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PHARMACOLOGY OF SILYBUM MARIANUM AND ITS ACTIVE CONSTITUENTS. THERAPEUTIC ACTIVITY – PART 1

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ABSTRACT
In the last years a lot of effort has been made in demonstrating the role of plant extracts in medicine. The usage of herbal drugs for the treatment of several diseases has increased all over the world. The global popularity of herbal supplements and the promise they hold in treating various disease states has caused an unprecedented interest in understanding the molecular basis of the biological activity of traditional remedies. The herbal drugs are believed to be harmless without causing adverse reactions, as they are obtained from nature and are easily available. There are about 600 commercial herbal formulations available only for liver disease, which are claimed to have hepatoprotective effects. As the need for effective, affordable health promotion and treatment increases, especially in the growing ageing population, there is a need for rigorous scientific examination of herbal medicines. This article reviews on published reports pertaining to milk thistle's benefits.
KEYWORDS: Silymarin, disease, pharmacokinetics, regeneration, herbal medicine

INTRODUCTION

Silybum marianum (Milk Thistle) benefits and side effects have been studied extensively. Silymarin is a mix of flavonoligans from which silibin is the major component. Traditionally, milk thistle is commonly used for liver cirrhosis, alcoholic hepatitis, alcoholic fatty liver, liver poisoning, and viral hepatitis. *Silybum marianum* (SM) plays a role in displacing toxins binding to the liver and causing the liver cells to regenerate at a faster rate. Standardized extracts form fruits and seeds of *Silybum marianum*, sylimarin have been employed for the treatment of various disseases in humans, mainly liver-related disorders among those with different ethiologies. Silymarin is included in the pharmacopoeia of many countries under the trademark (Legalon) and its often used as a supportive therapy in food poisoning caused by fungi and in other chronic liver disorders. Besides its hepatoprotective acitivity, several studies have shown that silymarin is a strong antioxidant that is capable of scavenging both free radicals and reactive oxygen species. One of the important issues regarding the SM is that it may be a safe herbal product since no health hazard or side effects are known in conjuction with the proper administration of designed therapeutic dosages.

1. GASTROINTESTINAL EFFECTS

There are many studies where evaluated the efficacy of sylimarin/silibinin to treat a range of liver, gallbladder and intestinal disorders. The most extensively and disseminated property of sylimarin is its hepatoprotective activity against acute and chronic hepatitis, cirrhosis and toxin-induced hepatitis.

1.1. Hepatoprotective properties

Liver plays a major role in homeostasis as well as in detoxification of drugs and xenobiotics. Environmental exposure to drugs, pharmaceuticals and toxic substances cause liver damage, one of the major causes of mortality and morbidity.

Silymarin has been reported to protect liver cells from a wide variety of toxins, including acetaminophen (Avizeh et al., 2009, Girish et al., 2009, Das et al., 2011), ethanol, arsenic (Jain et al., 2011), carbon tetrachloride (Yadav et al., 2008, Tsai et al., 2008, Mohamed et al., 2010, Cho et al.,2009, Yadav et al., 2008). Silymarin has also been found to protect liver cells from ischemic injury (Wu, 1993), radiation (Kropacova et al., 1998), iron toxicity (Pietrangelo etal., 1949) and viral hepatitis (Mcpartland, 1996).

Mechanisms of Action

Bibliographic sources state that sylimarin act in several different ways: as an antioxidant, absorber and regulator of the intracellular glutathione; as a stabiliser and regulator of cell membrane permeability that prevents the entering of hepatotoxic substances into hepatocytes; as the ribosomal RNA synthesis promoter stimulating regeneration of the liver; as an inhibitor of the transformation of liver stellate cells into myofibroblasts – the process responsible for deposition of collagen fibers in liver.

Stimulation of Liver Regeneration:

Silymarin has been shown to exert profoung effects on hepatocellular plasma membrane that affect its stability and is able to increase the synthesis rate of rrna by activating RNA polymerase (Machicao and Sonnenbichler, 1997) which increase structural and functional proteins production in hepatocytes.

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Sylimarin administration influenced membrane-lipid composition by inhibiting synthesis of cholesterol (Nassuato et al., 1991) and certain phospholipids, such as phosphatidylcholine and phosphatidylethanolamine (Schriewer and Weinhold, 1979), which could improve membrane integrity. Sylimarin can also directly stabilize the plasma membrane against both mechanical stress (e.g. By osmotic swelling) and chemical stress (e.g. By detergents) in different cell types. Basiglio et al (2009) shown a differential effect of whole silymarin and its active component siibinin on plasma membrane solubilisation and hepatocellular lysis induced by micelle-forming tensioactive agents.

Interaction of silymarin with membranes may be also relevant to its well-known antioxidant properties. The interactions of flavonoid compounds with the polar head groups of phospholipids at the lipid-water interface of the membrane contribute to the protective role against lipid peroxidation (Erlejman et al., 2004) due to antioxidant system of the cells.

Silymarin stimulates the regenerative ability of the liver to form new hepatocytes by stimulating the activity of DNA-dependent RNA-polymerase I (Sonnenbichler, 1986,1987). This results in an increase in rrna synthesis and increased protein synthesis. In both *in vivo* and *in vitro* experiments, significant increases in the formation of ribosomes and DNA synthesis were measured in addition to the increase in protein synthesis. Interestingly, the increased protein synthesis was only measured in damaged livers (partial hepectomy), not in controls (Sonnenbichler et al., 1986).

The hepatoprotective properties of milk thistle extracts in acute and chronic liver injury is probably related to inhibition of leukotriene B formation by silibinin (Dehmlow, 1996).

The protective effects of *Silybum marianum* on liver injury may be related to the recovery of the membrane fluidities of liver microsome and mitochondria (Wu et al., 2003). A double-blinded trial of 141 subjects demonstrated that milk thistle extract, silymarin, could improve symptoms and general well-being of patients suffered from hepatitis C (Tanamly et al., 2004)

Anti-inflammatory Effects: In general, severe inflammation is known as one of the major pathophysiological consequences of liver diseses such as viral hepatitis and cirrhosis. Silymarin has been shown to have significant anti-inflammatory effects on hepatic tissue. The anti- inflammatory effect seems to involve blocking the activation of intrahepatic Nuclear Factor kappa B (NF-kb), and consequent dim- inution of Tumour Necrosis Factor-alpha (TNF-a), Interferon (IFN- g), IL-2 and inducible Nitric Oxide Synthase (inos) (Colturato et al., 2012). Haddad et al. (2011) verified in an experimental model of NASH (non-alcoholic steatohepatitis) that treatment with 200 mg/kg of silibinin for 5 weeks caused an improvement of liver steatosis and inflammation and decreased the levels of plasma insulin and TNF-α.

Sylimarin administration, particularly in the chronic model, induces inhibition of expression of inos and HO-1, both higly induced in the proinflamatory response against ccl4 treatment (Cho et al., 2009). Silibinin has been shown to be an immune response modifier *in vivo*, modulated signaling cascades in hepatocytes and Kupffer cells causing inhibition of nitric oxide producton and tumor necrosis factor-α (TNF-α) release (Schumann et al., 2003). Al-Anati et al. (2009) shown hepatoprotective effects of silibinin against ochratoxin due to inhibition of TNF-α release from Kupffer cells toghether with a significant reduction of cellular cytotoxicity makers into the perfusate liver.

Several studies have demonstrated a variety of anti-inflammatory effects, including mast cell stabilization (Fantozzi et al., 1986), inhibition of neutrophil migration (De La Puerta et al., 1996), Kuppfer cell inhibition (Dehmlow, 1996), strong inhibition of leukotriene synthesis, and prostaglandin formation (Sonnenbichler, 1987, Dehmlow, 1996).

Anti-apoptotic effects: Silymarin treatment caused down-regulation of Tnk2, caspase 9, c1s, cytochrome c and up-regulation of Bag4 and PCNA in mouse liver compared with pyrogallol-treated animals (Upadhyay et al., 2010). This suggests that silymarin offer hepatoprotection especially by reducing apoptosis. Furthemore, augmentation of PCNA which is a marker of cell proliferation is an argumement in this respect.

Antifibrotic Effects: Hepatic stellate cells play a central pathogenic role in liver fibrogenesis. In response to some fibrotic influences (e.g., chronic ethanol exposure, carbon tetrachloride, thioacetamide, etc.), they proliferate and transform into myofibroblasts, which are responsible for the deposition of collagen fibers in the liver. α-SMA is considered as important marker for the detection of myofibroblast-like cells (Nouchi et al., 1991) and a reliable marker of activated hepatic stellate cells before fibrous tissue deposition (Carpino et al., 2005). One recent study investigated the effect of silymarin on the transformation of hepatic stellate cells into myofibroblasts (Tsai et al., 2008). Sylimarin at high dose (200mg/kg) was capable to reverse fibrosis which was previously established by prolonged ccl4 administration in rats. α-SMA content in liver tissues of sylimarin treated group was significantly increased compared with control. The anti-fibrotic activity of sylimarin in thioacetamide (TAA) intoxication for 8 weeks was various in dose dependent manner (Shaker et al., 2011). Shafik et al (2011) showed the synergistic antifibrotic effect of sylimarin with an therapeutic antifibrotic drug (verapamil) on pig serum-induced rat-liver fibrosis. In other study it was shown that silymarin retarded the development of alcohol-induced hepatic fibrosis in 12

baboons, consistent with several positive clinical trials (Lieber et al., 2003).

Matrix mettalloproteinases (mmps) have been reported to pay a role in some cellular cascades of hepatic inflammation and fibrosis.

Interference with cytocrome P450: Silybum marianum extract or silymarin may have an inhibitory effect on the cytochrome P450 (Phase I) detoxification system at dose dependent manner. At lowest applied concentration of 1,5 μg/ml of dry extract from *S.marianum* did not inhibit any of the nine cytochromes P450 tested (Doehmer et al., 2011). At higher concentration of 15 and 150 μg/ml of dry extract of *S.marianum* was detected moderate to strong inhibition of cyt. P450. In other study was shown that silibinin inhibits cytochrome p4502e1 – dependent ROS generation and ethanol metabolism in hepatocellular carcinoma (HCC) cells *in vitro* (Brandon-Warner et al., 2010).

Oxidative stress is a main mechanism which contribute to initiation and progression of hepatic damage in a variety of liver disorders. Jain et al. (2011) suggest that sylimarin scavenges free radical generation by arsenic. Administration of sylimarin significantly protected SOD, catalase and gpx activities by directly scavenging ROS as well as by inhibiting lipid peroxidation. In other study, silymarin prevented lipid peroxidation and augmentation of antioxidant defense against paracetamol in dose-dependent manner (Girish et al., 2009).In other study it was found that silibinin improved liver steatosis and decreased nonalcoholic steatosis (NASH) – induced lipid peroxidation, plasma insulin, ROS and returned the relative liver weight as well as GSH back to normal (Haddad et al., 2011).

1.2. Gallbladder protection

Milk thistle's effects on the bile ducts and hepatic-bile synthesis may be clinically important. Silymarin (420 mg per day, for 30 days) has reduced biliary cholesterol concentrations with a significant decrease in the bile saturation index in 15 cholecystectomized patients compared to placebo controls, probably by decreasing the synthesis of liver cholesterol (Passera et al., 1991) *Silybum marianum* prevents cholestasis induced by estrogens and taurolithocholate via inhibiting camp-phosphodiesterase (Crocenzi et al., 2005). However, in an other study with patients presenting symptoms of acute clinical hepatitis, treatment with 420mg/day of silymarin for 4weeks was able to reduce the symptoms related to biliary retention, including dark urine, jaundice and scleras icterus, but not modify the levels of aminotransferases and direct bilirubin.

In 1985, Koch (1985) reported that *Silybum marianum* was a very potent inhibitor of cyclic AMP phosphodiesterase. Milk thistle's constituents, silybin, silydianin and silychristin, are 12.66 to 52.06 times more active than theophylline. Silymarin protected against ethinyl estradiol-induced cholestasis by normalizing the bile salt pool size and HCO3 output in rats (Crocenzi et al., 2001)

1.3. Intestinal protection

Silymarin reduced colonic damage, lipid peroxidation, inflammatory cytokines, increasing of total antioxidant capacity of colonic tissue against trinitrobenzene sulphonic acid (TNBS) in rats (Esmaily et al., 2009).

Silibinin in co-administration with ursodeoxycholic acid (UDCA) was found to be effective in treating of intestinal inflammation and attenuating the degree of colonic tissue injury in rats by inhibition of NF-kb and neutrophil infiltration/activation in inflamed colon in addition to a favorable effect on proinflamatory cytokines (TNF-α and IL-1β) (Esmaily et al., 2010).

2. ANTI-TUMORAL EFFECTS

The aim of the present study is to describe and mention the effects of silymarin and its subcompounds on different types of cells both in humans and laboratory animals, the antitumor, antimetastatic effect and the mechanisms by which the active compound of silimarin is interposed in cellular, intercellular signaling pathways.

From the literature it is noted that studies on the effect of silymarin as antitumor agents were conducted on various cell types both in vitro and in vivo, namely: epithelial cells **(Soria et al., 2010)**, prostate tumor cells **(Flaig et al., 2010)**, lung cell tumor **(Mateen et al., 2010)**, mammary tumor cells **(Kim et al., 2009)**, the melanoma tumor cells **(Jiang et al., 2009)**, glioma cells **(Kim et al., 2009)**, hepatocytes from rat tumor **(Ramakrishnan et al., 2009)**, hepg2 cells (Chen **et al., 2009),** enterocytes from colon tumor (**Colombo et al., 2011),** ht1080 human fibrosarcoma cells (**Duan et al., 2011**), ovarian carcinoma cells (**Zhou et al., 2008),** a375 human melanoma cell-s2 (**Jiang et al., 2011**), 786-o renal carcinoma cells (**Chang et al., 2011**), and human colon adenocarcinoma-derived metastatic cells (**Kauntz et al., 2011**).

From selection of articles underlying in this work is remarkable that a considerably higher number of studies / articles with the subject silymarin and its antitumor effect were performed on prostate, colon, liver, lung, cns, skin.

Silymarin / silybinin and prostate cells

It is known that epidermal growth factor (egf) and transforming and growth factor alpha (tgfα) are potential factors by regulating mitogen-division process of prostate cancer cells through autocrine and paracrine cycles, stimulating their tumor metastasis. These two factors manifest their activity by binding itself to cell surface receptors with an activating effect of erk1 / 2

as the signal generation process of division in human prostate cancer. The treat cells (lncap and du145) with silymarin occurred tgfα protein levels decreased at intracellular and secretory levels with a decrease in mrna levels also(**Tyagi et al., 2008**). Silymarin has also cover that inhibits egfr activation erk1 / 2 without any changes in values of these proteins. Kinase activity of erk1 / 2 to elk1 was inhibited by silymarin in du145 cells. In other situations it inhibited activation of jnk1 / 2 in lncap cells while in du145 cells was observed a strong activation of jnk1 / 2. These results suggest that silymarin affects both signaling pathway tgfα-egfr signaling-erk1 / 2 in prostate tumor cells in the androgen-dependent cells (lncap) and androgen independent cells (du145) also. Other scientists suggests that silibinin`s activity inhibit hif-1α protein expression in association with the suppression of global protein translation (**Jiang et al., 2009**).

Another study describe the differential effect of the chemical constituents of silymarin in the cell cycle regulatory molecules in human prostate tumor cells (**Deep et al., 2008**). Thus, we have shown 7 flavonolignans named components: silybin a , silybin b, isosilybin a isosilybin b, silydianin, isosilydianin, silychristin and isosilychristrin. Using cell growth factor and mortality it was found that isosilybin isomer b has the strongest effect. Facs analysis (fluorescence activated cell sorting) on cell cycle showed that treatment with silybin a, silybin b, isosilybin a, isosilybin b, silybinin, silymarin for 72 h caused has an effect of cell cycle arrest. Westernblot test showed different impact on regulatory cyclins (d, e, a and b) in the cell cycle depending on chemical constituents of silymarin used in the study. Other studies (**Verschoyle et al., 2008**) has shown that conjugated metabolites of silybin with phospholipids, namely silipid could delayed tumor development in tramp mice (mouse as a model for prostate cancer, a genetic model of intestinal malignancy of prostate adenocarcinoma) and apcmin. He also revealed that silybin can inhibit the processes of invasion, degree of motility and migration of tumor cell types arcapm (**Wu et al., 2009**) by adjusting to a low level of vimentin and mmp-2 and following may be considered as antimetastatic agent between prostate tumor cells and metastatic derived cells in bone marrow.

Concerning the silybin biodisponibility by high oral dose, research study reveals that high blood concentrations of silybin (**Flaig et al., 2010**) could be noted just transiently. The low level concentrations of silybin penetration into tissue may be explained by its short half-life, a short period of tissue treatment with silybin or the existence of an active process of removing silybin from prostate tissue.

Silymarin / silybinin and enterocytes of the colon

Simylarin anti-tumoral effects it was observed in-vivo and in-vitro studies. These studies follow pathways and mechanisms by which silybin induce cell death, studies were performed on primary tumor cells of colon (sw480) and metastatic derivatives (sw620). Silybin on these cells produced death by apoptosis evidenced by dna fragmentation and caspase 3 activation in both cell lines. Apoptotic signaling mechanism is achieved by expression of tnf mrna via activation of apoptosis inducing ligand death receptors (dr4/dr5). Caspases 8 and 10 were activated indicating an extrinsic apoptosis signaling pathway. Bid protein in sw480 cells was also split this demonstrating an intersection between internal signaling pathway with a external pathway. it was also demonstrated that silybin is interposed in producing intracellular signaling apoptosis by disrupting mitochondrial membrane potential, cytochrome release into the cytosol and activation caspase 9. In cells treated with silybin demonstrated a cytoprotective effect by autophagy inhibition (**Kauntz et al., 2011**). Another study highlights the modulator effect of silybin on β-catetina wnt signaling is involved in presenting a disorder in colorectal cancer (**Sangeetha et al., 2010**). The experimental protocols were performed (**Velmurugan et al., 2010**) to follow the silybin effect on growth of xenografe tuomorale colonorectale cells in nude mice. Effect of silybin was a potent inhibitor of growth of these xenografe cells. The experiment analyze showed that silybin exhibit an antiproliferative effect, pro-apoptotic and antineoangiogenesis. Moreover, silybin-β reduces the expression and phospho-gsk3β catetine xenografe tissues. Other studies followed potential mechanisms of synergy between silymarin and chemotherapy (doxorubicin and paclitaxel type) tumor cell lines presenting drug resistance (**Colombo et al., 2011**).

Silymarin / silybinin and hepatocytes

A few studies investigated the dietary supplementation of silymarin has any role in mast cell density (mcd) and in the expressions of mmp-2 and mmp-9 in n-nitrosodiethylamine induced (ndea) liver cancer in wistar albino male rats. After the ndea administration rats showed increased mcd observed by toluidine blue staining along with upregulated expressions of mmp-2 and mmp-9 (**Ramakrishnan et al., 2009**). Silymarin treatment inhibited this increase of mcd and decrease the regulation of the expressions in mmp-2 and mmp-9. These effects was revealed by western blotting and immunohistochemistry. Silymarin manifest a few beneficial effects on liver carcinogenesis by attenuating the recruitment of mast cells and after that in decreasing the expressions of mmp-2 and mmp-9. Another study investigate mechanisms involved in the growth inhibitory effect of silymarin, in humanhepatocellular carcinoma (**Ramakrishnan et al., 2009**), in a dose-dependent manner. The percentage of apoptotic cells was

increased after treatment with 50 and 75 μ g/ml silymarin for 24 h. Also the silymarin treatment increased the proportion of cells with reduced dna content (sub- g 0 /g 1 or a 0 peak). The silymarin also decreased mitochondrial transmembrane potential of the cells, by increasing levels of cytosolic cytochrome c with concomitant decrease in anti-apoptotic proteins (bcl-2 and survivin) and proliferation-associated proteins (β -catenin, cyclin d1, c-myc and pcna). A different study was made on the effect of baicalein, silymarin, and their combination, on two human liver cell lines, hepg2 (hepatocellular carcinoma) and chang liver (non-tumor liver cells) (**Chen et al., 2009**). The results of the sudies indicates that the combination of baicalein and silymarin eradicates tumor cells efficiently and offers mechanistic insight for further exploitation of hcc treatment.

Silymarin / silybinin and melanoma cells

An experimental model showed that silibinin protected cells from mitomycin c (**Jiang et al., 2009**) induced apoptosis mainly through suppressing the mitochondria-mediated intrinsic apoptosis pathway, but not in an extrinsic pathway. The preincubation with silibinin before to mitomycin c treatment substantially suppressed cell apoptosis, attenuated the change of p53 and bcl-2 expressions by stop the translocation of bax to mitochondrial external membrane, and ameliorated the loss of mitochondrial membrane potential. Also the same author (**Jiang et al., 2011**) showed in another experiment that silibinin induced the generation of large amount of superoxide anion (O_2) and small amount of hydrogen peroxide (H_2O_2) through down-regulating the activity of mitochondrial complex iv and the protein level of cytochrome c.

Another experiment showed that the treatment of mel 1241 cells with silymarin or fh535, an inhibitor of wnt/b-catenin pathway, significantly inhibited cell migration of mel 1241 cells, which was associated with the elevated levels of casein kinase 1a and glycogen synthase kinase-3b, and decreased accumulation of nuclear b-catenin and inhibition of mmp-2 and mmp-9 levels. This effect of silymarin and fh535 was not found in mel 1011 melanoma cells. These results indicate for the first time that silymarin inhibits melanoma cell migration by targeting b-catenin signaling pathway (**Vaid et al., 2011**).

Silymarin / silybinin and mammary cells

The consumption of silibinin or silipide, a silibinin formulation with pharmaceutical properties superior to the unformulated agent, affect breast cancer development in the c3(1) sv40 t,t antigen transgenic multiple mammary adenocarcinoma mouse model. The result of the study suggests that promotion of carcinogenesis is not a feature of silibinin consistent across rodent models of mammary carcinogenesis (**Verschoyle et al., 2008**).

Another study showed that presence of matrix metalloproteinase-9 (mmp-9) and cyclooxygenase-2 (cox-2) are important steps in breast cancer pathogenesis. Silibinin has a down-regulates tpa-induced mmp-9 expression through inhibition of cox-2 expression in breast cancer cells (**Kim et al., 2009**).

Silymarin / silybinin and the cells of the central nervous system

Oral administration of silibinin in animals with subcutaneous u87mg glioma cells reduced tumor volume. Tumor tissue analysis showed a decrease in ki-67 positive cells, an increase in tunel-positive cells, and caspase activation (**Kim et al., 2009**). These results indicate that silibinin induces a caspase-dependent cell death via ca2?/ros/ mapk-mediated pathway in vitro and inhibits glioma growth in vivo. Another study (**Jeong et al., 2011**) showed that the silibinin induces apoptotic cell death through a calpain-dependent mechanism involving pkc, ros, and aif nuclear translocation in u87mg human glioma cells.

Silymarin / silybinin and lung cell tumor

An experimental model showed that silibinin treatment inhibited cell growth and targeted cell-cycle progressing causing a prominent g_1 arrest in dose and time dependent way. Concerning the level values, the silibinin (50-70 μm) modulated the protein of cyclin-dependent kinases(cdks), cyclins(d1, d3, e) in all three tumoral lines (h1299, h460 and bronchioalveolar carcinoma cell line) (**Mateen et al., 2010**). Another study showed that the silibinin has an anti-tumorigenesis effect of lung cancer in wild-type mice and no effect in inos-/- mice (**Ramasamy et al., 2010**). The lack of effect of silibinin in inos-/- mice may suggests that silibinin exerts most of its chemopreventive and angiopreventive effects through its inhibition of inos expression in lung tumors.

Silymarin / silybinin and epithelial cells

Experimental results showed that the use of silymarin increases the possibility of designing better arsenic-based cancer chemotherapies with less toxicity to normal cells (**Soria et al., 2010**). Arsenic (as) has a paradoxical biomedical role: it causes oxidative damage to normal cells leading to death or malignant transformation, but can be used, for the same reason, as an anticancer pro-apoptotic agent at high doses. Silymarin administration during arsenic based cancer chemotherapy (quercetin) is therapeutically useful in order to selectively decrease collateral toxicity in normal cells.

Silymarin / silybinin studies on various cell types in vitro and in vivo

Experimental results suggest that silibinin might induce p53-mediated autophagic cell death by activating ros-p38 and jnk pathways, as well as inhibiting mek/erk

and pi3k/akt pathways in fibrosarcoma cells (**Duan et al., 2011**). Another study showed that silibinin enhanced the sensitivity of a2780/taxol cells to paclitaxel, increased paclitaxel-induced apoptosis and g2/m arrest consistent with the down-regulation of survivin and p-glycoproteins (**Zhou l. et al., 2008**). A2780/taxol cells demonstrated a two-fold increase in invasiveness ability compared to a2780 cells, whereas the invasive potential was reduced dramatically by silibinin. Another experimental model with paclitaxel on renal carcinoma 786-o cells in vitro showed that combination treatment with silibinin and 5-fluorouracil, paclitaxel, vinblastine enhanced the chemosensivity of 5-fluorouracil and paclitaxel (**Chang et al., 2011**). Other study investigate the effects os silymarin, an inhibitor of the p-glycoprotein efflux pump, on oral bioavailability of paclitaxel (taxol) and a paclitaxel microemulsion (**Park et al., 2012**). Based on this experiment, the results showed that oral bioavailability of paclitaxel is significantly improved by co-administration with silymarin.

Concerning the anti-metastatic effect, detailed mechanistic analyses revealed that silibinin targets signaling molecules involved in the regulation of epithelial-to-mesenchymal transition, protease activation, adhesion, motility inhibiting metastasis (**Deep and Agarwal, 2010**).

Silybin was identified as a novel hsp90 inhibitor of heme-regulated elf2α kinase (hri) by hsp90-dependent firefly luciferase refolding and hsp90-dependent. A library of silybin analogues was designed, synthesized and evaluated (**Zhao et al., 2011**) using the identification of the essential, non-essential and detrimental functionalities on silybin that contribute to hsp90 inhibition.

It was noted also, that silibinin enhances the effect of egfr-tkis to overcome t790m-mediated drug resitance in non-small-cell lung carcinoma (nsclc) by suppression of egfr dimerization (**Rho et al., 2010**).

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