells.
2017 ³³⁷	Silymarin’s anticancer effects were due to inhibition of Akt and MAPK pathway.
2021 ³³⁸ X	Silymarin decreased proliferation and viability of MDA-MB-231 and MCF-7 cells in a concentration-dependent manner, inducing apoptosis. These results were obtained <i>in vitro</i> and <i>in vivo</i> .

Silymarin has the ability to inhibit clathrin-dependent trafficking at least in the case of certain viruses such as Hepatitis C virus, reovirus, influenza virus,^{269,270} and Hepatitis B virus.²⁷¹ The mechanism behind this inhibition is through interference with the clathrin endocytic pathway. Actually, silymarin interferes with all the clathrin-dependent endocytic processes. Taxifolin, a close relative of silybin, was also found to inhibit receptor-mediated endocytosis of β -hexosaminidase in normal fibroblast culture. There were similar findings with other flavonoids.²⁷²

Although there is no experimental evidence in this sense, we may presume that silymarin decreases endocytic trafficking in cancer cells too. Additionally, clathrin has protumoral effects beyond endocytosis: it switches TGF- β into a procancer role.²⁷³

Figure 6 shows a simplified overview of the clathrin-dependent endocytosis.

Silymarin and Renal Carcinoma

When targeting renal carcinoma cells with silymarin, migration and invasion were significantly decreased by inhibition of the

EGFR/MMP-9 pathway: silymarin blocked phosphorylation of EGFR and ERK1/ERK2 and reduced expression of MMP-9.²⁷⁵ This was confirmed by Liang et al²⁷⁶ and by Chang et al *in vivo*²⁷⁷ and in our unpublished observations with 2 patients with grade IV clear cell renal carcinoma, who experienced no new metastasis after they started on silymarin.

Silymarin and Pancreatic Cancer

Silymarin has not been extensively tested in pancreatic ductal adenocarcinoma (PDAC) (see Table 6). However, it does have an important antifibrotic effect. One of the major problems in PDAC is the intense stromal reaction with abundant production of stromal collagen fibers.²⁷⁸ These impede delivery of the chemotherapeutic drug to the tumor mass and create interstitial hypertension through the strongly hydrophilic hyaluronan. Therefore, silymarin’s antifibrotic effects may provide an interesting complement to standard treatment.²⁷⁹

Desmoplastic tumors are the consequence of the intense activity of cancer-associated fibroblasts (CAFs) producing

Table 8. Colon.

Year, Ref.	Findings
2002 ³³⁹ X	Silymarin inhibited chemically induced carcinogenesis of the colon in mice.
2013 ³⁴⁰ X	Silibinin blocked TNF α -induced NF- κ B activation <i>in vitro</i> and <i>in vivo</i> . Tumor growth and progression were concomitantly inhibited. Bcl2, COX2, VEGF, and MMPs levels were also diminished by silibinin feeding of xenotransplanted mice.
2015 ³⁴¹	Silymarin induced proteasomal degradation of cyclin D1 and inhibited growth of colon cancer cells.
2016 ³⁴²	Treatment with silymarin increased the efficacy of ionizing radiation on colon cancer cells causing increased cell death.
2017 ³⁴³	Silibinin inhibited proliferation and increased apoptosis in colon cancer cells.
2017 ³⁴⁴ X	The combination of regorafenib and silybin had synergistic antiproliferative and proapoptotic effect. This combination was tested in 22 patients with metastatic colon cancer. No control group was available.
2020 ³⁴⁵	Silymarin, associated with other nutraceuticals, reduced intestinal polyp growth in an animal model.

Table 9. Liver.

Year, Ref.	Findings
2005 ³⁴⁶	Silibinin strongly inhibited growth of hepatocellular cancer cells. It also increased apoptosis with inhibition of CDK2, CDK4, and CDC2 kinases.
2006 ³⁴⁷ X	Silymarin inhibited hepatocarcinogenesis induced by nitrosodiethylamine.
2008 ³⁴⁸ X	Silymarin decreased the expression of MMP2 and MMP9 and decreased recruitment of mast cells <i>in vivo</i> in a rat liver carcinogenesis model.
2008 ³⁴⁹	Silibinin decreased cell proliferation and migration of human hepatocellular cancer cells by inhibiting the Erk 1/2 cascade.
2009 ³⁵⁰	Silymarin decreased growth of hepatocellular carcinoma (HCC) cells and induced apoptosis.
2009 ³⁵¹ X	In a xenograft mouse model of HCC, silibinin reduced growth and proliferation through reduction of Akt/Erk signaling and increased histone acetylation.
2015 ³⁵²	Silibinin increased growth inhibition of hepatocarcinoma cells by either sorafenib or gefitinib.
2020 ³⁵³	Silymarin showed antimetastatic and proapoptotic effects on HepG2 cells through the Slit-2/Robo-1 pathway.

collagen fibers. PDAC and the liver have specialized CAFs known as stellate cells considered the producers of the desmoplastic reaction. Silymarin has been found to inhibit/decrease the desmoplastic reaction through 2 mechanisms:

- (a) it inhibits TGF β 2 that induces the desmoplastic phenotype of naïve fibroblasts,²⁸⁰
- (b) it increases E-cadherin expression^{281,282} decreasing the invasive nature of the desmoplastic reaction.

Silymarin decreased fibrosis not only in 2 models of induced liver fibrosis^{37,283} but also in lung fibrosis induced by cigarette smoke.²⁸⁴ In this last case, this occurred by downregulation of the TGF- β 1/Smad 2/3 pathway signaling.

Although we could not find any publication showing that silymarin could reduce the desmoplastic reaction in pancreatic cancer, we may assume that it has the potential to do so, because the mechanisms behind this are similar to those found in liver and lung cancers. Long et al suggested this possibility, however they did not incorporate any evidence in their review.²⁷⁹

Tables 2 to 12: Evidence of silymarin's anticancer effects. *In vivo* experiments are marked with an X in column 1.

In spite of the long list of publications mentioned in Tables 2 to 12, 5 cautionary notes should be added:

1. In a mouse model of induced mammary carcinogenesis, the administration of silymarin, slightly increased mammary tumor incidence.³⁶⁹ This may be due to silymarin's estrogenic effects,^{115,261,370} however, the issue remains controversial because silymarin increases ER β and decreases ER α expression.²⁶⁴
2. In a model of mouse hepatic carcinogenesis (with diethyl-nitrosamine), silymarin showed no effects at all.³⁷¹
3. In a mouse model of alcohol-dependent hepatocarcinoma, silibinin increased tumor progression if chronic alcohol intake continued.³⁷²
4. Many of the *in vitro* experiments described in Table 2 to 12 were performed at very high concentrations that are difficult or impossible to achieve *in vivo*. On the other hand, *in vivo* experiments (marked with an X in Tables 2 to 12) were mainly conducted with oral administration of silymarin or silibinin, so those results should have a more significant impact on future clinical research.
5. Most of the published literature on silymarin and cancer does not mention the p53 status of the cells and this information is of capital importance (silymarin shows apoptotic effects on p53 positive cells but not on mutated p53).

Pharmacokinetics

Flavolignans (silymarin is a mixture of flavolignans) generally have poor bioavailability. This is the consequence of:

Table 10. Ovary.

Year, Ref.	Findings
2014 ³⁵⁴	Silymarin suppressed cell growth and induced caspase-dependent apoptosis with increased p53, p21, and p27, and decreased CDK2.
2003 ³⁵⁵ X	A silybin-phosphatidylcholine complex decreased tumor growth in xenografted mice (tumor weight inhibition of 78%).
2013 ³⁵⁶ X	Silibinin decreased tumor growth <i>in vitro</i> and <i>in vivo</i> through downregulation of Erk and Akt signaling.

Table 11. Hematologic.

Year, Ref.	Disease	Findings
2001 ¹⁴³	Promyelocytic leukemia	Silymarin inhibited proliferation and induces differentiation into monocytes. It showed synergy with vitamin D3.
2010 ³⁵⁷	Acute myeloid leukemia	Silibinin induced differentiation of acute myeloid leukemia cells <i>ex vivo</i> (only in cases in which there were no chromosome aberrations).
2016 ³⁵⁸	Lymphoma	Silibinin induced apoptosis in Alk-positive anaplastic large cell lymphoma by suppressing the phosphorylation of NPM/ALK.
2020 ³⁵⁹	Lymphoma	Epstein-Barr positive lymphoma cell proliferation was inhibited and apoptosis induced through NF-kB inhibition by silymarin.
2016 ³⁶⁰	Multiple myeloma	Silybin suppressed myeloma cell proliferation and induced apoptosis by inhibiting the PI3K/Akt/mTOR pathway.

1. their strongly hydrophobic nature that does permit dilution to more than 50 µg/mL in water. Some organic solvents have a much better performance for this purpose. For example, ethanol shows a solubility of 225 mg/mL;³⁷³
2. the fact that they are quickly metabolized;
3. the fact they are poorly absorbed in the intestine.

The pharmacokinetic considerations we shall make refer to the standardized form of silymarin with known amounts of silybin.

Absorption. silymarin is not soluble in water and oral administration shows poor absorption in the alimentary tract (approximately 1% in rats,³⁷⁴ however, other authors mention a higher absorption around 30%). In spite of this low absorption, according to Janiak et al, a plasma level of 500 mg/L (500 µg/mL) is achievable 90 min after oral administration of 200 mg/kg of silymarin in mice.³⁷⁵

Excretion. Silymarin is mainly excreted in the bile and half-life is 6 h.

Toxicity. Toxicity is almost absent³⁷⁶ and therefore high oral doses can be administered with negligible side effects.

Dose/absorption studies in humans. A number of other studies have administered various doses and studied the plasma concentration. For example, with oral administration of 240 mg of silybin to 6 healthy volunteers the following results were obtained³⁷⁷:

maximum plasma concentration 0.34 ± 0.16 µg/mL

and time to maximum plasma concentration 1.32 ± 0.45 h. Absorption half life 0.17 ± 0.09 h, elimination half life 6.32 ± 3.94 h.³⁷⁷ Beckmann-Knopp et al³⁷⁸ also found: “*Mean maximum plasma concentration after an oral dose of 700 mg silymarin, containing 254 mg of silibinin, is 317 ng/ml or 0.6 mM. Accumulation in plasma during three daily medications is negligible. Plasma protein binding is reported to reach about*

90–95%.” After feeding volunteers with a smaller dose of 80 mg of a lipophilic silybin-phosphatidylcholine complex (silipide) Gatti et al³⁷⁹ found that free unconjugated silybin reached a maximum concentration of 141 ng/mL after 2.4 h. The level of conjugated silybin peaked after 3.8 h reaching 255 ng/mL. Another study on 6 healthy volunteers used a larger dose of 560 mg of silymarin and attained concentrations starting at 0.18 and going as high as 0.64 µg/mL.³⁷⁷ These results are quite different and to some extent controversial.

Absorption studies in animals. Administration of silybin to animals also showed divergent results. In dogs,³⁸⁰ the silybin-phosphatidylcholine complex (SPC) showed increased concentrations when compared with silymarin extract, however, the results showed a low level in general: SPC: 1.310 ± 880 ng/ml; silymarin: 383 ± 472 ng/ml. While Morazzoni et al³⁸¹ found higher peak levels of silybin in the form of silipide when administered to rats: “*After oral silipide, silybin reached peak plasma levels within 2 h, with a C_{max} of 9.0 ± 3.0 µg/ml for unconjugated drug and 93.4 ± 16.7 µg/ml for total (free + unconjugated drug).*”

Pharmacodynamic conclusions: The above studies show that the achievable concentration in humans (with a low dose) is far lower than what was found in rodents (with a high dose). The important issue is that most of the experiments found in the literature at cellular level used a concentration around 100 µg/mL. Even in the study by Morazzoni et al,³⁸¹ the level of 100 µg/mL was not achieved and in any case it is a peak level that cannot be sustained. Therefore, is the experimental level of 100 µg/mL achievable at the bedside?

We think that there is no evidence that it can be. Oral administration of silymarin in humans achieves nanogram, but not microgram levels. Furthermore, we should not extrapolate Morazzoni’s findings in rats to humans as their pharmacokinetics may differ.

Table 12. Miscellaneous.

Year, Ref.	Organ/tumor	Findings
2013 ³⁶¹	Pharynx	Silymarin increased PTEN, reduced Akt phosphorylation, and induced apoptosis in pharynx squamous cell carcinoma cells.
2015 ³⁶²	Glioma	Silibinin induced apoptosis in glioma cells through downregulation of PI3K pathway and decreased FoxM1 transcription factor expression.
2018 ³⁶³ X	Oral cancer	<i>In vivo</i> , silymarin reduced tumor growth and volume; <i>in vitro</i> it produced apoptosis through induction of the DR5 (death receptor 5)-caspase 8-truncated Bid pathway.
2017 ³⁶⁴	Salivary glands	Silymarin increased proapoptotic Bim protein in mucoepidermoid carcinoma cells, inducing apoptosis.
2012 ³⁶⁵	Cervical	Silymarin induced apoptosis in cervical cancer cells through increase of PTEN, inhibition of Akt phosphorylation, and decreased expression of MMP9.
2007 ³⁶⁶	Osteosarcoma	Silibinin decreased human osteosarcoma cell invasion through Erk inhibition of a FAK/ERK/uPA/MMP2 pathway.
2017 ³⁶⁷	Rabdoid tumor	Silibinin inhibited rabdoid tumor cell migration and invasion through inhibition of the PI3K/Akt pathway.
2019 ³⁶⁸	Human gastric cancer cells	Silymarin downregulated the MAP kinase pathway inhibiting growth and inducing apoptosis.

Therefore, the evidence based on these high concentration experiments should be viewed with caution. On the other hand, experiments with xenograft models are more reliable (Tables 2 to 12, xenograft results are marked with an X).

Tissue concentration. For cancer treatment purposes the important data to know are the concentrations achievable in tissues. Zhao and Agarwal³⁸² found the following results in mice 30 min after administration:

Liver: 8.8 µg per gram of tissue
 Lung: 4.3 µg per gram of tissue
 Stomach: 123 µg per gram of tissue
 Pancreas: 5.8 µg per gram of tissue
 Prostate: 2.5 µg per gram of tissue after 1 h.

After an oral intake, silipide (the lipophilic SPC), achieved a maximum concentration of silybin in bile within 4 h and then declined with a mean time of approximately 10 h.³⁸³ Silibinin complexed with the amino-sugar meglumine is water soluble and can reach a tissue concentration high enough to show clear antigrowth effects in NSCLC xenografts.³⁰⁷ The distribution in different tissues also varies widely according to the type of tissue considered. It is higher in the liver and diminishes in lungs, pancreas, and prostate.³⁸² A relatively high concentration is achievable in colorectal mucosa (20-141 nmol/g of tissue).³⁸⁴

The tissue levels obtainable compare unfavorably with those used in cell studies. To achieve apoptosis in cell studies, a concentration of more than 20 µM was necessary,³⁸⁵ and this concentration does not seem easy to achieve by oral intake of standard preparations. It was also necessary to use a concentration of 100 µg/mL to induce apoptosis in Ramos cells (B lymphocytes).³⁸⁶ Kamrani et al³⁸⁷ used concentrations between 50 and 100 µg/mL to induce apoptosis in colon cancer cells. Therefore, while only a nanomolar concentration can be attained in tissues, micromolar concentrations were needed to induce apoptosis in these studies (the molecular weight of silybin is 482, 100 µg/ml = 207 µM).

In spite of this difficulty, Sing and Agarwal²⁹⁸ found an important decrease in tumor volume in xenografted mice with human prostate carcinoma cells when the mice were orally fed with silymarin.

There are also different requirements for effects on cell migration versus proliferation. For endothelial cells, it was necessary to use a concentration of 48.1 µg/mL of silymarin to achieve a 20% reduction in proliferation and 16.1 µg/mL to achieve the same reduction in proliferation of LoVo colon cancer cells¹⁵⁶ to achieve a reduction of migration of 50%, it is necessary a concentration of 1.15 µg/mL on endothelial cells (with silibinin instead of silymarin 0.66 µg/mL were enough to achieve the same).¹⁵⁶ Our conclusion is that, from a bioavailability standpoint, it is much easier to achieve migration inhibition, than proliferative reduction.

In Europe one of the most used brands of silymarin is Legalon® L (silybin 3,23-O-bis-hemisuccinate) that comes in capsules of 150 mg. It also comes in vials containing 350 mg of silibinin for intravenous use. In the United States, silymarin is considered a nutritional supplement.³⁸⁸ The intake of 5 of these capsules in 6 human volunteers, showed no adverse events. The concentration in plasma correlated with the dose and only 10% of it was unconjugated silymarin. A half life of 6 h was estimated.³⁸⁹

In experimental conditions, many researchers dilute silybin in DMSO, a polar solvent in which silymarin is highly soluble. Unfortunately, this is not possible at the bedside.

Pharmaceutical Methods to Increase Bioavailability

Silymarin's low solubility, rapid metabolism, and quick excretion, led researchers and pharmaceutical industry to develop methods that could solve these very important drawbacks. Therefore, many compounds have been formulated mainly using nanotechnology. These compounds include nanosuspensions, solid dispersions, complexes with cyclodextrins and phospholipids, microemulsions, nanoemulsions, liposomes, polymer nanocarriers, solid-lipid nanoparticles and nanostructured

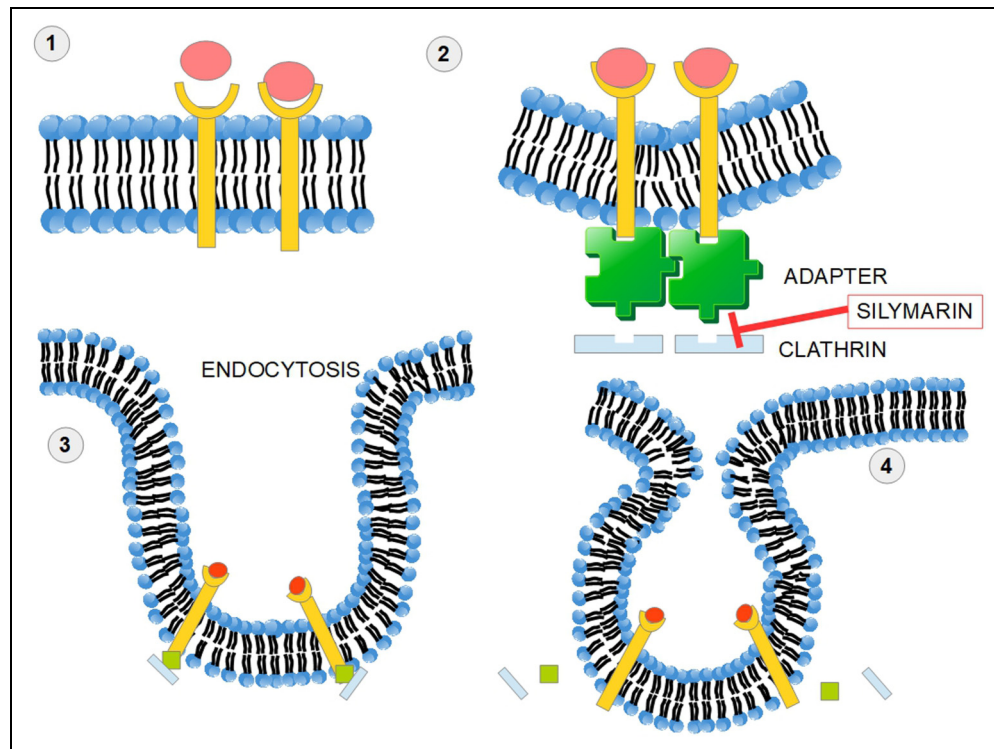


Figure 6. Silymarin impedes clathrin-dependent endocytosis. In receptor-mediated endocytosis, or clathrin-mediated endocytosis, cells incorporate hormones, proteins, and sometimes viruses in a selective manner that depends on a ligand-receptor interaction. (1) The ligand-receptor interaction takes place on the cell surface. (2) An adapter protein and clathrin “coat” the internal surface of the receptors and a process of membrane invagination starts. (3) Advanced invagination process. (4) The adaptor protein and clathrin are released. Silymarin impedes this mechanism of endocytosis. Clathrin-dependent endocytosis of surface receptors participates in cellular signaling pathways. Clathrin seems to be a valid target in cancer therapy.²⁷⁴

lipid carriers, and polymer-based nanocarriers.³⁹⁰ We shall discuss only a few of them.

- ▶ **Combination with succinate:** is available on the market under the trade mark Legalon® (bis hemisuccinate silybin).
- ▶ **Combination with phosphatidylcholine:** this was the first system developed for a better bioavailability: it consists of the combination of 2 molecules of phosphatidylcholine with one of silybin. It has been registered under the name Siliphos®, but is also known as Idb1016, silypide, or phytosome.^{391–394} This method increased bioavailability 10-fold.³⁹⁵
- ▶ **Silybin-cyclodextrin complex:** adding cyclodextrin considerably enhances silymarin’s water solubility.
- ▶ **Other combinations with:** meglumine, 23-O-phosphate. The problem with silybin combinations is that although they increase water solubility at the same time, they may reduce other effects such as antioxidant properties.²³
- ▶ **Nanosuspensions:** are colloidal dispersions of drug particles with surfactants on the surface or other kind of synthetic stabilizers. This method improves dissolution and prolongs drug half life.³⁹⁶

- ▶ **Polymeric micelles:** are nano-sized particles in which a hydrophobic substance is fully covered by a hydrophilic external layer. Wu et al³⁹⁷ developed a silybin core included in amphiphilic chitosan micelles.
- ▶ **Self micro-emulsifying drug delivery systems (SMEDDS):** are mixtures of oil and surfactants. Liu et al³⁹⁸ developed a silybin SMEDD that significantly increased its bioavailability.
- ▶ **Liposomes:** are lipid bilayer structures with a silybin core. This composition substantially improves bioavailability.³⁹⁹
- ▶ **Inclusion in polymeric matrices** that carry and protect the drug.⁴⁰⁰

There are many other mechanisms based on nano-particles that increase absorption, prolong half life, and improve water solubility of silymarin, that escape the scope of this article. For a review of the issue, see Di Costanzo et al⁴⁰¹ and Piazzini et al⁴⁰² (Figure 7).

Dosage and side Effects

A phase I study of silymarin in prostate cancer patients showed that 13 g daily per os divided into 3 doses was well tolerated.

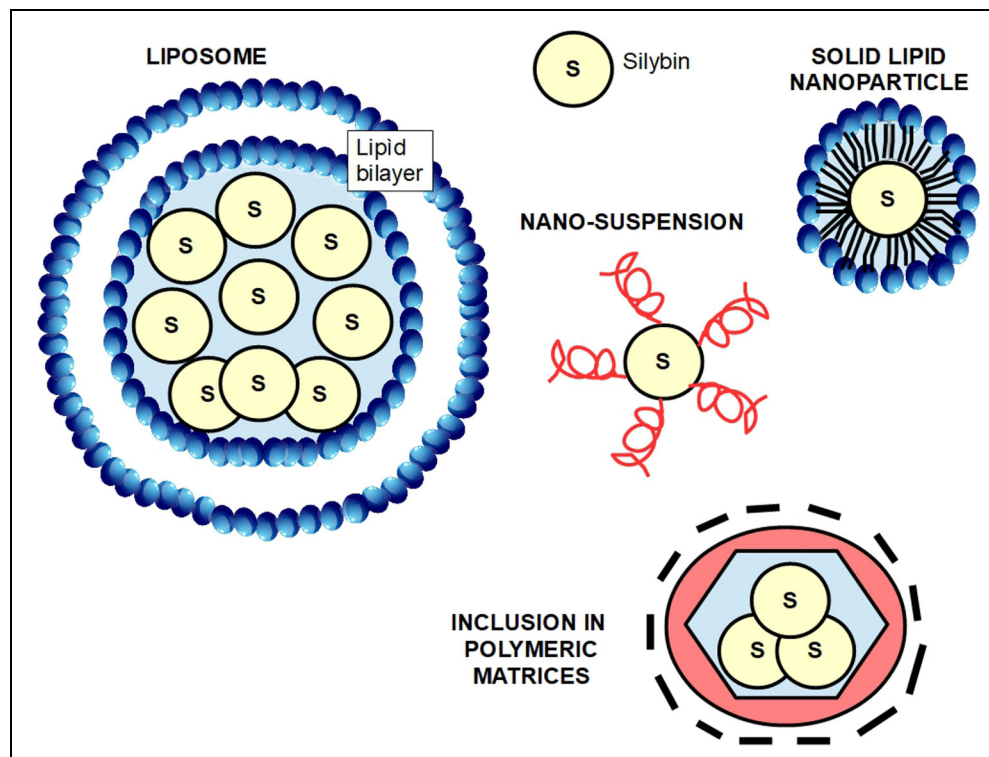


Figure 7. Methods to increase silymarin's bioavailability.

The most frequent adverse event was asymptomatic liver toxicity.⁴⁰³ Side effects, although rare, were mainly related to the gastrointestinal tract, such as diarrhea, bloating, and nausea.⁴⁰⁴ Abenavoli et al⁴⁰⁵ found that daily doses beyond 1500 mg had laxative effects and increased bile flow. The usual dose of 400 or 800 mg a day is probably insufficient to achieve anticancer effects. It may be necessary to administer 800 mg 4 times a day because the half-life is short. However, the dose of silymarin for cancer treatment remains controversial. In one study, a high dose of silybinin was administered to patients prior to prostatectomy (13 g daily). They achieved high plasma concentrations, but nevertheless, low levels of silybinin were found in prostate tissue.⁴⁰⁶ In an attempt to circumvent some of these problems one group used a silymarin-phosphatidylcholine compound administered orally as a daily dose of 2.8 g for 4 weeks prior to surgery. They achieved high levels in human breast cancer tissue.⁴⁰⁷ This high bioavailability in this breast cancer study is an encouraging signal for a phase II clinical trial. It should also be noted that silymarin constituents have different anticancer abilities,²² therefore a formulation of the strongest combination would represent a fundamental step in order to incorporate this flavonoid into standard treatments.

The Main Problems with Silymarin

Problem 1: Bioavailability. The evidence gathered in Tables 2 to 12 clearly shows that silymarin should have a place in cancer treatment. The main problem is its bioavailability. Many of the *in vitro* investigations have used concentrations

that are very difficult to achieve at the bedside. The combination of silymarin with phosphatidylcholine (silipide) has a better bioavailability, however this combination is not available for clinical use.

Problem 2: Dual nature of silymarin's effects. Silymarin has protumoral and antitumoral effects. For example, in pancreatic cancer it promotes growth arrest and apoptosis (see Table 6) and decreases CD44 signaling. However, Lee et al⁴⁰⁸ found that in addition to the antitumoral actions, silymarin also upregulated cancer stemness-related genes, namely TWIST1, Snail, and c-Jun. At the same time, it decreased p53 wild type and increased Ki-67 (a marker of proliferation). This is a powerful call for caution. On the other hand, in bladder cancer, silymarin seems to decrease stemness through inhibition of the β -catenin/ZEB1 signaling (Wu 178). In pancreatic tumors (PANC1), it was also found that silymarin targeted stem cells decreasing proliferation and increasing apoptosis,⁴⁰⁹ and had similar effects in breast cancer cells.^{410,411} These controversies on silymarin prostemness or antistemness effects may be due to context or tumor dependency. The question remains unsolved.

Problem 3: DNA intercalation. In 2020, Pawar and Jaldappagari⁴¹² reported that flavolignans had the ability to intercalate into the DNA double helix with moderate binding affinity. Other authors have vehemently contradicted this finding.⁴¹³ However, if this silymarin effect on DNA is confirmed, it may have unthought consequences which are favorable (modulating gene activities against cancer) or undesirable (genotoxicity and or mutations). The issue is important enough to encourage further basic research in this area.

Clinical Trials

The United States Clinical Trials web-page lists the following trials for silymarin in cancer:

1. NCT03130634: The Efficacy of Silymarin as Adjuvant Therapy on Colorectal Cancer patients Undergoing FOLFIRI Treatment.
State: recruiting since 2017.
Kaohsiung Medical University Chung-Ho Memorial Hospital. Taiwan.
Study Design: This is an open-label, randomized, comparative, double arm, single center study to assess efficacy of Silymarin (150 mg 3 times a day) as adjuvant therapy on metastatic colorectal cancer patients undergoing FOLFIRI chemotherapy in Taiwan.
2. NCT00487721: The Effect of High-dose Silybin-phytosome in Men With Prostate Cancer. (A Pilot Biomarker Study of Oral Silybin-Phytosome Followed by Prostatectomy in Patients With Localized prostate cancer).
State: completed 2014
University of Colorado. Denver
Subjects will take Silibin-Phytosome for 2 to 10 weeks. The dose of Silibin-Phytosome is 13 g daily, in 3 divided doses.
Outcome: To determine if measurable silibinin tissue levels are detectable in the prostate glands of men treated with Silybin-Phytosome administered according to the protocol.
Results: low concentration of silymarin in prostatic tissue.¹¹⁷
3. NCT01829178: Evaluation of Effects of Silymarin on Cisplatin Induced Nephrotoxicity in Upper Gastrointestinal Adenocarcinoma.
State: completed 2015
University of Tehran
This study looked for possible protective effects of silymarin on kidney injury in patients receiving cisplatin.
No results posted.
4. NCT00055718: Silymarin (Milk Thistle Extract) in Treating Patients with Acute Lymphoblastic Leukemia Who Are Receiving Chemotherapy.
State: completed 2013
Miami Children's Hospital, Winthrop University Hospital, Mount Sinai Medical School.
This study looked for hepatoprotective effects of silymarin in patients receiving chemotherapy.
No results posted.
5. NCT02146118: A Phase II Study to Assess Efficacy of Combined Treatment with Erlotinib (Tarceva) and Silybin-phytosome (Siliphos) in Patients With EGFR Mutant Lung Adenocarcinoma.
State: unknown
Gosin University. Busan, Korea.
No results.

6. NCT01402648: Estrogen Receptor Beta Agonists (Eviendep) and Polyp Recurrence
State: completed 2011
Ospedale Policlinico Consorziiale—Gastroenterology Unit. Bari, Italy
No results.

The conclusion we reach regarding clinical trials is:

1. There were only 2 clinical trials (4 and 5) to determine therapeutic possibilities of silymarin against cancer. Neither have published results.
2. The dose used in the clinical trials showed differences of up to 1000% which clearly means that there is no standard dose.

Schröder et al⁴¹⁴ conducted a randomized double-blind, cross-over placebo-controlled trial (with 2 periods of 10 weeks with a wash out period in the middle) with 49 patients that showed rising PSA levels after radical prostatectomy (34) or radiotherapy (15). They received a supplement containing soy, different isoflavones, silymarin, vitamins, minerals, and antioxidants. While receiving the treatment the doubling time for PSA was 1150 days compared with 445 days with the placebo. The fact that the supplement contained many other components besides the silymarin makes it impossible to draw conclusions about this compound. But it is evident that the supplement modified the biochemical evolution of the disease, delaying PSA progression.

Four Clinical Cases

Four clinical case reports are available, which though they cannot in themselves constitute a proof for the efficacy of silymarin, are nonetheless interesting and suggest a need for further studies. Hsu et al⁴¹⁵ describe the case of a 66-year-old Taiwanese patient with a regression of an 11 cm diameter hepatocellular carcinoma. The patient was receiving 450 mg of silymarin daily, and no other medication. Even if we cannot consider this regression as a consequence of silymarin treatment, the fact that spontaneous regression of hepatomas is quite infrequent, makes us think of some intervention of silymarin in this unusual event. Moroni and Zanlorenzi⁴¹⁶ published another case of complete regression of an advanced unresectable hepatocellular carcinoma treated with sorafenib and silymarin. Additionally, Bosch-Barrera et al⁴¹⁷ presented 2 cases of brain metastases from lung cancer in which the treatment with silymarin decreased edema and the size of metastases, without improvement of the primary tumor.

Discussion

The concept of a tumor as a consequence of the mutation of one gene, and with one driver signaling or metabolic pathway, is flawed in most cases with the exception of cases such as chronic myeloid leukemia. Usually many genes and pathways

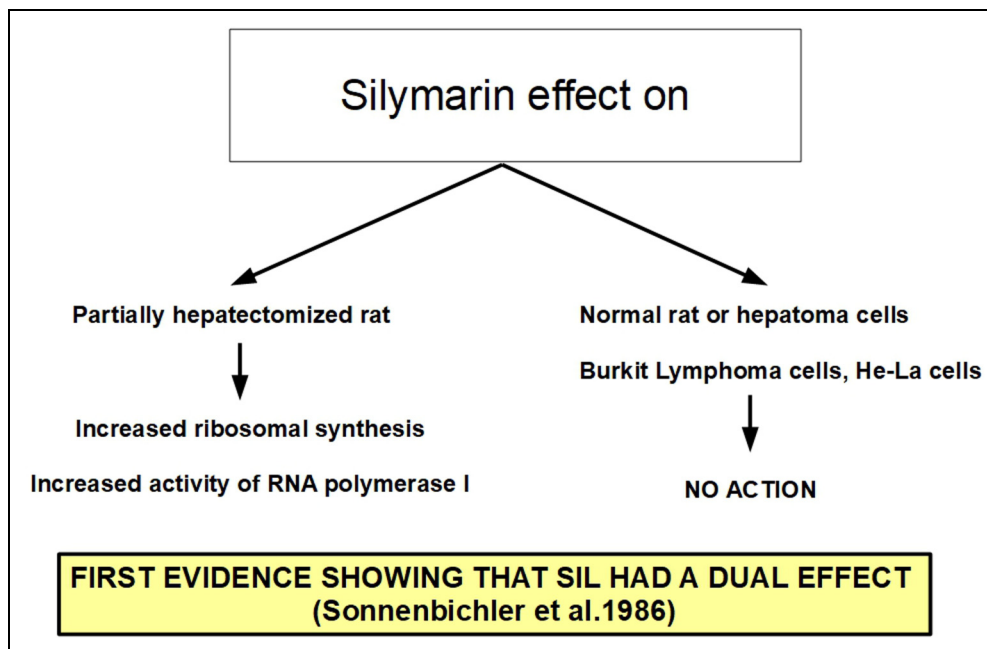


Figure 8. Different effects of silymarin according to context.

are involved. The approach of attacking only one of the many hallmarks of cancer is also flawed.⁴¹⁸ Recent evidence suggests that multiple genes are usually involved along with many signaling pathways, all interconnected, and interdependent and generating an extraordinary ability of tumor cells to survive and resist internal and external threats.⁴¹⁹ This is one of the reasons why treatments made up of many different drugs are implemented in most treatment protocols.

Silymarin and its derivatives, through its multipronged attacks, allow one drug to reach many targets at the same time. Of course, we cannot expect silymarin to “cure” cancer all by itself, and it cannot replace any conventional chemotherapeutic treatment, but it is rather a privileged companion to therapeutic schemes in which it may develop useful complementary activity. This activity entails 3 concepts:

- (a) cancer prevention;
- (b) synergy with some treatment protocols;
- (c) decrease of collateral damage induced by chemotherapeutic drugs.

Silymarin’s clinically achievable concentration in serum and at the tumor site, with the possible exception of the liver, seems insufficient for inducing apoptosis. However, xenograft model experiments showed that even with this low bioavailability drawback, silymarin could stop tumor growth.^{288,351,356}

The first studies on silymarin activity in cancer were performed in hepatic cells showing some characteristics that cannot be really considered antitumoral such as increased ribosomal synthesis and RNA polymerase I activation. This did not happen in hepatoma cells or in other malignant cells (Figure 8).

Antiproliferative activity was found against almost all types of tumors, whether solid or nonsolid (Tables 2 to 12). These findings were confirmed not only at cellular level but also *in vivo*.

Silymarin has many other antitumor effects that can complement mainstream treatment protocols, such as:

- reduction of cell motility and invasion through TGF- β 2 inhibition;
- inhibition of HIF-1 α translation;
- decreased TIMP1 expression, thus decreasing metalloproteases activation;
- inhibition of the EGFR-MMP9 pathway;
- decreasing the accumulation of MDSCs in the tumor;
- inhibition of ERK and AKT signaling;
- protection against off-target toxicity of chemotherapeutic drugs;
- synergistic or added effects with some chemotherapeutics;
- reduction of extracellular fibronectin production.

To this short list we must add that there is evidence sustaining clear benefits in clinical cases such as hepatocarcinoma and clear cell renal carcinoma.

However, silymarin also has some effects that work against classical chemotherapy. For example, its ability as an antioxidant reduces ROS production. Many of the drugs currently used against cancer are precisely based on the creation of an oxidative stress with increased ROS that induces apoptosis of malignant cells.

Therefore, we must ask: why is silymarin a useful complement to chemotherapy?

Evidence indicates that there may be 2 possible answers:

1. silymarin has context-dependent effects: its behavior is different in normal and malignant cells as can be seen in BOX 2.
2. Its anticancer effects overwhelm those that seem favorable for cancer cell survival.

Box 2. built on references.^{420–423}

Silymarin effects in normal cells

- Increased protein synthesis through increased ribosome formation and RNA polymerase I stimulation.
- Antioxidant effects through increased glutathione production and ROS scavenging.
- Decreased electron leak in the electron transport chain through uncoupling.
- Increased level of mitochondrial membrane potential.
- Anti-apoptotic effect under cellular stress.

Silymarin effects in malignant cells

- Decreased cancer cell migration
- Decreased proliferation
- Decreased angiogenesis
- Decreased expression of HIF-1 α
- Decreased expression of TIMP1
- Anti-desmoplastic effects
- Reduced/inhibited NF- κ B activation
- Inhibited T-cell inflammatory cytokines
- Decreased expression of TGF- β 2
- Decreased expression of LPR1
- Inhibited Wnt signaling
- Reduced activation of prostaglandin E2
- Modulation of integrin β 1 signaling
- Reduced activation of NHE1
- Reduced/inhibited STAT3 activation
- Activated autophagy
- Reversed chemoresistance

The dual effects of silymarin can be clearly seen in an experiment by Su et al.⁴²⁴ Here nasopharyngeal carcinoma cells were exposed to low concentrations of silymarin and this showed protumoral effects: “*Silymarin increased the expressions of superoxide dismutase 1, catalase, and glutathione peroxidase. Consequently, the cell apoptosis was reduced markedly. An increase of Bcl-2 expression and a decrease of activated caspase-3....*” Additionally, there is the problem of increased stemness induced by silymarin in pancreatic adenocarcinoma cells.

In spite of these isolated discouraging report,⁴²⁴ the overwhelming sum of experimental evidence shows that silymarin has clear anticancer effect, and at the same time protects normal cells from the collateral damage of chemotherapy. There is reasonable doubt that the concentration that can be reached *in vivo* is enough to induce apoptosis. However, xenografted tumors were reduced in size suggesting that somehow silymarin induces tumor apoptosis. In a small cohort prospective study there were no benefits found when silymarin was added to standard chemotherapy. On the other hand, a slight tendency to lower metastatic behavior was seen in the cohort receiving silymarin.⁴²⁵

As already mentioned, silymarin is not a stand-alone compound against cancer and it should be used alongside other medications. On this point, it is important to note that silymarin is an inhibitor of the Cytochrome P450 system, particularly silychristin. Therefore, caution should be exercised in this respect.⁴²⁶

Silybin could be used as a scaffold or structure that can be modified improving its antitumoral effects. For example, Manivannan et al⁴²⁷ have synthesized silybin analogues with increased anticancer capacity. One of these compounds

named “15k,” was very potent and selective for ovarian cancer cells, where it bound to tubulin with high affinity. Subsequent experiments found that 15k induced growth arrest and apoptosis of ovarian cancer cells at a much lower concentration than silymarin. Furthermore, it showed no toxicity in animals.⁴²⁸

Finally, it is important to note that many of silymarin’s multipronged antitumoral actions are equally, or sometimes even better conveyed by other flavonoids such as genistein and epigallocatechin gallate.^{429,430}

What Remains to be Done?

In the first place, some of silymarin’s protumoral effects demand further research with the objective of ascertaining if they need to be counteracted. Then, the precise silymarin concentrations required for the different antitumoral effects need to be established. And finally, the tumor concentration achievable with the different pharmaceutical preparations has to be determined. Once these 3 pieces of information are combined silymarin will be ready for serious clinical trials as a complement to classical chemotherapeutic schedules.

Conclusions

Silymarin compounds have considerable antitumoral effects. Well-planned clinical trials should be necessary to finally assess its bedside indications. Its dual, antitumoral and protumoral, effects merit further research.

Silymarin should be used at very high doses because low concentrations may induce protumoral effects. There is no toxicity even with very high doses. Silymarin’s low absorption and bioavailability make it preferable to use modified pharmaceutical forms, such as nanoparticles or conjugated with compounds that increase its water solubility. These combinations already exist even if they have not been marketed as yet. The abundant existing evidence shows that silymarin has a definite place in cancer treatment. It has the ability to interfere with the expression of proteins related to cell cycle regulation, apoptosis, angiogenesis, and multidrug resistance. These characteristics define an anticancer drug. On the other hand, its strong antioxidant activity makes it a useful drug in cancer prevention.

Silymarin’s lack of toxicity, even at very high doses, and the lack of effects on normal cells are important reasons for its further development.

Is there any other drug, that with no toxicity at all that can:

1. Inhibit EGFR signaling
2. Upregulate CDK inhibitors such as p21 and p27
3. Downregulate CDKs
4. Induce growth arrest by interfering MAPkinases cascade
5. Induce apoptosis
6. Inhibit TGF- α
7. Reduce the expression of VEGF and VEGFR
8. Inhibit the AKT axis
9. Decrease tumoral fibrosis

10. and have many other antitumoral effects?

Probably no other drug can achieve all these results without adverse events or high toxicity. We cannot expect that such a nontoxic pharmaceutical work as a stand-alone drug against cancer. But it can be an important factor in a multidrug anticancer schedule. Having mentioned this, the reports of increased stemness problem remain an unsolved issue which needs further investigation.

The inability to patent the compound is no doubt a drawback for the pharmaceutical industry and will restrict investment in these types of compounds. In its bioavailable formulations, silymarin deserves to be tested on clinical grounds, not as a stand-alone pharmaceutical, but as part of a treatment schedule.

Finally, the low concentrations that can be achieved with silymarin extracts at the bedside (in the order of ng/mL) hints to a serious bias in much of the past and present research at the cellular level where the average concentration range used was between 50 and 100 µg/mL. As a precondition for repurposing silymarin, newer pharmaceutical formulations should be screened in order to establish whether they can reach the necessary therapeutic concentrations.

Abbreviations

AR	androgen receptor
BCRP	Breast cancer resistance protein.
CDK	cyclin dependent kinase
CA	carbonic anhydrase
CAF	cancer associated fibroblast
COX2	cyclooxygenase 2
CXCR4	C-X-C chemokine receptor type 4
DMBA	dimethylbenzanthracene
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EMT	epithelial–mesenchymal transition
ER	estrogen receptor.
ERB1	eukaryotic ribosome biogenesis protein 1
ERK	extracellular signal-regulated kinases
FDA	Food and Drug Administration (USA)
FKBP5	FK506 binding protein 5
HCC	hepatocellular carcinoma
HIF-1 alpha	hypoxia inducible factor 1 alpha
HDAC	histone deacetylase
IGFBP3	insulin like growth factor binding protein 3
IL	interleukin
MDSC	myeloid derived suppressor cell
MAPK	mitogen-activated protein kinase
MMP	metalloproteases
MDR	multidrug resistance protein
MRP1	multidrug resistance-associated protein 1
NF-kB	nuclear factor-kappa B
NSCLC	nonsmall cell lung cancer
PDAC	pancreatic ductal adenocarcinoma
PDGF	platelet derived growth factor
PD-L1	programmed death ligand 1
PSA	prostate specific antigen

Rb	retinoblastoma protein
ROS	reactive oxygen species
SCLC	small cell lung cancer
SIRT1	NAD-dependent deacetylase sirtuin-1
SLIT2	slit homolog 2 protein
SMEDDS	self micro-emulsifying drug delivery systems
SPC	silybin phosphatidyl coline
SREBP1	sterol regulatory element binding protein 1
STAT3	signal transducer and activator of transcription 3
TGF	transforming growth factor
TPA	tetradecanoylphorbol acetate
TRAIL	tumor necrosis factor (TNF)-related apoptosis-inducing ligand
TRAMP	transgenic adenocarcinoma of the mouse prostate
UPAR	urokinase plasminogen activator receptor
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
ZEB1	zinc finger E-box-binding homeobox 1

Acknowledgments

Ms. Julia Hanna Weiss cooperated in the revision and correction of the article.

Author Contributions

Both authors equally contributed in the writing of the article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.


Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects

ORCID iD

Tomas Koltai  <https://orcid.org/0000-0002-7398-5096>

Trial Registration

Not applicable, because this article does not contain any clinical trials.

Plagiarism

All the figures, tables, and boxes are original and were developed by the authors.

References

1. Delmas D. Silymarin and derivatives: from biosynthesis to health benefits. *Molecules*. 2020;25(10):2415. <https://doi.org/10.3390/molecules25102415>

2. Young JA, Evans RA, Hawkes RB. Milk thistle (*Silybum marianum*) seed germination. *Weed Sci.* 1978;26(4):395-398. <https://doi.org/10.1017/S0043174500050189>
3. Letteron P, Labbe G, Degott C, et al. Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice. *Biochem. Pharmacol.* 1990;39:2027-2034. [https://doi.org/10.1016/0006-2952\(90\)90625-U](https://doi.org/10.1016/0006-2952(90)90625-U)
4. Křen V, Walterova D. Silybin and silymarin—new effects and applications. *Biomed Papers.* 2005;149(1):29-41. <https://doi.org/10.5507/bp.2005.002>
5. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. *Drugs.* 2001;61(14):2035-2063. <https://doi.org/10.2165/00003495-200161140-00003>
6. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González Á, et al. Hepatoprotective effect of silymarin. *World J Hepatol.* 2014;6(3):144. <https://doi.org/10.4254/wjh.v6.i3.144>
7. Flora K, Hahn M, Rosen H, Benner K. Milk thistle (*Silybum marianum*) for the therapy of liver disease. *Am J Gastroenterol.* 1998;93(2):139-143. [https://doi.org/10.1016/S0002-9270\(97\)00082-8](https://doi.org/10.1016/S0002-9270(97)00082-8)
8. Theodosiou E, Purchartová K, Stamatis H, Kolisis F, Křen V. Bioavailability of silymarin flavonolignans: drug formulations and biotransformation. *Phytochem Rev.* 2014;13(1):1-18. <https://doi.org/10.1007/s11101-013-9285-5>
9. Polyak SJ, Ferenci P, Pawlowsky JM. Hepatoprotective and antiviral functions of silymarin components in hepatitis C virus infection. *Hepatology.* 2013;57(3):1262-1271. <https://doi.org/10.1002/hep.26179>
10. Federico A, Dallio M, Loguerzio C. Silymarin/silybin and chronic liver disease: a marriage of many years. *Molecules.* 2017;22(2):191. <https://doi.org/10.3390/molecules22020191>
11. Schadewaldt H. The history of silymarin. Contribution to the history of liver therapy. *Med Welt.* 1969;20(15):902-914. PMID: 5784380.
12. Andrzejewska J, Sadowska K, Mielcarek S. Effect of sowing date and rate on the yield and flavonolignan content of the fruits of milk thistle (*Silybum marianum* L. Gaertn.) grown on light soil in a moderate climate. *Ind Crops Prod.* 2011;33(2):462-468. <https://doi.org/10.1016/j.indcrop.2010.10.027>
13. Takemoto T, Ikegawa S, Nomoto K. Studies on constituents of *Silybum marianum* (L.) Gaertn. I. New flavonolignans named 2,3-dehydrosilymarin and 2,3-dehydrosilychristin. *AGRIS (FAO of the UN).* 1975; 95(8):1017 PMID: 123760. <https://doi.org/10.1248/yakushi1947>
14. Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME. Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*). *Org Biomol Chem.* 2003;1:1684-1689. <https://doi.org/10.1039/B300099K>
15. Möschlin G. Phd thesis, Karlsruhe University, 1959, mentioned in Biedermann, D., Vavříková, E., Cvak, L., & Křen, V. Chemistry of silybin. *Natural Prod Rep.* 2014;31(9):1138-1157. <https://doi.org/10.1039/C3NP70122K>
16. Wagner H, Hörhammer L, Seitz M. Chemical evaluation of a silymarin-containing flavonoid concentrate from *Silybum marianum* (L.) Gaertn. *Arzneimittelforschung.* 1968;8(6):696-698. PMID: 5755806.
17. Wagner H, Hörhammer L, Münster R. On the chemistry of silymarin (silybin), the active principle of the fruits from *Silybum marianum* (L.) Gaertn. (*Carduus marianus* L.). *Arzneimittelforschung.* 1968;18(6):688-696. PMID: 5755805.
18. Pelter A, Hansel R. The structure of silybin (silybum substance E6), the first flavonolignan. *Tetrahedron Lett.* 1968;9(25):2911-2916. [https://doi.org/10.1016/S0040-4039\(00\)89610-0](https://doi.org/10.1016/S0040-4039(00)89610-0)
19. Cardone RA, Alfarouk KO, Elliott RL, et al. The role of sodium hydrogen exchanger 1 in dysregulation of proton dynamics and reprogramming of cancer metabolism as a sequela. *Int J Mol Sci.* 2019;20(15):3694. <https://doi.org/10.3390/ijms20153694>
20. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. *Nat Rev Cancer.* 2011;11(9):671-677. <https://doi.org/10.1038/nrc3110>
21. Simánek V, Křen V, Ulrichová J, Vicar J, Cvak L. Silymarin: what is in the name...? An appeal for a change of editorial policy. *Hepatology.* 2000;32(2):442-444. <https://doi.org/10.1053/jhep.2000.9770>
22. Biedermann D, Vavříková E, Cvak L, Křen V. Chemistry of silybin. *Nat Prod Rep.* 2014;31(9):1138-1157. <https://doi.org/10.1039/C3NP70122K>
23. Gažák R, Svobodová A, Psotová J, et al. Oxidised derivatives of silybin and their antiradical and antioxidant activity. *Bioorg Med Chem.* 2004;12(21):5677-5687. <https://doi.org/10.1016/j.bmc.2004.07.064>
24. Kroll DJ, Shaw HS, Oberlies NH. Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies. *Integr Cancer Ther.* 2007;6(2):110-119. <https://doi.org/10.1177/1534735407301825>
25. Deep G, Oberlies NH, Kroll DJ, Agarwal R. Identifying the differential effects of silymarin constituents on cell growth and cell cycle regulatory molecules in human prostate cancer cells. *Int J Cancer.* 2008;123(1):41-50. <https://doi.org/10.1002/ijc.23485>
26. Ding TM, Tian SJ, Zhang ZX, et al. Determination of active component in silymarin by RP-LC and LC/MS. *J Pharm Biomed Anal.* 2001;26(1):155-161. [https://doi.org/10.1016/S0731-7085\(01\)00364-8](https://doi.org/10.1016/S0731-7085(01)00364-8)
27. Desplaces A, Choppin J, Vogel G, Trost W. The effects of silymarin on experimental phalloidine poisoning. *Arzneim-Forsch.* 1975;25(1):89-96. PMID: 125090.
28. Tuchweber B, Sieck R, Trost W. Prevention by silybin of phalloidin-induced acute hepatotoxicity. *Toxicol Appl Pharmacol.* 1979;51(2):265-275. [https://doi.org/10.1016/0041-008X\(79\)90469-1](https://doi.org/10.1016/0041-008X(79)90469-1)
29. Mayer KE, Myers RP, Lee SS. Silymarin treatment of viral hepatitis: a systematic review. *J Viral Hepat.* 2005;12(6):559-567. <https://doi.org/10.1111/j.1365-2893.2005.00636.x>
30. Fried MW, Navarro VJ, Afdhal N, et al. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: a randomized controlled trial. *Jama.* 2012;308(3):274-282. <https://doi.org/10.1001/jama.2012.8265>

31. Hawke RL, Schrieber SJ, Soule TA, Wen Z, Smith PC, Reddy KR, SynCH Trial Group. Silymarin ascending multiple oral dosing phase I study in noncirrhotic patients with chronic hepatitis C. *J Clin Pharmacol*. 2010;50(4):434-449. <https://doi.org/10.1177/0091270009347475>
32. Wagoner J, Negash A, Kane OJ, et al. Multiple effects of silymarin on the hepatitis C virus lifecycle. *Hepatology*. 2010; 51(6):1912-1921. doi : <https://doi.org/10.1002/hep.23587>
33. Trinchet JC, Coste T, Levy VG, et al. Treatment of alcoholic hepatitis with silymarin. A double-blind comparative study in 116 patients. *Gastroenterol Clin Biol*. 1989;13(2):120-124. PMID: 2707520.
34. Soliman NA, El-Dardiry SA. Taraxacum officinale and Silybum marianum alone or combined orchestrate experimentally induced hepatic steatosis through lipogenesis, glucose tolerance and oxidant/antioxidant status. *Int J Biol Chem Sci*. 2015; 9(4):1918-1928. <https://doi.org/10.4314/ijbcs.v9i4.17>
35. Ni X, Wang H. Silymarin attenuated hepatic steatosis through regulation of lipid metabolism and oxidative stress in a mouse model of nonalcoholic fatty liver disease (NAFLD). *Am J Transl Res*. 2016;8(2):1073. PMID: 27158393.
36. Zhu SY, Jiang N, Yang J, et al. Silybum marianum oil attenuates hepatic steatosis and oxidative stress in high fat diet-fed mice. *Biomed Pharmacother*. 2018;100:191-197. <https://doi.org/10.1016/j.biopha.2018.01.144>
37. Clichici S, Olteanu D, Nagy AL, Oros A, Filip A, Mircea PA. Silymarin inhibits the progression of fibrosis in the early stages of liver injury in CCl4-treated rats. *J Med Food*. 2015; 18(3):290-298. <https://doi.org/10.1089/jmf.2013.0179>
38. Ferenci P, Dragosics B, Dittrich H, et al. Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. *J Hepatol*. 1989;9(1):105-113. [https://doi.org/10.1016/0168-8278\(89\)90083-4](https://doi.org/10.1016/0168-8278(89)90083-4)
39. Tighe SP, Akhtar D, Iqbal U, Ahmed A. Chronic liver disease and silymarin: a biochemical and clinical review. *J Clin Transl Hepatol*. 2020;8(4):454. <https://doi.org/10.14218/JCTH.2020.00012>
40. Basaga H, Poli G, Tekkaya C, Aras I. Free radical scavenging and antioxidative properties of 'silibin' complexes on microsomal lipid peroxidation. *Cell Biochem Funct*. 1997;15(1):27-33. [https://doi.org/10.1002/\(SICI\)1099-0844\(199703\)15:1<27::AID-CBF714>3.0.CO;2-W](https://doi.org/10.1002/(SICI)1099-0844(199703)15:1<27::AID-CBF714>3.0.CO;2-W)
41. Machicao F, Sonnenbichler J. Mechanism of the stimulation of RNA synthesis in rat liver nuclei by silybin. *Hoppe Seylers Z Physiol Chem*. 1977;358(2):141-147. <https://doi.org/10.1515/bchm2.1977.358.1.141>
42. Shriewer H, Krämer U, Rukowski G, Borjjs KJ. Influence of silybin-dihemisuccinate on fatty acid synthesis in rat liver. *Arzneimittelforschung*. 1979;29(3):524-526. PMID: 39576.
43. Fiebrich F, Koch H. Silymarin an inhibitor of prostaglandin synthetase. *Experientia*. 1979;35(12):1550-1552. <https://doi.org/10.1007/BF01953185>
44. Fiebrich F, Koch H. Silymarin an inhibitor of lipooxygenase. *Experientia*. 1979;35(12):1548-1560. <https://doi.org/10.1007/BF01953184>
45. Bindoli A, Cavallini L, Siliprandi N. Inhibitory action of silymarin of lipid peroxide formation in rat liver mitochondria and microsomes. *Biochem Pharmacol*. 1977;26(24):2405-2409. [https://doi.org/10.1016/0006-2952\(77\)90449-X](https://doi.org/10.1016/0006-2952(77)90449-X)
46. Sonnenbichler J, Goldberg M, Hane L, Madubunyi I, Vogl S, Zetl I. Stimulatory effect of silibinin on the DNA synthesis in partially hepatectomized rat livers: non-response in hepatoma and other malign cell lines. *Biochem Pharmacol*. 1986;35(3):538-541. [https://doi.org/10.1016/0006-2952\(86\)90233-9](https://doi.org/10.1016/0006-2952(86)90233-9)
47. Koch HP, Bachner J, Löffler E. Silymarin: potent inhibitor of cyclic AMP phosphodiesterase. *Methods Find Exp Clin Pharmacol*. 1985;7(8):409-413. PMID: 3001454.
48. Miadonna A, Tedeschi A, Leggieri E, Lorini M, Froidi M, Zanussi C. Effects of silybin on histamine release from human basophil leucocytes. *Br J Clin Pharmacol*. 1987;24(6):747-752. <https://doi.org/10.1111/j.1365-2125.1987.tb03241.x>
49. Moroni PL, Barcellini W, Borghi M. Silybinin inhibition of human T lymphocyte. Activation. *Int J Tissue React*. 1988; 10(3):177-181. PMID: 3265704.
50. Chávez E, Bravo C. Silymarin-induced mitochondrial Ca²⁺ + release. *Life Sci*. 1988;43(12):975-981. [https://doi.org/10.1016/0024-3205\(88\)90542-5](https://doi.org/10.1016/0024-3205(88)90542-5)
51. Valenzuela A, Guerra R, Videla LA. Antioxidant properties of the flavonoids silybin and (+)- cyanidanol-3: comparison with butylated hydroxyanisole and butylated hydroxytoluene. *Planta Me*. 1986;52(6):438-440. <https://doi.org/10.1055/s-2007-969247>
52. Wilasrusmee C, Kittur S, Shah G, et al. Immunostimulatory effect of Silybum Marianum (milk thistle) extract. *Med Sci Monit*. 2002;8(11):BR439-BR443. PMID: 12444368.
53. Esmail N, Anaraki SB, Gharagozloo M, Moayedi B. Silymarin impacts on immune system as an immunomodulator: one key for many locks. *Int Immunopharmacol*. 2017;50:194-201. <https://doi.org/10.1016/j.intimp.2017.06.030>
54. Wu ZS, Wu Q, Yang JH, et al. Prognostic significance of MMP-9 and TIMP-1 serum and tissue expression in breast cancer. *Int J Cancer*. 2008;122(9):2050-2056. <https://doi.org/10.1002/ijc.23337>
55. Stolf AM, Cardoso CC, Acco A. Effects of silymarin on diabetes mellitus complications: a review. *Phytother Res*. 2017; 31(3):366-374. <https://doi.org/10.1002/ptr.5768>
56. Tuorkey MJ, El-Desouki NI, Kamel RA. Cytoprotective effect of silymarin against diabetes-induced cardiomyocyte apoptosis in diabetic rats. *Biomed Environ Sci*. 2015;28(1):36-43. <https://doi.org/10.3967/bes2015.004>
57. Rafieian-Kopaie M, Nasri H. Silymarin and diabetic nephropathy. *J Renal Inj Prev*. 2012;1(1):3. <https://doi.org/10.12861/jrip.2012.02>
58. García-Ramírez M, Turch M, Simó-Servat O, Hernández C, Simó R. Silymarin prevents diabetes-induced hyperpermeability in human retinal endothelial cells. *Endocrinologia, diabetes y nutricion*. 2018;65(4):200-205. <https://doi.org/10.1016/j.endinu.2017.12.004>
59. Krečman V, Škottová N, Walterová D, Ulrichová J, Šimánek V. Silymarin inhibits the development of diet-induced

- hypercholesterolemia in rats. *Planta Med.* 1998;64(02):138-142. <https://doi.org/10.1055/s-2006-957391>
60. Milić N, Milošević N, Suvajdžić L, Žarkov M, Abenavoli L. New therapeutic potentials of milk thistle (*Silybum marianum*). *Nat Prod Commun.* 2013;8(12). <https://doi.org/10.1177/1934578X1300801236>
 61. Skottova N, Krecman V, Walterova D, Ulrichová J, Simanek V. Effects of silymarin and silybin on lipoprotein cholesterol levels and oxidizability of low density lipoproteins in rats. *Atherosclerosis.* 1997;134(1-2):134-134. eLIBRARY ID: 279046. ISSN0021-9150.
 62. Haddadi R, Shahidi Z, Eyvari-Brooshghalan S. Silymarin and neurodegenerative diseases: therapeutic potential and basic molecular mechanisms. *Phytomedicine.* 2020;79:153320. <https://doi.org/10.1016/j.phymed.2020.153320>
 63. Borah A, Paul R, Choudhury S, et al. Neuroprotective potential of silymarin against CNS disorders: insight into the pathways and molecular mechanisms of action. *CNS Neurosci Ther.* 2013;19(11):847-853. <https://doi.org/10.1111/cns.12175>
 64. Wang MJ, Lin WW, Chen HL, et al. Silymarin protects dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation. *Eur J Neurosci.* 2002;16(11):2103-2112. <https://doi.org/10.1046/j.1460-9568.2002.02290.x>
 65. Murata N, Murakami K, Ozawa Y, et al. Silymarin attenuated the amyloid β plaque burden and improved behavioral abnormalities in an Alzheimer's disease mouse model. *Biosci, Biotechnol, Biochem.* 2010;74(11):2299-2306. <https://doi.org/10.1271/bbb.100524>
 66. Yaghmaei P, Azarfar K, Dezfulian M, Ebrahim-Habibi A. Silymarin effect on amyloid- β plaque accumulation and gene expression of APP in an Alzheimer's disease rat model. *DARU J Pharmaceut Sci.* 2014;22(1):1-7. <https://doi.org/10.1186/2008-2231-22-24>
 67. Sato M, Murakami K, Uno M, et al. Structure-activity relationship for (+)-taxifolin isolated from silymarin as an inhibitor of amyloid β aggregation. *Biosci, Biotechnol, Biochem.* 2013;77(5):1100-1103. <https://doi.org/10.1271/bbb.120925>
 68. Shariati M, Shaygannejad V, Abbasirad F, et al. Silymarin restores regulatory T cells (tregs) function in multiple sclerosis (MS) patients in vitro. *Inflammation.* 2019;42(4):1203-1214. <https://doi.org/10.1007/s10753-019-00980-9>
 69. Navabi F, Shaygannejad V, Abbasirad F, et al. Immunoregulatory effects of silymarin on proliferation and activation of Th1 cells isolated from newly diagnosed and IFN- β 1b-treated MS patients. *Inflammation.* 2019;42(1):54-63. <https://doi.org/10.1007/s10753-018-0872-x>
 70. Yön B, Belviranlı M, Okudan N. The effect of silymarin supplementation on cognitive impairment induced by diabetes in rats. *J Basic Clin Physiol Pharmacol.* 2019;30(4). <https://doi.org/10.1515/jbcpp-2018-0109>
 71. Jin G, Bai D, Yin S, et al. Silibinin rescues learning and memory deficits by attenuating microglia activation and preventing neuroinflammatory reactions in SAMP8 mice. *Neurosci Lett.* 2016;629:256-261. <https://doi.org/10.1016/j.neulet.2016.06.008>
 72. Soto CP, Perez BL, Favari LP, Reyes JL. Prevention of alloxan-induced diabetes mellitus in the rat by silymarin. *Comparative Biochemistry and Physiology Part C: pharmacology. Toxicol Endocrinol.* 1998;119(2):125-129. [https://doi.org/10.1016/S0742-8413\(97\)00198-9](https://doi.org/10.1016/S0742-8413(97)00198-9)
 73. Matsuda T, Ferreri K, Todorov I, et al. Silymarin protects pancreatic β -cells against cytokine-mediated toxicity: implication of c-Jun NH2-terminal kinase and janus kinase/signal transducer and activator of transcription pathways. *Endocrinology.* 2005;146(1):175-185. <https://doi.org/10.1210/en.2004-0850>
 74. Huseini HF, Larijani B, Heshmat R, et al. The efficacy of *Silybum marianum* (L.) gaertn.(silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial. *Phytother Res, Int J Devoted Pharmacol Toxicol Eval Nat Prod Derivat.* 2006;20(12):1036-1039. <https://doi.org/10.1002/ptr.1988>
 75. Hussain SAR. Silymarin as an adjunct to glibenclamide therapy improves long-term and postprandial glycemic control and body mass index in type 2 diabetes. *J Med Food.* 2007;10(3):543-547. <https://doi.org/10.1089/jmf.2006.089>
 76. Voroneanu L, Nistor I, Dumea R, Apetrii M, Covic A. Silymarin in type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *J Diabetes Res.* 2016; 20(12) Volume 2016. Article ID 5147468 <https://doi.org/10.1155/2016/5147468>
 77. Sobolová L, Škottová N, Večeřa R, Urbánek K. Effect of silymarin and its polyphenolic fraction on cholesterol absorption in rats. *Pharmacol Res.* 2006;53(2):104-112. <https://doi.org/10.1016/j.phrs.2005.09.004>
 78. Škottová N, Večeřa R, Urbánek K, Váňa P, Walterová D, Cvak L. Effects of polyphenolic fraction of silymarin on lipoprotein profile in rats fed cholesterol-rich diets. *Pharmacol Res.* 2003;47(1):17-26. [https://doi.org/10.1016/S1043-6618\(02\)00252-9](https://doi.org/10.1016/S1043-6618(02)00252-9)
 79. Škottová N, Krečman V. Silymarin as a potential hypocholesterolaemic drug. *Physiol Res.* 1998;47(1):1-7. PMID: 9708694.
 80. Nassuato G, Iemmolo RM, Strazzabosco M. Effect of silibinin on biliary lipid composition experimental and clinical study. *J Hepatol.* 1992;12:290-295. [https://doi.org/10.1016/0168-8278\(91\)90829-Z](https://doi.org/10.1016/0168-8278(91)90829-Z)
 81. Somogyi A, Ecsedi GG, Blazovics A, Miskolczi K, Gergely P, Feher J. Short term treatment of type II hyperlipoproteinaemia with silymarin. *Acta Med Hung.* 1989;46:289-295. PMID: 2699920.
 82. Zima T, Kamenikova L, Janebova M, Buchar E, Crkovska J, Tesar V. The effect of silibinin on experimental cyclosporine nephrotoxicity. *Renal Fail.* 1998;20(3):471-479. <https://doi.org/10.3109/08860229809045136>
 83. Soto C, Pérez J, García V, Uría E, Vadillo M, Raya L. Effect of silymarin on kidneys of rats suffering from alloxan-induced diabetes mellitus. *Phytomedicine.* 2010;17(14):1090-1094. <https://doi.org/10.1016/j.phymed.2010.04.011>
 84. Fallahzadeh MK, Dormanesh B, Sagheb MM, et al. Effect of addition of silymarin to renin-angiotensin system inhibitors on proteinuria in type 2 diabetic patients with overt nephropathy: a randomized, double-blind, placebo-controlled trial. *Am J Kidney Dis.* 2012; 60(6):896-903. <https://doi.org/10.1053/j.ajkd.2012.06.005>

85. Akbari-Kordkheyli V, Abbaszadeh-Goudarzi K, Nejati-Laskokalayeh M, Zarpou S, Khonakdar-Tarsi A. The protective effects of silymarin on ischemia-reperfusion injuries: a mechanistic review. *Iran J Basic Med Sci.* 2019;22(9):968. <https://doi.org/10.22038/ijbms.2019.34284.8147>
86. Rao PR, Viswanath RK. Cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial infarction in albino rats. *Exper Clin Cardiol.* 2007;12(4):179. PMID: 18651002.
87. Hou YC, Liou KT, Chern CM, et al. Preventive effect of silymarin in cerebral ischemia-reperfusion-induced brain injury in rats possibly through impairing NF- κ B and STAT-1 activation. *Phytomedicine.* 2010;17(12):963-973. <https://doi.org/10.1016/j.phymed.2010.03.012>
88. Moghaddam AH, Sangdehi SRM, Ranjbar M, Hasantabar V. Preventive effect of silymarin-loaded chitosan nanoparticles against global cerebral ischemia/reperfusion injury in rats. *Eur J Pharmacol.* 2020;877:173066. <https://doi.org/10.1016/j.ejphar.2020.173066>
89. Senturk H, Kabay S, Bayramoglu G, et al. Silymarin attenuates the renal ischemia/reperfusion injury-induced morphological changes in the rat kidney. *World J Urol.* 2008;26(4):401-407. <https://doi.org/10.1007/s00345-008-0256-1>
90. Turgut F, Bayrak O, Catal F, et al. Antioxidant and protective effects of silymarin on ischemia and reperfusion injury in the kidney tissues of rats. *Int Urol Nephrol.* 2008;40(2):453-460. <https://doi.org/10.1007/s11255-008-9365-4>
91. Demir M, Amanvermez R, Polat AK, et al. The effect of silymarin on mesenteric ischemia-reperfusion injury. *Med Princ Pract.* 2014;23(2):140-144. <https://doi.org/10.1159/000356860>
92. de la Lastra CA, Martin MJ, Motilva V, Jimenez M, La Casa C, Lopez A. Gastroprotection induced by silymarin, the hepatoprotective principle of *Silybum marianum* in ischemia-reperfusion mucosal injury: role of neutrophils. *Planta Med.* 1995;61(02):116-119. <https://doi.org/10.1055/s-2006-958028>
93. Jin Y, Zhao X, Zhang H, Li Q, Lu G, Zhao X. Modulatory effect of silymarin on pulmonary vascular dysfunction through HIF-1 α -iNOS following rat lung ischemia-reperfusion injury. *Exp Ther Med.* 2016;12(2):1135-1140. <https://doi.org/10.3892/etm.2016.3370>
94. Younis NN, Shaheen MA, Mahmoud MF. Silymarin preconditioning protected insulin resistant rats from liver ischemia-reperfusion injury: role of endogenous H₂S. *J Surg Res.* 2016;204(2):398-409. <https://doi.org/10.1016/j.jss.2016.04.069>
95. Rolo AP, Oliveira PJ, Moreno AJ, Palmeira CM. Protection against post-ischemic mitochondrial injury in rat liver by silymarin or TUDC. *Hepatol Res.* 2003;26(3):217-224. [https://doi.org/10.1016/S1386-6346\(03\)00108-6](https://doi.org/10.1016/S1386-6346(03)00108-6)
96. Koçarslan A, Koçarslan S, Aydin MS, et al. Intraperitoneal administration of silymarin protects end organs from multivisceral ischemia/reperfusion injury in a rat model. *Brazilian J Cardiovasc Surg.* 2016;31:434-439. <https://doi.org/10.5935/1678-9741.20160072>
97. Svobodová A, Zdařilová A, Mališková J, Mikulková H, Walterová D, Vostalová J. Attenuation of UVA-induced damage to human keratinocytes by silymarin. *J Dermatol Sci.* 2007;46(1):21-30. <https://doi.org/10.1016/j.jdermsci.2006.12.009>
98. Katiyar SK, Mantena SK, Meeran SM. Silymarin protects epidermal keratinocytes from ultraviolet radiation-induced apoptosis and DNA damage by nucleotide excision repair mechanism. *PLoS ONE.* 2011;6(6):e21410. <https://doi.org/10.1371/journal.pone.0021410>
99. Vostálová J, Tinková E, Biedermann D, Kosina P, Ulrichová J, Rajnochová Svobodová A. Skin protective activity of silymarin and its flavonolignans. *Molecules.* 2019;24(6):1022. <https://doi.org/10.3390/molecules24061022>
100. Altaei T. The treatment of melasma by silymarin cream. *BMC Dermatol.* 2012;12(1):1-6. <https://doi.org/10.1186/1471-5945-12-18>
101. Berardesca E, Cameli N, Cavallotti C, Levy JL, Piérard GE, de Paoli Ambrosi G. Combined effects of silymarin and methylsulfonylethane in the management of rosacea: clinical and instrumental evaluation. *J Cosmet Dermatol.* 2008;7(1):8-14. <https://doi.org/10.1111/j.1473-2165.2008.00355.x>
102. Meeran SM, Katiyar S, Elmets CA, Katiyar SK. Silymarin inhibits UV radiation-induced immunosuppression through augmentation of interleukin-12 in mice. *Mol Cancer Ther.* 2006;5(7):1660-1668. <https://doi.org/10.1158/1535-7163.MCT-06-0095>
103. Palit P, Mukhopadhyay A, Chattopadhyay D. Phyto-pharmacological perspective of silymarin: a potential prophylactic or therapeutic agent for COVID-19, based on its promising immunomodulatory, anti-coagulant and anti-viral property. *Phytother Res.* 2021;2021:1-12. <https://doi.org/10.1002/ptr.7084>
104. Gorla US, Rao K, Kulandaivelu US, Alavala RR, Panda SP. Lead finding from selected flavonoids with antiviral (SARS-CoV-2) potentials against COVID-19: an in-silico evaluation. *Comb Chem High Throughput Screening.* 2021;24(6):879-890. <https://doi.org/10.2174/1386207323999200818162706>
105. Camini FC, da Silva TF, da Silva Caetano CC, et al. Antiviral activity of silymarin against mayaro virus and protective effect in virus-induced oxidative stress. *Antiviral Res.* 2018;158:8-12. <https://doi.org/10.1016/j.antiviral.2018.07.023>
106. Lani R, Hassandarvish P, Chiam CW, et al. Antiviral activity of silymarin against chikungunya virus. *Sci Rep.* 2015;5(1):1-10. <https://doi.org/10.1038/srep11421>
107. da Silva TF, Ferraz AC, Almeida LT, et al. Antiviral effect of silymarin against Zika virus in vitro. *Acta Trop.* 2020;211:105613. <https://doi.org/10.1016/j.actatropica.2020.105613>
108. Rakelly de Oliveira D, Relison Tintino S, Morais Braga MFB, et al. In vitro antimicrobial and modulatory activity of the natural products silymarin and silibinin. *BioMed Res Int.* 2015;2015. <https://doi.org/10.1155/2015/292797>
109. Vereckeí AS, Besch HR, Zipes DP. Combined amiodarone and silymarin treatment, but not amiodarone alone, prevents sustained atrial flutter in dogs. *J Cardiovasc Electrophysiol.* 2003;14:861-867. <https://doi.org/10.1046/j.1540-8167.2003.02446.x>
110. Ágoston M, Örsi F, Free E, et al. Silymarin and vitamin E reduce amiodarone-induced lysosomal phospholipidosis in rats. *Toxicology.* 2003;190:231-241. [https://doi.org/10.1016/S0300-483X\(03\)00188-4](https://doi.org/10.1016/S0300-483X(03)00188-4)

111. Rastegarpanah M, Malekzadeh R, Vahedi H, et al. A randomized, double blinded, placebo-controlled clinical trial of silymarin in ulcerative colitis. *Chin J Integr Med.* 2015;21(12):902-906. <https://doi.org/10.1007/s11655-012-1026-x>
112. Nguyen THT, Trinh NT, Tran HN, et al. Improving silymarin oral bioavailability using silica-installed redox nanoparticle to suppress inflammatory bowel disease. *J Controlled Release.* 2021;331:515-524. <https://doi.org/10.1016/j.jconrel.2020.10.042>.
113. Hagan M, Hayee BH, Rodriguez-Mateos A. (Poly) phenols in inflammatory bowel disease and irritable bowel syndrome: a review. *Molecules.* 2021;26(7):1843. <https://doi.org/10.3390/molecules26071843>
114. Monfared ME, Jadidi A, Hosseini SM, Ashtiani AR. Investigating the effectiveness of silymarin in treatment of migraine patients referred to medical centers affiliated to arak university of medical sciences. *J Biochem Tech.* 2018;9(2):98-101.
115. Capasso R, Aviello G, Capasso F, et al. Silymarin BIO-C®, an extract from *Silybum marianum* fruits, induces hyperprolactinemia in intact female rats. *Phytomedicine.* 2009;16(9):839-844. <https://doi.org/10.1016/j.phymed.2009.02.007>
116. Saberi Z, Gorji N, Memariani Z, Moeini R, Shirafkan H, Amiri M. Evaluation of the effect of *Silybum marianum* extract on menopausal symptoms: a randomized, double-blind placebo-controlled trial. *Phytother Res.* 2020;34(12):3359-3366. <https://doi.org/10.1002/ptr.6789>
117. Kayedpoor P, Mohamadi S, Karimzadeh-Bardei L, Nabiuni M. Anti-inflammatory effect of silymarin on ovarian immunohistochemical localization of TNF- α associated with systemic inflammation in polycystic ovarian syndrome. *Int J Morphol.* 2017;35(2):723-732. <https://doi.org/10.4067/S0717-95022017000200054>
118. Mehta RG, Moon RC. Characterization of effective chemopreventive agents in mammary gland in vitro using an initiation-promotion protocol. *Anticancer Res.* 1991;11(2):593-596. PMID: 1905902.
119. Ruy YC. Advances in pharmacological studies of silymarin. *Mem Inst Oswaldo Cruz.* 1991;86, Suppl 2, 79-85. <https://doi.org/10.1590/s0074-02761991000600020>.
120. Agarwal R, Katiyar SK, Lundgren DW, Mukhtar H. Inhibitory effect of silymarin, an antihepatotoxic flavonoid, on 12-O-tetradecanoylphorbol-13-acetate-induced epidermal ornithine decarboxylase activity and mRNA in SENCAR mice. *Carcinogenesis.* 1994;15(6):1099-1103. <https://doi.org/10.1093/carcin/15.6.1099>
121. Gershbein LL. Action of dietary trypsin, pressed coffee oil, silymarin and iron salt on 1,2- dimethylhydrazine tumorigenesis by gavage. *Anticancer Res.* 1994;14(3A):1113-1116. PMID: 8074460.
122. Kim DH, Jin YH, Park JB, Kobashi K. Silymarin and its components are inhibitors of betaglucuronidase. *Biol Pharm Bull.* 1994;17(3):443-445. <https://doi.org/10.1248/bpb.17.443>
123. Valenzuela A, Garrido A. Biochemical bases of the pharmacological action of the flavonoid silymarin and of its structural isomer silibinin. *Biol Res.* 1994;27(2):105-112. PMID: 8640239.
124. Bartholomaeus AR, Bolton R, Ahokas JT. Inhibition of rat liver cytosolic glutathione S transferase by silybin. *Xenobiotica.* 1994;24(1):17-24. <https://doi.org/10.3109/00498259409043217>
125. Dehmlow C, Murawski N, de Groot H. Scavenging of reactive oxygen species and inhibition of arachidonic acid metabolism by silibinin in human cells. *Life Sci.* 1996;58(18):1591-1600. [https://doi.org/10.1016/0024-3205\(96\)00134-8](https://doi.org/10.1016/0024-3205(96)00134-8)
126. De la Puerta R, Martinez E, Bravo L, Ahumada MC. Effect of silymarin on different acute inflammatory models and leukocyte migration. *J Pharm Pharmacol.* 1996;48(9):968-970. <https://doi.org/10.1111/j.2042-7158.1996.tb06014.x>
127. Zi X, Mukhtar H, Agarwal R. Novel cancer chemopreventive effects of a flavonoid antioxidant silymarin: inhibition of mRNA expression of an endogenous tumor promoter TNF alpha. *Biochem Biophys Res Commun.* 1997;239(1):334-339. <https://doi.org/10.1006/bbrc.1997.7375>
128. Ahmad N, Gali H, Javed S, Agarwal R. Skin cancer chemopreventive effects of a flavonoid antioxidant silymarin are mediated via impairment of receptor tyrosine kinase signaling and perturbation in cell cycle progression. *Biochem Biophys Res Commun.* 1998;247(2):294-301. <https://doi.org/10.1006/bbrc.1998.8748>
129. Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. *J Natl Cancer Inst.* 1997;89(8):556-566. <https://doi.org/10.1093/jnci/89.8.556>
130. Saliou C, Kitazawa M, McLaughlin L, et al. Antioxidants modulate acute solar ultraviolet radiation-induced NF-kappa-B activation in a human keratinocyte cell line. *Free Radical Biology and Medicine.* 1999;26(1-2):174-183. [https://doi.org/10.1016/S0891-5849\(98\)00212-3](https://doi.org/10.1016/S0891-5849(98)00212-3)
131. Lahiri-Chatterjee M, Katiyar SK, Mohan RR, Agarwal R. A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. *Cancer Res.* 1999;59(3):622-632. PMID: 9973210.
132. Scambia G, De Vincenzo R, Ranelletti PO, et al. Antiproliferative effect of silybin on gynaecological malignancies: synergism with cisplatin and doxorubicin. *Eur J Cancer.* 1996;32(5):877-882. [https://doi.org/10.1016/0959-8049\(96\)00011-1](https://doi.org/10.1016/0959-8049(96)00011-1)
133. Zi X, Grasso AW, Kung HJ, Agarwal R. 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. *Cancer Res.* 1998;58(9):1920-1929. PMID: 9581834.
134. Zi X, Feyes DK, Agarwal R. 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. *Clin Cancer Res.* 1998;4(4):1055-1064. PMID: 9563902.
135. Sharma Y, Agarwal C, Singh AK, Agarwa IR. Inhibitory effect of silibinin on ligand binding to erbB1 and associated mitogenic signaling, growth, and DNA synthesis in advanced human prostate carcinoma cells. *Mol Carcinog.* 2001;30(4):224-236. <https://doi.org/10.1002/mc.1032>

136. Saliou C, Rihn B, Cillard J, Okamaoto T, Packer L. Selective inhibition of NF- κ B activation by the flavonoid hepatoprotector silymarin in HepG2. Evidence for different activating pathways. *FEBS Lett.* 1998;440:8-12. [https://doi.org/10.1016/S0014-5793\(98\)01409-4](https://doi.org/10.1016/S0014-5793(98)01409-4)
137. Manna SK, Mukhopadhyay A, Van NT, Aggarwal BB. Silymarin suppresses TNF-induced activation of NF- κ B, c-Jun N-terminal kinase, and apoptosis. *J Immunol.* 1999; 163(12):6800-6809. PMID: 10586080.
138. Zi X, Agarwal R. Modulation of mitogen-activated protein kinase activation and cell cycle regulators by the potent skin cancer preventive agent silymarin. *Biochem Biophys Res Commun.* 1999;263(2):528-536. <https://doi.org/10.1006/bbrc.1999.1398>
139. Agarwal R, Agarwal C, Ichikawa H, Singh RP, Aggarwal BB. Anticancer potential of silymarin: from bench to bed side. *Anticancer Res.* 2006;26(6B):4457-4498. PMID: 17201169.
140. Zhao J, Sharma Y, Agarwal R. Significant inhibition by the flavonoid antioxidant silymarin against 12-O-tetradecanoylphorbol 13-acetate-caused modulation of antioxidant and inflammatory enzymes, and cyclooxygenase 2 and interleukin-1 α expression in SENCAR mouse epidermis: implications in the prevention of stage I tumor promotion. *Mol Carcinog.* 1999;26(4):321-333. [https://doi.org/10.1002/\(SICI\)1098-2744\(199912\)26:4<321::AID-MC11>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1098-2744(199912)26:4<321::AID-MC11>3.0.CO;2-9)
141. Zi X, Zhang J, Agarwal R, Pollak M. Silibinin up-regulates insulin-like growth factor-binding protein 3 expression and inhibits proliferation of androgen-independent prostate cancer cells. *Cancer Res.* 2000;60(20):5617-5620. PMID: 11059749.
142. Zhu W, Zhang JS, Young CY. Silymarin inhibits function of the androgen receptor by reducing nuclear localization of the receptor in the human prostate cancer cell line LNCaP. *Carcinogenesis.* 2001;22(9):1399-1403. <https://doi.org/10.1093/carcin/22.9.1399>
143. Kang SN, Lee MH, Kim KM, Cho D, Kim TS. Induction of human promyelocytic leukemia HL-60 cell differentiation into monocytes by silibinin: involvement of protein kinase C. *Biochem Pharmacol.* 2001;61(12):1487-1495. [https://doi.org/10.1016/S0006-2952\(01\)00626-8](https://doi.org/10.1016/S0006-2952(01)00626-8)
144. Verdura S, Cuyàs E, Llorach-Parés L, et al. Silibinin is a direct inhibitor of STAT3. *Food Chem Toxicol.* 2018;116:161-172. <https://doi.org/10.1016/j.fct.2018.04.028>
145. Bosch-Barrera J, Queralt B, Menendez JA. Targeting STAT3 with silibinin to improve cancer therapeutics. *Cancer Treat Rev.* 2017;58:61-69. <https://doi.org/10.1016/j.ctrv.2017.06.003>
146. Mao J, Yang H, Cui T, et al. Combined treatment with sorafenib and silibinin synergistically targets both HCC cells and cancer stem cells by enhanced inhibition of the phosphorylation of STAT3/ERK/AKT. *Eur J Pharmacol.* 2018;832:39-49. <https://doi.org/10.1016/j.ejphar.2018.05.027>
147. Thelen P, Wuttke W, Jarry H, Grzmil M, Ringert RH. Inhibition of telomerase activity and secretion of prostate specific antigen by silibinin in prostate cancer cells. *J Urol.* 2004;171(5):1934-1938. <https://doi.org/10.1097/01.ju.0000121329.37206.1b>
148. Katiyar SK, Roy AM, Baliga MS. Silymarin induces apoptosis primarily through a p53-dependent pathway involving Bcl-2/Bax, cytochrome c release, and caspase activation. *Mol Cancer Ther.* 2005;4(2):207-216. PMID: 15713892.
149. Singh T, Prasad R, Katiyar SK. Therapeutic intervention of silymarin on the migration of non-small cell lung cancer cells is associated with the axis of multiple molecular targets including class I HDACs, ZEB1 expression, and restoration of miR-203 and E-cadherin expression. *Am J Cancer Res.* 2016;6(6):1287. PMID: 27429844.
150. Trappoliere M, Caligiuri A, Schmid M, et al. Silybin, a component of silymarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells. *J Hepatol.* 2009;50(6):1102-1111. <https://doi.org/10.1016/j.jhep.2009.02.023>
151. Vaid M, Prasad R, Sun Q, Katiyar SK. Silymarin targets β -catenin signaling in blocking migration/invasion of human melanoma cells. *PLoS ONE.* 2011;6(7):e23000. <https://doi.org/10.1371/journal.pone.0023000>
152. Woo SM, Min KJ, Chae IG, Chun KS, Kwon TK. Silymarin suppresses the PGE2-induced cell migration through inhibition of EP2 activation; G protein-dependent PKA-CREB and G protein-independent Src-STAT3 signal pathways. *Mol Carcinog.* 2015;54(3):216-228. <https://doi.org/10.1002/mc.22092>
153. Wu KJ, Zeng J, Zhu GD, et al. Silibinin inhibits prostate cancer invasion, motility and migration by suppressing vimentin and MMP-2 expression. *Acta Pharmacol Sin.* 2009;30(8):1162-1168. <https://doi.org/10.1038/aps.2009.94>
154. Eo HJ, Park GH, Jeong JB. Inhibition of wnt signaling by silymarin in human colorectal cancer cells. *Biomol Ther.* 2016;24(4):380. <https://doi.org/10.4062/biomolther.2015.154>
155. Dastpeyman M, Motamed N, Azadmanesh K, et al. Inhibition of silibinin on migration and adhesion capacity of human highly metastatic breast cancer cell line, MDA-MB-231, by evaluation of β 1-integrin and downstream molecules, Cdc42, Raf-1 and D4GDI. *Med Oncol.* 2012;29(4):2512-2518. <https://doi.org/10.1007/s12032-011-0113-8>
156. Yang SH, Lin JK, Chen WS, Chiu JH. Anti-angiogenic effect of silymarin on colon cancer LoVo cell line. *J Surg Res.* 2003; 113(1):133-138. [https://doi.org/10.1016/S0022-4804\(03\)00229-4](https://doi.org/10.1016/S0022-4804(03)00229-4)
157. Yang SH, Lin JK, Huang CJ, Chen WS, Li SY, Chiu JH. Silibinin inhibits angiogenesis via Flt-1, but not KDR, receptor up-regulation. *J Surg Res.* 2005;128(1):140-146. <https://doi.org/10.1016/j.jss.2005.04.042>
158. <http://www.uniprot.org/uniprot/P17948> Accessed 10/20/2020
159. Kaipa JM, Starkuviene V, Erfle H, Eils R, Gladilin E. Transcriptome profiling reveals silibinin dose-dependent response network in non-small lung cancer cells. *PeerJ.* 2020;8:e10373. <https://doi.org/10.7717/peerj.10373>
160. Adhikari M, Kaushik N, Ghimire B, et al. Cold atmospheric plasma and silymarin nanoemulsion synergistically inhibits human melanoma tumorigenesis via targeting HGF/c-MET downstream pathway. *Cell Commun Signal.* 2019;17(1):1-14. <https://doi.org/10.1186/s12964-019-0360-4>
161. Kim DH, Park SJ, Lee SY, Yoon HS, Park CM. Silymarin attenuates invasion and migration through the regulation of

- epithelial-mesenchymal transition in Huh7 cells. *Korean J Clin Lab Sci.* 2018;50(3):337-344. <https://doi.org/10.15324/kjcls.2018.50.3.337>
162. Li J, Hu L, Zhou T, et al. Taxifolin inhibits breast cancer cells proliferation, migration and invasion by promoting mesenchymal to epithelial transition via β -catenin signaling. *Life Sci.* 2019;232:116617. <https://doi.org/10.1016/j.lfs.2019.116617>
 163. McCarthy K, Maguire T, McGreal G, McDermott E, O'Higgins N, Duffy MJ. High levels of tissue inhibitor of metalloproteinase-1 predict poor outcome in patients with breast cancer. *Int J Cancer.* 1999;84(1):44-48. [https://doi.org/10.1002/\(SICI\)1097-0215\(19990219\)84:1<44::AID-IJC9>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1097-0215(19990219)84:1<44::AID-IJC9>3.0.CO;2-P)
 164. Ylissimio S, Höyhty M, Turpeenniemi-Hujanen T. Serum matrix metalloproteinases-2,-9 and tissue inhibitors of metalloproteinases-1,-2 in lung cancer--TIMP-1 as a prognostic marker. *Anticancer Res.* 2000;20(2B):1311-1316. PMID: 10810441.
 165. Gong Y, Scott E, Lu R, Xu Y, Oh WK, Yu Q. TIMP-1 Promotes accumulation of cancer associated fibroblasts and cancer progression. Kyprianou N, ed. *PLoS ONE.* 2013;8(10):e77366. <https://doi.org/10.1371/journal.pone.0077366>
 166. Jia JD, Bauer M, Cho JJ, et al. Antifibrotic effect of silymarin in rat secondary biliary fibrosis is mediated by downregulation of procollagen $\alpha 1$ (I) and TIMP-1. *J Hepatol.* 2001;35(3):392-398. [https://doi.org/10.1016/S0168-8278\(01\)00148-9](https://doi.org/10.1016/S0168-8278(01)00148-9)
 167. Chen IS, Chen YC, Chou CH, Chuang RF, Sheen LY, Chiu CH. Hepatoprotection of silymarin against thioacetamide-induced chronic liver fibrosis. *J Sci Food Agric.* 2012;92(7):1441-1447. <https://doi.org/10.1002/jsfa.4723>
 168. Di Sario A, Bendia E, Taffetani S, et al. Hepatoprotective and antifibrotic effect of a new silybin-phosphatidylcholine-vitamin E complex in rats. *Dig Liver Dis.* 2005;37(11):869-876. <https://doi.org/10.1016/j.dld.2005.05.011>
 169. Ward Y, Lake R, Yin JJ, et al. LPA Receptor heterodimerizes with CD97 to amplify LPA-initiated RHO-dependent signaling and invasion in prostate cancer cells. *Cancer Res.* 2011;71(23):7301-7311. <https://doi.org/10.1158/0008-5472.CAN-11-2381>
 170. Kato K, Yoshikawa K, Tanabe E, et al. Opposite roles of LPA 1 and LPA 3 on cell motile and invasive activities of pancreatic cancer cells. *Tumor Biol.* 2012;33(5):1739-1744. <https://doi.org/10.1007/s13277-012-0433-0>
 171. Wei JS, Johansson P, Chen L, et al. Massively parallel sequencing reveals an accumulation of de novo mutations and an activating mutation of LPAR1 in a patient with metastatic neuroblastoma. *PLoS ONE.* 2013;8(10):e77731. <https://doi.org/10.1371/journal.pone.0077731>
 172. Sahay D, Leblanc R, Grunewald TG, et al. The LPA1/ZEB1/miR-21-activation pathway regulates metastasis in basal breast cancer. *Oncotarget.* 2015;6(24):20604. <https://doi.org/10.18632/oncotarget.3774>
 173. Eraky SM, El-Mesery M, El-Karef A, Eissa LA, El-Gayar AM. Silymarin and caffeine combination ameliorates experimentally-induced hepatic fibrosis through down-regulation of LPAR1 expression. *Biomed Pharmacother.* 2018;101:49-57. <https://doi.org/10.1016/j.biopha.2018.02.064>
 174. Kim S, Han J, Jeon M, et al. Silibinin inhibits triple negative breast cancer cell motility by suppressing TGF- $\beta 2$ expression. *Tumor Biol.* 2016;37(8):11397-11407. <https://doi.org/10.1007/s13277-016-5000-7>
 175. Zare Z, Dizaj TN, Lohrasbi A, et al. Silibinin inhibits TGF- β -induced MMP-2 and MMP-9 through smad signaling pathway in colorectal cancer HT-29 cells. *Basic Clin Cancer Res.* 2020;12(2):81-90.
 176. El-Lakkany NM, Hammam OA, El-Maadawy WH, Badawy AA, Ain-Shoka AA, Ebeid FA. Anti-inflammatory/anti-fibrotic effects of the hepatoprotective silymarin and the schistosomicide praziquantel against schistosoma mansoni-induced liver fibrosis. *Parasit Vectors.* 2012;5(1):1-14. <https://doi.org/10.1186/1756-3305-5-9>
 177. Malekinejad H, Taheri-Brurjerdi M, Janbaz-Acyabar H, Amniattalab A. Silymarin regulates HIF-1 α and iNOS expression in the brain and gills of hypoxic-reoxygenated rainbow trout *oncorhynchus mykiss*. *Aquatic Biology.* 2012;15(3):261-273. <https://doi.org/10.3354/ab00427>
 178. Jung HJ, Park JW, Lee JS, et al. Silibinin inhibits expression of HIF-1 α through suppression of protein translation in prostate cancer cells. *Biochem Biophys Res Commun.* 2009;390(1):71-76. <https://doi.org/10.1016/j.bbrc.2009.09.068>
 179. Kim S, Han J, Kim JS, et al. Silibinin suppresses EGFR ligand-induced CD44 expression through inhibition of EGFR activity in breast cancer cells. *Anticancer Res.* 2011;31(11):3767-3773. PMID: 22110198.
 180. Handorean AM, Yang K, Robbins EW, Flaig TW, Iczkowski KA. Silibinin suppresses CD44 expression in prostate cancer cells. *Am J Transl Res.* 2009;1(1):80. PMID: 19966941.
 181. Wang B, He G, Xu G, Wen J, Yu X. miRNA-34a inhibits cell adhesion by targeting CD44 in human renal epithelial cells: implications for renal stone disease. *Urolithiasis.* 2019;48:1-8. <https://doi.org/10.1007/s00240-019-01155-9>
 182. Merzak A, Koocheckpour S, Pilkington GJ. CD44 Mediates human glioma cell adhesion and invasion in vitro. *Cancer Res.* 1994;54(15):3988-3992. PMID: 7518347.
 183. Dohadwala M, Luo J, Zhu L, et al. Non-small cell lung cancer cyclooxygenase-2-dependent invasion is mediated by CD44. *J Biol Chem.* 2001;276(24):20809-20812. <https://doi.org/10.1074/jbc.C100140200>
 184. Jothy S. CD44 And its partners in metastasis. *Clin Exp Metastasis.* 2003;20(3):195-201. <https://doi.org/10.1023/A:1022931016285>
 185. Rutnam ZJ, Yang BB. The non-coding 3' UTR of CD44 induces metastasis by regulating extracellular matrix functions. *J Cell Sci.* 2012;125(8):2075-2085. <https://doi.org/10.1242/jcs.100818>
 186. Troness B, Spartz A, Sharma U, et al. CD44 Facilitates metastasis by promoting co-clustering of breast cancer cells and cancer associated fibroblasts. *Cancer Res.* 2019;79(13 Supplement):2044. <https://doi.org/10.1158/1538-7445.AM2019-2044>
 187. Tyagi A, Agarwal C, Dwyer-Nield LD, Singh RP, Malkinson AM, Agarwal R. Silibinin modulates TNF- α and IFN- γ mediated signaling to regulate COX2 and iNOS expression in tumorigenic mouse lung epithelial LM2 cells. *Mol Carcinog.* 2012;51(10):832-842. <https://doi.org/10.1002/mc.20851>
 188. Lu W, Lin C, King TD, Chen H, Reynolds RC, Li Y. Silibinin inhibits Wnt/ β -catenin signaling by suppressing Wnt co-receptor

- LRP6 expression in human prostate and breast cancer cells. *Cell Signal*. 2012;24(12):2291-2296. <https://doi.org/10.1016/j.cellsig.2012.07.009>
189. Kauntz H, Bousserouel S, Gossé F, Raul F. The flavonolignan silibinin potentiates TRAIL-induced apoptosis in human colon adenocarcinoma and in derived TRAIL-resistant metastatic cells. *Apoptosis*. 2012;17(8):797-809. <https://doi.org/10.1007/s10495-012-0731-4>
 190. Kauntz H, Bousserouel S, Gossé F, Raul F. Silibinin triggers apoptotic signaling pathways and autophagic survival response in human colon adenocarcinoma cells and their derived metastatic cells. *Apoptosis*. 2011;16(10):1042. <https://doi.org/10.1007/s10495-011-0631-z>
 191. Son YG, Kim EH, Kim JY, et al. Silibinin sensitizes human glioma cells to TRAIL-mediated apoptosis via DR5 up-regulation and down-regulation of c-FLIP and survivin. *Cancer Res*. 2007;67(17):8274-8284. <https://doi.org/10.1158/0008-5472.CAN-07-0407>
 192. Bousserouel S, Bour G, Kauntz H, Gosse F, Marescaux J, Raul F. Silibinin inhibits tumor growth in a murine orthotopic hepatocarcinoma model and activates the TRAIL apoptotic signaling pathway. *Anticancer Res*. 2012;32(7):2455-2462. PMID: 22753701.
 193. Hagelgans A, Nacke B, Zamaraeva M, Siegert G, Menschikowski M. Silibinin down-regulates expression of secreted phospholipase A2 enzymes in cancer cells. *Anticancer Res*. 2014;34(4):1723-1729. PMID: 24692702.
 194. Chen YH, Chen CL, Lu DW, Liang CM, Tai MC, Chen JT. Silibinin inhibits platelet-derived growth factor-driven cell proliferation via downregulation of N-glycosylation in human Tenon's Fibroblasts in a proteasome-dependent manner. *PLoS ONE*. 2016;11(12):e0168765. <https://doi.org/10.1371/journal.pone.0168765>
 195. Inoue K, Slaton JW, Eve BY, et al. Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. *Clin Cancer Res*. 2000;6(5):2104-2119. PMID: 10815938.
 196. Mizukami Y, Jo WS, Duerr EM, et al. Induction of interleukin-8 preserves the angiogenic response in HIF-1 α -deficient colon cancer cells. *Nat Med*. 2005;11(9):992. <https://doi.org/10.1038/nm1294>
 197. Kozłowski L, Zakrzewska I, Tokajuk P, Wojtukiewicz MZ. Concentration of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. *Roczniki Akademii Medycznej w Białymstoku (1995)*. 2003;48:82-84. PMID: 14737948.
 198. Inoue K, Slaton JW, Kim SJ, et al. Interleukin 8 expression regulates tumorigenicity and metastasis in human bladder cancer. *Cancer Res*. 2000;60(8):2290-2299. PMID: 10786697.
 199. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. *J Clin Invest*. 1996;97(12):2792-2802. <https://doi.org/10.1172/JCI118734>
 200. Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I. Curcumin induces glutathione biosynthesis and inhibits NF- κ B activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. *Antioxid Redox Signaling*. 2005;7(1-2):32-41. <https://doi.org/10.1089/ars.2005.7.32>
 201. Gerritsen ME, Carley WW, Ranges GE, et al. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. *Am J Pathol*. 1995;147(2):278. PMID: 7543732.
 202. Hussain SA, Jassim NA, Numan IT, Al-Khalifa II, Abdullah TA. Anti-inflammatory activity of silymarin in patients with knee osteoarthritis. *Saudi Med J*. 2009;30(1):98-103. PMID: 19139781.
 203. Au AY, Hasenwinkel JM, Frondoza CG. Silybin inhibits interleukin-1 β -induced production of pro-inflammatory mediators in canine hepatocyte cultures. *J Vet Pharmacol Ther*. 2011;34(2):120-129. <https://doi.org/10.1111/j.1365-2885.2010.01200.x>
 204. Shi Z, Zhou Q, Gao S, et al. Silibinin inhibits endometrial carcinoma via blocking pathways of STAT3 activation and SREBP1-mediated lipid accumulation. *Life Sci*. 2019;217:70-80. <https://doi.org/10.1016/j.lfs.2018.11.037>
 205. Si L, Liu W, Hayashi T, et al. Silibinin-induced apoptosis of breast cancer cells involves mitochondrial impairment. *Arch Biochem Biophys*. 2019;671:42-51. <https://doi.org/10.1016/j.abb.2019.05.009>
 206. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases structure, function, and biochemistry. *Circ Res*. 2003;92(8):827-839. <https://doi.org/10.1161/01.RES.0000070112.80711.3D>
 207. Yousefi M, Ghaffari SH, Soltani BM, et al. Therapeutic efficacy of silibinin on human neuroblastoma cells: akt and NF- κ B expressions may play an important role in silibinin-induced response. *Neurochem Res*. 2012;37(9):2053-2063.
 208. Tang TC, Poon RT, Lau CP, Xie D, Fan ST. Tumor cyclooxygenase-2 levels correlate with tumor invasiveness in human hepatocellular carcinoma. *World J Gastroenterol*. 2005;11(13):1896-1902. <https://doi.org/10.3748/wjg.v11.i13.1896>
 209. Ramakrishnan G, Elinos-Báez CM, Jagan S, et al. Silymarin downregulates COX-2 expression and attenuates hyperlipidemia during NDEA-induced rat hepatocellular carcinoma. *Mol Cell Biochem*. 2008;313(1):53-61. <https://doi.org/10.1007/s11010-008-9741-5>
 210. Wyllie AH. Apoptosis and carcinogenesis. *Eur. J. Cell Biol*. 1997;73(3):189-197.
 211. Rugamba A, Kang DY, Sp N, et al. Silibinin regulates tumor progression and tumorsphere formation by suppressing PD-L1 expression in Non-small cell lung cancer (NSCLC) cells. *Cells*. 2021;10(7):1632. <https://doi.org/10.3390/cells10071632>
 212. Sellam LS, Zappasodi R, Chettibi F, et al. Silibinin down-regulates PD-L1 expression in nasopharyngeal carcinoma by interfering with tumor cell glycolytic metabolism. *Arch Biochem Biophys*. 2020;690:108479. <https://doi.org/10.1016/j.abb.2020.108479>
 213. Yang C, Li F, Ma J, et al. Evaluation of anti-cancer potency of silibinin on murine renal carcinoma RenCa cells in an animal model with an intact immune system. *Anti-Cancer Drugs*. 2020;31(8):785-791. <https://doi.org/10.1097/CAD.0000000000000931>
 214. Capaccione KM, Pine SR. The notch signaling pathway as a mediator of tumor survival. *Carcinogenesis*. 2013;34(7):1420-1430.

215. Zhang S, Yang Y, Liang Z, et al. Silybin-mediated inhibition of notch signaling exerts antitumor activity in human hepatocellular carcinoma cells. *PLoS ONE*. 2013;8(12):e83699. <https://doi.org/10.1371/journal.pone.0083699>
216. Ahmed LA, Abd El-Rhman RH, Gad AM, Hassaneen SK, El-Yamany MF. Dibenzazepine combats acute liver injury in rats via amendments of notch signaling and activation of autophagy. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2021;394(2):337-348. <https://doi.org/10.1007/s00210-020-01977-0>
217. Kim TH, Woo JS, Kim YK, Kim KH. Silibinin induces cell death through reactive oxygen species-dependent downregulation of notch-1/ERK/Akt signaling in human breast cancer cells. *J Pharmacol Exp Ther*. 2014;349(2):268-278. <https://doi.org/10.1124/jpet.113.207563>
218. Lin Z, Fang D. The roles of SIRT1 in cancer. *Genes Cancer*. 2013;4(3-4):97-104. <https://doi.org/10.1177/1947601912475079>
219. Yang H, Bi Y, Xue L, et al. Multifaceted modulation of SIRT1 in cancer and inflammation. *Critical Reviews™ in Oncogenesis*. 2015;20(1-2). <https://doi.org/10.1615/CritRevOncog.2014012374>
220. Feng B, Huang B, Jing Y, et al. Silymarin ameliorates the disordered glucose metabolism of mice with diet-induced obesity by activating the hepatic SIRT1 pathway. *Cell Signal*. 2021;84:110023. <https://doi.org/10.1016/j.cellsig.2021.110023>
221. Sarubbo F, Ramis MR, Kienzer C, et al. Chronic silymarin, quercetin and naringenin treatments increase monoamines synthesis and hippocampal Sirt1 levels improving cognition in aged rats. *J Neuroimmune Pharmacol*. 2018;13(1):24-38. <https://doi.org/10.1007/s11481-017-9759-0>
222. Wu WT, Chen YR, Lu DH, Senatov FS, Yang KC, Wang CC. Silymarin modulates catabolic cytokine expression through Sirt1 and SOX9 in human articular chondrocytes. *J Orthop Surg Res*. 2021;16(1):1-9. <https://doi.org/10.1186/s13018-021-02305-9>
223. Zhou B, Wu LJ, Li LH, et al. Silibinin protects against isoproterenol-induced rat cardiac myocyte injury through mitochondrial pathway after up-regulation of SIRT1. *J Pharmacol Sci*. 2006;102(4):387-395. <https://doi.org/10.1254/jphs.FPJ06005X>
224. Liang Z, Yang Y, Wang H, et al. Inhibition of SIRT1 signaling sensitizes the antitumor activity of silybin against human lung adenocarcinoma cells in vitro and in vivo. *Mol Cancer Ther*. 2014;13(7):1860-1872. <https://doi.org/10.1158/1535-7163.MCT-13-0942>
225. Jiang C, Agarwal R, Lü J. Anti-angiogenic potential of a cancer chemopreventive flavonoid antioxidant, silymarin: inhibition of key attributes of vascular endothelial cells and angiogenic cytokine secretion by cancer epithelial cells. *Biochem Biophys Res Commun*. 2000;276(1):371-378. <https://doi.org/10.1006/bbrc.2000.3474>
226. Attia GM, Elmansy RA, Algaidi SA. Silymarin decreases the expression of VEGF-A, iNOS and caspase-3 and preserves the ultrastructure of cardiac cells in doxorubicin induced cardiotoxicity in rats: a possible protective role. *Int J Clin Exp Med*. 2017;10(2):4158-4173. www.ijcem.com/ISSN:1940-5901/IJCEM0043306
227. Kaya MD, Başer E, Kaya S, et al. The effect of silymarin on VEGF, VEGFR-1 and IL-1 α levels in placental cultures of severe preeclamptic women. *J Turk Ger Gynecol Assoc*. 2014;15(1):30. <https://doi.org/10.5152/jtgga.2014.81592>
228. Zhang J, Ma H, Yang L. Silymarin inhibits invasion and migration of hepatoma cell line MHCC97. *Basic & Clinical Medicine*. 2019;39(12):1741. http://journal11.magtechjournal.com/Jwk_jcyxylc
229. Miller DM, Thomas SD, Islam A, Muench D, Sedoris K. c-Myc and cancer metabolism. *Clin Cancer Res*. 2012;18(20):5546-5553. <https://doi.org/10.1158/1078-0432.CCR-12-0977>
230. Rainone F. Milk thistle. *Am Fam Physician*. 2005;72(7):1285-1288. PMID: 16225032.
231. He Q, Osuchowski MF, Johnson VJ, Sharma RP. 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. *Planta Med*. 2002;68(01):676-679. <https://doi.org/10.1080/713611034>
232. Mastron JK, Siveen KS, Sethi G, Bishayee A. Silymarin and hepatocellular carcinoma: a systematic, comprehensive, and critical review. *Anti-Cancer Drugs*. 2015;26(5):475-486. <https://doi.org/10.1097/CAD.0000000000000211>
233. Rajamanickam S, Kaur M, Velmurugan B, Singh RP, Agarwal R. Silibinin suppresses spontaneous tumorigenesis in APC min/+ mouse model by modulating beta-catenin pathway. *Pharm Res*. 2009;26(12):2558-2567. <https://doi.org/10.1007/s11095-009-9968-1>
234. Rajamanickam S, Velmurugan B, Kaur M, Singh RP, Agarwal R. Chemoprevention of intestinal tumorigenesis in APCmin/+ mice by silibinin. *Cancer Res*. 2010;70(6):2368-2378. <https://doi.org/10.1158/0008-5472.CAN-09-3249>
235. Şentürk M, Gülçin İ, Beydemir Ş, Küfrevioğlu Öİ, Supuran CT. In vitro inhibition of human carbonic anhydrase I and II isozymes with natural phenolic compounds. *Chem Biol Drug Des*. 2011;77(6):494-499. <https://doi.org/10.1111/j.1747-0285.2011.01104.x>
236. Koz Ö, Ekinci D, Perrone A, et al. Analysis of saponins and phenolic compounds as inhibitors of α -carbonic anhydrase isoenzymes. *J Enzyme Inhib Med Chem*. 2013;28(2):412-417. <https://doi.org/10.3109/14756366.2011.651464>
237. Jaggi AS, Singh N. Silymarin and its role in chronic diseases. *Drug discovery from mother nature*. 2016; 929:25-44. https://doi.org/10.1007/978-3-319-41342-6_2
238. Feng B, Meng R, Huang B, Bi Y, Shen S, Zhu D. Silymarin protects against renal injury through normalization of lipid metabolism and mitochondrial biogenesis in high fat-fed mice. *Free Radical Biology and Medicine*. 2017;110:240-249. <https://doi.org/10.1016/j.freeradbiomed.2017.06.009>
239. Singh RP, Raina K, Sharma G, Agarwal R. Silibinin inhibits established prostate tumor growth, progression, invasion, and metastasis and suppresses tumor angiogenesis and epithelial-mesenchymal transition in transgenic adenocarcinoma of the mouse prostate model mice. *Clin Cancer Res*. 2008;14(23):7773-7780. <https://doi.org/10.1158/1078-0432.CCR-08-1309>
240. Deep G, Agarwal R. Antimetastatic efficacy of silibinin: molecular mechanisms and therapeutic potential against cancer. *Cancer Metastasis Rev*. 2010;29(3):447-463. <https://doi.org/10.1007/s10555-010-9237-0>
241. Sadighi S, Dashti-Khavidaki S, Shahbazi F, et al. The effects of concomitant use of silymarin and chemotherapy on solid tumors:

- a pilot randomized controlled trial. *Basic & Clinical Cancer Research*. 2017;9(2):13-19.
242. Fanoudi S, Alavi MS, Karimi G, Hosseinzadeh H. Milk thistle (*Silybum Marianum*) as an antidote or a protective agent against natural or chemical toxicities: a review. *Drug Chem Toxicol*. 2020;43(3):240-254. <https://doi.org/10.1080/01480545.2018.1485687>
243. Comelli MC, Mengs U, Schneider C, Prosdociami M. Toward the definition of the mechanism of action of silymarin: activities related to cellular protection from toxic damage induced by chemotherapy. *Integr Cancer Ther*. 2007;6(2):120-129. <https://doi.org/10.1177/1534735407302349>
244. Hagag AA, Elgamsy MA, El-Asy HM, Mabrouk MM. Protective role of silymarin on hepatic and renal toxicity induced by MTX based chemotherapy in children with acute lymphoblastic leukemia. *Mediterr J Hematol Infect Dis*. 2016;8(1): e2016043. <https://doi.org/10.4084/MJHID.2016.043>
245. Invernizzi R, Bernuzzi S, Ciani D, Ascari E. Silymarin during maintenance therapy of acute promyelocytic leukemia. *Haematologica*. 1993;78(5):340-341. PMID: 8314167.
246. Bokemeyer C, Fels LM, Dunn T, et al. Silibinin protects against cisplatin-induced nephrotoxicity without compromising cisplatin or ifosfamide anti-tumour activity. *Br J Cancer*. 1996;74(12):2036-2041. <https://doi.org/10.1038/bjc.1996.673>
247. Gaedeke J, Fels LM, Bokemeyer C, Mengs U, Stolte H, Lentzen H. Cisplatin nephrotoxicity and protection by silibinin. *Nephrol Dial Transplant*. 1996;11(1):55-62. <https://doi.org/10.1093/ndt/11.1.55>
248. Psotová J, Chlopčíková Š, Grambal F, Šimánek V, Ulrichová J. Influence of silymarin and its flavonolignans on doxorubicin-iron induced lipid peroxidation in rat heart microsomes and mitochondria in comparison with quercetin. *Phytother Res*. 2002;16(S1):63-67. <https://doi.org/10.1002/ptr.811>
249. Yardım A, Kucukler S, Özdemir S, et al. Silymarin alleviates docetaxel-induced central and peripheral neurotoxicity by reducing oxidative stress, inflammation and apoptosis in rats. *Gene*. 2021;769:145239. <https://doi.org/10.1016/j.gene.2020.145239>
250. Chang TK, Yin TC, Su WC, et al. Pilot study of silymarin as supplementation to reduce toxicities in metastatic colorectal cancer patients treated with first-line FOLFIRI Plus bevacizumab. *Oncol Res Featuring Preclin Clin Cancer Ther*. 2021; 7-8: 801-809. <https://doi.org/10.3727/096504021X16218531628569>
251. Moezian GSA, Javadinia SA, Sales SS, Fanipakdel A, Elyasi S, Karimi G. Oral silymarin formulation efficacy in management of AC-T protocol induced hepatotoxicity in breast cancer patients: a randomized, triple blind, placebo-controlled clinical trial. *J Oncol Pharm Pract*. 2021;10781552211006182. <https://doi.org/10.1177/10781552211006182>
252. Rho JK, Choi YJ, Jeon BS, et al. Combined treatment with silibinin and epidermal growth factor receptor tyrosine kinase inhibitors overcomes drug resistance caused by T790 M mutation. *Mol Cancer Ther*. 2010;9(12):3233-3243. <https://doi.org/10.1158/1535-7163.MCT-10-0625>
253. Sun Y, Guan Z, Zhao W, et al. Silibinin suppresses bladder cancer cell malignancy and chemoresistance in an NF- κ B signal-dependent and signal-independent manner. *Int J Oncol*. 2017; 51(4):1219-1226. <https://doi.org/10.3892/ijo.2017.4089>
254. Zhou L, Liu P, Chen B, et al. Silibinin restores paclitaxel sensitivity to paclitaxel-resistant human ovarian carcinoma cells. *Anticancer Res*. 2008;28(2A):1119-1127. PMID: 18507063.
255. Zhang S, Morris ME. Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. *J Pharmacol Exp Ther*. 2003;304(3):1258-1267. <https://doi.org/10.1124/jpet.102.044412>
256. Maitrejean M, Comte G, Barron D, El Kirat K, Conseil G, Di Pietro A. The flavanolignan silybin and its hemisynthetic derivatives, a novel series of potential modulators of P-glycoprotein. *Bioorg Med Chem Lett*. 2000;10(2):157-160. [https://doi.org/10.1016/S0960-894X\(99\)00636-8](https://doi.org/10.1016/S0960-894X(99)00636-8)
257. Džubák P, Hajdúch M, Gažák R, et al. New derivatives of silybin and 2, 3-dehydrosilybin and their cytotoxic and P-glycoprotein modulatory activity. *Bioorg Med Chem*. 2006;14(11):3793-3810. <https://doi.org/10.1016/j.bmc.2006.01.035>
258. Leslie EM, Mao Q, Oleschuk CJ, Deeley RG, Cole SP. Modulation of multidrug resistance protein 1 (MRP1/ABCC1) transport and ATPase activities by interaction with dietary flavonoids. *Mol Pharmacol*. 2001;59(5):1171-1180. <https://doi.org/10.1124/mol.59.5.1171>
259. Nguyen H, Zhang SZ, Morris ME. Effect of flavonoids on MRP1-mediated transport in Panc-1 cells. *J Pharm Sci*. 2002;92:250-257. <https://doi.org/10.1002/jps.10283>
260. Viktorová J, Dobiasová S, Řehořová K, et al. Antioxidant, anti-inflammatory, and multidrug resistance modulation activity of silychristin derivatives. *Antioxidants*. 2019;8(8):303. <https://doi.org/10.3390/antiox8080303>
261. Seidlova-Wuttke D, Becker T, Christoffel V, Jarry H, Wuttke W. Silymarin is a selective estrogen receptor β (ER β) agonist and has estrogenic effects in the metaphysis of the femur but no or anti-estrogenic effects in the uterus of ovariectomized (ovx) rats. *J Steroid Biochem Mol Biol*. 2003;86(2):179-188. [https://doi.org/10.1016/S0960-0760\(03\)00270-X](https://doi.org/10.1016/S0960-0760(03)00270-X)
262. Plišková M, Vondráček J, Křen V, et al. Effects of silymarin flavonolignans and synthetic silybin derivatives on estrogen and aryl hydrocarbon receptor activation. *Toxicology*. 2005;215(1-2):80-89. <https://doi.org/10.1016/j.tox.2005.06.020>
263. El-Shitany NA, Hegazy S, El-Desoky K. Evidences for antiosteoporotic and selective estrogen receptor modulator activity of silymarin compared with ethinylestradiol in ovariectomized rats. *Phytomedicine*. 2010;17(2):116-125. <https://doi.org/10.1016/j.phymed.2009.05.012>
264. Atawia RT, Tadros MG, Khalifa AE, Mosli HA, Abdel-Naim AB. Role of the phytoestrogenic, pro-apoptotic and anti-oxidative properties of silymarin in inhibiting experimental benign prostatic hyperplasia in rats. *Toxicol Lett*. 2013; 219(2):160-169. <https://doi.org/10.1016/j.toxlet.2013.03.002>
265. Vostalova J, Vidlar A, Ulrichova J, Vrbkova J, Simanek V, Student V. Use of selenium-silymarin mix reduces lower urinary tract symptoms and prostate specific antigen in men. *Phytomedicine*. 2013;21(1):75-81. <https://doi.org/10.1016/j.phymed.2013.07.018>

266. Khalil EA. Hormonal profile and histopathological study on the influence of silymarin on both female and male albino rats. *Egyptian J Hosp Med.* 2003;13(1):112-122. <https://doi.org/10.21608/ejhm.2003.18236>
267. Abedi H, Jahromi HK, Hashemi SMA, Jashni HK, Jahromi ZK, Pourahmadi M. The effect of silymarin on spermatogenesis process in rats. *Int J Med Res Health Sci.* 2016;5(6):146-150.
268. Mellman I, Yarden Y. Endocytosis and cancer. *Cold Spring Harbor Perspect Biol.* 2013;5(12):a016949. <https://doi.org/10.1101/cshperspect.a016949>
269. Blaising J, Lévy PL, Gondeau C, et al. Silibinin inhibits hepatitis C virus entry into hepatocytes by hindering clathrin-dependent trafficking. *Cell Microbiol.* 2013;15(11):1866-1882. <https://doi.org/10.1111/cmi.12155>
270. Polyak SJ, Oberlies NH, Pécheur EI, Dahari H, Ferenci P, Pawlotsky JM. Silymarin for hepatitis C virus infection. *Antiviral Ther.* 2013;18(2):141. <https://doi.org/10.3851/IMP2402>
271. Umetsu T, Inoue J, Kogure T, et al. Inhibitory effect of silibinin on hepatitis B virus entry. *Biochem Biophys Rep.* 2018;14:20-25. <https://doi.org/10.1016/j.bbrep.2018.03.003>
272. Vladutiu GD, Middleton Jr E. Effects of flavonoids on enzyme secretion and endocytosis in normal and mucopolipidosis II fibroblasts. *Life Sci.* 1986;39(8):717-726. [https://doi.org/10.1016/0024-3205\(86\)90019-6](https://doi.org/10.1016/0024-3205(86)90019-6)
273. Caballero-Díaz D, Bertran E, Peñuelas-Haro I, et al. Clathrin switches transforming growth factor- β role to pro-tumorigenic in liver cancer. *J Hepatol.* 2020;72(1):125-134. <https://doi.org/10.1016/j.jhep.2019.09.012>
274. Smith CM, Haucke V, McCluskey A, Robinson PJ, Chircop M. Inhibition of clathrin by pitstop 2 activates the spindle assembly checkpoint and induces cell death in dividing HeLa cancer cells. *Mol Cancer.* 2013;12(1):4. <https://doi.org/10.1186/1476-4598-12-4>
275. Li L, Gao Y, Zhang L, Zeng J, He D, Sun Y. Silibinin inhibits cell growth and induces apoptosis by caspase activation, down regulating survivin and blocking EGFR-ERK activation in renal cell carcinoma. *Cancer Lett.* 2008;272(1):61-69. <https://doi.org/10.1016/j.canlet.2008.06.033>
276. Liang L, Li L, Zeng J, et al. Inhibitory effect of silibinin on EGFR signal-induced renal cell carcinoma progression via suppression of the EGFR/MMP-9 signaling pathway. *Oncol Rep.* 2012;28(3):999-1005. <https://doi.org/10.3892/or.2012.1874>
277. Chang HR, Chen PN, Yang SF, et al. Silibinin inhibits the invasion and migration of renal carcinoma 786-O cells in vitro, inhibits the growth of xenografts in vivo and enhances chemosensitivity to 5-fluorouracil and paclitaxel. *Mol Carcinog.* 2011;50(10):811-823. <https://doi.org/10.1002/mc.20756>
278. Koltai T, Reshkin SJ, Carvalho T, Cardone RA. Targeting the stromal pro-tumoral hyaluronan-CD44 pathway in pancreatic cancer. *Int J Mol Sci.* 2021;22(8):3953. <https://doi.org/10.3390/ijms22083953>
279. Long J, Zhang Y, Yu X, et al. Overcoming drug resistance in pancreatic cancer. *Expert Opin Ther Targets.* 2011;15(7):817-828. <https://doi.org/10.1517/14728222.2011.566216>
280. Ting HJ, Deep G, Jain AK, et al. Silibinin prevents prostate cancer cell-mediated differentiation of naïve fibroblasts into cancer-associated fibroblast phenotype by targeting TGF β 2. *Mol Carcinog.* 2015;54(9):730-741. <https://doi.org/10.1002/mc.22135>
281. Amawi H, Hussein NA, Karthikeyan C, et al. HM015k, A novel silybin derivative, multi-targets metastatic ovarian cancer cells and is safe in zebrafish toxicity studies. *Front Pharmacol.* 2017;8:498. <https://doi.org/10.3389/fphar.2017.00498>
282. Song Y, Ye M, Zhou J, Wang ZW, Zhu X. Restoring E-cadherin expression by natural compounds for anticancer therapies in genital and urinary cancers. *Mol Therapy-Oncol.* 2019;14:130-138. <https://doi.org/10.1016/j.omto.2019.04.005>
283. Lieber CS, Leo MA, Cao Q, Ren C, DeCarli LM. Silymarin retards the progression of alcohol-induced hepatic fibrosis in baboons. *J Clin Gastroenterol.* 2003;37(4):336-339. <https://doi.org/10.1097/00004836-200310000-00013>
284. Ko JW, Shin NR, Park SH, et al. Silibinin inhibits the fibrotic responses induced by cigarette smoke via suppression of TGF- β 1/smad 2/3 signaling. *Food Chem Toxicol.* 2017;106:424-429. <https://doi.org/10.1016/j.fct.2017.06.016>
285. Lao CD, Demierre MF, Sondak VK. Targeting events in melanoma carcinogenesis for the prevention of melanoma. *Expert Rev Anticancer Ther.* 2006;6(11):1559-1568. <https://doi.org/10.1586/14737140.6.11.1559>
286. Li LH, Wu LJ, Jiang YY, et al. Silymarin enhanced cytotoxic effect of anti-Fas agonistic antibody CH11 on A375-S2 cells. *J Asian Nat Prod Res.* 2007;9(7):593-602. <https://doi.org/10.1080/10286020600882502>
287. Jones V, Katiyar SK. Emerging phytochemicals for prevention of melanoma invasion. *Cancer Lett.* 2013;335(2):251-258. <https://doi.org/10.1016/j.canlet.2013.02.056>
288. Lee MH, Huang Z, Kim DJ, et al. Direct targeting of MEK1/2 and RSK2 by silybin induces cell-cycle arrest and inhibits melanoma cell growth. *Cancer Prev Res.* 2013;6(5):455-465. <https://doi.org/10.1158/1940-6207.CAPR-12-0425>
289. Vaid M, Singh T, Prasad R, Katiyar SK. Silymarin inhibits melanoma cell growth both in vitro and in vivo by targeting cell cycle regulators, angiogenic biomarkers and induction of apoptosis. *Mol Carcinog.* 2015;54(11):1328-1339. <https://doi.org/10.1002/mc.22208>
290. Gajos-Michniewicz A, Czyz M. Modulation of WNT/ β -catenin pathway in melanoma by biologically active components derived from plants. *Fitoterapia.* 2016;109:283-292. <https://doi.org/10.1016/j.fitote.2016.02.002>
291. Mokhtari MJ, Motamed N, Shokrgozar MA. Evaluation of silibinin on the viability, migration and adhesion of the human prostate adenocarcinoma (PC-3) cell line. *Cell Biol Int.* 2008;32(8):888-892. <https://doi.org/10.1016/j.cellbi.2008.03.019>
292. Davis-Searles PR, Nakanishi Y, Kim NC, et al. Milk thistle and prostate cancer: differential effects of pure flavonolignans from *Silybum marianum* on antiproliferative end points in human prostate carcinoma cells. *Cancer Res.* 2005;65(10):4448-4457. <https://doi.org/10.1158/0008-5472.CAN-04-4662>
293. Kacar S, Aykanat NEB, Sahinturk V. Silymarin inhibited DU145 cells by activating SLIT2 protein and suppressing expression of CXCR4. *Med Oncol.* 2020;37(3):1-9. <https://doi.org/10.1007/s12032-020-1343-4>

294. Zi X, Agarwal R. Silibinin decreases prostate-specific antigen with cell growth inhibition via G1 arrest, leading to differentiation of prostate carcinoma cells: implications for prostate cancer intervention. *Proc Natl Acad Sci USA*. 1999;96:7490-7495. <https://doi.org/10.1073/pnas.96.13.7490>
295. Deep G, Singh RP, Agarwal C, Kroll DJ, Agarwal R. Silymarin and silibinin cause G1 and G2-M cell cycle arrest via distinct circuitries in human prostate cancer PC3 cells: a comparison of flavanone silibinin with flavanolignan mixture silymarin. *Oncogene*. 2006;25(7):1053. <https://doi.org/10.1038/sj.onc.1209146>
296. Tyagi A, Bhatia N, Condon MS, Bosland MC, Agarwal C, Agarwal R. Antiproliferative and apoptotic effects of silibinin in rat prostate cancer cells. *Prostate*. 2002;53(3):211-217. <https://doi.org/10.1002/pros.10146>
297. Deep G, Oberlies NH, Kroll DJ, Agarwal R. Isosilybin B and isosilybin A inhibit growth, induce G1 arrest and cause apoptosis in human prostate cancer LNCaP and 22Rv1 cells. *Carcinogenesis*. 2007;28(7):1533-1542. <https://doi.org/10.1093/carcin/bgm069>
298. Singh RP, Agarwal R. A cancer chemopreventive agent silibinin, targets mitogenic and survival signaling in prostate cancer. *Mutation Res/Fund Mol Mech Mutagenesis*. 2004;555(1):21-32. <https://doi.org/10.1016/j.mrfmmm.2004.05.017>
299. Flaig TW, Su LJ, Harrison G, Agarwal R, Glodé LM. Silibinin synergizes with mitoxantrone to inhibit cell growth and induce apoptosis in human prostate cancer cells. *Int J Cancer*. 2007;120(9):2028-2033. <https://doi.org/10.1002/ijc.22465>
300. Kohno H, Suzuki R, Sugie S, Tsuda H, Tanaka T. Dietary supplementation with silymarin inhibits 3, 2'-dimethyl-4-aminobiphenyl-induced prostate carcinogenesis in male F344 rats. *Clin Cancer Res*. 2005;11(13):4962-4967. <https://doi.org/10.1158/1078-0432.CCR-05-0137>
301. Wu K, Jin Z, Lei L, et al. Silibinin reverses epithelial-to-mesenchymal transition in metastatic prostate cancer cells by targeting transcription factors. *Oncol Rep*. 2010;23(6):1545-1552. https://doi.org/10.3892/or_00000794
302. Deep G, Gangar SC, Oberlies NH, Kroll DJ, Agarwal R. Isosilybin A induces apoptosis in human prostate cancer cells via targeting Akt, NF- κ B, and androgen receptor signaling. *Mol Carcinog*. 2010;49(10):902-912. <https://doi.org/10.1002/mc.20670>
303. Singh RP, Sharma G, Dhanalakshmi S, Agarwal C, Agarwal R. 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. *Cancer Epidemiol Prev Biomarkers*. 2003;12(9):933-939. PMID: 14504208.
304. Singh RP, Dhanalakshmi S, Tyagi AK, Chan DC, Agarwal C, Agarwal R. Dietary feeding of silibinin inhibits advance human prostate carcinoma growth in athymic nude mice and increases plasma insulin-like growth factor-binding protein-3 levels. *Cancer Res*. 2002;62:3063-3069. PMID: 12036915.
305. Tyagi A, Agarwal C, Agarwal R. 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. *Mol Cancer Ther*. 2002;1(7):525-532. PMID: 12479270.
306. Chu SC, Chiou HL, Chen PN, Yang SF, Hsieh YS. Silibinin inhibits the invasion of human lung cancer cells via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2. *Mol Carcinog*. 2004;40(3):143-149. <https://doi.org/10.1002/mc.20018>
307. Cufi S, Bonavia R, Vazquez-Martin A, et al. Silibinin meglumine, a water-soluble form of milk thistle silymarin, is an orally active anti-cancer agent that impedes the epithelial-to-mesenchymal transition (EMT) in EGFR-mutant non-small-cell lung carcinoma cells. *Food Chem Toxicol*. 2013;60:360-368. <https://doi.org/10.1016/j.fct.2013.07.063>
308. Mateen S, Tyagi A, Agarwal C, Singh RP, Agarwal R. Silibinin inhibits human nonsmall cell lung cancer cell growth through cell-cycle arrest by modulating expression and function of key cell-cycle regulators. *Mol Carcinog*. 2010;49(3):247-258. <https://doi.org/10.1002/mc.20595>
309. Li W, Mu D, Song L, et al. Molecular mechanism of silymarin-induced apoptosis in a highly metastatic lung cancer cell line anip973. *Cancer Biother Radiopharm*. 2011;26(3):317-324. <https://doi.org/10.1089/cbr.2010.0892>
310. Sharma G, Singh RP, Chan DC, Agarwal R. Silibinin induces growth inhibition and apoptotic cell death in human lung carcinoma cells. *Anticancer Res*. 2003;23(3B):2649-2655. PMID: 12894553.
311. Mateen S, Raina K, Jain AK, Agarwal C, Chan D, Agarwal R. Epigenetic modifications and p21-cyclin B1 nexus in anticancer effect of histone deacetylase inhibitors in combination with silibinin on non-small cell lung cancer cells. *Epigenetics*. 2012;7(10):1161-1172. <https://doi.org/10.4161/epi.22070>
312. Corominas-Faja B, Oliveras-Ferraras C, Cuyas E, et al. Stem cell-like ALDHbright cellular states in EGFR-mutant non-small cell lung cancer: a novel mechanism of acquired resistance to erlotinib targetable with the natural polyphenol silibinin. *Cell Cycle*. 2013;12(21):3390-3404. <https://doi.org/10.4161/cc.26417>
313. Tyagi A, Singh RP, Ramasamy K, et al. Growth inhibition and regression of lung tumors by silibinin: modulation of angiogenesis by macrophage-associated cytokines and nuclear factor- κ B and signal transducers and activators of transcription 3. *Cancer Prev Res*. 2009;2(1):74-83. <https://doi.org/10.1158/1940-6207.CAPR-08-0095>
314. Cuyas E, Perez-Sanchez A, Micol V, Menendez JA, Bosch-Barrera J. STAT3-targeted Treatment with silibinin overcomes the acquired resistance to crizotinib in ALK-rearranged lung cancer. *Cell Cycle*. 2016;15:3413-3418. <https://doi.org/10.1080/15384101.2016.1245249>
315. de Oliveira DT, Sávio ALV, de Castro Marcondes JP, et al. Cytotoxic and toxicogenomic effects of silibinin in bladder cancer cells with different TP53 status. *J Biosci*. 2017;42(1):91-101. <https://doi.org/10.1007/s12038-016-9654-5>
316. Vinh PQ, Sugie S, Tanaka T, et al. Chemopreventive effects of a flavonoid antioxidant silymarin on N-butyl-N-(4-hydroxybutyl) nitrosamine-induced urinary bladder carcinogenesis in male ICR mice. *Jpn J Cancer Res*. 2002;93(1):42-49. <https://doi.org/10.1111/j.1349-7006.2002.tb01199.x>

317. Imai-Sumida M, Chiyomaru T, Majid S, et al. Silibinin suppresses bladder cancer through down-regulation of actin cytoskeleton and PI3K/Akt signaling pathways. *Oncotarget*. 2017;8(54):92032. <https://doi.org/10.18632/oncotarget.20734>
318. Zeng J, Sun Y, Wu K, et al. Chemopreventive and chemotherapeutic effects of intravesical silibinin against bladder cancer by acting on mitochondria. *Mol Cancer Ther*. 2011;10(1):104-116. <https://doi.org/10.1158/1535-7163.MCT-10-0577>
319. Wu K, Ning Z, Zeng J, et al. Silibinin inhibits β -catenin/ZEB1 signaling and suppresses bladder cancer metastasis via dual-blocking epithelial-mesenchymal transition and stemness. *Cell Signal*. 2013;25(12):2625-2633. <https://doi.org/10.1016/j.cellsig.2013.08.028>
320. Ge Y, Zhang Y, Chen Y, et al. Silibinin causes apoptosis and cell cycle arrest in some human pancreatic cancer cells. *Int J Mol Sci*. 2011;12(8):4861-4871. <https://doi.org/10.3390/ijms12084861>
321. Feng W, Cai D, Zhang B, Lou G, Zou X. Combination of HDAC inhibitor TSA and silibinin induces cell cycle arrest and apoptosis by targeting survivin and cyclinB1/Cdk1 in pancreatic cancer cells. *Biomed Pharmacother*. 2015;74:257-264. <https://doi.org/10.1016/j.biopha.2015.08.017>
322. Shukla SK, Dasgupta A, Mehla K, et al. Silibinin-mediated metabolic reprogramming attenuates pancreatic cancer-induced cachexia and tumor growth. *Oncotarget*. 2015;6(38):41146. <https://doi.org/10.18632/oncotarget.5843>
323. Nambiar D, Prajapati V, Agarwal R, Singh RP. In vitro and in vivo anticancer efficacy of silibinin against human pancreatic cancer BxPC-3 and PANC-1 cells. *Cancer Lett*. 2013;334(1):109-117. <https://doi.org/10.1016/j.canlet.2012.09.004>
324. Zhang X, Liu J, Zhang P, et al. Silibinin induces G1 arrest, apoptosis and JNK/SAPK upregulation in SW1990 human pancreatic cancer cells. *Oncol Lett*. 2018;15(6):9868-9876. <https://doi.org/10.3892/ol.2018.8541>
325. Zhang S, Yang X, Morris ME. Combined effects of multiple flavonoids on breast cancer resistance protein (ABCG2)-mediated transport. *Pharm Res*. 2004;21(7):1263-1273. <https://doi.org/10.1023/B:PHAM.0000033015.84146.4c>
326. Tyagi AK, Agarwal C, Chan DC, Agarwal R. Synergistic anticancer effects of silibinin with conventional cytotoxic agents doxorubicin, cisplatin and carboplatin against human breast carcinoma MCF-7 and MDA-MB468 cells. *Oncol Rep*. 2004;11(2):493-499. <https://doi.org/10.3892/or.11.2.493>
327. Kim S, Choi JH, Lim HI, et al. Silibinin prevents TPA-induced MMP-9 expression and VEGF secretion by inactivation of the Raf/MEK/ERK pathway in MCF-7 human breast cancer cells. *Phytomedicine*. 2009;16(6-7):573-580. <https://doi.org/10.1016/j.phymed.2008.11.006>
328. Rastegar H, Ashtiani HA, Anjarani S, Bokae S, Khaki A, Javadi L. The role of milk thistle extract in breast carcinoma cell line (MCF-7) apoptosis with doxorubicin. *Acta Med Iran*. 2013;591-598.
329. Kalla PK, Chitti S, Aghamirzaei ST, Senthilkumar R, Arjunan S. Anti-cancer activity of silymarin on MCF-7 and NCIH-23 cell lines. *Adv Biol Res*. 2014;8(2):57-61. <https://doi.org/10.5829/idosi.abr.2014.8.2.82286>
330. Forghani P, Khorramizadeh MR, Waller EK. Silibinin inhibits accumulation of myeloid-derived suppressor cells and tumor growth of murine breast cancer. *Cancer Med*. 2014;3:215-224. <https://doi.org/10.1002/cam4.186>
331. Jiang K, Wang W, Jin X, Wang Z, Ji Z, Meng G. Silibinin, a natural flavonoid, induces autophagy via ROS-dependent mitochondrial dysfunction and loss of ATP involving BNIP3 in human MCF7 breast cancer cells. *Oncol Rep*. 2015;33(6):2711-2718. <https://doi.org/10.3892/or.2015.3915>
332. Pirouzpanah MB, Sabzichi M, Pirouzpanah S, Chavoshi H, Samadi N. Silibinin-induced apoptosis in breast cancer cells by modulating p53, p21, Bak and Bcl-XL pathways. *Asian Pac J Cancer Prev*. 2015;16(5):2087-2092. <https://doi.org/10.7314/apjcp.2015.16.5.2087>
333. Zheng N, Zhang P, Huang H, et al. ER α down-regulation plays a key role in silibinin-induced autophagy and apoptosis in human breast cancer MCF-7 cells. *J Pharmacol Sci*. 2015;128(3):97-107. <https://doi.org/10.1016/j.jphs.2015.05.001>
334. Jahanafrooz Z, Motameh N, Bakhshandeh B. Comparative evaluation of silibinin effects on cell cycling and apoptosis in human breast cancer MCF-7 and T47D cell lines. *Asian Pac J Cancer Prev*. 2016;17(5):2661-2665. PMID: 27268647.
335. Molavi O, Narimani F, Asiaee F, et al. Silibinin sensitizes chemoresistant breast cancer cells to chemotherapy. *Pharm Biol*. 2017;55(1):729-739. <https://doi.org/10.1080/13880209.2016.1270972>
336. Paulpandi M. 78P In vivo activation of mitochondrial pathway and cell cycle arrest through silymarin loaded iron nanoparticles as proficient nanocomplex system for triple negative breast cancer therapy. *Ann Oncol*. 2017;28(suppl_10):mdx655-mdx020. <https://doi.org/10.1093/annonc/mdx655.020>
337. Sik CG, Seon YE, Jung KY, et al. Anticancer effect of silymarin on breast cancer cells through inhibition of Akt and MAPK pathway expression. 151-151. *KALAS Int Symp*. 2017;8:151-151.
338. Kim SH, Choo GS, Yoo ES, et al. Silymarin inhibits proliferation of human breast cancer cells via regulation of the MAPK signaling pathway and induction of apoptosis. *Oncol Lett*. 2021;21(6):1-10. <https://doi.org/10.3892/ol.2021.12753>
339. Kohno H, Tanaka T, Kawabata K, et al. Silymarin, a naturally occurring polyphenolic antioxidant flavonoid, inhibits azoxymethane-induced colon carcinogenesis in male F344 rats. *Int J Cancer*. 2002;101(5):461-468. <https://doi.org/10.1002/ijc.10625>
340. Raina K, Agarwal C, Agarwal R. Effect of silibinin in human colorectal cancer cells: targeting the activation of NF- κ B signaling. *Mol Carcinog*. 2013;52(3):195-206. <https://doi.org/10.1002/mc.21843>
341. Eo HJ, Park GH, Song HM, et al. Silymarin induces cyclin D1 proteasomal degradation via its phosphorylation of threonine-286 in human colorectal cancer cells. *Int Immunopharmacol*. 2015;24(1):1-6. <https://doi.org/10.1016/j.intimp.2014.11.009>
342. Lal M, Gupta D. Studies on radiation sensitization efficacy by silymarin in colon carcinoma cells. *Discoveries*. 2016;4(1):1. <https://doi.org/10.15190/d.2016.3>

343. Horita MA, Ezekiel U. The effects of silibinin on colorectal cancer cell line. *FASEB J*. 2017;31(1 Supplement):766. https://doi.org/10.1096/fasebj.31.1_supplement.766.5
344. Belli V, Sforza V, Cardone C, et al. Regorafenib in combination with silybin as a novel potential strategy for the treatment of metastatic colorectal cancer. *Oncotarget*. 2017;8(40):68305. <https://doi.org/10.18632/oncotarget.20054>
345. Girardi B, Pricci M, Giorgio F, et al. Silymarin, boswellic acid and curcumin enriched dietetic formulation reduces the growth of inherited intestinal polyps in an animal model. *World J Gastroenterol*. 2020;26(14):1601. <https://doi.org/10.3748/wjg.v26.i14.1601>
346. Varghese L, Agarwal C, Tyagi A, Singh RP, Agarwal R. Silibinin efficacy against human hepatocellular carcinoma. *Clin Cancer Res*. 2005;11(23):8441-8448. <https://doi.org/10.1158/1078-0432.CCR-05-1646>
347. Ramakrishnan G, Raghavendran HRB, Vinodhkumar R, Devaki T. Suppression of N-nitrosodiethylamine induced hepatocarcinogenesis by silymarin in rats. *Chem-Biol Interact*. 2006;161(2):104-114. <https://doi.org/10.1016/j.cbi.2006.03.007>
348. Ramakrishnan G, Jagan S, Kamaraj S, Anandakumar P, Devaki T. Silymarin attenuated mast cell recruitment thereby decreased the expressions of matrix metalloproteinases-2 and 9 in rat liver carcinogenesis. *Invest New Drugs*. 2009;27(3):233-240. <https://doi.org/10.1007/s10637-008-9163-y>
349. Momeny M, Khorramizadeh MR, Ghaffari SH, et al. Effects of silibinin on cell growth and invasive properties of a human hepatocellular carcinoma cell line, HepG-2, through inhibition of extracellular signal-regulated kinase 1/2 phosphorylation. *Eur J Pharmacol*. 2008;591(1-3):13-20. <https://doi.org/10.1016/j.ejphar.2008.06.011>
350. Ramakrishnan G, Lo Muzio L, Elinos-Báez CM, et al. Silymarin inhibited proliferation and induced apoptosis in hepatic cancer cells. *Cell Prolif*. 2009;42(2):229-240. <https://doi.org/10.1111/j.1365-2184.2008.00581.x>
351. Cui W, Gu F, Hu KQ. Effects and mechanisms of silibinin on human hepatocellular carcinoma xenografts in nude mice. *World J Gastroenterol*. 2009;15(16):1943. <https://doi.org/10.3748/wjg.15.1943>
352. Gu HR, Park SC, Choi SJ, et al. Combined treatment with silibinin and either sorafenib or gefitinib enhances their growth-inhibiting effects in hepatocellular carcinoma cells. *Clin Mol Hepatol*. 2015;21(1):49. <https://doi.org/10.3350/cmh.2015.21.1.49>
353. Aykanat NEB, Kacar S, Karakaya S, Sahinturk V. Silymarin suppresses HepG2 hepatocarcinoma cell progression through downregulation of slit-2/robo-1 pathway. *Pharmacol Rep*. 2020;72(1):199-207. <https://doi.org/10.1007/s43440-019-00040-x>
354. Fan L, Ma Y, Liu Y, Zheng D, Huang G. Silymarin induces cell cycle arrest and apoptosis in ovarian cancer cells. *Eur J Pharmacol*. 2014;743:79-88. <https://doi.org/10.1016/j.ejphar.2014.09.019>
355. Gallo D, Giacomelli S, Ferlini C, et al. Antitumour activity of the silybin-phosphatidylcholine complex, IdB 1016, against human ovarian cancer. *Eur J Cancer*. 2003;39(16):2403-2410. [https://doi.org/10.1016/S0959-8049\(03\)00624-5](https://doi.org/10.1016/S0959-8049(03)00624-5)
356. Cho HJ, Suh DS, Moon SH, et al. Silibinin inhibits tumor growth through downregulation of extracellular signal-regulated kinase and Akt in vitro and in vivo in human ovarian cancer cells. *J Agric Food Chem*. 2013;61(17):4089-4096. <https://doi.org/10.1021/jf400192v>
357. Zhang J, Harrison JS, Uskokovic M, Danilenko M, Studzinski GP. Silibinin can induce differentiation as well as enhance vitamin D3-induced differentiation of human AML cells *ex vivo* and regulates the levels of differentiation-related transcription factors. *Hematol Oncol*. 2010;28:124-132. <https://doi.org/10.1002/hon.929>
358. Molavi O, Samadi N, Wu C, Lavasanifar A, Lai R. Silibinin suppresses NPM-ALK, potently induces apoptosis and enhances chemosensitivity in ALK-positive anaplastic large cell lymphoma. *Leuk Lymphoma*. 2016;57(5):1154-1162. <https://doi.org/10.3109/10428194.2015.1068306>
359. Lu S, Zhang J, Wang Y. Silymarin inhibits proliferation and induces apoptosis in epstein-barr virus-positive lymphoma cells by suppressing nuclear factor-kappa B pathway. *Curr Top Nutraceutical Res*. 2020;18(4).
360. Feng N, Luo J, Guo X. Silybin suppresses cell proliferation and induces apoptosis of multiple myeloma cells via the PI3K/Akt/mTOR signaling pathway. *Mol Med Rep*. 2016;13:3243-3248. <https://doi.org/10.3892/mmr.2016.4887>
361. Su CH, Chen LJ, Liao JF, Cheng JT. Increase of phosphatase and tensin homolog by silymarin to inhibit human pharynx squamous cancer. *J Med Food*. 2013;16(9):778-784. <https://doi.org/10.1089/jmf.2012.2534>
362. Zhang M, Liu Y, Gao Y, Li S. Silibinin-induced glioma cell apoptosis by PI3K-mediated but Akt-independent downregulation of FoxM1 expression. *Eur J Pharmacol*. 2015;765:346-354. <https://doi.org/10.1016/j.ejphar.2015.08.057>
363. Won DH, Kim LH, Jang B, et al. In vitro and in vivo anti-cancer activity of silymarin on oral cancer. *Tumor Biol*. 2018;40(5), 1010428318776170. <https://doi.org/10.1177/1010428318776170>
364. Choi ES, Oh S, Jang B, et al. Silymarin and its active component silibinin act as novel therapeutic alternatives for salivary gland cancer by targeting the ERK1/2-Bim signaling cascade. *Cell Oncol*. 2017;40(3):235-246. <https://doi.org/10.1007/s13402-017-0318-8>
365. Yu HC, Chen LJ, Cheng KC, Li YX, Yeh CH, Cheng JT. Silymarin inhibits cervical cancer cell through an increase of phosphatase and tensin homolog. *Phytother Res*. 2012;26(5):709-715. <https://doi.org/10.1002/ptr.3618>
366. Hsieh YS, Chu SC, Yang SF, Chen PN, Liu YC, Lu KH. Silibinin suppresses human osteosarcoma MG-63 cell invasion by inhibiting the ERK-dependent c-Jun/AP-1 induction of MMP-2. *Carcinogenesis*. 2007;28:977-987. <https://doi.org/10.1093/carcin/bgl221>
367. Li Y, Zhang C, Cai D, Chen C, Mu D. Silibinin inhibits migration and invasion of the rhabdoid tumor G401 cell line via inactivation of the PI3K/Akt signaling pathway. *Oncol Lett*. 2017;14(6):8035-8041. <https://doi.org/10.3892/ol.2017.7246>
368. Kim SH, Choo GS, Yoo ES, et al. Silymarin induces inhibition of growth and apoptosis through modulation of the MAPK signaling pathway in AGS human gastric cancer cells. *Oncol Rep*. 2019;42(5):1904-1914. <https://doi.org/10.3892/or.2019.7295>

369. Malewicz B, Wang Z, Jiang C, et al. Enhancement of mammary carcinogenesis in two rodent models by silymarin dietary supplements. *Carcinogenesis*. 2006;27(9):1739-1747. <https://doi.org/10.1093/carcin/bgl032>
370. Kummer V, Maskova J, Canderle J, Zraly Z, Neca J, Machala M. Estrogenic effects of silymarin in ovariectomized rats. *VETERINARNI MEDICINA-PRAHA*. 2001;46(1):17-23. <https://doi.org/10.17221/7846-VETMED>
371. Imamoto R, Okano JI, Sawada S, Fujise Y, Abe R, Murawaki Y. Null anticarcinogenic effect of silymarin on diethylnitrosamine-induced hepatocarcinogenesis in rats. *Exp Ther Med*. 2014;7(1):31-38. <https://doi.org/10.3892/etm.2013.1391>
372. Brandon-Warner E, Eheim AL, Foureau DM, Walling TL, Schrum LW, McKillop IH. Silibinin (milk thistle) potentiates ethanol-dependent hepatocellular carcinoma progression in male mice. *Cancer Lett*. 2012;326(1):88-95. <https://doi.org/10.1016/j.canlet.2012.07.028>
373. Javed S, Kohli K, Ali M. Reassessing bioavailability of silymarin. *Altern Med Rev*. 2011;16:239-249. PMID: 21951025.
374. Wu JW, Lin LC, Hung SC, Chi CW, Tsai TH. Analysis of silibinin in rat plasma and bile for hepatobiliary excretion and oral bioavailability application. *J Pharm Biomed Anal*. 2007;45:635-641. <https://doi.org/10.1016/j.jpba.2007.06.026>
375. Janiak B, Kessler B, Kunz W, Schnieders B. Die wirkung von silymarin auf gehalt und function einiger durch einwirkung von tetrachlorkohlenstoff bzw. Halothan beeinflussten mikrosomalen leberenzyme. *Arzneimittelforschung*. 1973;23:1322-1326. PMID: 4801229.
376. Fraschini F, Demartini G, Esposti D. Pharmacology of silymarin. *Clin Drug Investig*. 2002;22(1):51-65. <https://doi.org/10.2165/00044011-200222010-00007>
377. Lorenz D, Lucker PW, Mennicke WH, Wetzelsberger N. Pharmacokinetic studies with silymarin in human serum and bile. *Methods find. Exp Clin Pharmacol*. 1984;6(10):655-661. PMID: 6513680.
378. Beckmann-Knopp S, Rietbrock S, Weyhenmeyer R, et al. Inhibitory effects of silibinin on cytochrome P-450 enzymes in human liver microsomes. *Pharmacol Toxicol*. 2000;86(6):250-256. <https://doi.org/10.1111/j.0901-9928.2000.860602.x>
379. Gatti G, Perucca E. Plasma concentrations of free and conjugated silybin after oral intake of a silybin-phosphatidylcholine complex (silipide) in healthy volunteers. *Int J Clin Pharm Ther*. 1994;32(11):614-617. PMID: 7874377.
380. Filburn CR, Kettenacker R, Griffin DW. Bioavailability of a silybin-phosphatidylcholine complex in dogs. *J Vet Pharmacol Ther*. 2007;30(2):132-138. <https://doi.org/10.1111/j.1365-2885.2007.00834.x>
381. Morazzoni P, Montalbetti A, Malandrino S, Pifferi G. Comparative pharmacokinetics of silipide and silymarin in rats. *Eur J Drug Metab Pharmacokinet*. 1993;18(3):289-297. <https://doi.org/10.1007/BF03188811>
382. Zhao J, Agarwal R. 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. *Carcinogenesis*. 1999;20(11):2101-2108. <https://doi.org/10.1093/carcin/20.11.2101>
383. Schandalik R, Gatti G, Perucca E. Pharmacokinetics of silybin in bile following administration of silipide and silymarin in cholecystectomy patients. *Arzneimittelforschung*. 1992;42:964-968. PMID: 1329780.
384. Hoh C, Boocock D, Marczylo T, et al. Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences. *Clin Cancer Res*. 2006;12(9):2944-2950. <https://doi.org/10.1158/1078-0432.CCR-05-2724>
385. Tyagi AK, Singh RP, Agarwal C, Chan DC, Agarwal R. Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth inhibition, G2-M arrest, and apoptosis. *Clin Cancer Res*. 2002;8(11):3512-3519. PMID: 12429642.
386. Ranjbar N, Saravani R, Faezizadeh Z. Silymarin inhibits toll-like receptor 8 gene expression and apoptosis in ramos cancer cell line. *Avicenna J Phytomed*. 2020;10(2):161. PMID: 32257888.
387. Kamrani Z, Heshmati M, Babashah S. Evaluation effects of silymarin on cytotoxicity and apoptosis on SW480 colon cancer cell line. *Research in Karyotic Cell & Tissue*. 2021;1(3).
388. Pradhan SC, Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. *Indian J Med Res*. 2013;124(5):491-504. PMID: 17213517.
389. Weyhenmeyer R, Mascher H, Birkmayer J. Study on dose-linearity of the pharmacokinetics of silibinin diastereomers using a new stereospecific assay. *Int J Clin Pharmacol Ther Toxicol*. 1992;30(4):134-138. PMID: 1572758.
390. Di Costanzo A, Angelico R. Formulation strategies for enhancing the bioavailability of silymarin: the state of the art. *Molecules*. 2019;24(11):2155. <https://doi.org/10.3390/molecules24112155>
391. Kidd P, Head K. A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex (siliphos). *Altern Med Rev*. 2005;10:193-203. PMID: 16164374.
392. Comoglio A, Tomasi A, Malandrino S, Poli G, Albano E. Scavenging effect of silipide, a new silybin-phospholipid complex, on ethanol-derived free radicals. *Biochem Pharmacol*. 1995;50(8):1313-1316. [https://doi.org/10.1016/0006-2952\(95\)02001-S](https://doi.org/10.1016/0006-2952(95)02001-S)
393. Tvrdý V, Pourová J, Jirkovský E, Křen V, Valentová K, Mladěnka P. Systematic review of pharmacokinetics and potential pharmacokinetic interactions of flavonolignans from silymarin. *Med Res Rev*. 2021;41(4):2195-2246. <https://doi.org/10.1002/med.21791>
394. Pawar HA, Bhangale BD. Phytosome as a novel biomedicine: a microencapsulated drug delivery system. *J Bioanal Biomed*. 2015;7(1):6-12. <https://doi.org/10.4172/1948-593X.1000116>
395. Morazzoni P, Magistretti MJ, Giachetti C, Zanolo G. Comparative bioavailability of silipide, a new flavanolignan complex, in rats. *Eur J Drug Metab Pharmacokinet*. 1992;117:39-44. <https://doi.org/10.1007/BF03189986>
396. Zhang ZB, Shen ZG, Wang JX, et al. Micronization of silybin by the emulsion solvent diffusion method. *Int J Pharm*. 2009;376(1-2):116-122. <https://doi.org/10.1016/j.ijpharm.2009.04.028>
397. Wu W, Wang Y, Que L. Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. *Eur J Pharm*

- Biopharm.* 2006;63(3):288-294. <https://doi.org/10.1016/j.ejpb.2005.12.005>
398. Liu L, Pang X, Zhang W, Wang S. Formulation design and in vitro evaluation of silymarin loaded self micro emulsifying drug delivery systems. *Asian J Pharm Sci.* 2007;2:150-160.
399. Maheshwari H, Aggarwal R, Patil C, Katare OP. Preparation and pharmacological evaluation of silibinin liposomes. *Arzneimittelforschung.* 2003;53(06):420-427. <https://doi.org/10.1055/s-0031-1297130>
400. Nguyen MH, Yu H, Dong B, Hadinoto K. A supersaturating delivery system of silibinin exhibiting high payload achieved by amorphous nano-complexation with chitosan. *Eur J Pharm Sci.* 2016;89:163-171. <https://doi.org/10.1016/j.ejps.2016.04.036>
401. Di Costanzo A, Angelico R. Formulation strategies for enhancing the bioavailability of silymarin: the state of the art. *Molecules.* 2019;24(11):2155. <https://doi.org/10.3390/molecules24112155>
402. Piazzini V, D'Ambrosio M, Luceri C, et al. Formulation of nanomicelles to improve the solubility and the oral absorption of silymarin. *Molecules.* 2019;24(9):1688. <https://doi.org/10.3390/molecules24091688>
403. Flaig TW, Gustafson DL, Su LJ, et al. A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. *Invest New Drugs.* 2007;25(2):139-146. <https://doi.org/10.1007/s10637-006-9019-2>
404. Jacobs PB, Dennehy C, Ramirez G, Sapp J, Lawrence VA. Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. *Am J Med.* 2002;113:506-515. [https://doi.org/10.1016/S0002-9343\(02\)01244-5](https://doi.org/10.1016/S0002-9343(02)01244-5)
405. Abenavoli L, Capasso R, Milic N, Capasso F. Milk thistle in liver diseases: past, present, future. *Phytother Res.* 2010;24(10):1423-1432. <https://doi.org/10.1002/ptr.3207>
406. Flaig TW, Glodé M, Gustafson D, et al. A study of high-dose oral silybin-phytosome followed by prostatectomy in patients with localized prostate cancer. *Prostate.* 2010;70(8):848-855. <https://doi.org/10.1002/pros.21118>
407. Lazzeroni M, Guerrieri-Gonzaga A, Gandini S, et al. A presurgical study of oral silybin-phosphatidylcholine in patients with early breast cancer. *Cancer Prev Res.* 2016;9(1):89-95. <https://doi.org/10.1158/1940-6207.CAPR-15-0123>
408. Lee SM, Lee GW, Park SY, et al. 2020. Dual Effects of Silibinin on Human Pancreatic Cancer Cells. Preprint downloaded from <https://assets.researchsquare.com/files/rs-130714/v1/6f2d72c0-7e77-448d-85b5-b2bdeea220a0.pdf?c=1608755626> accessed 7/7/2021.
409. Tehrani FK, Ranji N, Kouhkan F, Hosseinzadeh S. PANC-1 cancer stem-like cell death with silybin encapsulated in polymericosomes and deregulation of stemness-related miRNAs and their potential targets. *Iran J Basic Med Sci.* 2021;24(4):514. <https://doi.org/10.22038/ijbms.2021.54001.12136>
410. Abdollahi P, Ebrahimi M, Motamed N, Samani FS. Silibinin affects tumor cell growth because of reduction of stemness properties and induction of apoptosis in 2D and 3D models of MDA-MB-468. *Anti-cancer Drugs.* 2015;26(5):487-497. <https://doi.org/10.1097/CAD.0000000000000205>
411. Firouzi J, Ebrahimi M, Sotoodehnejadnematollahi F. Evaluation of inhibitory effect of silibinin on growth and stemness property of MCF-7 cell line derived mammospheres. *SSU_Journals.* 2017;25(2):111-122. <http://jssu.ssu.ac.ir/article-1-3944-en.html>
412. Pawar SK, Jaldappagari S. Intercalation of a flavonoid, silibinin into DNA base pairs: experimental and theoretical approach. *J Mol Recognit.* 2020;33:e2812. <https://doi.org/10.1002/jmr.2812>
413. Biedermann D, Hurtová M, Biedermannová L, Valentová K, Křen V. Flavonolignans from silymarin do not intercalate into DNA: rebuttal of data published in the paper J. Mol. Recognit. e2812 (2019). *J Mol Recognit.* 2021;34(7):e2888. <https://doi.org/10.1002/jmr.2888>
414. Schröder FH, Roobol MJ, Boevé ER, et al. Randomized, double-blind, placebo-controlled crossover study in men with prostate cancer and rising PSA: effectiveness of a dietary supplement. *Eur Urol.* 2005;48(6):922-931. <https://doi.org/10.1016/j.euro.2005.08.005>
415. Hsu CY, Sun PL, Chang HC, Perng DS, Chen YS. Spontaneous regression of advanced hepatocellular carcinoma: a case report. *Cases J.* 2009;2(1):6251. <https://doi.org/10.4076/1757-1626-2-6251>
416. Moroni M, Zanlorenzi L. Complete regression following sorafenib in unresectable, locally advanced hepatocellular carcinoma. *Future Oncol.* 2013;9(8):1231-1237. <https://doi.org/10.2217/fon.13.86>
417. Bosch-Barrera J, Sais E, Cañete N, et al. Response of brain metastasis from lung cancer patients to an oral nutraceutical product containing silibinin. *Oncotarget.* 2016;7(22):32006. <https://doi.org/10.18632/oncotarget.7900>
418. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5):646-674.
419. Gladilin E. Graph-theoretical model of global human interactome reveals enhanced long-range communicability in cancer networks. *PLoS ONE.* 2017;12(1):e0170953. <https://doi.org/10.1371/journal.pone.0170953>
420. Polyak SJ, Morishima C, Shuhart MC, Wang CC, Liu Y, Lee DYW. Inhibition of T-cell inflammatory cytokines, hepatocyte NF- κ B signaling, and HCV infection by standardized silymarin. *Gastroenterology.* 2007;132(5):1925-1936. <https://doi.org/10.1053/j.gastro.2007.02.038>
421. Zhong X, Zhu Y, Lu Q, Zhang J, Ge Z, Zheng S. Silymarin causes caspases activation and apoptosis in K562 leukemia cells through inactivation of Akt pathway. *Toxicology.* 2006;227(3):211-216. <https://doi.org/10.1016/j.tox.2006.07.021>
422. Křen V, Walterová D. Silybin and silymarin-new effects and applications. *Biomed Papers.* 2005;149(1):29-41. PMID: 16170386.
423. Saller R, Brignoli R, Melzer J, Meier R. An updated systematic review with meta-analysis for the clinical evidence of silymarin. *Complement Med Res.* 2008;15(1):9-20. <https://doi.org/10.1159/000113648>
424. Su CH, Chen LJ, Liao JF, Cheng JT. Dual effects of silymarin on nasopharyngeal carcinoma cells (NPC-TW01). *Complement Med Res.* 2013;20(4):261-266. <https://doi.org/10.1159/000354594>
425. Sadighi S, Dashti-Khavidaki S, Shahbazi F, et al. The effects of concomitant use of silymarin and chemotherapy on solid tumors: a pilot randomized controlled trial. *Basic Clin Cancer Res.* 2017;9(2):13-19.
426. Faisal Z, Mohos V, Fliszár-Nyúl E, et al. Interaction of silymarin components and their sulfate metabolites with human serum

- albumin and cytochrome P450 (2C9, 2C19, 2D6, and 3A4) enzymes. *Biomed Pharmacother.* 2021;138:111459. <https://doi.org/10.1016/j.biopha.2021.111459>
427. Manivannan E, Amawi H, Hussein N, et al. Design and discovery of silybin analogues as antiproliferative compounds using a ring disjunctive-based, natural product lead optimization approach. *Eur J Med Chem.* 2017;133:365-378. <https://doi.org/10.1016/j.ejmech.2017.03.033>
428. Amawi H, Hussein NA, Karthikeyan C, et al. HM015k, A novel silybin derivative, multi-targets metastatic ovarian cancer cells and is safe in zebrafish toxicity studies. *Front Pharmacol.* 2017;8:498. <https://doi.org/10.3389/fphar.2017.00498>
429. Bhatia N, Agarwal R. Detrimental effect of cancer preventive phytochemicals silymarin, genistein and epigallocatechin 3-gallate on epigenetic events in human prostate carcinoma DU145 cells. *Prostate.* 2001;46(2):98-107. [https://doi.org/10.1002/1097-0045\(20010201\)46:2<98::AID-PROS1013>3.0.CO;2-K](https://doi.org/10.1002/1097-0045(20010201)46:2<98::AID-PROS1013>3.0.CO;2-K)
430. Fallah M., Davoodvandi A., Nikmanzar S., et al. (2021). Silymarin (milk thistle extract) as a therapeutic agent in gastrointestinal cancer. *Biomed Pharmacother.*, 142, 112024.