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

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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-1α expression, inducing apoptosis in some malignant cells, and inhibiting promitotic 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

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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

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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*, 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, metals, allylalcohol) and alcohol-induced damage, including hepatitis, cirrhosis, and jaundice. 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 *Cardus 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;
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, however, we do not know exactly for what purpose. 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.

**Chemistry.** The standardized extract obtained from the seeds of *Silybum marianum* is known as silymarin which contains between 70% and 80% of silymarin flavolignans. Silymarin is a mixture of 8 flavolignan structurally related isomers: silybin (or silibinin), isosilibinin, silydianin, silychristin, isosilychristin, and taxifolin. 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.**

| Component | Percentage |
|-----------|------------|
| Silybin    | 60% to 70% |
| Silychristin | 20%       |
| Silydianin  | 10%        |
| Isosilybin  | 5%         |
| Taxifolin   | 1%         |

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

In 1959, Möschlin isolated silybin, and then in 1968 silymarin chemistry was described in detail by Wagner et al.,16, 17 and Pelter and Hansel. 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). 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, 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
ethyl acetate. After a second lipid and impurities extraction, what is left is a mixture of flavolignans called silymarin. Silybin is obtained from silymarin through methanolic extraction.

**Biological activity.** In 1975, Desplaces et al 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. 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. 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. No transaminase lowering was found with silymarin in hepatitis C virus infection in another study with very high doses of silymarin. Other authors arrived to completely different results: silymarin had antiviral actions by blocking hepatitis C virus cellular entry and transmission. 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 al 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.
4. **Reduction/inhibition of hepatic fibrosis:** silymarin showed the ability to reduce hepatic fibrosis in the early stages of liver injuries.
5. **Cirrhosis:** a large population study showed that silymarin decreased mortality in patients with hepatic cirrhosis.

In spite of the evidence favoring its benefits in chronic liver disease, “the overall efficacy of silymarin remains unclear”
according to Tighe et al.\textsuperscript{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.\textsuperscript{40} It is considered 10-fold more antioxidant than vitamin E. In 1977, Machicao and Sonnenbichler\textsuperscript{41} showed that silybin increased RNA synthesis in rat liver cells and mainly increased the production of ribosomal RNA and polymerase A. Shriever et al.\textsuperscript{42} found that silymarin inhibited fatty acid synthesis in rat liver: fatty-acid-synthetase and ATP-citrate-lyase, 2 of the main lipogenic enzymes, were diminished by about 50%. Fiebrich and Koch\textsuperscript{43,44} described silymarin as a blocker of prostaglandin production \textit{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.\textsuperscript{45}

A few years later Sonnenbichler et al.\textsuperscript{46} 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 \textit{(in vitro)} by a
phosphodiesterase preparation, an inhibitor of histamine release from human basophil leukocytes, dose-dependent downregulator of in vitro lymphocyte blastogenesis and alters the mitochondrial electron transport chain through mitochondrial calcium release, in addition to its antioxidant properties. Immunostimulatory effects of silymarin were also described in experimental models, but not in the context of cancer treatment.

**Silymarin and Other Diseases (Table 1)**

Silymarin has been investigated and proposed for the treatment of many different diseases, from Alzheimer dementia to SARS Covid-19, including diabetes, diabetic complications, hyperlipidemia, and hypercholesterolemia 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. 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 did not mention anticancer activities. But in 1994, Agarwal et al 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. Silymarin and its components also inhibit beta-glucuronidase.

Valenzuela and Garrido proposed 3 levels for silymarin’s action in experimental animals:

(a) as an antioxidant, by scavenging prooxidant free radicals and by increasing the intracellular concentration of the tripeptide glutathione;
(b) through a regulatory action of cell membrane permeability and increase in its stability against xenobiotic injury;
(c) 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, although this function does not clearly hint towards anticancer activity. On the other hand, silymarin scavenges reactive oxygen species as noted above, and inhibits...
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. \(^{129}\) These publications strongly suggest a cancer-preventive activity and silymarin is slowly emerging as an anticancer drug. For example, Scambia et al. \(^{132}\) 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 Erb1 \(^{135}\) 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\(\alpha\) 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. \(^{147}\)

### Table 1. 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 | Cyclosporine nephrotoxicity | 82 |
| Renal diseases | 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 | 97-99 |
| 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 | Escherichia coli | 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 |

### 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 that \(^{149}\):

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; \(^{149}\)
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 β1 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 VGEFA 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 al 159 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 (lisophosphatidic receptors 1 and 3) are related to cancer invasiveness. 169–172 Silymarin has the ability to downregulate LPAR1. 173

Silymarin and TGF β2

Silibinin reduces the expression of TGF β2 in different tumors such as triple negative breast, 174 prostate, and colorectal cancers. 175 TGF β2 downregulation impedes the TGF β2/Smad pathway reducing cellular motility and MMP2 and MMP9 (metalloproteases) reducing invasion. In the liver, TGF β2 downregulation results in an antifibrotic effect, preventing hepatic fibrosis induced by inflammatory liver diseases. 166,176
**Silymarin and Hypoxia Inducible Factor-1α (HIF-1α)**

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

**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. In prostate cancer, silibinin decreased/inhibited CD44 expression as well. 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 molecule 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 and metastasis.

**Silymarin Modulation of TNFα (Tumor Necrosis Factor Alpha)**

Tyagi et al. showed that silibinin pretreatment of lung cancer cells inhibited TNFα induced “phosphorylation of STAT3, STAT1, and Erk1/2, NF-κB-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 al. 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. 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 cells and in hepatocarcinoma.

**Silymarin and Phospholipase A2**

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

**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.

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

Interleukin 8 has been identified as a protumoral cytokine and there is evidence showing that inhibition of IL-8 reduces tumorigenesis. Flavonoids, in general, reduce levels of IL-8. Curcumin, apigenin, and silybin showed the ability to decrease IL-8 levels.

**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.

**Silibinin Acts as a Mitochondrial “Poison” in Malignant Cells**

Si et al. 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. Yousefi et al. 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. Silymarin decreased the expression of COX2 in a model of chemically induced hepatocarcinoma in rats.

**Silybinin 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. Silybin inhibits PD-L1 by impeding STAT5 binding in NSCLC. This hints at the possible usefulness of silymarin as a complement to immune checkpoint inhibitors. A similar effect was found in nasopharyngeal carcinoma. In renal carcinoma cells, silybin decreased PD-L1 in murine renal cancer cells in vitro and in vivo.

**Silybin and Notch Signaling**

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

**Silymarin and SIRT1**

SIRT1 can deacetylare histones and other substrates and may act in a dual manner: as tumor suppressor or tumor promoter. Silymarin has the ability to increase hepatic SIRT1 expression. Silymarin can also increase SIRT1 expression in other tissues, such as hippocampus, articular chondrocytes, and heart muscle. 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.

**Silymarin and VEGF/VEGFR**

The angiogenic cytokine vascular endothelial growth factor (VEGF) and its receptor (VEGFR) play critical roles in vasculo genesis and angiogenesis. Jiang et al. 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 toxicity as well as other manifestations of cardiotoxicity. A similar decrease in VEGF and VEGFR levels was found with silymarin in preclampsic placenta, however the effect was very modest.

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

**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. There is direct and indirect evidence that silymarin interacts with c-Myc in some cases increasing its expression in liver cells as a response to hepatic chemical injuries or decreasing it in malignancies. Rajamanickam et al. found that silymarin could prevent spontaneous tumorigenesis in an APCmin/+ mouse model (prone to develop intestinal tumors) by decreasing β-catenin, cyclin D1, c-Myc, phosho-glycogen synthase kinase-3β expression, phosho-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. 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. Silymarin increases mitochondrial release of Ca++ and lowers mitochondrial membrane potential in cancer cells and increases the transmembrane potential in toxic aggressions. 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. The antimetastatic effect was due to less invasion, less EMT, less collagen I-cancer cell adhesion, and less expression of CD44.

In a randomized clinical study with patients harboring solid tumors, silymarin was added to standard chemotherapy. Although
silymarin failed to improve the results, there was a slight— not significant— trend towards reduced metastasis. 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: it protects against liver and kidney toxicities induced by methotrexate in children and adults treated for leukemia. Silybin decreased cisplatin’s nephrotoxicity without affecting its antitumoral effectiveness. There is also evidence that it protects the heart from doxorubicin toxicity, however, it is less potent than quercetin in this effect. Silymarin reduced docetaxel central and peripheral neurotoxicity. Silymarin was able to decrease diarrhea produced by irinotecan treatment. Silymarin reduced hepatotoxicity in patients with nonmetastatic breast cancer receiving doxorubicin /cyclophosphamide-paclitaxel.

**Silymarin and Resistance to Treatment**

Rho et al found that adding silymarin to epidermal growth factor receptor tyrosine kinase inhibitors could overcome
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. A similar result was obtained with ovarian cancer cells resistant to paclitaxel. The mechanism involved in resensitization to chemotherapeutic drugs is not fully known, however possible factors are: inhibition of NF-κB nuclear migration, inhibition of survival protein levels, downregulation of Pgp (MDR 1), and multidrug resistance-associated protein 1 (MRP 1). Silychristin (a component of silymarin) and silychristin derivatives have shown the particular ability to inhibit Pgp activity in a concentration-dependent manner (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 3-N-butyl-1-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. However, it also has some estrogenic effects through ER-α. Silymarin has strong binding affinity to ER-β and a mild affinity for ER-α. 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. Contrario sensu, it may be advantageous in benign prostate hyperplasia and prostate cancer. However, in an experiment carried out in albino rats, silymarin increased testosterone and LH. It also increased spermatogenesis in rats. 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. There is also endocytosis without clathrin coating.
Table 6. Pancreas.

| Year, Ref. | Findings |
|-----------|----------|
| 2011320 | Silibinin induced cell cycle arrest and apoptosis in certain pancreatic cancer cell lines. |
| 2015321 | The combination of an HDAC inhibitor and silibinin had additive effects on growth inhibition and apoptosis of pancreatic cancer cells. |
| 2015322 | In an orthotopic model of pancreatic cancer, silibinin reduced glycolytic activity of cancer cells, proliferation, and cachexia. |
| 2013323 | Dose-dependent cell growth inhibition was produced by silibinin concentrations between 25 and 100 µM. In xenograft in nude mice, tumor weight was significantly decreased by dietary silibinin. |
| 2018324 | SW1990 pancreatic cancer cells showed G1 arrest with decreased cyclins and CDKs and apoptosis with silibinin. |

Table 7. Breast.

| Year, Ref. | Findings |
|-----------|----------|
| 2004325 | Silymarin, as part of a mixture of flavonoids, downregulated the Breast Cancer Resistance Protein (BCRP). The authors propose a “flavonoid cocktail” for this purpose. |
| 2004326 | Silibinin synergized with conventional chemotherapeutic drugs in anticancer effects on breast cancer cells. |
| 2009327 | Silibinin decreased MMP9 and VEGF expression induced by TPA through downregulation of the Raf/Mek/Erk pathway. |
| 2013328 | Silymarin showed synergy with doxorubicin in producing MCF7 cell apoptosis |
| 2014329 | Silymarin showed much higher proapoptotic gene induction in a lung cancer cell line than in a breast cancer cell line. |
| 2014330 | Silibinin inhibited the accumulation of myeloid derived suppressor cells (MDSC) in murine breast cancer and increased overall survival. Silibinin decreased tumor volume. |
| 2015331 | Silibinin induced autophagic death in breast cancer cells. Silibinin treatment decreased ATP levels and altered mitochondrial electric potential with increased ROS accumulation. |
| 2015332 | Silibinin induced apoptosis in breast cancer cells. (Comment: the concentrations used were too high and are not achievable in human use). |
| 2015333 | ERα inhibition was a key factor in silibinin-induced autophagy and apoptosis. Using ERα inhibitors with silibinin, both apoptosis and autophagia were further increased. |
| 2016334 | Silibinin decreased BCL2 proteins in breast cancer cells and normal breast cells and uniformly increased PTEN in different cancer cell lines. |
| 2017335 | Silibinin sensitized breast cancer cells to doxorubicin treatment. (Comment: The concentrations used were excessively high and difficult to achieve in the clinical setting). |
| 2017336 | Silymarin-loaded iron nanoparticles produced cell cycle arrest in triple negative breast cancer cells. |
| 2017337 | Silymarin’s anticancer effects were due to inhibition of Akt and MAPK pathway. |
| 2021338 | Silibinin decreased proliferation and viability of MDA-MB-231 and MCF-7 cells in a concentration-dependent manner, inducing apoptosis. These results were obtained in vitro and in vivo. |

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 vivo277 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
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 β2 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 fibrosis37,283 but also in lung fibrosis induced by cigarette smoke.284 In this last case, this occurred by downregulation of the TGF-β1/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

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 ERβ and decreases ERα expression.264
2. In a model of mouse hepatic carcinogenesis (with nitrosodimethyamine), 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:

| Year, Ref. | Findings |
|------------|----------|
| 2005346 | Silibinin strongly inhibited growth of hepatocellular cancer cells. It also increased apoptosis with inhibition of CDK2, CDK4, and CDC2 kinases. |
| 2006347 X | Silymarin inhibited hepatocarcinogenesis induced by nitrosodimethyamine. |
| 2008348 X | Silymarin decreased the expression of MMP2 and MMP9 and decreased recruitment of mast cells in vivo in a rat liver carcinogenesis model. |
| 2008349 | Silibinin decreased cell proliferation and migration of human hepatocellular cancer cells by inhibiting the Erk 1/2 cascade. |
| 2009350 | Silymarin decreased growth of hepatocellular carcinoma (HCC) cells and induced apoptosis. |
| 2009351 X | In a xenograft mouse model of HCC, silibinin reduced growth and proliferation through reduction of Akt/Erk signaling and increased histone acetylation. |
| 2015352 | Silibinin increased growth inhibition of hepatocarcinoma cells by either sorafenib or gefitinib. |
| 2020353 | Silymarin showed antimetastatic and proapoptotic effects on HepG2 cells through the Slit-2/Robo-1 pathway. |

### Table 9. Liver.

| Year, Ref. | Findings |
|------------|----------|
| 2002339 X | Silymarin inhibited chemically induced carcinogenesis of the colon in mice. |
| 2013340 X | Silibinin blocked TNFα-induced NF-kB activation in vitro and in vivo. Tumor growth and progression were concomitantly inhibited. Bcl2, COX2, VEGF, and MMPs levels were also diminished by silibinin feeding of xenotransplanted mice. |
| 2015341 | Silymarin induced proteasomal degradation of cyclin D1 and inhibited growth of colon cancer cells. |
| 2016342 | Treatment with silymarin increased the efficacy of ionizing radiation on colon cancer cells causing increased cell death. |
| 2017343 | Silibinin inhibited proliferation and increased apoptosis in colon cancer cells. |
| 2017344 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. |
| 2020345 | Sylimarin, associated with other nutraceuticals, reduced intestinal polyp growth in an animal model. |

### Table 8. Colon.

| Year, Ref. | Findings |
|------------|----------|
| 2005346 | Silymarin inhibited chemically induced carcinogenesis of the colon in mice. |
| 2013340 X | Silibinin blocked TNFα-induced NF-kB activation in vitro and in vivo. Tumor growth and progression were concomitantly inhibited. Bcl2, COX2, VEGF, and MMPs levels were also diminished by silibinin feeding of xenotransplanted mice. |
| 2015341 | Silymarin induced proteasomal degradation of cyclin D1 and inhibited growth of colon cancer cells. |
| 2016342 | Treatment with silymarin increased the efficacy of ionizing radiation on colon cancer cells causing increased cell death. |
| 2017343 | Silibinin inhibited proliferation and increased apoptosis in colon cancer cells. |
| 2017344 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. |
| 2020345 | Sylimarin, associated with other nutraceuticals, reduced intestinal polyp growth in an animal model. |
**Table 10.** Ovary.

| Year, Ref. | Disease | Findings |
|-----------|---------|----------|
| 2014[354] | Promyelocytic leukemia | Silymarin suppressed cell growth and induced caspase-dependent apoptosis with increased p53, p21, and p27, and decreased CDK2. |
| 2003[355] X | Acute myeloid leukemia | A silybin-phosphatidylcholine complex decreased tumor growth in xenografted mice (tumor weight inhibition of 78%). |
| 2013[356] X | Lymphoma | Silibinin decreased tumor growth in vitro and in vivo 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 ex vivo (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] 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] 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 absent[376] 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 obtained[377]:

- 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 al[378] 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 al[379] 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 al[381] 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 Cmax 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.
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 Agarwal\(^{382}\) found the following results in mice 30 min after administration:

- Liver: 8.8 \(\mu\)g per gram of tissue
- Lung: 4.3 \(\mu\)g per gram of tissue
- Stomach: 123 \(\mu\)g per gram of tissue
- Pancreas: 5.8 \(\mu\)g per gram of tissue
- Prostate: 2.5 \(\mu\)g 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.\(^{383}\) Silybin 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 \(\mu\)M 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 \(\mu\)g/mL to induce apoptosis in Ramos cells (B lymphocytes).\(^{386}\) Kamrani et al.\(^{387}\) used concentrations between 50 and 100 \(\mu\)g/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 \(\mu\)g/ml = 207 \(\mu\)M).

In spite of this difficulty, Sing and Agarwal\(^{298}\) 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 \(\mu\)g/mL of silymarin to achieve a 20% reduction in proliferation and 16.1 \(\mu\)g/mL to achieve the same reduction in proliferation of LoVo colon cancer cells\(^{156}\) to achieve a reduction of migration of 50%, it is necessary a concentration of 1.15 \(\mu\)g/mL on endothelial cells (with silybin instead of silymarin 0.66 \(\mu\)g/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 silybin 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...
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, sili-pide, 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.
The most frequent adverse event was asymptomatic liver toxicity. Side effects, although rare, were mainly related to the gastrointestinal tract, such as diarrhea, bloating, and nausea. Abenavoli et al. 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 silibinin were found in prostate tissue. 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. 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, 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 al. 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, and had similar effects in breast cancer cells. These controversies on silymarin protostem or antistem effects may be due to context or tumor dependency. The question remains unsolved.

Problem 3: DNA intercalation. In 2020, Pawar and Jaldappagari reported that flavoglycans had the ability to intercalate into the DNA double helix with moderate binding affinity. Other authors have vehemently contradicted this finding. 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.

Figure 7. Methods to increase silymarin’s bioavailability.
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.

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
   - Goso University. Busan, Korea.
   - No results.

6. NCT01402648: Estrogen Receptor Beta Agonists (Eviendep) and Polyp Recurrence
   - State: completed 2011
   - Ospedale Policlinico Consorziale—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 al\textsuperscript{414} 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 al\textsuperscript{415} 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 Zanlorenzi\textsuperscript{416} published another case of complete regression of an advanced unresectable hepatocellular carcinoma treated with sorafenib and silymarin. Additionally, Bosch-Barrera et al\textsuperscript{417} 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...
are involved. The approach of attacking only one of the many hallmarks of cancer is also flawed. 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. 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. 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-β2 inhibition;
- inhibition of HIF-1α translation;
- decreased TIMP1 expression, thus decreasing metalloproteinases 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.**

| 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-1α |
| • Decreased expression of TIMP1 |
| • Anti-angiogenic effects |
| • Reduced/inhibited NF-κB activation |
| • Inhibited T-cell inflammatory cytokines |

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-alpha
7. Reduce the expression of VEGF and VEGFR
8. Inhibit the AKT axis
9. Decrease tumoral fibrosis

Silybin could be used as a scaffold or structure that can be modified improving its antitumoral effects. For example, Manivannan et al. 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
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, silimarin 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 silimarin 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 silimarin, 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
SPC self micro-emulsifying drug delivery systems
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

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Plagiarism

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

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