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Review

POTENTIAL THERAPEUTIC EFFECTS OF MILK THISTLE

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Summary

Milk thistle is a herbaceous plant and is part of the characteristic vegetation of all those lands that overlook the Mediterranean Sea. The plant is known for its use as a herbal medicine. This medicinal plant is known for its beneficial properties for the treatment of liver disorders and other diseases. Such effects are related to its ability to act as free radical scavenger as well as to the activation of antioxidant enzymatic system (i.e. heme oxygenase-1). The aim of the present review is to provide a tool for the clinicians in order to exploit the beneficial effects of milk thistle expecially for those conditions in which the modern pharmacology has not yet developed a successfult strategy of treatment (i.e. Nonalcoholic Liver Disease).

Introduction

The Milk Thistle L. Gaertner (Milk Thistle) is a grass species belonging to the family Asteraceae; the plant can reach 1-2 meters in height and is characterized by alternate bright green leaves and with yellow plugs on the lobes. The flowers are grouped in terminal inflorescences to peep, purple in color and consisting solely from tubular flowers with bracts involucrali ending in spines folded backwards. Fruits consist of achenes smooth and hairless, shiny black mottled yellow, with persistent pappus.

Milk thistle is a plant native of southern Europe, Asia Minor and the North- Africa and it spreads throughout the Italian territory with the exception of Friuli, much of the Plains Valley and the Alps. It prefers dry and sunny soils and often grows wild on the edges of the roads and on the ruins. Rich in phytochemicals, milk thistle is one of the

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herbs most commonly used in the traditional medicine.

Chemical components

Milk thistle contains a 1.5-3 % flavonolignan mixture collectively called silymarin, that is the main plant complex pharmacologically active (Figure 1). The major component of the silymarin or silybin is the silibinin, a 50:50 mixture of silybin A and silybin B, in addition to silidianin, silicristine, and small amounts of isosilibinin. Furthermore, it also contains a trans mixture of diastereoisomers fatty acid such as linoleic acid, the most abundant, followed by the oleic, myristic, palmitic, stearic and arachidonic acid (1).

Pharmacokinetic

Pharmacokinetic studies were performed on silymarin generally studying its main component silybin. Silybin is poorly soluble in water and tends spontaneously to form non-absorbable microcrystals. The absorption and its bioavailability are, however, depending on its preparation. In humans and in animals, silibinin is metabolized and converted into glucuronide and sulfate and it is subjected to enterohepatic circulation (2). In studies conducted in healthy subjects it was shown that the plasma levels of silybin are dose-dependent and that silybin is rapidly conjugated in the plasma (approximately 80% of silybin in the plasma is present in its conjugated form). However, studies carried out on patients undergoing to cholecystectomy with drainage (T) about 50% of the dose of silybin assumed is found in the bile. The bile concentrations of silybin (all-conjugated form) are approximately 100 times higher than plasma (3). The half-life of total silybin is relatively short. To this regard previous studies in healthy volunteers reported for the total silybin a half-life of approximately 6 hours after administration of single doses of 280-700 mg of dry ex-

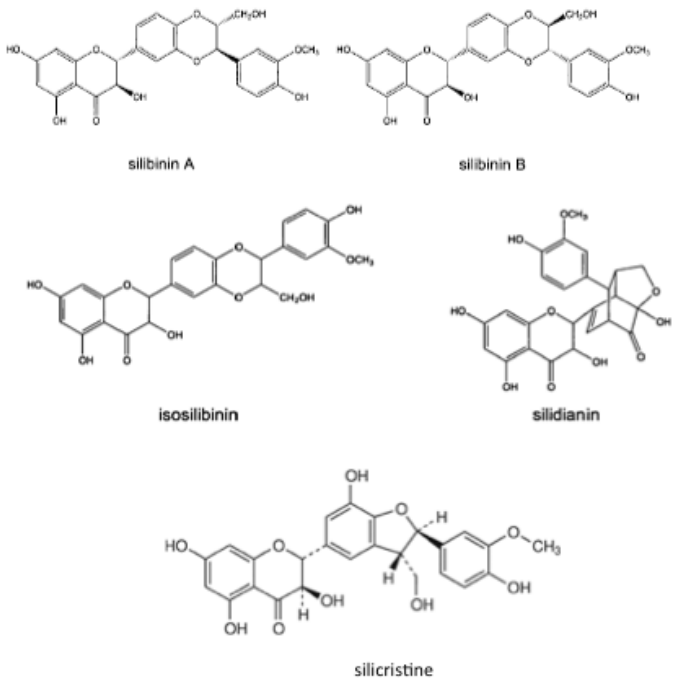

Figure 1

Figure 1. Chemical structures of the major pharmacologically active components of Milk Thistle.

tract of the fruits corresponding to 102-254 mg of silybin. Unconjugated silybin is rapidly eliminated from plasma with a half-life in plasma of approximately 1 hour. Biliary excretion is the major route of elimination of silybin (conjugated compound as glucuronide and sulfate), while the kidney eliminates less than 5% of the absorbed dose of total silybin.

Historical clinical uses

The Milk Thistle is a medicinal plant used for the treatment of liver disorders, spleen, gallbladder, hepatitis and gallstones since antiquity (4). Dioscorides advised the thistle as a remedy for snake bites, while Pliny the Elder in the first century BC used infusions made from the seeds of milk thistle for biliary disorders. In the XVII century, Nicholas Culpeper approved its use in the treatment of jaundice (5). Milk thistle was also used as a bitter tonic, as galactagogue, for the treatment of hemorrhoids and for dyspeptic disorders, in uterine hemorrhages, in the case of constipation, diabetes, in hay fever and for varicose veins.

Antioxidant Activity

Silymarin and silibinin exert antioxidant activity in vitro by reacting with reactive oxygen species in platelets, fibroblasts, and liver mitochondria (6). There is also an antioxidant effect indirectly linked to the capacity of the plant complex to inhibit the cytochrome P450 which in turns leads to oxygen free radicals formation (7, 8). Silymarin inhibits lipid peroxidation induced by oxygen free radicals in human erythrocytes causing the stabilization of the cell membrane structure (6). Furthermore, silymarin inhibits cAMP dependent phosphodiesterase, thus stabilizing lysosomal membranes and the degranulation of inflammatory cells (9). Finally, silibinin showed significant neuroprotective effects via its scavenging activity related to it peculiar chemical structure (10, 11). In fact, our previous studies showed that silibinin, as well as other polyphenols, upregulates the expression of heme oxygenase-1 (HO-1) which catalyze the conversion of heme to carbon monoxide and bilirubin both of which possess potent antioxidant activity under various experimental and clinical conditions (12-17). However, our studies showed that, HO activity inhibition by SnMP, did not reverse the antioxidant effects of silibinin.

Hepatoprotective Effects

There are many mechanisms of action, which are advocated to explain the hepatoprotective effects of silymarin (i.e. reduction of lipid peroxides and free radical scavenger activity) (18). Furthermore, the scientific literature reports the change of the properties of membrane and reduction of liver fibrogenesis are to be considered also as additional protective factors.

Silibinin binds to the regulatory subunit of RNA - dependent DNA polymerase I close to the binding site of estrogen thus acting as a natural steroid; the increase of ribosomal RNA in the liver stimulates the formation of ribosomes and therefore the protein synthesis and liver regeneration (19). Furthermore, the hepatoprotective effect of silybin was tested in rats with liver cirrhosis following prolonged administration of carbon tetrachloride (CCl_4): the result was that silybin is able to protect the structural and functional integrity of the membrane of the hepatocytes by preventing changes in the structure of their phospholipid produced by CCl_4 and restoring the activity of alkaline phosphatase and gamma-glutamyltranspeptidase (GGT) (20). Another important property of silybin and of silymarin is to regulate the content of glutathione (GSH) in various organs: treatment with silybin intravenously or intraperitoneally with silymarin was found to increase the GSH content in the liver, intestines and stomach, but no increase in the lungs, spleen and kidneys (21). The antioxidant action of silymarin is closely linked to its hepatoprotective function as oxidative reactions are the basis of various liver diseases, including inflammation, hepatic necrosis, fibrosis, and carcinogenesis mechanisms of hepatotoxicity of many substances.

Anti-inflammatory effects

The main anti-inflammatory effect of silymarin appears to be related to its ability

to inhibit transcriptional nuclear factor-kB (NFκB), which regulates the expression of several molecules involved in the inflammatory response, survival, growth and cell differentiation (18). In particular, NF-κB promotes the production of IL-1, IL-6, TNF, INF-γ and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF). In leukocytes basophils, silymarin inhibits the release of histamine mediated by neutrophils and mast cells. Furthermore, the four isomers of silymarin inhibited in vitro the activity of the lipoxygenase and prostaglandin synthetase (19).

Anti-fibrotic activity

All conditions of chronic liver injury are affected by the accumulation of scar tissue in the liver that can gradually change its architecture and functionality. This process, known as hepatic fibrosis, is a dynamic process of transition, between the chronic damage to the liver and cirrhosis. Stellate cells of the liver play a crucial role in fibrosis. In response to exogenous stimuli (for example in chronic exposure to ethanol or carbon tetrachloride) these cells proliferate and are transformed into myofibroblasts, responsible for the deposition of collagen fibers in the liver. Silybin, at the concentration of 10 mol/l reduces the proliferation of stellate cells isolated from rat liver by approximately 75% (22). It also reduces the speed of their conversion to myofibroblasts, and gene expression of extracellular matrix components indispensable for the fibrosis, such as TGF-β. Following occlusion of the biliary tract, liver inflammation that is generated causes a progressive expansion of fibrotic portal vessels that causes a secondary biliary cirrhosis. It was also shown that silymarin suppresses the expression of the profibrogenic procollagen a1 through the down-regulation of TGF-β1 gene expression ($p<0.01$) (23).

Anti-viral and anti-tumor activity

Although used as support therapy in liver diseases of viral origin, it was not detectable a direct activity on hepatitis C virus. Silymarin has proved capable of reducing the proliferation of tumor cells of various origin (Prostate, ovary, breast, lung, skin and bladder) (24). The main mechanism of action is due to the modulation of the expression of cyclins, CDK and CDK inhibitors (25). It has also been recently detected that silymarin reduces the increase of proliferation induced by UVB rays; additionally it inhibits microvessel density, inflammation and angiogenesis. In particular, the antiangiogenic activity has been demonstrated in several types of cancer, by a significant reduction in the secretion of Vascular Endothelial Growth Factor in the cancer cells. Nevertheless, silymarin interacts with the genome and modulates gene expression of molecules such as MMP-2, u-PA, ERK1/2, AP-1 and NF-κB, thus playing an important role in metastasis formation (26).

Other applications and mechanisms of action

The protective properties of silymarin and silybin have been also studied both in vitro and in vivo in respect of carcinogens using several experimental models. Silymarin has been shown to inhibit the proliferation of tumor cells of various origin (prostate, kidneys, liver, pancreas, lung, breast, bladder) and it was shown that silymarin reduces the proliferation of cancer cells induced by UVB radiation. In addition to its cytoprotective effects, mediated by the antioxidant and free radical scavenging activity, other mechanisms of action were identified based on the interaction with specific receptors. These latter have been studied on a molecular basis and through adjustment of the various routes of cellular communication with silybin; for example, it has been described inhibition of the transcription factor NF-kB inhibition signal EGFR-MAPK/ ERK1/2 activity on Rb and E2F. It was also suggested that silymarin can act by modulating the activation of substances that regulate the cell cycle (cyclins) and mitogen-activated protein kinase. The in vitro proapoptotic activities of silybin in pre- and/or carcinogenic activity with anti -angiogenic are other interesting properties highlighted by the most recent studies active ingredients of milk thistle that suggest new therapeutic applications for

the preparations of silymarin. Because of its antioxidant activity and ability to increase the concentrations of glutathione in plasma and in the pancreas, it has been shown that silymarin in rats with diabetes mellitus induced with alloxan may improve pancreatic function (27). Similarly, silymarin has been proven effective in preventing ulcer experimentally induced in animals (28). Silibinin does not alter the volume nor gastric acidity while significantly reduces the concentration of histamine, suggesting a mechanism of inhibition of peroxidation of lipoxygenase. In addition, it has been proposed that silybin prevents the diabetic neuropathy of the retina. In diabetic rats the silibinin (50 mg / kg administered as a complex of the b-cyclodextrin in water), administered for a period of 7-13 weeks, has been shown to inhibit the mono-ADP-ribosylation of proteins of the retina, a related factor peripheral neuropathy induced by diabetes (29). In a test carried out under the same experimental conditions it was also demonstrated that the increase of the mono-ADP-ribosylation of a membrane fraction of Schwann cells of the sciatic nerve of diabetic rats was normalized by silybin.

Conclusions

Silibinin has been proven to be pharmacologically active in various experimental models and possess clear clinical indications. Furthermore, its use in a clinical setting is justified also for its good tolerability since there are no description of possible interactions with drugs or foods. The safety data extrapolated from clinical trials show that preparations of milk thistle are associated with rare and generally mild adverse effects, generally gastrointestinal. Previous studies conducted on 4718 patients treated with dry extract of milk thistle fruit, the data revealed only a low level of adverse effects (0.8-2 %), most of which related to mild gastrointestinal distress. Some authors recommend , finally, a certain caution in hypertensive patients , due to the not negligible tyramine content of the drug.

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