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Silymarin as Herbal Medicine- A Review

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Review Article

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Abstract: Silymarin, a flavonolignan contain phenolic and flavanoid component from Silybum marianum plant, is used for the strengthening against various liver conditions in both experimental models and clinical settings. Silybum marianum (milk thistle) has been used for centuries as an herbal medicine for the treatment of liver disease. Its use for various liver disorders due to drug as well as environmental factors. Silybum marianum contains silymarin, which is composed of the flavanolignans silybin, silydianin, and silychristine, with silybin being the most biologically active. Number of studies has established the cancer chemo preventive role of silymarin in both in vitro and in vivo models. Silymarin modulates imbalance between cell survival and apoptosis through intervention with the expressions of cell cycle regulators and proteins involved in apoptosis. Silymarin was found to be effective in the illness of organs like brain, prostate, lungs, heart, kidneys, pancreas and others. Silymarin act mainly by its antioxidative and free radical-scavenging properties, recently new molecular mechanisms based on the specific receptor interaction were discovered – e.g., modulation and inhibition of drug transporters, estrogenic receptors, P-glycoprotein, nuclear receptors and some others.
Keywords: Silymarin, Silibinin, Hepatoprotecive, cancer, Inflammation.

INTRODUCTION

Silymarin, a flavonolignan from 'milk thistle' (Silybum marianum) plant is used from ancient times as a hepatoprotective drug. Along with hepatoprotective action other actions includes antioxidant, antifibrotic, anti-lipid peroxidative, immunomodulatory, anti-inflammatory and liver regenerating.

Silymarin has clinical applications in alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, fatty liver, toxic and drug induced liver diseases, Psoriasis, neuroprotective and neurotropic activity

INTRODUCTION & HISTORICAL ASPECTS

Silybum marianum, commonly known as 'milk thistle' (Family: Asteraceae /Compositae) is one of the oldest and methodically researched plants in the treatment of liver diseases [1]. It is an annual or bi-annual plant up to 2 meters high. It has large, bright leaves with characteristic white stains along the nerves and with waved and spiny margins. At the bottom of the stem and branches appears the flower capitulum between 4 and 8 cm diameter, poised by tubular purple flowers wrapped in several rows of coriaceous bracts. The plant has strong spine. The fruit is achenium type, brown-black colour with grey stains, bright and non-striated. Its size is about 7 mm length coronated by a white down of simple and deciduous hair. Its fruits may be confounded with the seeds, due to its size and shape. The leaves can be easily identified by the presence of milky veins, from which the plant derives its name [2]. The extracts of milk thistle is being used as a general medicinal herb from as early as 4th century B.C. and first reported by Theophrastus [3]. This plant is used as emetic in 1st century A.D. by Dioskurides and also became favored medicine for hepatobiliary diseases in 16th century and the drug was revived again in 1960 in central euripi [4]. The principle chemical constituents of the plant are obtained from the dried seeds and consist of four flavonolignans which are cooperatively known as silymarin. Wagner *et al.* [5] characterized these active compounds and Flora *et al.*, [6] reviewed its history, properties and the clinical effects. Silymarin is broadly prescribed by herbalists and has almost no side effects. The plant is native to the Mediterranean and grows throughout Europe and North America. It also grows in India, china, South America, Africa and Australia.

CHEMISTRY OF SILYMARIN

Silymarin is extracted from the dried seeds of milk thistle plant [2]. The active principle was first isolated and chemically characterized during 1968-1974. Shortly the biochemical effects of silymarin on RNA, protein and DNA synthesis was reported by

Sonnenbichler and Zetl [7]. Silymarin is a multipart mixture of four flavonolignan isomers, namely isosilybin (Fig-1), silydiani , silychristin with an empirical formula $C_{25}H_{22}O_{10}$ (Fig-2) and silybin (Fig-3), The structural resemblance of silymarin to steroid hormones is thought to be responsible for its protein synthesis facilitatory actions. Silybin is the major and most active component and represents about 60-70 per cent, followed by silychristin (20%) (Fig-4), silydianin (10%), and isosilybin (5%) [8].

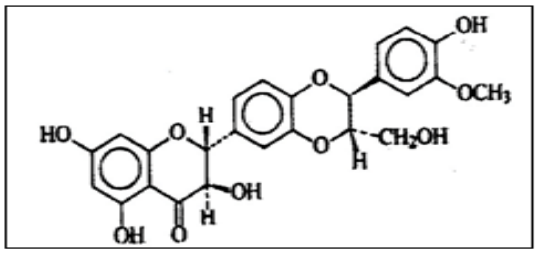

Fig-1: isosilybin

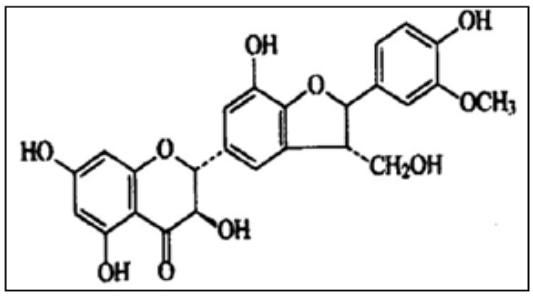

Fig-2: silychristin

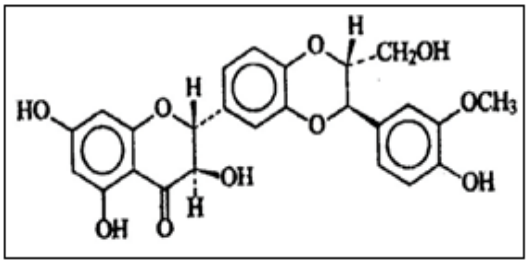

Fig-3: Silybin

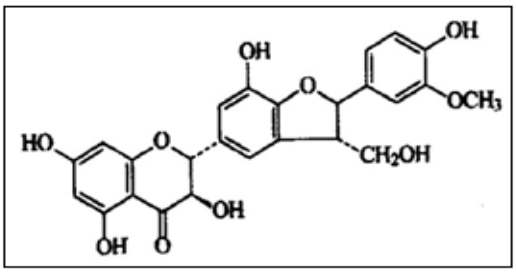

Fig-4: Silychristin

PHARMACOKINETICS

Silymarin is insoluble in water and usually administered as a sugar coated tablet or as an encapsulated standardized extract[1]. The absorption of silymarin from gastrointestinal tract is moderate (23-47%) as 2-3 per cent of the silybin recovered from rat bile in 24 h. About 20-40 per cent of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates in human beings [8]. The peak plasma levels after an oral dose are achieved in 4-6 h in experimental animals and in human beings [9-11], and elimination half-life is approximately 6 h [8, 12]. The studies on pharmacokinetic variables (mean ± SD), after an oral administration of 240 mg silybin in 6 healthy volunteers has been produced the data as follows: Absorption half life 0.17 ± 0.09 h, maximum plasma concentration 0.34 ± 0.16 µg/ml, time to maximum plasma concentration 1.32 ± 0.45 h [12], elimination half life 6.32 ± 3.94 h respectively .

PHARMACOLOGICAL ASPECTS

Hepatoprotective activity of silymarin has been confirmed by various researchers from all over the world against partial hepatectomy models and toxic models in experimental animals by using acetaminophen, D-galactosamine, ethanol, carbon tetrachloride and Amanita phalloides toxin. The antioxidant and antiinflammatory effect of silymarin is responsible for hepatoprotection during metabolism of xenobiotics with toxic metabolites excreted via bile in the form of glucuronides by preventing their enterohepatal circulation.

Carbon tetrachloride and silymarin

Various chemical agents are studied for their hepatotoxic effect among them carbon tetrachloride (CCl_4) has been thoroughly studied for its hepatotoxic properties [13]. Various hepatoprotective (both herbal and synthetic) drugs have been studied to observe the advantageous effects against the chemically induced liver injury produced by carbon tetrachloride [14]. Silymarin when evaluated in comparision to various polyherbal formulations in CCl4 induced hepatotoxicity in rats has led to absolute normalization of elevated transaminases levels [15]. Mouriel and Mourelle [16] found that silymarin treatment confined completely against harmful increase in the membrane ratios of cholesterol: phospholipids and sphingomyelin: phosphatidylcholine in rats with carbon tetrachloride induced cirrhosis.

Acetaminophen and hepatic injury

Acetaminophen is a widely used analgesic and antipyretic agent known to cause centrilobular liver necrosis at dose level above the therapeutic window. Silymarin has been studied for its protective action against acetaminophen induced toxicity in animal models. Ramellini & Meldolesi [17] in their in vitro studies on rat hepatocyte showed that silymarin accomplishment normalized the elevated biochemical parameters of liver and serum, caused by acetaminophen, by its stabilizing action on plasma membrane. A comparative study of andrographolide and silymarin on acetaminophen induced cholestasis

has produced the dose dependent choleretic and anticholestatic effects of these drugs [18].

Ethanol induced liver damage

Acute and chronic administration of ethanol produces extreme decrease in the liver reduced glutathione (GSH); an significant biomolecule against chemically induced cytotoxicity [19]. The hepatoprotective activity of silymarin against ethanol-induced damage has been evaluated in different animal models. The administration of ethanol cause increase in serum alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (γ-GT levels), with a disturbance in reduced and oxidized glutathione ratio. The animal group which received silymarin did not show any significant changes in these parameters, presenting its protective role against ethanol induced damage to liver [20].

Galactosamine related changes in liver and silymarin

Galactosamine produces liver damage and cause histopathological changes similar to human viral hepatitis. Cholestasis was observed after Galactosamine administration and was due to inhibition of the bile acids synthesis and also their conjugation with proteins or to damage in the biliary system. Saraswat *et al.,* [21] reported the significant anticholestatic effect of silymarin.

SILYMARIN AS ANTICANCER AGENT

Carcinogenesis is typical process comprises of various complex pathways like altered expression of transcriptional factors and proteins involved in cell cycle regulation, differentiation, invasion, apoptosis, angiogenesis and metastasis. Deregulated cell cycle progression, apoptosis and mutations together with increased angiogenic potential, invasion and metastasis have been described as hallmarks of cancer. Accordingly, the agents that could target one or more of these processes should be effective and ideal cancer chemopreventive agents. Silymarin regulate imbalance between defencive and invading (cell survival and apoptosis) through interference with the expressions of cell cycle regulators and proteins involved in apoptosis. In addition, silymarin also showed anti-inflammatory as well as anti-metastatic activity by modulating specific proteins [22]. Both silymarin and silibinin are particularly effective in inhibiting epidermal growth factor receptor (EGFR) signaling with suppression of cyclin-dependent kinase (CDK) expression and up-regulation of the CDK-inhibitors p21CIP1 and p27KIP1, with concomitant increase in their binding to CDKs. Silymarin induces growth seize at the G1 and G2 checkpoints. Silymarin, in subordinate doses induces the growth arrest through extracellular signal-regulated kinases (ERK1/2) inhibition and in higher doses leads to apoptosis through mitogen activated protein kinase (MAPK)/c-Jun N-terminal kinase (JNK) pathway [23-25].The studies have shown that silymarin inhibits both constitutively active and transforming growth factor (TGF)-a-mediated tyrosine phosphorylation of EGFR in advanced human prostate cancer DU145 cells [26]. Studies have shown that silymarin and silibinin down-regulate EGFR signaling via the inhibition in the expression and secretion of growth factors, and by inhibiting growth factor binding to and activation of EGFR and subsequent impairment of downstream mitogenic events causing anti-cancer efficacy in tumor cell lines [27].

MODULATION OF CELL CYCLE PROGRESSION BY SILYMARIN

Disruption of the normal regulation of cell cycle sequence and division is an central event in malignant transformation. The regulation of the cell cycle is controlled by a family of cyclins, CDKs, and CDK inhibitors (CDKIs). Silymarin has been reported to suppress the proliferation of tumor cells in various cancers including prostate, ovarian, breast, lung, skin, and bladder [22]. Numerous reports indicate that silymarin inhibits proliferation of cells by inhibiting cell cycle progression at different stages of the cell cycle. Studies indicate that silymarin induces G1 arrest and/or G2-M arrest in human prostate cancer LNCaP, PC3, and DU145 cells. Silymarin caused an induction of the CDK inhibitors Cip1/p21 and Kip1/ p27, and a decrease in CDK2 and CDK4 and associated kinase activities that led to G1 arrest. Treatment with silymarin showed dose- and time-dependent growth inhibition together with a G1 arrest in bladder transitional cell carcinoma (TCC) cells, T-24 (high-grade tumor) and TCC-SUP (high-grade invasive tumor). Silymarin treatment has been found to inhibit the growth of androgen dependent (LNCaP) and androgen independent (PC3 and DU145) prostate cancer cells [28]. Silymarin also induces G1 arrest and a decrease in the kinase activity of CDK and its associated cyclins in human breast cancer MDAMB 468 cells [29]. Silymarin treatment induced binding of Cip1/p21 with CDK2 and CDK6 paralleled a significant decrease in CDK2-, CDK6-, cyclin D1-, and cyclin E-associated kinase activities, along with a decrease in cyclins D1 and E. Studies have also shown that silymarin and silibinin transform G1 phase cyclins–CDKs–CDKIs for G1 arrest, and the Chk2–Cdc25C–Cdc2/cyclin B1 pathway for G2-M arrest, together with an altered subcellular localization of critical cell cycle regulators. Silymarin and silybin inhibits UVB-caused increase in cell proliferation and micro-vessel density and down-regulation of inflammatory and angiogenic responses in SKH-1 hairless mice [30]. Studies on hepatic cell resulted that silibinin significantly pregulated p21/CDK4 and p27/CDK4 complexes and down-regulated Rb-phosphorylation and E2F1/DP1 complex thereby inhibiting human hepatoma HuH7 cell growth [31]. Various experimental studies demonstrated the anti-cancer activity of isosilybin B and isosilybin A, isolated from silymarin, in human prostate carcinoma LNCaP and 22Rv1 cells that is mediated via cell cycle arrest and apoptosis induction [27]. These studies

suggested that regulation of cell cycle is one of the important mechanisms of action of silymarin in the prevention and therapeutics of cancer.

NEUROPROTECTIVE AND NEUROTROPIC ACTIVITIES OF SILYBIN/SILYMARIN

Silybin or silymarin may be useful in treatment and prevention of some neurodegenerative and neurotoxic processes, partly due to its antioxidative activity and various other unknown, mechanisms. Wang *et al*., [20] confirmed that silymarin could effectively protect dopaminergic neuron against lipopolysaccharide (LPS)-induced neurotoxicity by inhibiting an activation of microglia that represent resident macrophage-like population of brain cells acting in host defence and tissue repair in the CNS. Evidence from experimental models confirmed that activated microglia contribute to neuropathological changes in neurodegenerative diseases. Silymarin also inhibits the production of inflammatory mediators, such as tumor necrosis factor-α (TNF-α) and nitric oxide and thus reduces damage to dopaminergic neurons. Further on, studies revealed that silymarin at different doses reduced the production of inducible nitric oxide synthase in LPS stimulated BV-2 cells (model of microglia activation). It is evident from studies that the inhibitory effect of silymarin on microglia is regulated through the inhibition of nuclear factor κB (NF-κB) activation. An extract from Silybum marianum seeds was tested on the differentiation and survival of cultured neural cells (rat PC-12 pheochromocytoma cell line). The extract enhanced the differentiation of PC-12 cells and prevented apoptosis following nerve growth factor (NGF) withdrawal. Various flavonoids and hydroxyl-cinnamates was found to control neuronal damage induced by oxidized low-density lipoproteins that are normally able to enter neuronal cells and in a dose-dependent manner elicit neurotoxicity (DNA fragmentation and cell lysis) [32]. Silymarin has partly protective activity on brain and liver in ethanol treated pregnant rats [33]. No significant protective effects of silymarin on N-methyl-4-phenylpyridinium ion induced-neurotoxicity and on L-glutamate induced cell death in PC-12 neuronal cells were found by Mazzio *et al*., [34].

An interesting study targeting at neuro-immunomodulation regulated by silybin was carried out by Sakai *et al*., [35] and found that Major histocompatibility complex (MHC) I is usually suppressed in neuronal cells and neuroblastoma cells and this may lead to persistent viral infections. Induction of MHC I molecules in neuronal cells can stimulate the immune system to be able quickly to identify intracellular pathogens by cytotoxic T cells and remove the viruses from the central nervous system. Silymarin treatment resulted in the expression of MHC I in cells. Therefore, it was proposed that silymarin may be useful in the treatment of encephalitis. More studies, both in vitro and in vivo are, however, required.

SILYBIN/SILYMARIN IN TREATMENT AND PREVENTION OF GASTROINTESTINAL PROBLEMS

Silybin is known from ancient times as beneficial agent for liver and biliary system, however, other gastrointestinal problems can be treated and/or prevented by its preparations. In pancreas silybin can act mainly as cytoprotective aginst chemical damage and can also stimulate recovery after intoxication leading to damages. Silymarin was used in rats treated with alloxan [36, 37]. Alloxan causes severe necrosis of pancreatic β-cells, with the consequent lack of insulin secretion. For this reason it has been widely used to induce experimental diabetes mellitus, and many studies have been performed using this model to explore pancreatic damage. It was suggested that alloxan induces the production of H_2O_2 and of some-free radicals such as O_2 - and OH**.** , which produce cellular damage followed by cell death. There is a strong support for the suggestion that reactive oxygen species play a relevant role in the etiology and pathogenesis of diabetes and its long-term effects. Therefore, the above model was considered adequate for the study of pathology such as diabetes mellitus. It was found that silymarin was able to prevent a rise in both plasma glucose and pancreatic lipid peroxidation in the hyperglycemic rats [36]. Thus, it was suggested that the protective effect could be ascribed to silymarin either due to its antioxidant properties or to an increase of plasma and pancreatic glutathione concentrations, or both. Silymarin also stimulated pancreatic activity of antioxidant enzymes: glutathione peroxidase, superoxide dismutase and catalase [38]. Silymarin had not only a protective effect on rat alloxan-induced diabetes mellitus but it also induced pancreas recovery [39]. The seriousness of human diabetes mellitus as the world health problem is growing due to the fact that at least 150 million people are affected; therefore, there is the necessity to search for new drugs. The existing ones only favor insulin release or control blood glucose level but do not recover the endocrine pancreatic function. Silymarin represents a new possibility in the treatment of diabetes mellitus, not only for the enhanced insulin levels but also for the pancreatic function recovery. Nevertheless, more studies are required to prove its beneficial properties in human diabetes mellitus.

Matsuda *et al*., [38] recently studied another damaging mechanism of pancreatic β-cells in relation to silymarin. They investigated effect of silymarin on interleukin 1β (IL-1β) and/or interferon-γ (IFN-γ)-induced β-cell damage using RINm5F cells (insulinoma cell line) and human islets. IL-1β and/or IFN-γ brought about β-cell damage in a time-dependent manner in the insulinoma cells. Silymarin dose-dependently inhibited both cytokine-induced nitric oxide (NO) production and cell death. Also in the human islets silymarin prevented IL-1β+IFN-γ-induced NO production and β-cell dysfunction. These cytoprotective effects of silymarin appeared to be mediated through the suppression of c-

Jun NH2-terminal kinase and Janus kinase/signal transducer and activator of transcription pathways. Silymarin also inhibits production of inflammatory cytokines, such as IL-1β, IFN-γ, and IFN-α from macrophages or T-lymphocytes [39, 40], which probably initiate the destruction of β-cells in the development of type 1 diabetes. Therefore, silymarin may be useful as a therapeutic agent for the type 1 diabetes mellitus.

Silybin was also explored for the protection of cyclosporine A toxicity (10 mg/kg/day i. p.) in both endocrine and exocrine pancreas in rats [41]. In this context it is interesting to state that decoction from aerial parts of Silybum marianum is used in conventional medicine in Morocco in the treatment of diabetes mellitus and Maghrani *et al*., [42] deep-rooted its action in rats with experimental type 1 diabetes. The intestinal anti-inflammatory activity of a number of doses of silymarin was tested in the acute stage of trinitrobenzenesulfonic acid (TNBS) model of rat colitis [43] and results show that the pre-treatment with 50 mg/kg/p.o. of silymarin significantly reduced the macroscopic colonic damage and significantly reduced colonic myeloperoxidase (MPO) activity compared to nontreated colitic animals. This suggests that silymarin can participate in intestinal anti-inflammatory activity and have beneficiary effect in colitis.

The antioxidant activity of silymarin can be responsible for the protective effect in the colon and intestine. Silybin and other component of silymarin can also act in colon as specific inhibitors of intestinal bacterial β-glucuronidase [60]. Silymarin and pure silybin significantly inhibit the in vitro cell growth of colon cancer (LoVo cell line) and endothelial cell lines (EA.hy 926) [44].

SILYMARIN IN TREATMENT AND PREVENTION OF NEPHROPATHY

Use of silybin in kidney disorder has equivalent potential as mentioned under gasterointestinal activity part, i.e. chemoprotectant and antioxidant. Silymarin has found to be effective in the prevention of Cold ischaemia and reperfusion injury during kidney transplantation. It also appears important to improve the long-term allograft outcome after kidney transplantation. A number of flavonoids were tested for enhancement of cell survival. Pretreatment with quercetin considerably improved the survival rate of cells but many other flavonoids including silybin were ineffective. Silybin was evaluated and found to have a similar stimulatory effect to the kidney cells (Vero line, nonmalignant kidney cells from monkeys) as described in liver cells [44]. Comparable effects of silymarin and picroliv (standardized iridoid glycoside fraction of Picrorhiza kurroa) against aflatoxin B1 intoxication of rat kidney and liver were found by Rastogi *et al*., [45]. Silymarin prevents this intoxication by regulation of TNF-α expression or signal downstream of the inhibition of ceramide synthase whose deregulation represents the main toxigenic effect of fumonisin B1. Silybin was used in the treatment of 30 patients suffering from end-stage diabetic nephropathy (ESDN). Diabetic and especially hemodialysis patients are at increased risk of oxidative cell damage. The patient treated with silymarin led to a restoration of the thiol status within 72 h both in vitro and in vivo. In similar, the T-cell activation was improved significantly along with a noteworthy decrease in TNF-α release [46].

SILYBIN/SILYMARIN IN TREATMENT AND PREVENTION OF CARDIO-PULMONARY PROBLEMS

Amiodarone is a one of the few among the potent antiarrhythmic drugs with; however, its use is inadequate because of frequent and serious side effects. Free radical reactions remain the main culprit in the pathogenesis of amiodarone toxicity. Potential mechanisms of amiodarone toxicity include development of lysosomal phospholipidosis, direct cytotoxicity, indirect immunologically mediated toxic effects and membrane destabilization. Co-administration of silybin together with amiodarone decreases appreciably lysosomal phospholipidosis [47] and this effect is further enhanced in combination with vitamin E [48]. Silymarin in combination with vitamin E significantly decreased conjugated diene concentration [49] and same result were obtained with silymarin when administered alone. Silymarin did not attenuate antiarrhythmic activity of amiodarone [50]. Silybin can also be very valuable in the cardioprotective relevance during the cancer treatment with cardiotoxic drugs, as e. g., doxorubicin .The ability of silymarin and its isolated components to protect cardiomyocytes (rat) against doxorubicin-induced oxidative stress is mainly due to their cell membrane stabilizing effect and radical scavenging potency [51]. Silymarin also found to reduces damage to rat heart microsomes and mitochondria by a doxorubicin-Fe^{3+} complex [52]. Combination of doxorubicin with silybin was tested also in the xenograft growth (athymic BALB/c nu/nu mice) and enhanced the therapeutic response of doxorubicin together lung tumor treatment [53]. Moreover, silybin exhibits a dose- and time-dependent inhibitory effect on the invasion and motility of highly metastatic A549 cells in the absence of cytotoxicity [54].

SILYMARIN IN THE THERAPEUTICS OF ASTHMA

Silymarin has also found to have protective effect in the early phase of allergic asthma, an effect, which may be related to a negative influence of the flavonoid on bronchial responsiveness to histamine [55]. Silymarin showed a modest protection against the bronchospasm produced by aerosol antigen test in sensitized guinea-pigs. This advantageous effect on respiratory system thought to be because of the various biological effects of silymarin, e.g. its membrane-

stabilizing effect, inhibition of the arachidonic acid pathway and anti-inflammatory activity. Additional Protective effect of silymarin seems to be due to an indirect mechanism that reduces airway responsiveness to histamine, and consequently the immediate anaphylactic response.

SILYBIN/SILYMARIN IN SKIN PROTECTION

Exposure of skin to solar UV radiation induces a numeral of skin disorders, including sunburn cell formation, hyperplasia, erythema, photoaging, edema, immune suppression, DNA damage, melanogenesis and skin cancers. It is well accepted that UV irradiation, both its UVB (290–320 nm) and UVA (320–400 nm) component, induces the generation of reactive oxygen species (ROS), which create the oxidative stress in skin cells and play an vital role in the initiation, promotion of chain of skin aging and carcinogenesis process. Thus the use of antioxidants, to be exact naturally occurring herbal compounds, is being paid substantial interest to protect skin from unpleasant biological effects of solar UV radiation [56]. Both silymarin and silybin have been revealed to exhibit preventive effects against photo carcinogenesis in various animal tumor models. Topical application of silymarin to mouse skin (SKH-1 hairless mouse model) reduced UVB induced tumor multiplicity, tumor incidence and tumor size compared to those of non-treated animals. Silybin inhibited photo carcinogenesis in mice when applied topically or administered in the diet. Silymarin was found to reduce and suppress harmful effects of solar UV radiation, UV-induced oxidative stress, immune responses, inflammation, and DNA damage as well as induction of apoptosis. Topical application of silymarin suppressed intracellular production of hydrogen peroxide and nitric oxide and reduced depletion of catalase activity in UVB-irradiated mouse skin (SKH-1 hairless mice) and also significantly inhibited expression of cyclooxygenase-2 (COX-2) and its prostaglandin metabolites (PGE2, PGF2, PGD2), which have been concerned in tumor promotion [57]. In the SKH-1 hairless mice silymarin reserved UVB induced skin edema, prevented UVB-induced infiltration of inflammatory leukocytes, formation of sunburn and apoptotic cells and significantly reduced the activity of myeloperoxidase, a marker of tissue infiltration [46].

Topical application of silymarin prevented UVB induced skin changes in mouse skin [58]. Induction of apoptosis together with cell proliferation and cell cycle progression has been suggested as in vivo molecular mechanism of silybin efficacy against photocarcinogenesis by Mallikarjuna *et al*., [59]. 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) signaling [60]. Dual efficacy of silybin on apoptosis was observed also in human keratinocytes (HaCaT) [61].

SILYMARIN AND STEROID HORMONE RECEPTORS

A large number of natural compounds have been evaluated to transform nuclear hormone receptor-dependent gene expression. Silymarin Upon binding as ligands, can either activate nuclear receptors or compete with natural hormones. Some polyphenolic compounds lead to inhibition of steroid hormone receptor-dependent proliferation of cancer cells. Both silymarin and silybin produce antiandrogenic activity in the prostate cancer cell line LNCaP [46]. Several plant flavonoids or other polyphenolic compounds have been made known to elicit anti/estrogenic activity both in vitro and in vivo [62-64]. Silybin can bind to a purified steroid receptor [65] and estrogenic effects of silymarin have been observed in ovariectomised rats in the 30-day uterotrophic assay. However, this latter finding was not confirmed in the ovariectomised rats after subcutaneous treatment with silymarin [66]. Silymarin elicited partial ER activation and silybin B was probably responsible for a majority of the weak ER-mediated activity of silymarin, where as its diastereomer silybin A was found to be inactive [46]. This is possibly the most primary finding on the estrogenic activity of silybin and also the first study describing effects of separated silybin diastereoisomers A and B towards receptors in biological systems.

SILYMARIN AND DRUG TRANSPORTERS MODULATION

Multidrug resistance (MDR) represents an growing problem in the treatment of cancer and bacterial infections. It often appears after protracted exposure of cells to a single drug and is frequently characterized by its resistance to a series of structurally unrelated compounds. Pglycoprotein (Pgp) is the important player in the multidrug resistance pathway. Pgp is a 170 kDa phosphorylated glycoprotein encoded by human MDR1 gene. It is accountable for the systemic disposition of numerous structurally and pharmacologically unrelated amphipatic and lipophilic drugs, carcinogens, toxins and other xenobiotics in many organs, such as brain, intestine, liver and kidney. Like cytochrome P450, Pgp is vulnerable to inhibition, activation, or induction by herbal constituents [46]. Silymarin was found to be an inhibitor of Pgp function [67]. In laboratory studies Silymarin potentiated doxorubicin cyto-toxicity in Pgp-positive cells, while it inhibited Pgp ATPase activity and azidopine photoaffinity labeling of Pgp, suggesting a direct interaction with Pgp substrate binding [68]. Silymarin increased the accumulation of digoxin and vinblastin in human intestinal Caco-2 cells in a concetration dependent manner by inhibition of their Pgp mediated efflux [69]. Silybin potentiated doxorubicin-induced

growth inhibition and apoptosis in human prostate carcinoma DU145 cells. Silybin and its derivatives were identified as inhibitors of P-glycoprotein. This activity is mainly pronounced in the 2, 3-dehydrosilybin derivatives carrying prenyl- or geranyl substituents. These findings indicated that silymarin, silybin and its derivatives may inhibit Pgp-mediated cellular efflux, raising a potential for significant drug interactions with Pgp substrates. The effect of silymarin and its associated phytoconstituents on the pharmacokinetics of the known Pgp substrate indinavir was investigated in healthy volunteers [70, 71]. Pgp-like transporter in Leishmania spp. was also found to be inhibited by silybin that led to the parasite sensitization towards daunomycin [72]. Silybin also interacts with other drug transporters, e.g., with multidrug resistance-associated protein 1 (MRP1). Silymarin and other flavonoids ware tested in human pancreatic adenocarcinoma cell line (Panc-1) on the transport of daunomycin and vinblastin and was found that silymarin appreciably increases accumulation of daunomycin and vinblastin cells indicating the inhibition of MRP1. It is thought that GSH regeneration is involved in this process because in the other study with flavonoids [73] stimulation of GSH co-transport, ATPase and drug resistance- conferring properties of MPR1 were found to be modulated. Compound 5'-methoxyhydnocarpin-D (A flavonoid) is a potent inhibitor of the NorA MDR efflux pump in S. aureus. A figure of hydnocarpin type flavonolignans, derivatives of silybin, proved to have greater potency than the natural isolate, 5'-methoxyhydnocarpin- D [74]. Silybin itself had a medium inhibitory potency. Silybin also inhibited melarsen-induced lysis of blood stream form trypanosomes [75]. This makes silybin a good candidate for antiparasital and/or adjuvant antiparasite treatment.

SILYMARIN - REGULATOR OF APOPTOSIS AND INFLAMMATION PROCESS

Silymarin might produce its anti-inflammatory effect by inhibition of the transcription factor NF-κB, which regulates and coordinates the expression of various genes involved in the inflammatory process, in cytoprotection and carcinogenesis. NF-κB contributes to the production of interleukins IL-1 and IL-6, tumor necrosis factor (TNF-α), lymphotoxin, granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon (IFN-γ). Manna et al., [76] studied the effect of silymarin on NF-κB activation induced by various inflammatory agents. Silymarin blocked TNF-α-induced activation of NF-κB in a dose- and time-dependent manner. Silybin was found to cause a change in the ratio of Bax/Bcl-2 in a manner that favors apoptosis. Silybin also induced the cytochrome c release, activation of caspase-3 and caspase-9 and cleavage of poly (ADP-ribose) polymerase (PARP). These results suggest that silybin may exert its anticancer effect by inhibiting angiogenesis through induction of endothelial apoptosis via modulation of NF-κB, Bcl-2 family and caspases [77]. Silymarin also suppressed the TNF-α-induced protein and mRNA expression of adhesion molecules, such as VCAM-1, ICAM-1 and E-selectin, in HUVEC. Moreover, silymarin suppressed the TNF-α-induced DNA binding of NF-κB in HUVECs. Therefore, part of the silymarin anti-atherosclerotic activity is mediated by inhibiting the expression of adhesion molecules [78].

HYPOLIPIDEMIC EFFECT OF SILYMARIN

Various studies conducted on rats fed on high fat diet to evaluate silymarin as anti hyperlipidemic shows significant decrease total serum cholesterol, triglycerides, Very low density lipids with an increase in the level of high density lipids. The polyphenolic fraction of silymarin appeared to be a candidate of silymarin effect on plasma lipoproteins. Considering mechanism(s) of action of polyphenolic fraction on lipid metabolism, it has to be kept in mind that it contains, in addition to PolyPhenols, other components such as flavonolignan silibinin and its diastereoisomers, and a flavonoid taxifolin. The ability to reduce the liver cholesterol synthesis by suppressing 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity has been shown in vitro in silibinin [79]. The results from various studies indicate that the polyphenolic fraction is an active component of silymarin with positive effects on lipid metabolism and antioxidant status in the models of high exogenous intake of cholesterol and fat in rats. Namely, the polyphenolic fraction decreases cholesterol in liver, counteracts the development of fatty liver, positively modifies lipoprotein profile in plasma, above all it decreases VLDL-C and increases HDL-C/VLDL-C ratio, and ameliorates an antioxidant status in circulation by GSH increasing effect in blood. These Factors suggest that silymarin may modulate the process of atherosclerosis and hense might be a agent of future to prevent coronary artery disease.

ANTIFIBROTIC EFFECTS

Stellate hepatocytes have a crucial role in liver fibrogenesis. In response to fibrogenic influences (for example protracted exposure to ethanol or carbon tetrachloride), they proliferate and transform into myofibroblasts responsible for the deposition of collagen fibres in the liver. Recently, the effects of silibinin on the transformation of stellate cells into myofibroblasts have been investigated. The results have shown that silibinin, at a concentration of 100μmol/L reduce the proliferation of stellate cells isolated from fresh liver of rats by about 75%, reduce the conversion of such cells into myofibroblasts, and downregulates gene expression of extracellular matrix components indispensable for fibrosis [79]. Furthermore, it has been demonstrated that silymarin improves hepatic fibrosis in vivo in rats subjected to complete occlusion of the biliary duct, a manoeuvre that causes progressive hepatic fibrosis without inflammation. Silymarin, administered at a dosage of 50 mg/kg/day for 6 weeks,

is able to reduce fibrosis by 30 to 35% as compared with controls.

Mushroom poisoning

The most remarkable use of silymarin is in the treatment of Amanita phalloides (Death cap) poisoning, a toxic mushroom widespread in Europe and North America. Amanita phalloides possess two extremely powerful hepatotoxins, amanitin and phalloidin (LD50 of amanitin is 0.1 mg/ kg body weight) 1. Benzyl penicillin (3, 00,000 to 10, 00,000 U/kg/day) along with silybin (20-50 mg/ kg/day, iv) is shown to be effective against amanitin poisoning along with other supportive measures. There are no controlled trials available in the treatment of mushroom poisoning, other than a few case studies or individual case reports. Carducci *et al.*, [80] presented a report of a family of four poisoned by Amanita mushroom (amatoxin), admitted to a hospital in Naples with severe liver damage. Although they were treated with standard therapy, the clinical picture worsened till the third day when it was decided to add silybin hemisuccinate by intravenous route to the therapy. After silybin administration, the patients showed a favourable course with a rapid reduction of clinical picture. All patients were discharged on day 10-13. Subsequent investigations after 2 months revealed no morphological alterations in hepatobiliopancreatic echography. The investigators suggested that silybin may play a significant role in protecting hepatic tissue not yet injured by the toxins. The results of a 20 yr retrospective study from clinical data of 2108 patients hospitalized in North America and Europe with amatoxin poisoning due to 35 species of mushrooms and Chi square statistical comparison of survivors and dead versus treated individuals supported silybin use either alone or in combination [46].

ANTIOXIDANT ACTIVITY IN BRAIN

Recent studies have analyzed the antioxidant properties of silymarin in liver[1] and brain [81]. The brain has a high consumption of oxygen, large amounts of polyunsaturated fatty acids [82] high concentrations of free iron ions and low levels of antioxidants defenses compared to other organs [83]. This characterizes the fragility of the brain against reactive oxygen species. The reactive oxygen species (ROS) can modulate several pathway of cellular signal transduction. ROS can activate transcription factors; to increase the activity of proteins. Recent studies demonstrated a protective effect of silymarin on oxidative stress in brain [81] and experiments were conducted to measure cerebral concentration of glutathione (GSH), cerebral superoxide dismutase (SOD) activity, malondialdehyde (MDA), ascorbic acid (AA) and protein. The results demonstrated that silymarin induce an increase of GSH, AA levels, and SOD activity in brain of rats treated with 200 mg/kg/day for 3 days, showing a protective effect on antioxidant defense systems. In studies, the antioxidant capacity against peroxyl radicals has been shown to be reduced in hippocampus and cortex of young rats and also in the hippocampus, of aged animals at the highest employed dose. This is because potentially antioxidant molecules, such as silymarin, can change the redox state of the cellular ronment, altering the antioxidant defense system [84]. According to the free radicals theory of ageing [85], the rate of generation of reactive oxygen species (ROS) and accumulation of changes (damage) that cause these species, increase the risk of death, and cause deterioration over time progressive in biological systems, determined by the accumulation of mitochondrial ROS that causes a lowered metabolism during aging. This falls in metabolism leads to a decrease in the antioxidant defense system and an increase in ROS by dysfunctional mitochondria. This reduction of antioxidant defense system associated to the physiological processes of aging is visible between the animals treated with vehicle because aged animals have lower antioxidant defense in cortex compared to young rats. An increase in ACAP in the cortex of aged animals treated with SM 400 were measured and positive results suggest that this may be related with the reduction in LPO measured by TBARS assay and levels of protein oxidation, suggesting SM as a potential compound for the treatment of neurodegenerative diseases or diseases related to age, such as Alzheimer diseases.

As previously observed by Nencini *et al*., [81], through the levels of malondialdehyde, silymarin decreased lipid in the cortex of young and aged rats, but in a dose greater than that used by them (SM 400 mg/kg/day). In the study of Soto *et al*., [86], it was observed that SM lowered MDA few hours after the beginning of the experiment, concomitant with a subsequent increase that was gradually reduced after 2 days of treatment with SM 200 mg/kg/day. The presence of carbonylated proteins in tissue samples has become a widely accepted biomarker of oxidative stress [87]. An increase in carbonyl proteins under oxidizing conditions could create a high percentage of dysfunctional proteins that may be a major contributor to cell damage and death due to oxidative stress. The results of various studies indicate that both silymarin doses decreased protein oxidation in hippocampus and cortex tissues of animals, particularly for immune-reactive bands of molecular weight ranging from 50 to 120 kDa. Therefore, obtained results clearly demonstrated that silymarin exerts a strong protective effect against oxidative stress damage at the protein level. Silymarin at a dose of 200 mg/kg/day was more effective in the reduction of proteins oxidation in hippocampus and cortex of aged rats compared to the young them. The protein oxidation is an important early event in Alzheimer Disease brain [88] and, in this way, it can be proposed SM as a candidate compound against this disease.

VIRAL HEPATITIS

Studies show that silymarin is effective in both acute and chronic hepatitis. Studies showed that administration of silymarin shortens treatment time and lowers serum bilurubin, AST and ALT. In acute hepatitis silymarin 140mg dose three times daily for three weeks shows lower levels of AST then control groups. In patients with chronic hepatitis 420 mg of silymarin per day for six months resulted in a significant improvement in serum liver enzyme levels [88].

SAFETY AND SIDE EFFECTS

Silymarin is generally regarded to be safe, although allergic reactions, including anaphylaxis, have been reported in three cases. The most common side effect of silymarin is a mild laxative effect. Other reported adverse events include nausea, epigastric discomfort, arthralgia, pruritus, headache and urticaria. In one study of patients with alcoholic liver disease, side effects were reported in seven of 46 (15%) receiving silymarin compared with four of 29 (14%) receiving placebo over 2 years of use. Concern has been raised regarding alterations of drug metabolism by silymarin. For example, as a result of cytochrome P450 enzyme inhibition and decreased bilirubin conjugation, silymarin may lead to reduced clearance and possible toxicity in patients treated with drugs conjugated by UGT1A6/9. While silymarin appears to have few negative effects, it is not known whether it has any interactions with interferon, ribavirin, lamivudine, or other conventional treatments for hepatitis B or C.

FUTURE DIRECTIONS

Silymarin is widely studied Herbal drug which is presently used in the treatment of various disorders, with the advancement of medical specialities and research on the new topics various other beneficial effect of silymarin will be discovered in future. Silymarin might be a potential therapeutic agent for the neuroprotection, prevention of neurodegenerative disease progression and possibly a brain tonic. As the research will progress day by day silymarin may explored for other beneficial effects. Silymarin can be a potent cardioprotective agent of future because of its antioxidant, antiinflammatory, antifibotic effect. There is need of promoting clinical trials on silymarin to explore undiscovered benefits on human health. Silymarin may agent of future for treating disorders of organs in addition to liver related problems.

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