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New Therapeutic Potentials of Milk Thistle (*Silybum marianum*)

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Silymarin is a bioflavonoid complex extract derived from dry seeds of Milk thistle [(*Silybum marianum*(L.) Gaernt. (Fam. Asteraceae/Compositaceae)] whose hepatoprotective effect has clinically been proved. Low toxicity, favorable pharmacokinetics, powerful antioxidant, detoxifying, preventive, protective and regenerative effects and side effects similar to placebo make silymarin extremely attractive and safe for therapeutic use. The medicinal properties of silymarin and its main component silibinin have been studied in the treatment of Alzheimer's disease, Parkinson's disease, sepsis, burns, osteoporosis, diabetes, cholestasis and hypercholesterolemia. Owing to its apoptotic effect, without cytotoxic effects, silymarin possesses potential applications in the treatment of various cancers. Silymarin is being examined as a neuro-, nephro- and cardio-protective in the damage of different etiologies due to its strong antioxidant potentials. Furthermore, it has fetoprotective (against the influence of alcohol) and prolactin effects and is safe to be used during pregnancy and lactation. Finally, the cosmetics industry is examining the antioxidant and UV-protective effects of silymarin. Further clinical studies and scientific evidence that silymarin and silibinin are effective in the therapy of various pathologies are indispensable in order to confirm their different flavonolignan pharmacological effects.

Keywords: Milk thistle, Silymarin, Silibinin, Therapeutic, Protective.

Milk thistle [*Silybum marianum* (L.) Gaernt., family Asteraceae/Compositaceae] is probably the oldest and the best-studied plant in the treatment of liver diseases. The first records of milk thistle healing effects are recorded in the Old Testament [1]. Silymarin standardized dry extract is a bioflavonoid complex consisting of at least seven flavonolignans (Figure 1). Silibinin is the major component of this complex extract (about 60-70%) and is biologically the most active constituent of silymarin, followed by silicristin (20%), silidianin (10%), and isosilybin (5%). Silibinin is a mixture of two stereoisomers, silibinin A and silibinin B in the equimolar ratio 1:1 [2].

Modern Western medicine has confirmed hepatoprotective properties of silymarin by conducting animal studies and clinical trials [1]. Silymarin has antioxidant, anti-inflammatory, antifibrotic, detoxifying and regenerative properties. It stimulates protein synthesis and liver regeneration. Silymarin has numerous hepatoprotective effects: it prevents lipid peroxidation by scavenging free radicals and increases the levels of reduced glutathione (GSH). It regulates membrane permeability and membrane stability in damage caused by xenobiotics, regulates nuclear expression and inhibits the transformation of hepatocytes in myofibroblasts (with cirrhosis) [3, 4]. New *in vivo* studies have proved silymarin to be a strong hepatoprotective in hepatic fibrosis induced by carbon tetrachloride (CCl_4). After silymarin had been administered to the rats treated previously with CCl_4, the levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminases were normalized and the secretion of the connective tissue growth factor was decreased, indicating that silymarin ameliorates the destructive changes in the liver after hepatic fibrosis was provoked [5]. An insight has recently been published into the possible mechanism of silymarin defense capacity, as an antioxidant against various hepatotoxicants. Orally administered silymarin to the experimental mice in only three doses of 100 or 200 mg/kg increased the amount of metabolites generated from homocysteine in the transsulfuration pathway (cystathionine, cysteine, and glutathione), elevated the activity of cystathionine β-synthase, while down-regulated cysteine dioxygenase. This resulted in the augmented oxygen radical scavenging capacity of the liver cytosol and reduced lipid peroxidation. For the first time, it was demonstrated that the antioxidant capacity of silymarin was connected to the hepatic glutathione production in the liver by cysteine synthesis increment and inhibited degradation to taurin [6]. Silymarin inhibits the absorption of α-faloidine and *α*-amanitin toxins (from the mushroom *Amanita phalloides*) as it prevents their binding to the cell surface and limits the transportation systems on the cell membranes. Silymarin/silibinin (SIL) stimulates the regeneration of hepatocytes by nuclear A polymerase activation, increasing protein synthesis and inhibiting the expression of the adhesion molecules (E-selection) [7]. The anti-inflammatory effect of silymarin prevents the activation of the intrahepatic nuclear factor kappa B (NF-kB), reducing the levels of tumor necrosis factor alpha (TNF-α), interelukin-2 (IL-2), interferon gamma (IFN-γ) and the inducible nitric oxide synthase (iNOS) [8]. It was shown that silymarin was hepatoprotective [9], and it was used in the treatment of nonalcoholic fatty liver [4], alcoholic liver cirrhosis [10], and acute and chronic hepatitis [11].

SIL possesses some other potential remedying properties and the new therapeutic effects of milk thistle extract have been considered and proved in various preclinical and clinical studies which are presented in this paper.

Pharmacokinetics of SIL: Silymarin/silibinin is rapidly, but poorly absorbed due to its high liposolubility and poor water solubility. After an oral administration of silymarin (equivalent to 120 mg silibinin), the peak plasma concentration was reached within 1-2 h, but the maximum concentration was only 1.1 to 1.3 mg/mL [12]. The increased bioavailability of silymarin was achieved by complexation with phosphatidylcholine, β-dextran or a suitable

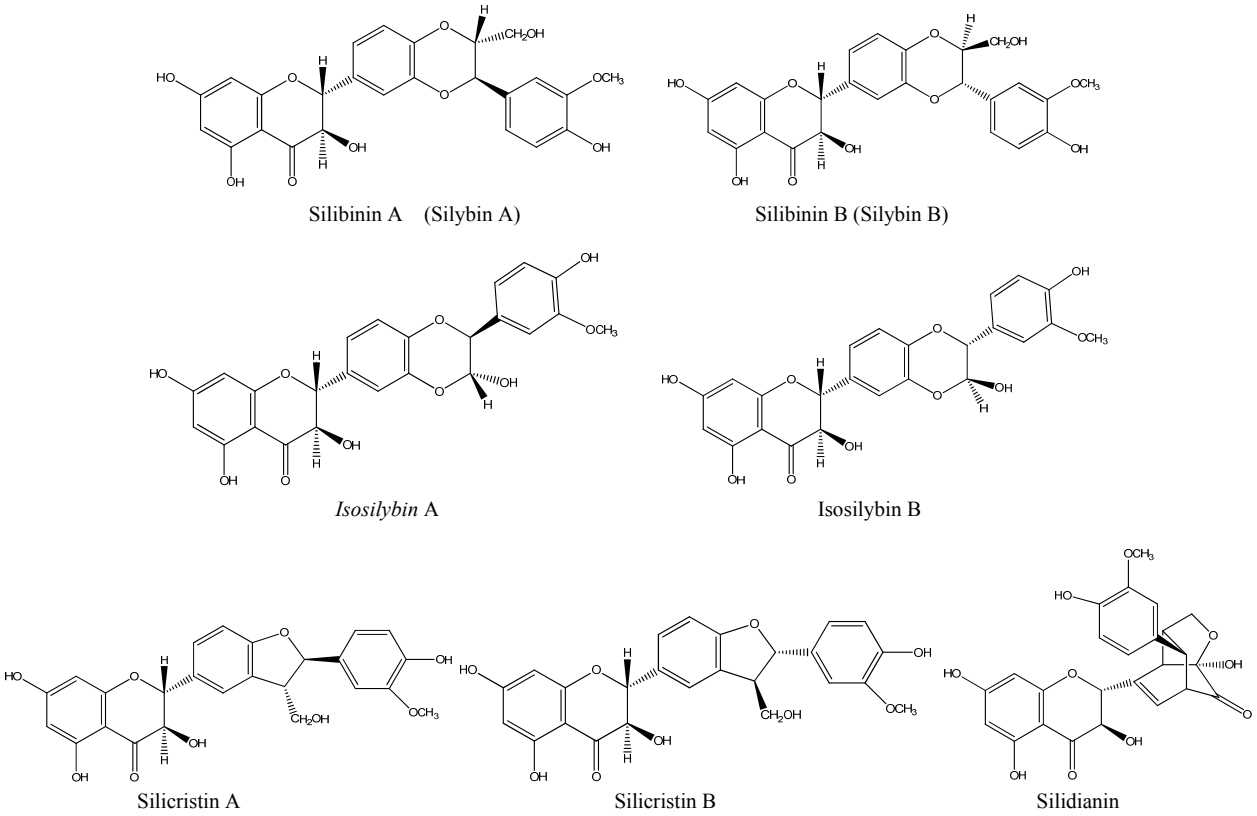

Figure 1. Flavonoligans of Milk thistle extracts.

material for capsulation. Silibinin in plasma is rapidly distributed in liver, lungs, skin, prostate, and pancreas, and the maximum level is achieved within just one hour after oral administration. About 70% of silibinin binds to plasma proteins, and the concentration in the bile is 100 times higher than in plasma. In humans, higher doses of silibinin are subjected to I and II metabolism phases, whereby the liver microsomes produce one dominant demethylated metabolite in the first phase, while three mono-hydroxyl metabolites and one dihydroxyl metabolite are generated in small amounts. Silibinin monoglucuronide, silibinin diglucuronide, silibinin monosulfate and silibinin glucuronide sulfate are produced in the second phase, and then *O*-demethyl silibinin glucuronide and silibinin triglucuronide. The halftime elimination of silymarin (silibinin equivalent to 25%) is 6.32 h and it is mainly excreted via bile. Only 1 to 7% of silymarin is excreted into the urine [13-15]. However, the urinary excretion of 31 detected metabolites was followed by clearance for 24 h while substantial amounts of glucuronides, sulfo-glucuronides and diglucuronides were excreted within 48 h. A non-marginal entero-hepatic recirculation, which preferentially occurs for silymarin sulfate–glucuronides and diglucuronides, may be the explanation for a delayed clearance of the conjugated metabolites. Although, polyphenols are drastically modified by the colon microbiota, no microbially derived compounds were detected after the oral administration of silymarin [16].

Silymarin side effects: German Commission E approved silymarin for various liver conditions and stated that it had no side effects at the recommended doses. It is safe for use in pregnancy (560 mg/day), in children (20-50 mg intravenously) and in elders over 75 years (420 mg/day). The toxic effects of the milk thistle extracts have not been recorded at a dose of 1200 mg/day, although it was administered at doses of 160-600 mg/day divided in three equal doses in most of the clinical studies. However, mild allergies were recorded at doses greater than 1500 mg a day. A few patients reported heartburn, diarrhea, bloating, flatulence and dyspepsia. It is proved that silymarin is well-tolerated and safe for use up to 41 months [17].

Silymarin interaction with drugs: Silymarin inhibited the enzymes of phase I metabolism: CYP2E1, CYP2D6, CYP2C19, CYP1A2 and CYP2A6 and the enzymes of the phase II metabolism such as uridine diphosphoglucuronyltransferase (UGT) 1A1 isozymes, and due to this ability it could cause interactions with the drugs that are metabolized with the same enzymes. It was also detected that silymarin, in a dose-dependent manner, inhibited P-glycoprotein, which is responsible for the excretion of the xenobiotics from the liver into the hepatobiliary duct and could cause the drug accumulation that is excreted via those routes. However, only the interactions of silymarin with metronidazole (increases the clearance of metronidazole by about 30%), pyrazinamide (prevents its excretion) [13] and warfarin (increases the risk of bleeding) have been detected so far [18].

Effect on Alzheimer's disease and dementia: Alzheimer's disease (AD) is characterized by progressive cognitive impairment and protein plaque deposits. The extracellular aggregation of amyloid β (Aβ-peptide), a protein plaque component, found in the brain cells of the patients with AD, presents the major histopathological change in this disease. It is assumed that Aβ-peptide generates H_2O_2, which is instantly converted into a highly reactive hydroxyl radical which causes an oxidative stress. In the *in vitro* study on human neuroblastoma SH-SZ5Y, it was proved that silibinin, in a dose-dependent manner, inhibited the aggregation of Aβ-peptide, decreased Aβ-peptide cytotoxicity, reduced hydrogen peroxide production and cell injury. Silibinin is a powerful anti-oxidative agent which increases GSH and superoxide dismutase (SOD) levels

and inhibits lipid peroxidation. The study confirmed the anti-oxidative effects of silibinin and assumed that the decrease of H_2O_2 production, induced by Aβ aggregation, was the main route for its neuroprotective activity [19].

Pretreatment with silibinin in a dose-dependent manner improved the spatial memory and energy metabolism in the brain, reduced cholinergic dysfunction and prevented the accumulation of lipid peroxides in mice after streptozotocin (STZ) injection had been administered. Several studies reported that the impaired brain energy metabolism was the key event in AD pathophysiology. It was demonstrated that dysregulated Ca^{2+} intracellular homeostasis and increased free radical formation disturbed the mitochondrial function and caused the changes in brain energy metabolism. The pretreatment with silibinin eliminated the disruption of the metabolism energy (the defects in the electron-transport chain of mitochondria and the reduced production of ATP in the mice brains that STZ caused), reduced the levels of reactive oxygen species (ROS) and malonyldialdehyde (MDA), and increased GSH and Ca^{2+} levels in the synaptic space. Loss or down-regulation of the neuronal nicotinic acetylcholine receptors (nAChRs) was also observed in AD. Silibinin reduced the anti-acetylcholinesterase action, restored the function of acetylcholinesterase and mRNA expression in a dose-dependent manner in the brain of the mice that were treated with STZ. In addition, it prevented the decreased expression of the nAChRs (particularly the subtypes which are predominant in the brain and cause the impairment of the cognitive functions) [20].

The cognitive deficit was observed in patients with diabetes and this disease was often cited as a risk factor for the development of vascular dementia and Alzheimer's disease. It is supposed that the stimulation of ubiquitous and inducible heme oxygenase (HO)-1, caused by silibinin, was accountable for the silibinin antioxidant and anti-inflammatory activity as the HO isoforms catalyzed the conversion of heme to carbon monoxide and bilirubin, and its increased expression was the response to the oxidative stress in the CNS. The level of HO-1 was reduced in the forebrain and cerebellum of the diabetic mice, while it was unexpectedly increased in the brainstem; treatment with silibinin caused a considerable induction of HO-1 in the forebrain and cerebellum, but it provoked inhibition in the brainstem. Silibinin decreased isoprostanes and 8-OH deoxyguanosine (markers of lipid peroxidation and DNA damage, respectively) in the hippocampus and hypothalamus of the treated diabetic animals. Silibinin protected DNA from the oxidative stress and therefore, it could be considered as an important phytotherapeutic agent in the treatment strategy of the accompanying diabetic side effects on the CNS [21].

Silibinin's favorable effects on the cognitive function was confirmed by *in vivo* studies on mice whose memory loss was caused by methamphetamine (METH), which reduced the ability to recognize a new object and the five-fold choice. METH provokes hallucinations and delusions in humans, and METH chronic use causes cognitive deficits after withdrawal. Silibinin neutralized the METH effects and reduced the time necessary to recognize the new items (impairment in the novel object recognition test). Silibinin administered to the healthy mice did not cause any changes in the serotonin and dopamine levels, but annulled the effects that METH had on these systems if it had been given as a pretreatment. It is believed that silibinin inhibited monoamine oxygenase (MAO) and reduced the dopamine and serotonin metabolisms. The silibinin antioxidant effect reduced the peroxynitrile concentration which caused the dopamine and serotonin concentrations to be maintained in the brain. Silibinin anti-inflammatory effects protected microglial activation in the midbrain, striatum, thalamus, orbitofrontal and insular cortices provoked by METH. It additionally gave protection from the neurotoxicity caused by lipopolysaccharides that initiated a pro-inflammatory cascade and released the cytokines. Therefore, silibinin can have a potentially important role in the therapy of drug addicts [22].

Effect on Parkinson's disease: The possible causes of the degeneration of the dopaminergic neurons in Parkinson's disease (PD) are: reduced GSH levels, DNA damage, iron deposition and primarily, the oxidative stress that impairs the oxidative phosphorylation and energy metabolism that lead to dopaminergic neuron death. Silymarin postponed the oxidative damage of the neurons (reducing the synthesis of ROS and maintaining SOD levels) that had been developed in many neurodegenerative diseases by inhibiting MAO in glial cells, by stimulating peroxidation product secretion and, probably, by stimulating the synthesis of ribosomal RNA as it bonds via the estradiol receptors [23].

Anticarcinogenic effects: The anti-tumor effect of silymarin was detected in tumors induced in the epidermis of mice in 1994 [24]; this led to a number of studies. Silymarin is classified in the group of effective chemoprotective and chemopreventive agents in the treatment of various cancer conditions. In 1999, it was reported that silibinin was probably responsible for its anti-proliferative effect since it inhibited DNA synthesis in prostate carcinoma LNCaP and DU145 cells, breast carcinoma MCF-7 cells and cervical carcinoma A431 cells [25].

Effect on skin cancer: Silymarin demonstrated the capacity to reduce the expression of the TNF-α endogenous promoter in SENCAR mice with chemically induced skin cancer. Orally administered silymarin not only inhibited the tumor growth, but it further decreased the volume of the existing tumors (80-97%) with no signs of toxicity to the animals. Silymarin inhibited all the proteins of the mitogen-activated protein kinase (MAPK) family: ERK1/2, JNK and p38 and induced apoptosis; it was irrelevant whether silymarin had been administered orally or topically [26-28]. It can be applied in a benign condition such as premalignant keratosis. It was found that silymarin possessed a protective effect in SKH-1 mice with UVB radiation-induced skin tumor initiation, tumor growth and complete carcinogenesis. Silymarin significantly affected the inhibition of UVB-induced sunburns and the formation of apoptotic cells, skin oedema, catalase activity reduction, induction of expression and activity of cyclooxygenase (COX) and ornithine decarboxylase (ODC), when applied either topically or orally before or after exposure to UVB. Regulating the p53-p21 cascade that protected the DNA from damage, silymarin reduced a number of the thymidine dimer-positive cells and slowed the kinetics of the DNA damage by stimulating its reparation after the UV exposure. Silymarin induced the level of Cip1/p21 protein in the epidermis of the mice during the acute radiation with UVB rays, thus inhibiting the cell proliferation and expression of the genes responsible for the cell growth in a cascade way [29-31]. In addition, in novel studies, it was found that the main constituent of silymarin, silibinin, saved the cells from apoptosis through inhibition of IGF-1R activation followed by repression of ERK1/2 and JNK phosphorylation [32] and induced autophagy protection of human carcinoma A431 cells from UVB-induced apoptosis [33] in UVB-irradiated A431 cells. Furthermore, silibinin contributed to the reduction of A, E and D1 cyclins, increased the protein levels of CDKIs, Cip1/p21 and Kip1/p27 in the tumor cells, inhibited the activity of cyclin-dependent kinase (CDK) and thus decreased the proliferation and growth of the tumor cells. Therefore, it is expected that the silibinin non-toxic photo-protective effects will be

considered as a potentially useful chemo-preventive agent for the treatment of skin cancer [34].

Effect on hepatocellular carcinoma: The positive effects of silymarin in the treatment of hepatocellular carcinoma were reported in several *in vivo* and *in vitro* studies. In the study conducted on Wistar rats with induced hepatocellular carcinoma, treatment with silymarin (1000 ppm orally, 16 weeks before or 5 weeks after tumor induction) regulated the increased levels of aminotransferases (AST and ALT), phosphatases (ACP and ALP), lactate dehydrogenase, gamma-glutamyl transferase and 5'-nucleotidase, tumor marker alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA). The reduction in MDA-DNA formation was recorded in the animals with tumors that had received silymarin compared with the control group [35]. The research on H4IIE hepatocytes, where the tumor had been induced with a high ethanol concentration, confirmed the hepatoprotective effects of SIL: silibinin inhibited CYP2E1 (but not alcohol dehydrogenase) and ethanol metabolism, reduced the ROS level, while it also reduced the proliferation and progression of the cell cultures in the rats suffering from hepatocellular carcinoma at a dose of 10 μM [36], which was consistent with the findings that silibinin inhibited cell proliferation, matrix metalloproteinase 2 enzymatic activity, NO production and ERK 1/2 phosphorylation in a dose-dependent manner in the HepG-2 cell line [37]. Furthermore, recent studies confirmed that pretreatment with silymarin down-regulated the cytokines-gene expression in the human hepatocellular carcinoma cell lines susceptible to amphotericin B-induced oxidative stress [38].

Effect on breast cancer: Silymarin inhibited both anchorage-dependent and anchorage-independent cell growth in dose- and time-dependent manners in the treatment of human breast carcinoma. Its effects on the cell growth and proliferation were probably connected with an induction of up to 19-fold in the protein expression of CDK inhibitor Cip1/p21G1 which caused the arrest in the cell cycle progression [39]. In addition, silibinin had a synergistic effect with anticancer chemotherapeutics such as doxorubicin, cisplatin and carboplatin in the human breast cancer cells. Silibinin, in a dose-dependent manner, inhibited the expression of matrix metalloproteinase 9 (MMP-9), preventing the transcription of this protein by blocking the pathway that went through MAPK and by suppressing the AP-1 proteins with oligonucleotides containing the sequence for the AP-1 DNA at the binding site of the MMP-9 , preventing the expression of MMP-9 in the *in vitro* cultures of the human breast cancer cells [40, 41]. The results were even more impressive with the use of silibinin-loaded lipid nanoparticles containing D-α-tocopheryl-polyethyleneglycol 1000 succinate (TPGS) [42]. It was recently found that silibinin was a Wnt/β-catenin signaling inhibitor by suppressing Wnt co-receptor LRP6 expression at the transcription level, and that the anti-cancer activity of silibinin was associated with its inhibitory effect on Wnt/LRP6 signaling [43].

Effect on ovarian cancer: Silipide (IDB 1016 - silibinin complex with phosphatidylcholine), administered orally to hairless mice which had been embedded with the xenograft of human ovarian cancer cells, contributed to a significant reduction in the tumor mass by 78% [44]. It was found that silibinin enabled the overcoming of resistance to paclitaxel. It reduced the invasiveness of the paclitaxel-resistant tumor cells in another *in vitro* study on paclitaxel-sensitive and paclitaxel-resistant cells [45]. If these effects are proved in *in vivo* conditions, silibinin in combination with paclitaxel could become a part of the strategy in the treatment of tumors in patients resistant to paclitaxel.

Effect on cervical cancer: Silymarin has a possible application in chemo-prevention and in the treatment of cervical carcinoma. Silymarin caused the apoptosis of HeLa cells of human cervical cancer at low doses (80 μmol/L) and cell necrosis at high doses (160 μmol/L). The apoptosis caused by silymarin via MAPK activation was due to chromatin condensation and nuclear fragmentation of the apoptotic cells [46]. It was found that silibinin inhibited hypoxia-induced HIF-1α accumulation and HIF-1 transcriptional activity in human cervical (HeLa) and hepatoma (Hep3B) cells, activated Akt, and reduced hypoxia-induced vascular endothelial growth factor (VEGF) released by HeLa and Hep3B cells, and finally, inhibited cell proliferation [47].

Effect on bladder cancer: Silibinin inhibited cell growth and caused cell cycle arrest in the G1 and G2/M phases of the highly invasive TCC-SUP cells (apoptosis), and the G1 phase of the cell cycle in T-24 of human bladder carcinoma cells (without apoptosis), respectively. Silibinin acted in the T-24 cells through erbB1-mediated mitogenic signalization, inhibiting the cell growth without causing apoptosis [48]. It was found that orally administered silymarin, at a dose of 1000 ppm for 8 weeks during the initiation of cancer, or 24 weeks after the initiation, caused statistically significant reduction in the number of pre-neoplastic lesions and the rate of the disease progression of bladder cancer in the *in vivo* study on ICR mice with induced bladder cancer [49]. Furthermore, silibinin inhibited the tumor RT4 xenograft growth by decreasing the tumor volume and weight, reduced cell proliferation and increased apoptosis in the tumors in the *in vivo* studies. The assumed mechanism of action was decrement of the surviving protein expression and its nuclear localization [50].

Effect on lung cancer: Silibinin slowed the growth of A549 human lung cancer cells and enhanced the effect of doxorubicin modulation of the NF-kB route thus inhibiting the resistance to doxorubicin, significantly reduced the levels of iCOX-2 and diminished the adverse effects of the chemotherapeutics [51]. In addition, silibinin reversed the resistance to etoposide and to doxorubicin in human small-cell lung carcinoma and acted synergistically with the chemotherapeutics [52]. Oral pretreatment with silibinin statistically reduced the number of tumors and large tumors (> 1.5 mm) in relation to the control group in the study on A/J mice, where lung cancer had been provoked by urethane. This study evaluated the density of blood vessels where it was found that silibinin decreased the number of highly vascularized tumors and the density of the blood vessels in the vascularized tumors. Finally, silibinin decreased the levels of iNOS and COX-2 enzymes whose expression increased in the process of angiogenesis responsible for the tumor development [53]. The pretreatment with silibinin reduced the phosphorylation of STAT1 and STAT3 induced by cytokines responsible for the proliferation of the A549 human lung cancer cells under the *in vitro* conditions. Silibinin inhibited the AP-1 transcription factor of DNA, blocked the MAPK cascade and inhibited iNOS at the concentration of 200 mmol/L, indicating that it could be a potential chemo-preventive agent for lung cancer treatment [54]. Silibinin-meglumine, a water-soluble form, inhibited the growth of non-small-cell lung carcinoma mouse xenografts as efficiently as the "gold standard" gefitinib and reversed the resistance of gefitinib-unresponsive tumors [55].

Effect on prostate cancer: SIL possessed anti-carcinogenic activity both with hormone-dependent and hormone-independent prostate cancer and its component isosilybin B was demonstrated as the most effective suppressor of the topo IIa gene promoter activity. The inhibiting effect on the prostate cancer cells in the LNCaP, PC3 and DU145 cultures that SIL exhibited was not registered in the

normal prostate epithelial cells [56]. Silibinin induced increased expression of cytokeratins and chromogranin A and modulated the phosphorylation status of retinoblastoma (Rb) and Rb-related proteins in human prostate carcinoma LNCaP cells. Furthermore, it caused a considerable reduction in the prostate specific antigen (PSA) in the LNCaP cells, which was important for cell growth inhibition. SIL down-regulated epidermal growth factor receptor (EGFR) signaling in PCA, which consequently led to the cascade that inhibited CDK and cancer cell cycle arrest. Silibinin/silymarin inhibited tumor growth factor (TGF-α) mRNA expression and decreased secreted and cellular levels of TGF-α in both LNCaP and DU145 cells. It not only reduced NF-κB signaling activity in DU145 prostate cancer cells, but also increased the sensitivity of the cells to TNFα-induced apoptosis that was commonly inhibited [57]. Additionally, the silipide/silibinin effect in prostate cancer was accomplished by reducing the level of insulin-like growth factor-1 (IGF-1) and/or elevating the level of insulin-like growth factor-binding protein-3 (IGFBP-3) in TRAMP mice [58]. A dose-dependent decrease was also observed in cyclin B1, cyclin E, and cyclin A protein levels by silibinin, which suggested that oral silibinin blocked PCA growth and progression via modulation of the tumor IGF-IGFBP-3 axis and cell cycle regulation [59].

Effect on oral cancer: Silymarin was proved to decrease the viability of human pharynx squamous carcinoma (FaDu) cells in an *in vitro* study. Apoptosis of the FaDu cells was preceded by 10-fold diminished Akt phosphorylation and five to six-fold upregulated expression of the phosphatase and tensin homolog. This caused the inhibition of Bcl-2 expression and elevation of caspases activity which led to the apoptosis of the FaDu cells [60].

Effect on gastric cancer: Silibinin dose-dependently inhibited the growth of human gastric carcinoma SGC-7901 cells. Namely, it provoked G2 phase arrest in the cell-cycle progression of the SGC-7901 cell line. SGC-7901 cells growth inhibition was observed after silibinin significantly decreased the expression of p34cdc2 levels and induced p53 and p21 expression. A final common pathway, involving the activation of caspase, is usual for most chemotherapeutic agents. Surprisingly, silibinin caused apoptotic death of SGC-7901 cells in a caspase-independent manner [61].

Effect on colon cancer: Silibinin, in a dose-dependent manner, induced cell cycle arrest in the G2/M phase of the FET and GEO cell lines of human colon cancer and in the poorly differentiated HCT116 cells in the G1 phase. As with prostate cancer, silibinin inhibited CDK and the activity of D1, and E cyclins in colon carcinoma, but did not reduce COX-2 expression and activity. It also inhibited autocrine TGF-α secretion, and its binding to the EGFR and the EGFR expression [62]. Significant reduction in the frequency of colonic ACF (aberrant crypt foci) in a dose-dependent manner in F344 rats was achieved after dietary administration of silymarin (100, 500 and 1,000 ppm), either during or after 4 weeks of exposure to the carcinogens [63]. Orally administered silibinin, before or during the tumor initiation, caused statistically significant reduction in the number of formed tumors, while it prevented the development of macroscopically visible tumors (only polyps were developed) during the entire period of the study on Wistar rats with induced colon cancer, using 1.2-dimethylhydrazine (DMH). Silibinin modulated the enzyme activity of an intestinal micro flora, reduced oxidative stress in the colon and prevented the retoxification of DMH (it inhibited cytochrome P450 activity, the creation of the DMH carcinogenic metabolites, and also induced the enzyme induction of second phase metabolism which facilitates DMH excretion). Furthermore, silibinin facilitated the establishment of the antioxidant enzyme functions [64]. Another *in vivo* study reported that silibinin had an anti-proliferative effect against human colorectal carcinoma HT29 xenograft and an antiangiogenic effect, which is very important in preventing metastasis. The silibinin antiproliferative activity was achieved via down-regulation of ERK1/2 and Akt phosphorylation, as well as the cyclin D1 expression. The silibinin antiangiogenic activity *in vivo* was connected with the inhibition of iNOS, COX-1, COX-2, hypoxia-inducing factor-1α (HIF-1α) and VEGF [65]. Therefore, silibinin could be considered as a promising chemo-preventive agent for the treatment of colon cancer.

Effect on pancreatic cancer: Dietary silibinin suppressed the growth of human pancreatic carcinoma BxPC-3 and PANC-1 tumor xenografts (representing early and late clinical stages of the disease, respectively), inhibited the cell proliferation and induced the apoptosis in BxPC-3 tumors in *in vitro* and *in vivo* studies. It caused a strong G1 arrest in BxPC-3 cells in a dose-and/or time-dependent manner and a moderate arrest in PANC-1 cells. The authors assumed that silibinin inhibited the G1/S cell cycle progression via down-regulation of cyclin D1 and CDK4/2 or by up-regulation of CDKIs [66].

Effect on leukemia: Silibinin, in a dose-dependent manner, inhibited the proliferation of the human promyeolitic leukemia HL-60 cell line and induced cell differentiation, which was even more pronounced when combined with 1,25-(OH)2D3 vitamin via both protein kinase C α and β level elevation and enhanced the activity [67]. Silymarin strongly inhibited Akt protein levels in K562 leukemia cells, caused caspases activation and PARP cleavage, which inhibited the growth of the human leukemia cells and induced their apoptosis [68]. This was confirmed for both silibinin A and silibinin B on the same cell culture, which showed that the diastereoisomers were more potent than the silibinin racemic mixture [69].

Nephroprotective effect: Silibinin-hemisuccinate (also silicristin and silidianin) stimulated the proliferation of Vero cells similar to fibroblasts (African green monkey, *C. aethiops*) by about 23% more than the control group, increased the synthesis of DNA molecules in the cells, protein synthesis, and reduced the toxic effects of paracetamol. In addition, silibinin had a positive effect on cells chemically damaged by cisplatin and vincristine, but only when it had been administered before these drugs [70]. Silymarin treatment of diabetic nephropathy patients at the final phase caused the normalization of immunoregulatory defects in *in vitro* studies [71]. Silymarin, in a dose-dependent manner, acted preventively in ischemia and reperfusion after renal injury and decreased the morphological changes that included the dilation and vacuolization of tubules, pelvic inflammation, interstitial inflammation, perirenal fat infiltration, and glomerular and tubular necrosis caused by the nephrotoxic drugs: cisplatin, doxorubicin, aminoglycosides, vincristine, cyclosporine and acetaminophen in *in vitro* studies [72].

Neuroprotective effect: Silymarin had a neuroprotective effect in rats with brain damage caused by acetaminophen in an *in vivo* study. In the group of rats treated only with silymarin, increased GSH and ascorbic acid levels and slightly increased SOD levels were observed in relation to the control, while the MDA levels were significantly reduced in the group that had received silymarin three days before paracetamol was administered. It is supposed that silymarin binds nonenzymatic ROS into CNS, or it increases the protein synthesis involved in the antioxidant activity, and, therefore, the authors recommended the use of silymarin in the treatment of neurodegenerative and neurotoxic disorders [73]. Silibinin, administered intragastrically 30 min before a permanent middle

cerebral artery occlusion, significantly alleviated neurological deficit, reduced an infarct volume and suppressed a brain oedema, which was assumed to have been accomplished by up-regulation of pAkt, pmTOR, HIF-1α, Bcl-2 and by down-regulation of Bax, and NF-κB in the ischemic brain tissue after a stroke [74]. The pretreatment with silymarin had a statistically significant neuroprotective effect in the *in vivo* studies on the rats with the neurotoxicity caused by Na-fluoride since it increased the activity of the antioxidant systems (SOD, CAT and GSH) [75].

Cardioprotective effect: Fat infiltration in the myocardium of obese db/db mice was induced to correspond to myocardium steatosis of obese and diabetic patients or to patients with non-alcoholic steatosis. Silibinin decreased the ALT levels in the serum three times in comparison with the untreated group and reduced the insulin and glucose levels in the serum during the fasting period causing the elimination of the generated insulin resistance. The treatment with silibinin significantly decreased myocardial injury and mitigated morphological abnormalities of the majority of the myocardiocytes, reduced the damage caused by oxidative stress and maintained the GSH level. Silibinin completely eliminated the increased TNF-α expression and decreased the expression of genes for IL-6, which was observed in the obese mice [76]. Silibinin attenuated the phenylephrine-induced hypertrophic response in H9c2 cardiac cells, up-regulation of atrial natriuretic peptide and the increase of cellular protein levels by blocking ERK1/2 and Akt signaling pathways. Pretreatment of the H9c2 cells with silibinin also protected them from H_2O_2-induced cellular stress [77].

Hypocholesterolemic and hypolipidemic effects: Silymarin had the same effect on serum cholesterol as probucol (an antioxidant hypocholesterolemic drug) in rats on a high-cholesterol diet. Silymarin increased the HDL level mildly, reduced the cholesterol content in the liver and stimulated LDL cholesterol excretion from the liver, which contributed to the anti-atherogenic effect [78]. The results were confirmed in a study in which silymarin dose–dependently reduced total cholesterol, LDL and triglyceride levels in rabbits on a high cholesterol diet and thus inhibited the formation of atherosclerotic plaques [79]. In a four-month randomized double blind study silymarin, administered three times a day (200 mg tablet) with a standard therapy in non-insulin dependent hyperlipidemic patients caused a statistically significant reduction in total cholesterol, LDL and triglyceride levels compared with the control group which received only a standard therapy with placebo [80]. Silibinin-cyclodextrin significantly decreased triglyceride levels in relation to the placebo group in a double-blind six-month-study with patients with poorly controlled non-insulin dependent diabetes [81]. The treatment with silymarin, during one month, significantly reduced total cholesterol, LDL and VLDL levels, and increased HDL levels slightly (silymarin showed the same effect as lovastatin) in the randomized clinical study with 57 patients with dyslipidemia [82].

Effect on diabetes: In a study on Wistar rats, silibinin showed a significant hypoglycemic effect in the liver, inhibiting gluconeogenesis during fasting and glycolysis in satiety, respectively, opening the possibility of the clinical application of silibinin in the treatment of diabetes [8]. The blood glucose level of patients with diabetes mellitus II, in the group who received silymarin, was statistically significantly lower after four months of administration in relation to the placebo group, where the increased glucose level was recorded in a double-blind placebo-controlled clinical study. The patients treated with silymarin, compared with the control group, had a slight, but not statistically significant weight loss and reduction in blood pressure [80].

A long-term treatment with silymarin reduced lipoperoxidation and insulin resistance in the patients with alcohol liver cirrhosis who had similar disease histories and pathological characteristics, like patients with alcoholic liver disease and NASH. Namely, the significant decrease in fasting blood glucose levels, insulin level decrement (40%), reduction of exogenous insulin needs, decrease of mean daily blood glucose levels, reducing daily glycosuria and HbA1c levels, were recorded in the group that received silymarin together with the standard therapy after only 4 months of administration [83]. In addition, the rise in glucose and fatty acid levels in the diabetics led to ROS increment accompanied by insulin resistance, dysfunction of the pancreatic β-cells and insulin secretion – the effects that may be significantly mitigated due to the silymarin antioxidant action [84].

Effect on sepsis: The endotoxins (lipopolysaccharides) that activate macrophages and produce cytokines and lead to oxidative stress are considered to have the key role in sepsis development and multiple body function disorders. Pretreatment with silymarin had the same effect as pretreatment with antioxidant *N*-acetylcysteine (NAC) in a study on rats with induced septic lung and brain damage. The survival rate in the group treated with NAC was the same as in the group treated with silymarin after 72 h. Both silymarin and NAC effectively eliminated the increase of TNF-α, IL-1 and IL-6, LDH and MDA caused by sepsis. Silymarin inhibited the oxidative injury caused by the lungs and brain sepsis owing to its ability to balance the antioxidative status and to regulate the inflammatory mediators [85].

Effects on osteoporosis and osteoarthritis: Seidlova-Wuttke *et al.* were the first to observe that silymarin had a raloxiphen-like effect on bone structure [86]. In another study it was demonstrated that silymarin did not affect the luteinizing hormone (LH) level, or cholesterol, LDL and HDL levels in rats after ovariectomy, but it increased the uterine weight, height and hypertrophy of endometrial luminal epithelium compared with the control group, probably due to partial binding to ERα receptors in the uterus. However, silymarin augmented the density of the trabecular part of the long bones, did not affect the osteocalcin levels, but reduced the alkaline phosphatase levels and increased the calcium and phosphorus levels in the serum and stimulated the secretion of parathyroid hormone 5.5 times more than that with ethinyl estradiol. The potential use of silymarin against osteoporosis development was confirmed in this study, assuming that its effect could not be achieved only through the estrogen receptor, but some other mechanisms of action should be included [87].

The destructive changes of moniodoacetate-provoked osteoarthritis in rats were ameliorated with both celecoxib and silymarin. Celecoxib (100 mg/kg) and silymarin (50 mg/kg) applied separately or in parallel (celecoxib in a dose of 100 mg/kg and silymarin in a dose of 25 mg/kg) lowered the osteoarthritis MDA and NO levels. A combination therapy, containing both celecoxib and silymarin, resulted in IL-1β reduction in the serum more significantly than the application of each drug separately. Silymarin potentiated the effect of celecoxib, and applied together, they also amended the histopatological findings (fibrillated surface, presence of osteoclasts and connective tissue) in the rats with induced osteoarthritis. The authors proposed further testing of celecoxib and silymarin as a combined osteoarthritis therapy in humans [88].

Preventive effect for fetal alcohol syndrome: Research on the influence of silymarin on fetal alcohol syndrome was made on Fisher female rats with confirmed pregnancies. The hatchlings from the ethanolic group had significantly less mass, less total brain mass

and less mass of the corpus callosum than the control and in the group treated with silymarin, while the female group, along with ethanol was fed with silymarin during the pregnancy, gave birth to offspring of a nearly identical body mass as the control group [89]. In another study, exposure to alcohol during fetal development influenced the spatial orientation of the adult rats, and the spatial memory impairment was more pronounced in the female rats, but was significantly lower in the group pretreated with silymarin. It is assumed that silymarin scavenged the free radicals produced by ethanol oxidation thus preventing the potential negative effects on the migration of the cells, then, it acted on the hippocampus stimulating the DNA and proteins synthesis, maintaining the number of cerebral Purkinje cells, or it acted through the NMDA receptors [90].

Prolactive effect: Female rats were treated either with a standardized extract (silymarin BIO-C ® = Pilùtatte ® - Sil ®) or with metoclopramide intraperitoneally (the control). Sil ® caused a statistically significant increase in body weight and serum prolactin levels in the female rats compared with the control. After interruption of the treatment with Sil ® after 66 days, the prolactin level was still elevated. Bromocriptine, an agonist of D2-dopamine receptors, dose-dependently, significantly reduced the prolactin levels in the serum induced by Sil ® treatment. It is clear that silymarin increased the circulating prolactin levels in the female rats and that the dopamine D2 receptors were probably at least partially involved in this effect [91].

Immunomodulatory effect: Silymarin modulated CD4+ splenocytes proliferation in mice activated αCD3 mAb (anti-mouse CD3 monoclonal antibody) in the *in vitro* study, but did not have a direct mitogenic activity and cytotoxic effects. Silymarin, at the concentration of 50 μM, significantly inhibited the CD3-induced NF-kB nuclear translocation and production of IL-2 and IFN-γ in the activated splenocytes after 72 h in relation to the untreated cells and showed immunomodulatory potential [92]. Silibinin exerted anti-inflammatory and anti-fibrotic effects on the CD14+ cells by NF-kB mediated inhibition of TNF-α, IL-10, TNF-β1, PGE_2 and NO production [93]. The down-regulation of NF-kB, induced by pretreatment with silibinin, prevented mouse-ovalbumin induced allergic airway inflammation in the *in vivo* study [94]. Silibinin inhibited the leukotriene formation significantly by Kupffer cells *in vitro*, inhibiting the 5-lipo-oxygenase pathway [95], which is consistent with the studies indicating that sylimarin and its constituent silibinin inhibited arachidonic acid metabolism *in vitro* [96, 97].

Effect on burns: The burns caused by hot water in Wistar rats caused severe oxidative stress and tissue damage. The dermal or dermal/oral application of silymarin restored the increased LDH and MDA levels and decreased the GSH levels to the values of the control group. When the burns were treated with silymarin, the higher levels of TNF-α were reduced compared with the group without the silymarin treatment. Myeloperoxidase activity, which indicated the increased infiltration of neutrophils in the damaged tissue, was significantly increased 48 h after the burns had been caused, but was fully restored to the level of the control group after the silymarin treatment. Silymarin eliminated the increased thromboplastin activity observed in the burns and reduced the oxidative damage of the epidermis and dermis caused by heat [98].

Effect on cholestasis: It was shown that silibinin had a therapeutic application in cholestasis because it affected the transport of tauro- and glyco-conjugated bile salts through the canalicular membrane in isolated hepatocytes of Wistar rats. Silibinin possessed an anti-cholecystic effect on estradiol-17ß-D-glucuronide and taurolitocholates, and induced cholestasis by increasing the level of cytosolic Ca^{2+} through cAMP, which opened the possibility for further research of the silibinin activity in the prevention of gallstone formation [99].

SIL effect in cosmetics: Cosmetic products containing silymarin to treat rosacea and to maintain the skin moist and lips smooth are already on the market. As the aging processes are accompanied by oxidative stress, SIL is expected to be used in cosmetic preparations against wrinkles and aging skin. The ability of silymarin to protect the skin from epidermal hyperplasia and DNA epidermal cell damage caused by UVB radiation makes SIL appropriate and benefitting to be used in cosmetics preparations with a UV protection factor in sunscreens [100].

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