MILK THISTLE IN LIVER DISEASES: PAST, PRESENT, FUTURE

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PAST, PRESENT, FUTURE

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Abstract

Silybum marianum or Milk Thistle (MT) is the most well-researched plant in the treatment of liver disease. The active complex of MT is a lipophilic extract from the seeds of the plant and is composed of three isomer flavonolignans (silybin, silydianin, and silychrstine) collectively known as silymarin. Silybin is a component with the greatest degree of biological activity and makes up 50 to 70% of silymarin. Silymarin is found in the entire plant but it is concentrated in the fruit and seeds. Silymarin acts as an antioxidant by reducing free radical production and lipid peroxidation, has anti-fibrotic activity and may act as a toxin blockade agent by inhibiting binding of toxins to the hepatocyte cell membrane receptors. In animals, silymarin reduces liver injury caused by acetaminophen, carbon tetrachloride, radiation, iron overload, phenylhydrazine, alcohol, cold ischemia, and *Amanita phalloides*. Silymarin has been used to treat alcoholic liver disease, acute and chronic viral hepatitis and toxin-induced liver diseases.

Key words: Milk Thistle, silybin, silipide, silymarin, liver diseases, reactive oxygen species.

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The Past: History of Milk Thistle

Silybum marianum (L.) Gaernt. (= *Cardus marianum* L.) is an annual to biannual plant of the family *Asteraceae* growing to 1.5 m (Rambaldi et al., 2005). “Silybum” is the name Dioscorides gave to edible thistles and “marianum” comes from the legend that the white veins running through the plant's leaves were caused by a drop of the Virgin Mary's milk. While looking for a place to nurse the infant Jesus when leaving Egypt, Mary could only find a shelter in a bower formed from the thorny leaves of the Milk Thistle (MT) (Morazzoni and Bombardelli, 1995). According to this story the folk belief that the plant was good for nursing mothers was born. Other names that have been attributed to MT include Marian thistle, Mary thistle, St. Mary's thistle, Our Lady's thistle, Holy thistle, sow thistle, Blessed Virgin thistle, Christ's crown, Venue thistle, heal thistle, variegated thistle and wild artichoke. *S. marianum* is native to southern Europe, southern Russia, Asia Minor and northern Africa and is naturalized in North and South America as well in South Australia. The plant grows in warm, dry soil and blooms in July-August. The crude drug consists of the obliquely obovoid fruits (achenes) from which the silvery pappus is removed. Each fruit is about 5-7mm long, up to 2-3 mm wide and 1.5 mm thick, with a glossy, brownish black to greyish, brown husk. The commercial drug originates principally from the cultivated sources, partly from Germany, but primarily from China, Argentina, Romania, Hungary and from a few Mediterranean countries (Figure 1). The freshly milled fruits have a cocoa-like odour and an oily and bitter taste (Capasso et al., 2003).

MT has been used since the time of ancient physicians and herbalists to treat a range of liver and gallbladder disorders, including hepatitis, cirrhosis and jaundice, and to protect the liver against poisoning from chemical and environmental toxins, including snakebites, insect stings, mushroom poisoning, and alcohol (Rambaldi et al., 2005).

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One of the earliest records of MT is found in the Bible (Genesis 3:18). In this verse, God told Adam and Eve when they were banished from the Garden of Eden that "*thorns also and thistles shall it bring forth to thee*".

Some of the earliest people to use and write about MT were ancient Greek and Roman physicians and herbalists, each of whom seemed to have their own name for the herb. Dioscorides called it “sillybon”, Pliny the Elder called it “sillybum”, and Theophrastus called it “pternix”. Dioscorides’ use of MT is one of the oldest known references of the medicinal use of this plant. He suggested preparing it in a tea “for those that be bitten of serpents”. Another famous ancient herbalist, Pliny the Elder, wrote that mixing the juice of the plant with honey was good for “carrying off bile” (Ross, 2008).

More recently it has been discovered that Indian and Chinese medicines used MT in clinical practice (Abenavoli et al., 2008). Available records do not seem to offer us the insight how (i.e., by what anecdotal or empirical evidence) MT came to be advised for liver and gallbladder problems. Although MT is the most often associated with treating liver disorders, physicians have tried to apply its curative properties to other ailments, including the stimulation of breast-milk production and bile secretion, treatment of depression, the protection against the poisonous mushroom *Amanita phalloides* and other environmental toxins (Pradhan and Girish, 2006)

The Present use of Milk Thistle

Biochemistry

The crude drug contains 15-30% lipids, in the form of triglycerides [linoleic (about 60%), oleic (about 30%) and palmitic (about 9%) acid]; about 30% proteins, sugars (arabinose, rhamnose, xylose, glucose); tocopherol, (0.038%) sterols (0.063%) with cholesterol, compesterol and stigmasterol, and flavonoids including quercetin, taxifolin, eriodictyol and chrysoeriol (Table 1). However, the constituents responsible for the activity

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are flavanolignans (flavanone derivatives) initially isolated as a mixture of addition products of a coniferyl alcohol, phenylproponoid alcohol, and a 2,3-dihydroflavonol, taxifolin (Wu et al., 2009). This mixture, known as silymarin, represents 1.5-3 % of the dry drug weight and consists of (Figure 2): silybin (=silybinin, silibinin) (approximately 50 to 60%), isosilibyn (about 5%), silychristin (about 20%) and silydianin (about 10%), as well as silimonin, isosilychristin, isosilibinin, etc. The drug can be identified for its microscopic characteristics by Thin Layer Chromatography (TLC) or High Performance Liquid Chromatography (HPLC); spectrophotometry can also be used.

Pharmacokinetics

About 20-50% of silymarin is absorbed following an oral administration in humans and about 80% of the dose is excreted in the bile while about 10% enters the enterohepatic circulation. However, the pharmacokinetic studies have principally been performed on silibinin, the main component of silymarin.

The bioavailability of silibinin is low and seems to depend on several factors such as (i) the content of accompanying substances with a solubilising character such as other flavonoids, phenol derivates, aminoacids, proteins, tocopherol, fat, cholesterol and other substances found in the preparation and (ii) the concentration of the preparation itself (Voinovich et al., 2009). The systemic bioavailability can be enhanced by adding solubilising substances to the extract (Saller et al., 2001). The bioavailability of silybinin can also be enhanced by the complexation with phosphatidylcholine or β-cyclodextrin and possibly by the choice of the capsule material (Morazzoni et al., 1992; Arcari et al., 1992). The variations in the content, dissolution and (oral) bioavailability of silybinin between different commercially available silymarin products - despite the same declaration of content - are significant (Schulz et al., 1995). Therefore, the comparisons between studies should be carried out with the caution, considering the differences between the analytical

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methods used (TLC vs HPLC) and whether free, conjugated or total silybinin is the object of measurement. Systemic plasma concentrations are usually measured, even though the site of action of silymarin is the liver, as they provide an estimate on the quantity of the drug being absorbed from the gastrointestinal tract. The adequate bioavailability accounts for the dose-related oral activity of silymarin in the liver (Saller et al., 2001).

In male volunteers, after single oral administration of a standardised dose of silibinin 100 to 360mg, plasma silibinin Cmax was reached after approximately two hours and ranges between 200 and 1400 µg/L, of which approximately 75% was present in the conjugated form (Weyhenmeyer et al., 1992; Gatti and Perucca, 1994). For total silibinin, an elimination half-life of approximately six hours was estimated (Saller et al., 2008). Between 3% and 8% of an oral dose was excreted in the urine, while 20 to 40% was recovered from the bile as glucuronide and sulfate conjugates. The remaining part was excreted via faeces (unchanged, not absorbed). Silibinin concentrations in bile reached approximately 100 times those found in serum (10–5 to 10–4 mol/L of silibinin in bile) with peak concentrations reached within two to nine hours (Saller et al., 2008).

Barzaghi and co-workers investigated the pharmacokinetics of silybin phosphatidylcholine complex (Id B1016) and detected an increase in the oral bioavailability of silybin in healthy human subjects, probably by a facilitatory role of the drug complex on the passage of the drug across the gastrointestinal tract (Barzaghi et al., 1990). The bioavailability of Id B1016 was shown to be several times higher than that of silymarin in the patients with hepatic cirrhosis (Orlando et al., 1990).

Pharmacological studies

Several pharmacological studies have been carried out on the active components of MT, silymarin and silybinin. It has been found that these substances exert hepato-protective, anti-oxidant, anti-inflammatory and anti-fibrotic properties; in addition, they

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stimulate protein biosynthesis and liver regeneration, increase lactation and possess immuno-modulation activity. The following is the synthesis of the selected studies on this subject.

Anti-inflammatory and immuno-modulation activity

Silymarin shows anti-inflammatory and immuno-modulation activity in various structures and pathways of the cell. The tumour necrosis factor-α receptor (TNFα-R) super-family, located in the cell membranes, contains several members with homologous cytoplasmic domains known as death domains (DD), important in initiating apoptosis and other signalling pathways following ligand binding by the receptors (Saller et al., 2008).

A pre-clinical study showed that silibinin prevented the effect of TNF-α induced with α-amanitin in hepatocytes (El-Bahay et al., 1999), possibly by reactive oxygen species (ROS)-dependent mechanisms (Chovolou et al., 2003). In mice treated with 750 mg/kg/day silymarin by gavage and 2.25 mg/kg Fumonisin B1 (inhibitor of ceramide synthase), silymarin prevented the Fumonisin B1-induced increases in the TNF receptor 1 (TNF-R1) expression, TNF-R1–associated apoptosis (by caspases pathways) and the induction of lymphotoxin ß and interferon (IFN)-γ (He et al., 2004).

Some evidence suggested that silymarin inhibited the expression of adhesion molecules, such as E-selectin (Kang et al., 2003), another family of transmembrane molecules, expressed particularly on the surface of leukocytes, involved in inflammatory pathways. In human cells, silymarin caused "*in vitro*" a reduction of lectin-dependent and natural killer (NK) cell mediated cytotoxicity but not of antibody dependent cell-mediated cytotoxicity (ADCC) (Deák et al., 1990).

The inhibition of the 5-lipoxygenase pathway in the cytoplasm, in particular leukotriene B4 (LTB4), at the silibinin concentrations achieved "*in vivo*", could well represent a pivotal pharmacological property of silymarin. The study which evaluated the action of silibin in isolated Kupferr cells, indicated a strong inhibitory effect on LTB4

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formation with the IC50 of 15 µmol/L silibinin (Dehmlow et al., 1996a). Similar results were found in porcine basilar arteries (IC50 = 100 µmol/L), human blood cells and human omentum endothelial cells (Dehmlow et al., 1996b) This selective inhibition of leukotriene formation by Kupffer and possibly other cells could at least partly account for the hepatoprotective properties of silibinin.

Silymarin anti-inflammatory nuclear DNA/RNA mediated effects involve nuclear factor kappa B (NF-κB), a ubiquitous rapid response transcription factor in inflammatory cells. Also, many genes encoding the proteins of the hepatic acute phase response are under the control of the transcription factor NF-κB (Saliou et al., 1998). Inactive NF-κB is present in the cytoplasm complexed with an inhibitory protein I-κB. NF-κB is activated by a number of incoming signals from the cell surface. Released from I-κB inhibition, NF-κB translocates into the nucleus and binds to the kappa B motif of the target gene. The NF-κB activation process can be inhibited by pharmacologic agents at each activation step; that is glucocorticoids, cyclosporine, tacrolimus, and antioxidants (Lee and Burckart, 1998).

In vitro silymarin was found to suppress both the kappa B motif of NF-κB DNA binding activity and its dependent gene expression in hepatoma cells. In addition, silymarin blocked the translocation of NF-κB p65 protein (through phosphorylation) to the nucleus without affecting its ability to bind the DNA (Manna et al., 1999). Silymarin also inhibited the TNF-α- induced activation of mitogen-activated protein kinase and c-Jun N-terminal kinase and abrogated TNF-α-induced cytotoxicity and caspase activation (Saller et al., 2008). Kang et al. reported that silymarin had exerted an inhibitory effect on nitric oxide (NO) production and inductible nitric oxide synthase (iNOS) gene expression in macrophages, although at relatively high concentrations of 12.5–25 µg/ml (Kang et al., 2002). The oral administration at the dosage of 100 mg/kg attenuated NO production by peritoneal macrophages in lipopolysaccharide (LPS)-treated mice. Silymarin, also concentration-dependent, suppressed the LPS-induced production of NO in isolated

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mouse peritoneal macrophages. Moreover, iNOS mRNA and its protein expression were completely abrogated by silymarin in LPS-stimulated murine RAW 264.7 cells. These results suggest that silymarin inhibits NO production and iNOS gene expression by inhibiting NF-κB/Rel activation. This conclusion was subsequently confirmed by Schümann and co-workers (Schümann et al., 2003). The authors tested silibinin in the mouse model of concanavalin A (ConA)-induced, T cell-dependent hepatitis. Silibinin significantly inhibited ConA-induced liver disease. Silibinin proved to be an immune-response modifier *in vivo*, inhibiting intrahepatic expression of tumor necrosis factor, interferon-g, interleukin (IL)-4, IL-2, and iNOS, and augmenting synthesis of IL-10. In addition, silibinin inhibited intrahepatic activation of NF-kB. The major mechanism of this hepatoprotective activity appears to be the inhibition of intrahepatic NF-kB activation, which prevents the subsequent synthesis of TNF, IFN-g, IL-2, and iNOS. Furthermore, while the synthesis of IL-10 was augmented, the production of IL-4 was inhibited within the liver.

Enhanced protein synthesis

Regeneration of liver cells is necessary for hepatic recovery from acute or chronic conditions. In chronic disease, fibrosis occurs simultaneously with cellular regeneration. The ultimate outcome is determined by the type of the process which dominates. Sonnenbichler and Zetl reported that the intra-peritoneal administration of silibinin had caused a marked increase in the synthesis of ribosomal RNA (increased polymerase I) in rat liver. The same authors found that silibinin had stimulated the synthesis of DNA in partially hepatectomised rats, but neither in healthy controls nor in hepatoma or other neoplastic cells (Sonnenbichler and Zetl, 1986; Pradhan and Girish, 2006). The exact mechanism of the action, probably involving polymerase I activation, is unknown. Silybin appeared to stimulate ribonucleic acid (RNA) polymerase-I and ribosomal RNA as it was found in several preclinical studies. This effect leads to more rapid formation of ribosomes,

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which in turn increases protein synthesis. This action has important therapeutic implications in repairing the damaged hepatocytes and restoring the normal liver functions.

Antifibrotic activity

Liver fibrosis can result in remodelling of liver architecture leading to hepatic insufficiency, portal hypertension and hepatic encephalopathy. These processes involve complex interplay of cells and mediators (Gebhardt, 2002). In the initial phase the proliferation of hepatic parenchymal cells is developed. The conversion of hepatic stellate cells (HSC) into myofibroblast is considered as the central event in fibrogenesis. Silymarin inhibits NF-kB and also retards HSC activation. It also inhibits protein kinases and other kinases involved in signal transduction and may interact with the intracellular signalling pathways. The evidence for anti-fibrotic activity comes largely from animal studies. Favari and Perez-Alvez reported that orally administered silymarin (50 mg/kg) to the rats with chronic carbon tetrachloride (CCl4) liver damage could reduce the collagen content in the liver of these animals up to 55% (increased with CCl4 approximately 4-to 6-fold as compared to controls) (Favari and Perez-Alvarez, 1997). In similar experiments, there was a reduction of collagen and pro-collagen III contents after biliary obstruction in the rat by 30% with 50 mg/kg/day although not at the level of 25 mg/kg/day of silymarin (Mourelle et al., 1989; Boigk et al., 1997). Silymarin suppressed the expression of pro-fibrogenic pro-collagen-α1(I) and TIMP-1 most likely via down-regulation of TGF-β1 mRNA in the rats with biliary fibrosis (Jia et al. 2001). Silymarin, administered during three years, retarded the development of alcohol-induced hepatic fibrosis in baboons (Lieber et al., 2003).

Antioxidant effects

Free radicals, including the superoxide radical, hydroxyl radical (OH), hydrogen peroxide (H_2O_2), and lipid peroxide radicals were implicated in liver diseases (Urtasun et al., 2008). The mechanism of free radical damage included ROS-induced peroxidation of

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the polyunsaturated fatty acid in the bilayer cell membrane, which caused the chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane, lipids and proteins (Nagata et al., 2007). The antioxidant properties of silibinin were evaluated by studying the ability to react with relevant biological ROS or oxidants such as superoxide anion radical ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$) and hypochlorous acid (HOCl) (Detaille et al., 2008). Several pre-clinical studies reported that MT was not a good scavenger of $O_2^{\cdot}$ and no reaction with H_2O_2 was detected (IC50% >200 µmol/L). However, it reacted rapidly with $HO^{\cdot}$ radicals in free solution at approximately diffusion-controlled rate (IC50% = 1.2–7 µmol/L). It was reported that in vitro incubation with silymarin in the concentration equivalent to the usual therapeutic dosage markedly increased the expression of superoxide dismutase (SOD) in lymphocytes in the patients with alcoholic cirrhosis (Feher et al., 1988; Pradhan and Girish, 2006). Silymarin had no direct effect on ethanol metabolism and had no role in reducing ethanol levels or the rate at which ethanol was removed from the body. In fact, there is no evidence of the interaction of silymarin or silibinin with cytochrome P450–2E1, thus suggesting that these antitoxic effects are due to its antioxidant and free radical scavenging properties (Miguez et al., 1994).

The phenolic conformation of silymarin is thought to permit the formation of stable compounds from hydroxylic and oxygen radicals (Das and Vasudevan, 2006). The *in vivo* studies in rats indicate that silymarin can reduce the free radical load. The rats exposed to acetaminophen at toxic doses, had increased the levels of reduced glutathione and superoxide dismutase when treated with silymarin compared with the levels in controls (Singh et al., 2009). Furthermore, the other studies demonstrated that silymarin could inhibit cell lysis as measured by changes in alanine aminotransferase levels when exposing isolated hepatocytes to carbon tetrachloride and galactosamine (Tsai et al., 2008).

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The *in vitro* experiments with simian kidney cells damaged by paracetamol, cisplatin and vincristin demonstrated that administration of silibinin before or after the drug-induced injury could lessen or avoid the toxic effects. In the recently published study, the rats received cisplatin (3 mg/kg) intraperitoneally and silymarin (50 mg/kg) two hrs before or after cisplatin injection, during five days (Karimi et al., 2005). The pre-treatment with silymarin prevented the nephrotoxic effects of cisplatin while silymarin administered after the chemo-therapeutic agent reduced them to some extent.

Toxin blockade

Silymarin is a suitable candidate to treat iatrogenic and toxic liver diseases. It has a regulatory action on cellular and mitochondrial membrane permeability in association to an increase in membrane stability against xenobiotic injury. It can prevent the absorption of toxins into the hepatocytes by occupying the binding sites as well as inhibiting many transport proteins at the membrane. The phalloidin-transporting system, belonging to the hepatocyte-specific organic anion uptake transporters OATP2 (Fehrenbach et al., 2003), is inhibited in a competitive way by silymarin with no influence on membrane fluidity (Sebastian et al., 2004). The OATP2 may represent the part of a hepatic equipment which clears portal blood from bile acids, lipophilic hormones or xenobiotics. The transporting system incorporates phallotoxins into the hepatocytes leading to the death of these cells. Phalloidin (a bicyclic heptapeptide) and antamanide (a monocyclic decapeptide from *Amanita phalloides*) interact with bile-salt-binding polypeptides of the hepatocyte membrane. Similar or possibly identical transport mechanism inhibited by silibinin was described for amanitin (Wellington and Jarvis, 2001). Therefore, silymarin is able to reduce the cellular absorption of noxious xenobiotics other than mushroom poisons, thereby exerting cell-protection.

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MT and lactation

Traditionally MT has been used by nursing mothers for stimulating milk production and it was recently reported that MT increased the lactation in cows (Tedesco et al., 2004) and women (Di Pierro et al., 2008). However, the MT mode of action has not been established yet. Prolactin is the principal lactogenic hormone and it was recently shown that MT significantly increased circulating prolactin levels in female rats (Capasso et al., 2009). This effect seems to involve, at least partly, dopamine D_2 receptors. In the light of these results MT (or silymarin) could be considered a good candidate for the treatment of lactation insufficiency.

The Present Use of Milk Thistle: Overview of Evidence

The available clinical studies have generally investigated the effects of proprietary products containing silymarin/silybin in patients with hepatic disorders like cirrhosis, acute and chronic hepatitis and liver disease. However, there is also an interest in the use of silymarin in toxin- (mushroom *A. phalloides*) induced hepatitis.

The Agency for Healthcare Research and Quality data, published in 2002, analyzed sixteen prospective placebo-controlled trials. Twelve studies used Legalon®; eight of which used a dosage of 240 to 800 mg/day for the treatment period varying from seven days to six years. No information about doses or duration was reported in four studies. In remaining four of placebo-controlled studies, two used silymarin (no further characterization regarding preparation) and two studies used Silipide®, a silybin phosphatidyl cholin complex (240 mg/day). Seventeen additional trials did not include controls. Silymarin, silybin, silipide and silimarol were used with no standardized dose in nine studies. In the remaining two trials, silymarin was administered to the subjects with no liver disease diagnosed. These trials were probably aimed to determine the prophylactic

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use of silymarin in the subjects treated with concomitant anti-tuberculosis drugs and tacrine, but the results were contradictory, for the absence of hepatic illness.

A systematic meta-analysis, published in the last years, evaluated nineteen studies, eleven double blind and eight single blind (Saller et al., 2008). The authors concluded that it was reasonable to employ silymarin in the treatment scheme in the case of *Amanita phalloides* poisoning. Also, the available evidence supported the therapeutic use of silymarin, in view of its excellent safety profile, in alcohol-induced liver diseases, including liver cirrhosis (Child-Pugh classification grade A).

MT in alcohol-induced liver disease

Randomized, blinded, placebo-controlled studies assessed the effectiveness of MT in chronic alcoholic liver disease. The results of these trials might be conflicting and confounded because of heterogeneity of the degree of disease severity and alcoholic intake or abstinence. In a double-blind controlled study by Salmi and Sarna, one hundred and six patients with mild acute and sub-acute liver disease, of which ninety with confirmed histological diagnosis, were randomly allocated to either silymarin or placebo treatment, for four weeks (Salmi and Sarna, 1982). Alcohol was forbidden during the trial. The difference between the two groups was significant in an important decrease in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the silymarin group. No difference, instead, in reduction of serum bilirubin in both. Among the subgroup with the second histological work-up, the normalization of histological changes occurred more often in the silymarin group (eleven out of fifteen) than in controls (four out of fourteen).

Feher and co-workers, studied thirty six patients suffering from chronic alcoholic liver disease, treating them with an oral dose of 420 mg/day of silymarin or placebo for six months. Serum bilirubin, transaminase values and gamma glutamil transferase (GGT)

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activity were normalized in the silymarin group, with an improvement in histology of the liver and a decrease of pro-collagen III peptides (a marker of fibrotic progression in liver disease), but only a few patients had control histology (Feher et al., 1989).

In a double blind comparative study of 106 patients with histological proven alcoholic hepatitis, MT did not significantly influence the liver biopsy findings (Trichet et al., 1989).

MT in viral hepatitis

Even though silymarin does not affect viral replication, it might play a beneficial role in viral hepatitis by its inhibitory action on inflammatory cascade induced by viral infection. Lirussi and Okolicsanyi compared silymarin and ursodeoxicholic acid in a rather heterogeneous population of patients with active cirrhosis, the majority were HCV positive (Lirussi and Okolicsanyi, 1992). No efficacy was detected in this study. More recently, a new silybin-phosphatidylcholine complex was studied in a short pilot study on twenty patients with chronic active hepatitis (Buzzelli et al., 1993). Transaminase levels were reduced in silymarin group, but without consistent differences in other liver function tests (bilirubin, alkaline phosphatase, albumin).

Rambaldi et al. in the meta-analyses of randomized clinical trials valuated 915 patients with alcoholic hepatitis and hepatitis B or C (Rambaldi et al., 2005). The primary outcome measure was the number of patients dying. The secondary outcome measures were the development of clinical symptoms and complications analyzed separately and combined with liver biochemistry, liver biopsy findings, as well as the number and type of adverse events. The authors found no significant effect of MT on all-cause mortality and observed a potential beneficial effect of MT on mortality in patients with alcoholic liver disease, but this effect could not be confirmed in two high-quality trials. A potential

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beneficial effect of MT on liver-related mortality was observed, but again, this effect could not be demonstrated in three high quality trials.

Subsequently, Federico et al. evaluated the antioxidant and antifibrotic activity of Realsil® (complex silybin - vitamin E - phospholipids) to improve insulin resistance and liver damage in the patients with non-alcoholic fatty liver disease (NAFLD) and HCV chronic infection (Federico et al., 2006). This study enrolled eighty five patients; fifty nine were affected by primitive NAFLD (group A) and twenty six by HCV related chronic hepatitis C in combination with NAFLD, all HCV genotype-1b, and non-responders to previous antiviral treatment (group B). All the patients with a diagnosed liver disease two years prior to the study, according to histological criteria, were enrolled over six consecutive months and further divided into two subgroups using a systematic random sampling procedure: fifty three [thirty nine NAFLD and fourteen HCV) were treated with four tablets/day of Realsil® (one tablet contained 94 mg of silybin, 194 mg of phosphatidylcholine, and 90 mg of vitamin E) for six months followed by another six months of follow up, while the other thirty two patients (twenty NAFLD and twelve HCV) served as a control group (no treatment). For 0, 6, and 12 months, the outcomes were: body mass index, bright liver by ultrasonography, transaminase and GGT levels, blood glucose and insulin plasma levels with a contemporaneous determination of insulin resistance by Homeostasis Model Assessment (HOMA) test and, as indices of liver fibrosis, plasma levels of transforming growth factor β, hyaluronic acid, and metalloproteinase.

The results showed the improvement of liver enzyme levels in the treated group, but this only persisted in the group A. Hyperinsulinaemia, present in both groups, was significantly reduced only in the treated patients. The treatment with Realsil® significantly reduced all indices of liver fibrosis in both treated groups, with a persistent effect only in group B.

More recently El-Kamary et al. conducted a randomized controlled trial of silymarin *vs* placebo with 105 patients with symptoms compatible with acute clinical hepatitis and serum alanine aminotransferase levels > 2.5 times the upper limit of normal (El-Kamary et al., 2009). The patients received 420 mg/day of silymarin (Legalon®) or vitamin placebo for four weeks. The treatment with silymarin significantly reduced the symptoms related to biliary retention (dark urine, jaundice, scleral icterus), while the biomarkers considered (transaminase and direct bilirubin) were not significantly modified.

Liver cirrhosis

Clinical trials in liver cirrhosis are complicated by a series of confounding factors, such as concomitant therapies and complications of cirrhosis. The study by Ferenci et al. was performed to determine the effect of silymarin in patients with cirrhosis (Ferenci et al., 1989). It was a randomized controlled study comprising 170 patients with cirrhosis. Eighty seven patients (alcoholic forty, non-alcoholic forty-one) were treated with silymarin 140 mg oral dose, three times daily; eighty three patients (alcoholic forty five, non-alcoholic thirty eight) received a placebo. The mean observation period was forty one months. The survival rate, to four years of the treatment, was 58% in the first group and 39% in the controls. The analysis of subgroups indicated that the treatment was effective in the patients with alcoholic cirrhosis (p=0.01) and in the patients initially rated Child-Pugh A (p=0.03).

A French study group, conducted a randomized double-blind trial of silymarin versus placebo in 116 patients with histologically proven alcoholic hepatitis, fifty eight of them with cirrhosis (Trichet et al., 1989). Fifty seven patients received oral silymarin 420 mg/day and fifty nine received placebo for three months. The biological parameters were assessed in the serum, and the percutaneous liver biopsy was obtained both at the start of the trial and after three months (control histology available in thirty two silymarin and in

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thirty five placebo patients). The significant improvement in the score of alcoholic hepatitis and serum amino transferase activity was noted in both groups during the trial, irrespective of treatment with silymarin or placebo but clearly correlated with abstention from alcohol. However, this study is relatively short and half the population was not classified as having cirrhosis; since the deaths were not allocated to a diagnostic group, the entire group of the patients was considered "at risk". Bunout et al. conducted a placebo-controlled trial with silymarin in the patients with alcoholic liver cirrhosis. Seventy-one patients were randomly assigned to oral silymarin (n=34) at a relatively low dose of 280 mg/day or an equal number of placebo tablets (n=37) (Bunout et al., 1992). Both groups did not differ in their initial laboratory assessments and were followed up for an average of fifteen months. Ten patients died during the follow up (five in placebo and five in silymarin; liver-related death in nine). No details concerning distribution by the therapeutic group were provided. No significant differences were observed between these two groups. It was concluded that silymarin did not change the evolution in this trial. A subsequent Spanish study was specifically aimed to determine the effect of silymarin in the patients with alcoholic liver cirrhosis, respect to survival, clinical and laboratory changes (Parés et al., 1998). From February 1986 to June 1989, this randomized double-blind multicentre trial, comparing silymarin 450 mg/day in three divided doses (n=103) with placebo (n=97) enrolled 200 alcoholics with histologically- (n=191) or laparoscopically-proven (silymarin six, placebo three) liver cirrhosis. The primary outcome was the time of death and the secondary outcome was the progression of liver failure. The survival rate was similar in both groups and was not influenced by gender, persistence of alcoholic intake, severity of disease or by the presence of alcoholic hepatitis in the liver biopsies. Silymarin did not produced any significant effect on the progression of disease, but the frequency of complications was lower in the treated patients (p=0.06). The study by Bunout had the same end-point,

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considered by Ferenci and Parés, however a low dose was used, and it did not state whether it was properly blinded.

Toxic and iatrogenic liver disease

In spite of the large number of pre-clinical studies, the clinical trials in toxic liver disease are scarce and of poor quality. Only one study was set up to assess the ability of silymarin to antagonize or to prevent the hepatotoxic effects of tacrine (Allain et al., 1999). Tacrine is an anticholinesterase drug used for the treatment of Alzheimer's disease. In this randomized double-blind placebo controlled study on twenty three patients suffering from mild to moderate dementia of the Alzheimer type, the silymarin oral dose (420 mg/day) was given for one week, and then tacrine was added, first at 40 mg/day for six weeks, then at 80 mg for six additional weeks. The statistical difference was not observed for serum ALT, but the side effects, especially gastrointestinal disorders, were much less frequent in the silymarin group. Therefore, the co-administration in the initial phases of the treatment may improve tolerability.

In the study of prophylactic MT during the treatment for tuberculosis, twenty nine subjects with normal liver function tests received anti-tuberculosis drugs plus Hepabene®, a mixture of silymarin and *Fumaria officinalis* alkaloids. The differences in the AST and ALT reductions were statistically significant. Therefore, the combination of silymarin with such potentially hepatotoxic drugs may prevent such adverse reactions (Comelli et al., 2007).

Mushroom poisoning

The *Amanita phalloides* mushroom ("death cap") has been known and feared for at least two millennia and continues to cause serious illness and death. The most important, cytotoxin, out of many cytotoxins produced by mushrooms, is the potent amanitin found in

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some mushrooms belonging to the genera *Amanita* and *Galerina*. Amanitin is a cyclic octapeptide which inhibits RNA polymerase II, thus interfering with protein synthesis. Phalloidin, a cyclic heptapeptide that accompanies amanitin and may interfere with actin polymerization, is probably responsible for the initial gastrointestinal symptoms. *Amanita phalloides* intoxications are not very frequent. Unfortunately, there are no controlled studies available and so case-control studies collected over several decades, individual cases reports and "expert opinion" were reviewed (Saller et al., 2008).

Diabetic patients with chronic liver disease

Glucose intolerance in the patients with cirrhosis results from two abnormalities that occur simultaneously: insulin resistance in muscle and inadequate response of β-cells to secrete insulin appropriately in order to overcome the defect in insulin action (Petrides et al., 1994). Diabetes mellitus in the insulin-resistant patients with cirrhosis is the result of the progressive impairment in insulin secretion together with the development of hepatic insulin resistance leading to fasting hyperglycaemia and a diabetic glucose tolerance profile (Younossi and McCullough, 2009).

The prevalence of diabetes mellitus was reported to be higher in the patients with HCV-related cirrhosis (23.6%) than in those with HBV-related cirrhosis (9.4%) (Negro and Alaei, 2009). The prevalence of diabetes mellitus is also associated closely with the Child-Pugh score and increasing age. In a study by Velussi et al., the authors showed the effectiveness of a long-term treatment with MT to reduce lipoperoxidation and insulin resistance in the diabetic patients with cirrhosis (Velussi et al., 1997). A group of patients was enrolled to receive an oral dose of 600 mg/day of silymarin plus standard therapies, while the control group standard therapies alone. The trials outcomes were: fasting blood glucose levels, daily glucosuria, glycosylated haemoglobin (HbA1c) and malondialdehyde levels. There was a significant decrease in fasting blood glucose levels and in fasting

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insulin levels (-40%) already after four months of the treatment in the silymarin group, also seen for the mean daily blood glucose levels (-14.6%) and daily glucosuria (-32%). However, the HbA1c levels at the end of twelve months were lowered by 8.8%. In addition, there was a significant decrease in the mean exogenous insulin requirements in the treated group from 55 ± 5 IU/day to 45 ± 3 IU/day after six months and 42 ± 2 IU/day after twelve months, while the untreated group showed the significant increase in fasting insulin levels and a stabilized insulin requirement. The authors concluded that these results showed that silymarin might reduce the lipoperoxidation of cell membranes and insulin resistance, significantly decreasing endogenous insulin overproduction and the need for exogenous insulin administration, thus reducing the daily insulin dose required by almost 25% patients.

Preparations of Milk Thistle for humans

A water-soluble derivative of silybinin (silybinin dihemisccinate disodium) is available in Europe for *Amanita phalloides* poisoning. Madaus Legalon® is produced in tablets containing 70 or 140 mg silymarin and is given in a dose of one to two tablets up to three times daily, with a maximum dosage of 420 mg. The formulation includes extraction with alcohol, filtration and evaporation and may also include pressing, heat drying, and blending with other compounds. Some brands may add choline, inositol, tumeric extract, artichoke extract, whole herb powder, dandelion, licorice, curcuma, boldo, iron, or vtamins A and C.

One formulation is combined with kutkin, the roots and rhizome of *Picrorhiza kurroa*, a perennial herb found only in the higher mountains of the north-western Himalayas. Other concentrated oral formulations include tablets and softgel capsules. Silipide® is a complex of one part of silybin and two parts of phosphatidycholine from soybean phopholipids (lecithin), for which the standardization is expressed as silybin equivalents. It was more

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bio-available than standardized silymarin after oral ingestion in the normal volunteers, cirrhotics, and patients after cholecystectomy (Barzaghi et al., 1990; Federico et al., 2006). The bioavailability of silybin in Silipide® is approximately tenfold greater than the silybin content of standard MT preparations (Morazzoni and Bombardelli, 1995). Other preparations containing MT standardized to silymarin or to silybin are also found in commerce in different forms (granulates, tablets or coated tablets and drops).

Complications and adverse effects

Silymarin is reported to have a very good safety profile. No side-effects were observed in volunteers following a single oral dose of silymarin corresponding to 254 mg silybin (Weyhenmeyer et al., 1992). The patients with liver disorders of various origins who received oral silymarin (600-800 mg/day) for six months did not manifest the adverse effects (Velussi et al., 1993; Palasciano et al., 1994). The clinical trials involving about 3500 patients, of whom 2637 with liver disease, treated with Legalon (560 mg/day) for eight weeks reported that the frequency of adverse effects with silymarin was about 1%. The pooled adverse effects were transient gastrointestinal complaints like bloating, nausea, dyspepsia and diarrhea. (Leng-Pschow, 1996).

It was also shown that MT was generally non toxic and without side effects when administered to the adults in an oral dose range of 240-900 mg/day in two or three divided doses. At higher doses of more than 1500 mg/day silymarin may produce a laxative effect with an increased bile flow and secretion. Among the randomized controlled studies neither optimal nor maximal therapeutic doses were defined. In most of the trials the reportedly affective daily doses of silymarin were 420-600 mg (Saller et al., 2008).

However the case reported described an adverse reaction of the MT herbal medication. The symptoms included severe sweating, abdominal cramping, nausea, vomiting, diarrhea and weakness (Adverse Reaction Advisory Committee, 1999). Another

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report described an anaphylactic shock in a 54-year-old man with immediate-type allergy to kiwi fruit (Geier et al., 1990).

Preclinical data has not documented the acute toxicity for sylimarin and silybin. Silymarin, in particular, given orally to mice and dogs at doses of 20 or 1 g/kg did not cause adverse effects or mortality. However, a long-term oral administration of silymarin (100 mg/kg/day) to rats for 22 weeks did not also cause the adverse effects (El-Bahay et al., 1999, Saller, 2008). MT preparations are contra-indicated for the individuals with hypersensitivity to *Asteraceae.* None of the studies on cirrhosis, carried out with silymarin, considered clinical outcomes other than death as the primary end-point. Based on the available data, several issues could be partially examined in the above mentioned studies. Different issues were usually addressed in different trials; although these issues are interrelated, they have to be regarded individually and cannot be consolidated into one global picture.

Bleeding oesophageal varices constitute one of the most serious complications of cirrhosis. The total incidence of upper gastrointestinal bleedings (UGBs), both as a co-factor of death and as "last data", were reported in the two largest trials: 4.6% with silymarin vs. 9.6% with placebo, and 6.3% vs. 13.5% 79, respectively, and showed the differences in favour of the active treatment [p=0.042; Odds Ratio (OR) 0.44 (95% CI 0.20, 0.97)] (Ferenci et al., 1989). These findings suggest that the decreased rate of UGBs reflects an overall improvement in the patients, also evidenced by the lower liver-related mortality rate, rather than being a direct effect of silymarin.

The Future: New Prospective uses of Milk Thistle

The cancer chemopreventive role of MT flavonolignans has been reported in recent literature (Mazzio and Soliman, 2009). Silymarin modulates imbalance between cell survival and apoptosis through interference with the expressions of cell cycle regulators

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and proteins involved in apoptosis. In addition, silymarin also showed anti-inflammatory, anti-angiogenic and anti-metastatic effects (Ramasamy and Agarwal, 2008). Particularly, the protective effects of silymarin and its major active constituent, silibinin, studied in a variety of *in vitro* and *in vivo* cancer models, including liver cancer, suggest that they should be established in therapies as adjuncts in the clinical application in these patients to prevent or reduce chemotherapy as well as radiotherapy-induced toxicity. The molecular mechanisms of silibinin-mediated antiproliferative effects are mainly via receptor tyrosine kinases, androgen receptor, NF-κB, cell cycle regulatory and apoptotic signalling pathways in various cancer cells (Li et al. 2010). Additional studies are necessary to evaluate chemotherapeutic effects of silymarin.

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Table 1: Main constituents of Milk Thistle.

THE FRUITS	**silybin**
	silychristin
	silydianin
	3-deoxyderivatives of silychristin and silydianin (silymonin)
	neosilyhermin A
	neosilyhermin B
	2,3-dehydrosilybin
	taxifolin
	quercetin
	dihydrokaempferol
	kaempferol
	apigenin
	naringin
	eriodyctiol
	chrysoeriol
	5,7-dihydroxy chromone
	dehydroconiferyl alcohol
	silyhermin
	20-30% fixed oil (~60% linoleic acid; ~30% oleic acid; ~9% plamatic acid
	0.038% tocopherol
	0.63% sterols (cholesterol, campesterol, stigmasterol and sitosterol)
	25-30% protein, some mucilage
	flavonoids (apigenin and its 7-O- glucoside, 7-O- glucuronide; 4,7diglucoside, kaempferol and its 7-glucoside and 3-sulphate)
THE HERB	**luteolin and its 7-glucoside**
	sitosterol and its glucoside
	triterpene acetate
	fumaric acid
	polyacetylenes

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Figure 1: Distribution of Milk thistle in European countries (in black).



Figure 2: Chemical formulas of Silybin, Silydianin, Isosilibyn and Silychristin.







