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Role of Silymarin in Regeneration and Treatment of Skin Disorders; Progress in Signaling Pathways.

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ABSTRACT

Silymarin (SM) is the active component of milk thistle (MT) that is a flavonoid complex consisting of silibinin, silychristin, and silydianin. Silibinin (also known as silybin) is the major active component of the silymarin complex and is credited with giving MT its beneficial properties. Acting as a phyotonutrient silybin also provides a variety of benefits including anti-inflammatory, anti-carcinogenic and cardiovascular benefits. SM may involve suppression of NF-kappa B, a nuclear transcription factor, which regulates the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis. SM blocked TNF-induced activation of NF-kappa B in a dose- and time-dependent manner. This effect was mediated through inhibition of phosphorylation and degradation of Iota kappa B alpha, an inhibitor of NF-kappa B. SM significantly reduces apoptosis, skin oedema, and depletion of catalase activity and induction of cyclooxygenase activity. This provides protection against burn-induced oxidative skin injury and photocarcinogenesis. Furthermore, SM significantly decreased skin cancer number and size in a validated model of tumor promotion. These findings are supported by other studies that show similar effects for Silibinin including protects against UV light-induced DNA damage and cancer cell growth. Moreover, silibinin enhances the powerful tumor suppressor gene p53, a genetic factor that protects against cancer. Silibinin acts by other mechanisms to prevent UV light-induced skin cancer. In fact, some findings suggest that silibinin can help to repair DNA damage caused by previous exposure to UV light.
Keywords: Silymarin, milk thistle, cyclooxygenase, flavonoid complex, signaling pathways

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INTRODUCTION

Silymarin and pharmacological characteristics Milk thistle (MT) is an erect, stout, annual or biennial plant that grows to 1.5-3m in height. It has large prickly leaves, large purple flowering heads, and strongly spine scent stems (1). When broken, the leaves and stems exude a milky sap. The glabrous leaves are dark green, oblong, sinuate-lobed or pinnatified, with spiny margins (1, 2). The leaves have milk-white veins that give the leaves, which initially form a flat rosette, a diffusely mottled appearance. Silymarin (SM) known generally as poly phenolic compounds that isolated from the milk thistle plant (*Silybum marianum*). It is composed of primarily silibinin (90%) with small amounts of other silibinin stereoisomers, such as isosilybin and dihydrosilybin, etc (3, 4). SM, a flavonolignan complex that contains silibinin, is an antihepatotoxic substance and has a long tradition as herbal remedies for almost 200 years (1, 3, 4).

Signaling pathways of SM:

Silibinin is the major active constituent of SM and the anti-carcinogenic properties of SM and silibinin are almost identical. It should be noted that SM can contribute to the antioxidant (AO) defenses in different ways. Firstly, by direct free radical scavenging (1). Secondly, by preventing free radical formation by inhibiting specific enzymes responsible for free radical production, or by maintaining the integrity of electron-transport chain of mitochondria in stress conditions Thirdly, by participating in the maintenance of optimal redox status of the cell by activating a range of AO enzymes and non-enzymatic AOs, mainly via transcription factors, including Nrf2 and NF-κB (5-8) Finally, by activating an array of vitagenes, responsible for the synthesis of protective molecules, including HSP, thioredoxin (Trx), sirtuins, *etc.*, and providing additional protection in stress conditions. In most studies pure silybin, as the main component of SM, was used, however, in some cases SM also showed AO action *in vivo* (6, 8).

Silybin (10 µM and higher) is shown to have protective activity in ameliorating DNA damage in a model system. Treatment *in vitro* with silibinin significantly inhibited spontaneous O_2^- and H_2O_2 release and TNF-α production by monocytes from pre-eclamptic women. The main effect of silibinin was obtained at 50 µM concentration. Thus, the authors concluded that silibinin exerts anti-oxidative and anti-inflammatory effects on monocytes from pre-eclamptic pregnant women by inhibiting the *in vitro* endogenous release of ROS and TNF-α production (Figure 1) (8). One of the mechanisms responsible for the decrease in oxidative stress is the protective effect of SM/silibinin on mitochondrial structure and function. Indeed SM protects mitochondria from pathological events by triggering pro-survival cell signaling, *e.g.* silibinin supplementation is shown to optimize electron-transport chain, decreasing electron leakage and ROS formation and directly reducing activities of ROS-producing enzymes in the mitochondria (7, 9). In rats subjected to ischemia/reperfusion (I/R), compared with the control group, a severe impairment of mitochondrial bioenergetics was observed. The different behaviour of SM/silibinin in normal and cancerous cells should be mentioned (8, 9). In particular, SM is shown to have a protective effect against diabetes-induced cardiomyocyte apoptosis as well as apoptosis caused by various toxicants, while it causes apoptosis in cancerous cells (10). SM effectively suppressed cell growth in a dose- and time-dependent manner, and arrested cell cycle progression at G1/S phase in human ovarian cancer line A2780s and PA-1 cells via up-regulation of p53, p21, and p27 protein expression, and down-regulation of CDK2 protein expression (5, 8).

Furthermore, in the aforementioned study the SM treatment significantly induced apoptosis in A2780s and PA-1 cells by increasing Bax and decreasing Bcl-2 protein expression, and activation of caspase-9 and caspase-3. It has been shown that the oxidation of Keap1 cysteine thiols can be mediated by some polyphenols (10-12). It is an interesting fact that among flavonoids, the higher their intrinsic potential to generate oxidative stress and redox cycling, the stronger their potency as inducers of ARE-mediated gene expression. It was hypothesized that low concentrations of polyphenols could generate H_2O_2 and activates Nrf2 signaling, inducing cell adaptation to oxidative stress. Thus, polyphenols act as nutritional "medicines", which might have a preventative nature, rather than functioning as therapeutic agents (7, 12, 13). Therefore, the activation of Nrf2-ARE signaling by AO polyphenols to induce various AO molecules is probably attributable to their prooxidant activity. It seems likely that the same can be applied for silybin. In fact, pure silybin was found to be unstable whilst silybin in SM was stable in buffers from pH 1.0 to 7.8. The metabolism of silybin was more severe in its pure form compared to silybin in SM, as tested in a range of biological fluids including plasma, intestinal fluid and liver homogenates.

Silibinin has been shown to be a strong pro-oxidative agent, i.e., it was able to oxidize NADH *in vitro* in the presence of peroxidase and H2O2. This pro-oxidative action results from the production of free-radical derivatives and subsequent NADH oxidation (9, 13-16). Treatment with SM (50, 100, or 200 μM) for 24 h affected the cellular redox status and induced a dose-dependent increase in ROS generation in HepG2 cells. There was also a dose-dependent decrease in intracellular GSH level and decreased total AO potential in HepG2 cells. Silibinin induced cell death in human breast cancer cell lines MCF7 and MDA-MB-231 and it was attenuated by AOs, suggesting that the effect of silibinin was dependent on ROS generation. The nuclear factor-kappa B (NF-κB) is a widely expressed, inducible transcription factor that has been implicated in regulation of many cellular processes, including inflammation (12, 14). NF-κB, consisting of a group of five related proteins that are capable of binding to DNA, is activated by a wide range of stimuli including oxidative stress. It regulates the transcription of a range of genes, including pro-inflammatory cytokines and leukocyte adhesion molecules, acute phase proteins and anti-microbial peptides. Similar to Nrf2, in normal physiological conditions, NF-κB is found in cytoplasm in an inactive state associated with the inhibitory IκB proteins preventing its binding to target sites. Activation of NF-κB is considered to be an effective mechanism of host defense against infection and stress. Indeed, in response to stimuli, including cytokines and other stressors, IκB proteins are rapidly phosphorylated by IκB kinase on specific serine residues, with following ubiquitination, and degradation by the 26S proteasome (6, 14, 15). The resulting release of NF-κB and subsequent translocation to the nucleus orchestrates the transcription of target genes, responsible for cell survival and involved with inflammation, immunity, apoptosis, cell proliferation and differentiation (10, 15). The polyphenolic compounds express anti-inflammatory activity by modulating the expression of pro-inflammatory genes such as cyclooxygenase, lipoxygenase, nitric oxide synthases and several important cytokines, mainly acting through nuclear factor-κB and mitogen-activated protein kinase signaling (9, 10). Due to the large number of studies that have demonstrated regulatory effects of SM/silybin on the expression of NF-κB in various *in vitro* and *in vivo* models there is insufficient space in this review to analyze all of them, so we will focus only on recent investigations addressing the issue. It is well established that various plant-derived polyphenols can suppress TNF-α activated, NF-κB-associated inflammatory pathways both *in vitro* and *in vivo* (5, 10, 16). Sylimarin/silibinin is found in some high-end moisturizers to prevent cutaneous oxidative damage and photoaging. SM suppressed dust mite extract (DPE)-induced atopic dermatitis (AD)–like skin lesions in mice and reduced plasma level of IL-4 and ige. In other study was observed that SM causes inhibition of chemically induced messenger RNA expression of TNF- α and IL-1a in mouse skin (11, 12, 16). Several studies demonstrated the diverse effects of this substance such as chemoprotective and anticancer effects in the skin as well as playing a role in the prevention of radiodermatitis, chemical-induced irritant contact dermatitis, UVB-induced skin damage and also skin whitening effect (11).

Previous investigations also introduced SM – and its constituents – as an anti-inflammatory and AO agent which showed positive effects on the wound healing process and protected the skin against oxidative damage leading to the promotion of tissue regeneration particularly in burn induced oxidative skin injury in animal models (5, 8, 12). The topical administration of SM ointments in the concentration of 5, 10 and 20% were also reported to be effective in the treatment of diabetic wounds through inhibiting the inflammatory parameters and enhancing tissue regeneration. Furthermore, cutaneous photoprotection mechanisms triggered by SM and silybin are numerous and demonstrate mainly their ability to reduce and suppress harmful effects of solar UV radiation, such as UV-induced oxidative stress, inflammation, immune responses and DNA damage as well as induction of apoptosis (5, 11, 12). Topical application of SM suppressed intracellular production of hydrogen peroxide and nitric oxide and reduced depletion of catalase activity in UVB-irradiated mouse skin (SKH-1 hairless mice). In addition, SM inhibited expression of cyclooxygenase-2 (COX-2) and its prostaglandin metabolites (PGE2, PGF2, PGD2), which have been implicated in tumor promotion (11, 15, 16). SM and silybin have been also shown to prevent oxidative stress induced by UVA-irrradiation in human immortalized cutaneous cells (keratinocytes) (5, 15, 17). Studies have also indicated that topical treatment of SM to mouse skin prevents UVB-induced suppression of contact hypersensitivity response to contact sensitizer dinitrofluorobenzene. It has been shown that prevention of UVB-induced suppression of contact hypersensitivity by SM is mediated through the inhibition of interleukin IL-10 production and suppression of infiltration of skin cells after irradiation (15-18). Photochemical damage to DNA, predominantly in the form of cyclobutane-pyrimidine dimers (CPD), plays an important role in immune suppression and skin cancer initiation. Topical application of SM prevented UVB induced CPD formation in mouse skin (11, 15, 17). Induction of apoptosis together with the inhibition of DNA synthesis, cell proliferation and cell cycle progression has been suggested as *in vivo* molecular mechanism of silybin efficacy against photocarcinogenesis (15, 18). Silybin effect on UVB-induced apoptosis was examined in human epidermoid carcinoma A 431 cells. It

was shown, that silybin treatment prior to radiation causes a further increase in apoptosis, whereas post-treatment protects against apoptosis. Differential effects of silybin on UVB-induced apoptosis involved the modulation of mitochondrial apoptotic machinery (Bcl-2 family members, cytochrome c), caspases activation and mitogen-activated protein kinase (MAPK) signalin. Dual efficacy of silybin on apoptosis was observed also in human keratinocytes (11, 15, 19). Silybin afforded strong protection against UVB-induced apoptosis at lower doses (15 and 30 mJ/cm2), which was completely lost at a higher dose (120 mJ/cm2), and, in fact, increase in apoptosis together with strong down-regulation of activated protein-1 (AP-1) DNA binding activity were observed. These findings suggest that silybin could protect normal human skin keratinocytes against sunburn or apoptosis when the damage is moderate (15). When the UVB damage is severe, silybin causes apoptotic cell death, which might be of significance in deleting DNA damaged cells from cell cycle progression (11, 15, 20).
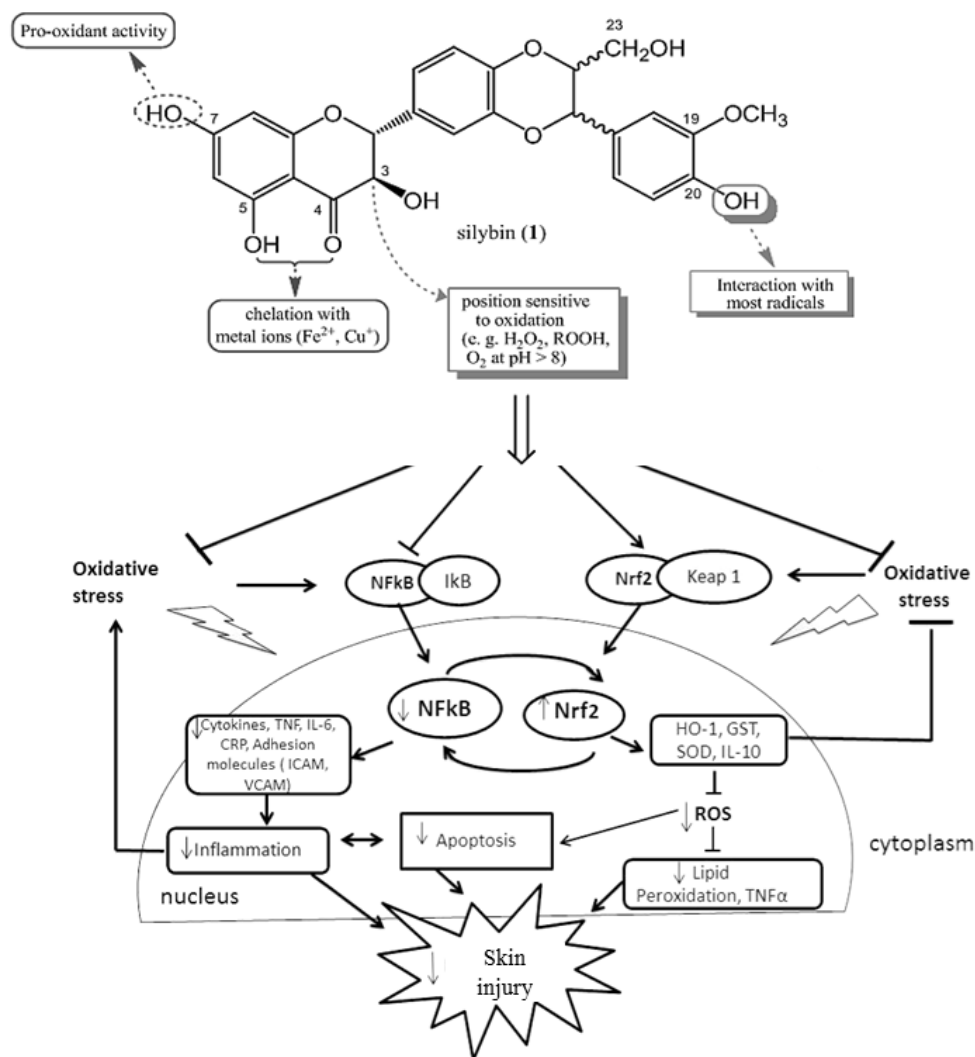

Figure1: Role of SM in regulation of the transcription of a range of genes, including pro-inflammatory cytokines and leukocyte adhesion molecules. Similar to Nrf2, in normal physiological conditions, NF-κB is found in cytoplasm in an inactive state associated with the inhibitory IκB proteins preventing its binding to target sites. SM is ameliorate and protect of skin structure against oxidative stress and free radicals.

CONCLUSION

Since flavonoids are not well absorbed in the gut, their active concentration in the plasma and target tissues are comparatively low, but probably sufficient for Nrf2 activation and NF-κB suppression as well as vitagene activation. Indeed, it seems very likely that activation of the Keap1/Nrf2/ARE pathway and inhibition of NF-κB pathway, rather than direct free radical scavenging activity, may be the main mechanisms of the health benefits of phytochemicals, including SM. Therefore, consumption of phytochemicals, including SM,

could have a pre-conditioning effect on the AO system of the body. This could explain the beneficial health-promoting effects of a diet rich in fruits and vegetables as important sources of the aforementioned chemicals (polyphenols and other phytochemicals) maintaining the body's ability to be highly adaptive to various stresses. SM and its main component silibinin are part of the dietary phytochemical mixture responsible for regulation of the AO defenses in the gut and in the whole body. The researches have demonstrated that SM is an effective skin cancer chemopreventive agent that exhibits no toxicity *in vivo*. It possesses strong AO and anti-inflammatory properties and has the ability to protect epidermal keratinocytes from UV radiation-induced apoptotic cell death through a mechanism involving repair of the damaged DNA. Topical treatment of mouse skin with SM, either before or after UVB exposure, prevents UVB-induced immunosuppression through a currently undefined mechanism that is associated with inhibition of interleukin (IL)-10 expressions and stimulation of IL-12 production in skin and draining lymph nodes. In conclusion, SM shows great promise as a superior herbal drug. Its good safety profile, better standardization and quality control, easy availability, and low cost are added advantages. Moreover, studies indicate that SM/silybin may be beneficial in skin photoprotection against sunburn response, DNA damage and immunosuppression. However, further studies are required in human system to determine cellular uptake, distribution and long term effects in the skin.

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