PHARMACOLOGY OF SILYBUM MARIANUM AND ITS ACTIVE
CONSTITUENTS. THERAPEUTIC ACTIVITY – PART 2

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ABSTRACT

Silymarin, a flavonolignan from ‘milk thistle’ (Silybum marianum) plant is used from ancient times as a hepatoprotective drug. Along the hepatoprotective action silymarin owns also other actions as antioxidant, anti-lipid peroxidative, antifibrotic, anti-inflammatory, immunomodulatory, liver regenerating, etc. In Part 1 of this article we reviewed the milk thistle’s benefic effects in gastrointestinal disorders and its antitumoral activities. Part 2 covers other applications of sylimarin such as antioxidant, antiinflammatory agent, nephroprotector, radiation and skin protector. It was also studied its neuro-psychiatric and cardiac action.

Keywords: silymarin, oxidative stress, antioxidant, neuroprotection, T cell activation

3. ANTIOXIDANT EFFECTS

Oxidative stress is a term relative to the elevated levels of reactive free radicals in an organism. Oxidative stress can occur from diminished antioxidants and/or increased production of reactive free radicals such as reactive oxygen species and/or reactive nitrogen species (ROS/RNS). Free radicals are molecules that contain one or more unpaired electrons and are capable of independent existence. They are formed wherever there is disease and cell damage and are especially produced in large quantities during infection. (Mandelker, 2011). Reactive oxygen compounds may attack and damage lipids, proteins and DNA and then be involved with pathogenesis of many diseases, such as central nervous system (CNS) diseases, cancer, cardiovascular diseases and liver damage. Antioxidants, including vitamins C, E and flavonoids, have positive effects on preventing or attenuating these diseases. Thus, there is considerable interest in the discovery and development of efficient synthetic or natural antioxidants (Huang et al. 2009).

Fruits of Silybum marianum (L.) Gaertn (milk thistle, Asteraceae), contain isomeric mixtures of flavonolignans, including silychristin, silydianin, silybin, and isosilybin, collectively known as silymarin (Abbasi et al., 2010). The phenolics rings structure of flavonolignans have shown antioxidant effect. Silymarin induces the increase of glutathione (GSH) content in the cell and the inhibition of lipid peroxidation (Valenzuela and Guerra, 1985). It has been reported that the comparison of the response of GSH, cyclooxygenase COX-2 and inducible nitric oxide synthase (inos) to silymarin in proliferating stem cells indicates that GSH is probably the first site of action of silymarin, as a consequence of which COX-2 and inos are changed during proliferation (Ahmadi-Ashshiani et al., 2012).

Recent studies have shown that silymarin has antioxidant effect in the central nervous system, being able to enter the CNS through the blood–brain barrier (BBB) (Nencini et al., 2007). The antioxidant capacity of silymarin 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 environment, altering the antioxidant defense system (Jones, 2006). It was clearly demonstrated that silymarin exerts a strong protective effect against oxidative stress damage at the protein level. Silymarin is more effective in the reduction of proteins oxidation in hippocampus and cortex of aged rats compared to the young them (Galhardi et al., 2009). The protein oxidation is an important early event in Alzheimer Disease brain (Chauhan and Chauhan, 2006) and, in this way, it can be proposed silymarin as a candidate compound against this disease.

It has been revealed that silymarin may be helpful in slowing down the progression of neurodegeneration in focal cerebral ischemia. These results suggest that the neuroprotective potential of silymarin is mediated through its antioxidative and anti-apoptotic properties (Raza et al., 2011).

It was demonstrated that addition of the hepatoprotective agent silibinin to the preservation medium completely suppresses the oxidative stress generated by cold preservation and warm reperfusion. In fact, silibinin restores glutathione (GSH) levels, diminishes oxidative radical (O2•−) and consequently, suppresses lipid peroxidation (Ligeret et al., 2008). This positive result suggests that silibinin can improve liver preservation, this could potentially enhance the outcome of liver transplantation.

Silibinin supplementation during the different stages of carcinogenesis (initiation, postinitiation and entire period) in the presence of the procarcinogen 1,2...
dimethylhydrazine (DMH) optimally modulates the tissue lipid peroxidation and the enzymatic antioxidant defenses (Sangeetha et al., 2009). Lipid peroxidation (LPO), a well-established mechanism of cellular injury, is used as an indicator of oxidative stress. The antiproliferative role of silibinin might be due to its ability to increase cell resistance to LPO (Kravchenko et al., 2003), to restore the activities of enzymatic antioxidants SOD and gpx (Altorjay et al., 1992), which help to scavenge both O2·− and H2O2 effectively and also its potential to effectively inhibit constitutive NF-кb activation which is a redox sensitive transcription factor (Yoo et al., 2004) responsible for cell growth. Silymarin modulates imbalance between cell survival and apoptosis through interference with the expressions of cell cycle regulators and proteins involved in apoptosis (Shaker et al., 2010).

Silybin and the analogues offer different ability to repair DNA base against radiation-induced damage. Silybin shows the highest efficiency compared with the others. The higher rapid repairation activity of silybin agrees well with the rate constant reacting with hydroxyl radical. (Fu et al., 2008). Protecting DNA from radiation damage might result in prevention of the cancer/ mutations induced by radiation.

Antioxidant activity of silybin is considered as one of its therapeutic properties. However, the one-electron reduction potential at neutral ph for silybin is relatively high at 0.76 V [Silybin-OU, H+/Silybin-OH]. Silybin is thus not considered to be a superb antioxidant in comparison to other antioxidants such as vitamins C and E. Recent studies have attempted to identify other silymarin active compounds which exhibit improved antioxidant and anticancer activities (Huber et al., 2008).

An oxidized form of silybin so-called 2,3-dehydroisilybin (DHS) (Scheme I) was found to exist in fruit of spotted milk thistle plants cultivated in Russia and its common independent states. A recent study has reported that DHS inhibited microsomal lipid peroxidation better than silybin (Gazak et al., 2004).

![Scheme I. Structures of silybin and 2,3-dehydroisilybin (DHS).](image)

It was demonstrated that DHS exhibited significant greater antioxidant and anticancer properties than silybin by a factor of three- and five-fold, respectively (Huber et al., 2008). Accordingly, DHS was reported to be a substantially better antioxidant than silybin owing to the presence of 3-OH and a 2,3- double bond in conjugation with the C-4 carbonyl group. Nevertheless, the 3-OH in DHS molecule is responsible not only for the electron delocalization of the B–C ring system, but also is able to react with radicals (Gažák et al., 2009).

In conclusion, Silymarin is known to own antioxidative (Singhal et al., 2011) and anti-apoptotic properties (Manna et al., 1999). Silymarin has been reported to decrease lipid peroxidation (Bosisio et al., 1992). Furthermore, it has been demonstrated that its anti-oxidative activity is related to the scavenging of free radicals (Rauen and de Groot, 1998) and activation of anti-oxidative defenses: increases in cellular glutathione (GSH) content (Valenzuela et al., 1989) and superoxide dismutase activity.

4. NEURO-PsYCHIATRIC EFFECTS

Silibinin provide DNA protection and reduced oxidative stress in a brain specific area, via the activation of the heme oxygenase (HO) system (Marrazzo et al., 2011). Silymarin enhanced the morphine produced antinociceptive effect and elevated the total thiol molecule level in the serum, which later antioxidant property was confirmed by the concentration-dependent radical scavenging activity (Malekinejad et al., 2011). In other study it shown that silibinin act as a novel dose-dependent inhibitor of amyloid β-peptide aggregation (amyloid plaque) which characterized Alzheimer disease (Yin et al., 2011). The amyloid β plaque was attenuated under silymarin in an Alzheimer disease mouse model due to Aβ oligomer production (Murata et al., 2010). Similar, was showed that silibinin prevents memory impairment and oxidative damage induced by amyloid Aβ in mice (Lu et al., 2009). Moreover, silymarin shows promise in protecting the spinal cord and cortical cells against oxidative stress and lipopolysaccharide stimulation under toxin action (Tsai et al., 2010). Cerebral cortex was protect to manganese action under the silymarin treatment in rats (Chtourou et al., 2010). Silymarin, but not its active constituent silibinin, protected rats against cerebrai ischemia-reperfusion-induced stroke injury by amelioration of the oxidative and nitrosative stresses and inflammation-mediated tissue injury through impeding the activation of proinflammatory transcription factor (e.g. NF-kB and STAT-1) in the upregulation of proinflammatory proteins and cytokines in stroke-damaged sites (Hou et al., 2010). Also, sylmarin shown neuroprotective effect in 6-hydroxydopamine hemi-parkinsonian rat, partly trough attenuating oxidative stress and via estrogenic pathway (Baluchnejadmojarad et al., 2010).

On the other hand, sylmarin treatments was efficient in reducing of obsessive-compulsive symptoms in duble
5. ANTI-INFLAMMATORY EFFECTS
The inflammatory response of the liver is trough to be triggered of pro-inflammatory cytokines from hepatic nonparenchymal (NP) cells such as resident monocytes, macrophages and Kupffer cells. Pro-inflammatory cytokines secreted locally by NP cells in the liver directly affect hepatocyte function. Silybin inhibits interleukin-1/β-induced production of pro-inflammatory mediators in canine hepatocyte cultures (Au et al., 2010). Silymarin produced a significant decrease in proinflammatory cytokines secreted by S100b, a specific ligand of receptor for advanced glycationendproducts (AGE). In vivo, sylimarin reduced tissue AGE accumulation, tail collagen crosslinking and concentrations of plasma glycated albumin, togther with decreased levels of oxidative and inflammatory biomarkers (Wu et al., 2011). Serum TNF-α was found to be decreased in peritoneal dialysis patients after 8 weeks of sylmarin administration (Nazemian et al., 2010). In other study was found that silymarin inhibited p65/NF-kB phosphorylation in CD4+T cell which suggest that this active biocompound is able to inhibit T cell activation and proliferation, notably acting on pathways of NF-kB activation/translocation (Gharagozloo et al., 2010).Silibinin down-regulates, TNF-α-induced MMP-9 expression through inhibition of the MEK/ERK pathway in gastric cancer cells (Kim et al., 2009).

6. NEPHROPROTECTIVE EFFECTS
Silymarin recovered the renal tissue damage induced by alloxan which was related to an increase in the activity and recovery of gene expression of antioxidant enzymes which in addition to the glutathione system constitute some of the most important defense mechanisms against free radicals damage (Soto et al., 2010). Also, sylmarin diet inhibits ferric nitrilotriacetate (Fe-NTA) induced renal carcinogenesis and nephro-inflammation through decreased of protein expression of iNOS and COX-2, secretion of proinflammatory cytokines, ODC activity, [3H]-thymidine incorporation into DNA (Kaur et al., 2010).

Renal cancer Caki-1 cell proliferation and induced apoptosis through inhibiting of EGFR and ERK was inhibited. Also, was decreased the expression of surviving, up-regulating the expression of p53 and triggering the cascades of caspase pathway (Li et all, 2008).

7. CARDIAC EFFECTS
Sylmarin prevents cytostatic – induced cardiotoxicity in rats. In this respect, sylmarin pretreatment inhibited Adriamycin-induced cardiotoxicity due to inhibition of lipid peroxidation and protection against GSH depletion (El-Shitany et al., 2008). Treatment with silymarin prevents increase in AST and CK serum activity and myocardial excitability of rats caused by doxorubicin. It also significantly reduces doxorubicinprooxidative activity and decreases histological changes in liver and heart tissue of animals treated with doxorubicin (Raskovic et al., 2011).

8. RADIOPROTECTIVE EFFECTS
Exposure of the skin to soar UV radiation generates reactive oxygen species (ROS) or oxidative stress, which is capable of oxidizing macromolecules, like lipids, proteins and DNA. Sylimarin was able to reduce UV-induced oxidative stress against UV radiation-induced oxidative stress-mediated skin disorders (Katiyar et al., 2008) through targeting infiltrating CD11b+ in mouse skin. In other study sylmarin has been shown to posses antioxidant effects against UV radiation in mouse skin model (Katiyar, 2002). It was observed that treatment of mouse skin with sylmarin inhibited UV-induced skin carcinogenesis in terms of tumor incidence and tumor growth (Katiyar et al., 1997). Becker-Schiebe et al. (2011) shown that silymarin-based cream could protect skin lesions caused by radiotherapy of breast cancer patients.Silibin and analogues showed inhibition of DNA damage in vitro correlated with the ability of the compounds to scavenge free radical after X-ray exposure (Fu et al., 2010). Oral administration of silybin to mice resulted in significant protection to radiation-induced mortality and DNA damage in blood leukocytes (Prabha et al., 2010). Sylimarin reduces the amount of UVB radiation-induced DNA damage as demonstrated by reduced amounts ocyclobutane pyrimidine dimers (cpds) and as measured by comet assay, and that ultimately may lead to reduced apoptosis of normal human epidermal keratinocytes (Katiyar et al., 2011).

9. SKIN PROTECTIVE EFFECTS
Silymarin/silibinin is found in some high-end moisturizers to prevent cutaneous oxidative damage and photoaging (Singh et al, 2009). Silymarin suppressed dust mite extract (DPE)-induced atopic dermatitis (AD) –like skin lesions in mice and reduced plasma level of IL-4 and ige (Kang et al, 2008). In other study was observed that silymarin causes inhibition of chemically induced messenger RNA expression of TNF-α and IL-1α in mouse skin (Singh et al, 2002). Silymarin inhibit YPA-caused lipid peroxidation in mouse skin epidermis, which supports its strong antioxidant activity (Lahiri_ Chatterjee, 1999).
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