

REVIEW

# Milk Thistle: Its Anti-Tumor Potential

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ABSTRACT

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Milk thistle has been used for treating liver disorders since approximately 2000 years. *Silybum marianum* and its seeds contain a whole family of natural compounds, called flavonolignans. Silimarin is a dry mixture of these compounds, which are extracted after processing with ethanol, methanol, and acetone. Silimarin contains mainly silibin A, silibin B, taxifolin, isosilibin A, isosilibin B, silichristin A and silidianin. Milk thistle has been suggested to inhibit cell proliferation and to induce apoptosis, while also having anti-angiogenic properties. Its mechanisms of action involve inhibition of tumor angiogenesis biomarkers (CD31 and nestin) and molecules regulating angiogenesis (VEGF, VEGFR1, VEGFR2, phospho-Akt and HIF-1a), while other pathways such as the Wnt/ $\beta$ -catenin pathway, cyclin-dependent kinases and MAPK have also been implicated in its actions. Ongoing research has focused on the improvement of milk thistle's bioavailability and its probable use as an adjuvant in standardized chemotherapy in the near future.

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**KEY WORDS:** *milk thistle; silibin;  
silimarin; molecular pathways,  
apoptosis*

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INTRODUCTION

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The Greek philosopher, physician and botanologist Theophrastus was the first to describe milk thistle, under the name “Pternix”, for its healing properties in the 4<sup>th</sup> century B.C. Milk thistle has been used for treating liver disorders since approximately 2000 years (Fig. 1).<sup>1</sup> Pliny the Elder (23-79 A.D.) suggested that a mixture of honey and the plant's juice could serve to “carry off the bile”.<sup>2</sup> *Silybum marianum* and its seeds contain a whole family of natural compounds, called flavonolignans. Silimarin is a dry mixture of these compounds, which are extracted after processing with ethanol, methanol, and acetone. Silimarin contains mainly silibin A, silibin B, taxifolin, isosilibin A, isosilibin B, silichristin A and silidianin. In 1959, the first member of this family was discovered, silibin, which is the most extensively researched one. Silibin is a mixture of silibin A and silibin B in approximately 1:1 ratio.<sup>3-7</sup>

**ABBREVIATIONS:**

CDK = cyclin dependent kinase  
MAPK = mitogen activated protein kinase  
PSA = prostate-specific antigen  
VEGF = vascular endothelial growth  
factor

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ANTICANCER ACTIVITY OF SILIMARIN,  
IN VITRO STUDIES

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Silimarin's non-toxicity for normal tissues, its lack of adverse side-effects even in high doses, and its being inexpensive and easily available have made silimarin an attractive candidate for combined use with doxorubicin in order to decrease adverse



**FIGURE 1.** Milk thistle in the Municipality of Marathonas, Athens, Greece.

side effects and increase treatment results of doxorubicin.<sup>8-11</sup> It has recently been documented that silimarin has a synergistic effect on apoptosis induced by doxorubicin in human breast carcinoma cell line (MCF-7), thus allowing for a dose reduction of doxorubicin and minimizing doxorubicin's adverse side effects.<sup>12</sup> In addition, silibin alone has been demonstrated to induce apoptotic cell death in MCF-7 cells. Furthermore, the combination of silibin and UVB resulted in an additive effect on apoptosis in MCF-7 cells. These *in vitro* results are suggestive of the role of silibin as a supplemental agent for the treatment of patients with breast cancer.<sup>13</sup> Also, the effect of silibin on human colon cancer HT-29 cells has been studied

and the results have indicated a loss of cell viability that was time dependent.<sup>14</sup> Reduction in serum prostate-specific antigen (PSA) levels has been proposed as an endpoint biomarker for hormone-refractory human prostate cancer intervention. Researchers have tested if silibin decreased PSA levels in hormone-refractory human prostate carcinoma LNCaP cells. Silibinin treatment of cells grown in serum resulted in a significant decrease in PSA together with an almost complete inhibition of cell growth by means of G1 arrest in cell cycle. Silibin-induced G1 arrest was accompanied by a significant decrease in the kinase activity of cyclin-dependent kinases (CDKs) and associated cyclins.<sup>15</sup> The role of neo-angiogenesis in prostate cancer growth and metastasis has been well established, but the development of effective and non-toxic pharmacological inhibitors of angiogenesis still remains an unaccomplished goal. Immunohistochemical analyses have confirmed inhibition of tumor angiogenesis biomarkers (CD31 and nestin) and molecules regulating angiogenesis (VEGF, VEGFR1, VEGFR2, phospho-Akt and HIF-1a).<sup>16</sup> It is widely known that the canonical Wnt/ $\beta$ -catenin signaling pathway has a crucial role in cell proliferation, migration, and differentiation.<sup>17</sup> Data from the above-mentioned study have implicated silibin as a novel small molecule Wnt/ $\beta$ -catenin signaling inhibitor by suppressing Wnt co-receptor LRP6 expression at the transcription level, and that the anti-cancer activity of silibin has been related to this inhibitory potential on Wnt/LRP6 pathway.<sup>18</sup> Treatment with active compounds of silimarin, isosilibin B and isosilibin A, has been demonstrated to result in growth inhibition and cell death together with a strong G1 arrest and apoptotic death in human prostate carcinoma LNCaP and 22Rv1 cells.<sup>19</sup> *In vitro* studies have also shown that silibin inhibits constitutively active Stat3 and induces apoptosis in DU145 cells, while other studies have documented that silibin synergizes human prostate carcinoma DU145 cells with doxorubicin, cisplatin and carboplatin induced growth inhibition and apoptotic death.<sup>20</sup> Synergistic effects of silibin with doxorubicin and cisplatin have also been reported in breast and ovarian cancer cell lines.<sup>21,22</sup> To sum up, anti-cancer activity of silimarin has been demonstrated in human breast cancer, skin cancer, androgen-dependent and independent prostate cancer, cervical cancer, colon cancer, ovarian cancer, hepatocellular carcinoma, bladder cancer and lung cancer cells.<sup>20-24</sup>

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#### ANTICANCER ACTIVITY OF SILIMARIN, IN VIVO STUDIES

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The efficacy of silimarin has been demonstrated using various transgenic models. Further studies suggested the important role of silimarin in inhibiting the chemical- and UV-induced skin carcinogenesis.<sup>24</sup> Recently, Gu et al have demonstrated that topical or dietary silibin treatment causes a strong protection against UVB-induced photo-carcinogenesis

by inhibiting cell proliferation, inflammation and angiogenesis in SKH-1 hairless mice.<sup>25</sup> It has also been suggested that dietary feeding of silibin prevents UVB radiation-induced skin damages including thymidine dimer-positive cells, proliferating cell nuclear antigen expression and apoptotic sunburn cells. Studies have documented silibin to inhibit the member of MAPKs family (ERK1/2, JNK and p38) and Akt activation induced by either acute or chronic UVB exposure of SKH-1 mouse skin.<sup>26,27</sup> Regarding prostate cancer, dietary administration of silimarin has decreased the incidence of 3,2-dimethyl-4-aminobiphenyl (DMBA)-induced prostatic adenocarcinoma in male F344 rats.<sup>28</sup> Studies have shown that dietary administration of silibin has resulted in inhibition of the advanced human prostate tumor xenograft growth in athymic nude mice by exhibiting anti-proliferative, pro-apoptotic and anti-angiogenic properties against prostate cancer.<sup>23,24</sup> Recently, dietary silibin has been shown to inhibit prostate tumor growth and progression in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice by modulating the expression of CDKs, CDKIs, insulin like growth factor (IGF)-1 and IGF binding protein-3.<sup>29</sup> Vinh et al have shown that administration of silimarin reduces the labeling index for BrdU and the cyclin D1-positive cell ratio in various bladder lesions.<sup>30</sup> Chemo-preventive efficacy of silibin against lung cancer has been suggested by a study that has found that silibin suppresses the growth of human non-small-cell lung carcinoma A549 xenograft growth in athymic BALB/c nu/nu mice.<sup>31</sup> Silibin also reduces systemic toxicity of doxorubicin with an enhanced therapeutic efficacy by modulating NF kappa B mediated signaling pathway in this model.<sup>32,33</sup> Anti-cancer properties of silimarin have also been documented in *in vivo* models of colon cancer. Silibin administered via feeding exhibited potent pro-apoptotic and multi-targeted effects at the molecular level. The effective reduction of pre-neoplastic lesions by silibin supports its use as a natural agent for colon cancer chemoprevention.<sup>34</sup> Different laboratories have also investigated the potential of silimarin against breast cancer and there are conflicting reports regarding the chemo-preventive efficacy of silimarin/silibin in mammary carcinogenesis.<sup>23,35</sup> Dietary supplementation of silimarin increased the number of mammary tumors in 1-methyl-1-nitrosourea (MNU)-induced mammary carcinogenesis and also increased incidence of mammary tumors in MMTVneu/HER2 transgenic mice.<sup>36</sup> On the other hand, silibin treatment was found to inhibit development of mammary tumors as well as lung metastasis in HER-2/neu transgenic mice.<sup>37</sup> Anti-cancer effects of silimarin have also been documented from xenograft models of ovarian cancer. Gallo et al have found that oral administration of Silipide to nude mice bearing tumor xenograft of human ovarian cancer cell line A2780 produces significant tumor inhibition. The down-regulation of VEGF receptor 3 and up-regulation of angiopoietin-2 were the plausible mechanisms for this anti-angiogenic activity.<sup>38</sup> Giacomelli et al have shown that Silipide

administration increased the anti-tumor activity of cisplatin in mice, while reducing the toxicity associated with this anti-cancer drug.<sup>39</sup> Anti-cancer effects of silibin have been reported in renal cell carcinoma, where oral administration of silibin was found to suppress the growth of local and metastatic tumors in xenograft model of renal cell carcinoma. Overall, the above-mentioned studies provide a rationale for silimarin's utility in future clinical trials.

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### SILIMARIN IN CLINICAL TRIALS

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The efficacy of silimarin is currently being evaluated in cancer patients alone or in combination with other chemotherapeutic agents. Several doses of silimarin have been tested. Silipide, a silibin formulation, was given orally to patients with colorectal adenocarcinoma at doses of 360, 720, or 1440 mg daily for 7 days and high levels of silibin were achieved in the colorectal mucosa of the patients.<sup>40</sup> Researchers have also recently completed a phase-I clinical trial with silibin in prostate cancer patients. Silibin phytosome (SiliphosR), a commercial preparation of silibin, at a dose of 13 g, divided in 3 daily doses, appears to be well tolerated in patients with advanced prostate cancer.<sup>41</sup> Now, they are starting a pilot Phase II clinical trial to estimate the effects of silibin administration on prostate cancer progression using surrogate biomarkers as endpoints. Silimarin has been used (along with soy, lycopene and antioxidants) in a phase III clinical trial to delay prostate specific antigen progression after prostatectomy and radiotherapy in prostate cancer patients.<sup>42</sup> Chemotherapeutic agents provide significant protective effects against many cancers; however, it has been shown that hepatotoxicity is a frequent adverse side effect caused by these drugs. Studies have shown that milk thistle has been used in treatment of chemotherapy induced hepatotoxicity and has been found to protect the liver during chemotherapy. Invernizzi et al have shown similar results with the use of silimarin in a 34-year-old woman with promyelocytic leukemia. The investigators administered 800 mg/d silimarin during the patient's methotrexate and 6-mercaptopurine chemotherapy. During the 4 months of treatment with silimarin, the patient had normal liver transaminase levels and there was no further interruption in her therapeutic process. Milk thistle supplementation in children with acute lymphoblastic leukemia (ALL) and higher hepatic toxicity has been related to decreases in serum transaminases and a greater than 50% reduction in total bilirubin.<sup>43</sup> Studies have demonstrated that silimarin may play a role in adjuvant cancer therapy. *In vitro* studies have shown that silimarin increased daunomycin accumulation, potentiated doxorubicin toxicity and inhibited efflux of these drugs from cancer cells. In a nonrandomized study, patients with brain metastases receiving stereotactic radiotherapy with omega 3 fatty acids and silimarin had longer survival times and a decreased number of radio-necroses.<sup>44</sup>

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### SILIBIN'S BIOAVAILABILITY

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Silibin is rapidly absorbed from the stomach, but absorption is low due to low water solubility. The use of labeled silibin in the rat has enabled to show that the intestinal absorption of 20 mg/kg amounts to approximately 35%. Peak radioactivity is found in the plasma 30 minutes after ingestion.<sup>45</sup> Bioavailability is also low due to high reactivity with phase II conjugation in the liver. Approximately 90% of the circulating silibin is conjugated with sulfates and glucuronides. Because of the potential inactivation of CYP3A4 and CYP2C9, silibin should be carefully co-administered with drugs (e.g. nifedipine, metronidazole, irinotecan, indinavir), and should be cleared by drugs, especially in high doses. Elimination of conjugated and unconjugated forms is equally fast with a mean elimination half-life of 6.32 hours. Also, silibin's excretion in the urine ranges from 1 to 7%.<sup>46</sup> Bioavailability of silibin can be enhanced up to three-fold eight hours after consumption of a phytosome form, i.e. a complex of a natural active ingredient and a phospholipid, in healthy individuals.<sup>47</sup> Parenteral administration of silibin seems to be better, while using a phosphatidyl choline-bile salt-mixed micelles formulation. Silibin B and silichristin demonstrate nonlinear pharmacokinetics compared to the rest of silimarin's compounds. This is attributed to the saturation of conjugating enzymes and delayed elimination as a result of the extensive entero-hepatic cycling of the aforementioned conjugates of the silibin compounds. Peak concentrations were achieved for all doses after 2 hours and silimarin compounds' half-life ranged from 0.8 to 2.4 hours. The highest bioavailability was documented for silibin A at the 700-mg dose.<sup>48</sup> Comparison of 3 silibin-containing preparations (liverman capsule, legalon capsule, and silimarin tablet) revealed best absorption and bioavailability for the liverman capsule.<sup>49</sup>

Silibin is known to have poor bioavailability for two main reasons, its multi-ring structure that is too large to be absorbed by simple diffusion and its poor miscibility with oils and other lipids, severely limiting its ability to cross the lipid-rich outer membrane of the enterocytes of the small intestine.<sup>50</sup> Comparative analyses of two clinical studies revealed high bioavailability of silibin in the colon tissue but poor levels into prostate tissue suggesting the organs specific differences in the silibin bioavailability following oral administration.<sup>51</sup> Recently, the bioavailability of silibin was reported to be significantly enhanced when administered in beagle dogs as silibin-nanosuspensions.<sup>52</sup> This study also suggests that uptake and bioavailability of silibin could be further enhanced through altering the nanoparticle size.<sup>52</sup> To sum up, recent efforts towards increasing the bioavailability of silibin are really encouraging.

Despite the overall safety of milk thistle seen in these few human studies, there are still no clear data to suggest anti-cancer activity among patients with cancer. Longer-term toxicology studies of milk thistle have not shown evidence of

cancer promotion and side-effects for milk-thistle components in human beings are usually mild (diarrhea, gastrointestinal upset, and transiently raised concentrations of liver enzymes).<sup>7</sup>

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### CONCLUSION

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Milk thistle has been suggested to inhibit cell proliferation and to induce apoptosis, while having anti-angiogenic properties, too. Its mechanisms of action involve inhibition of tumor angiogenesis biomarkers (CD31 and nestin) and molecules regulating angiogenesis (VEGF, VEGFR1, VEGFR2, phospho-Akt and HIF-1a), while other pathways such as the Wnt/ $\beta$ -catenin pathway, cyclin-dependent kinases and MAPK have also been implicated in its actions. In this view, the bioavailability of its major constitute silibin has to be improved in the near future, using forms such as nano-formulations for example. Nevertheless, several limitations should be mentioned and emphasized, including the lack of an official recommendation for its use, apparently due to lack of evidence-based information on clinical applications. Thus, on the basis of the existing data, milk thistle remains in the category of dietary supplements. Thus, the absence of adequate evidence for its recommendation as a treatment for cancer holds true for the time being. Further studies are warranted to further confirm or not milk thistle as a useful tool to decrease the adverse side effects of standardized chemotherapeutics.

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### DISCLOSURE

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There is no Conflict of Interest regarding this manuscript.

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