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Silybum marianum (L.) Gaertn. HERBAL DRUG IN CLINICAL PRACTICE: A REVIEW

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Abstract: *Silybum marianum* is a medicinal plant. It is very important for the cancer treatment and other diseases. *It is mention in our ancient text. Silymarin is the different solvent extract of Silybum marianum, or milk thistle, and its major medicinal active compound is silybin, which has a mention biological effect. It is used in different liver diseases, particularly chronic liver diseases, cirrhosis and liver cancer, because of its antioxidant, anti-inflammatory and antifibrotic power. Indeed, the anti-oxidant and anti-inflammatory effect of silymarin is oriented towards the reduction of virus-related liver damages through inflammatory cascade softening and immune system modulation. It also has a direct antiviral effect associated with its intravenous administration in hepatitis C virus infection. With respect to alcohol abuse, silymarin is able to increase cellular vitality and to reduce both lipid peroxidation and cellular necrosis. Furthermore, silymarin/silybin use has important biological effects in non-alcoholic fatty liver disease.*
It is commonly known as St Mary's Thistle and botanical name is Silybum marianum belong to family Asteraceae.
Keyword: *Silybum marianum, Milk thistle, silybin, Liver disorder, inflammatory, antifibrotic and antioxidant.*

Introduction: St Mary's Thistle is very important medicinal plant. Botanical name is Silybum marianum belong to family Asteraceae. It is commonly known as milk thistle, it has many active compounds like flavonoids, silymarin, and silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin are generally used interchangeably; however, each of these compounds has specific characteristics and actions, with an intrinsic beneficial or toxic effect. In the last 10 years, about many papers has been published on these substances, used as antioxidants or chemo-preventives and anticancer agents, and especially as hepatoprotectants. This publication volume indicates that scientific interest in these molecules, or classes of molecules, is high worldwide. In the US and Europe, about 65% of patients with liver disease take herbal preparations; in Europe, the cost of the use of silymarin reaches $180 million in Germany alone. Despite the wealth of literature, no firm clinical evidence exists to recommend the use of these substances in clinical practice [1-10]. This discrepancy is attributable to various factors, such as quality of clinical trials, heterogeneity of diagnoses, lack of standardized preparations, and frequently inconsistent dosing and outcome parameters. At a time when the use of herbal products is increasing, whether driven by individual choice or industry promotion, in our opinion it is necessary to focus more intently on these compounds that may have beneficial, placebo, or toxic effects.

Botanic Names

Carduus marianus,[11,12] Silybum marianum. [11]

Common Names

St Mary's Thistle, [11,12] Fructus Silybi Mariae, Milk Thistle.[11]

Botanical Description: Silybum marianum is indigenous to North Africa, South America, Australia, China and Central Europe. It is either an annual or biennial, growing up to 150 cm high. Other botanical features include: 1. Small

(6-7 mm long) one-seeded fruit, 6-8 hard dry skins, white silky pappus (15-20 mm diameter). At the apex Green, spiny, 20-150 cm stem with a single large flower. Leaves: alternate, cauline and basal, no petiole, large (25-50 cm long), broad (12-25 cm wide), Glossy green, variegated with white veins, glabrous Vibrant red purple tubular hermaphrodite florets, large flower heads (2.5-4.0 cm diameter), thorny bracts.

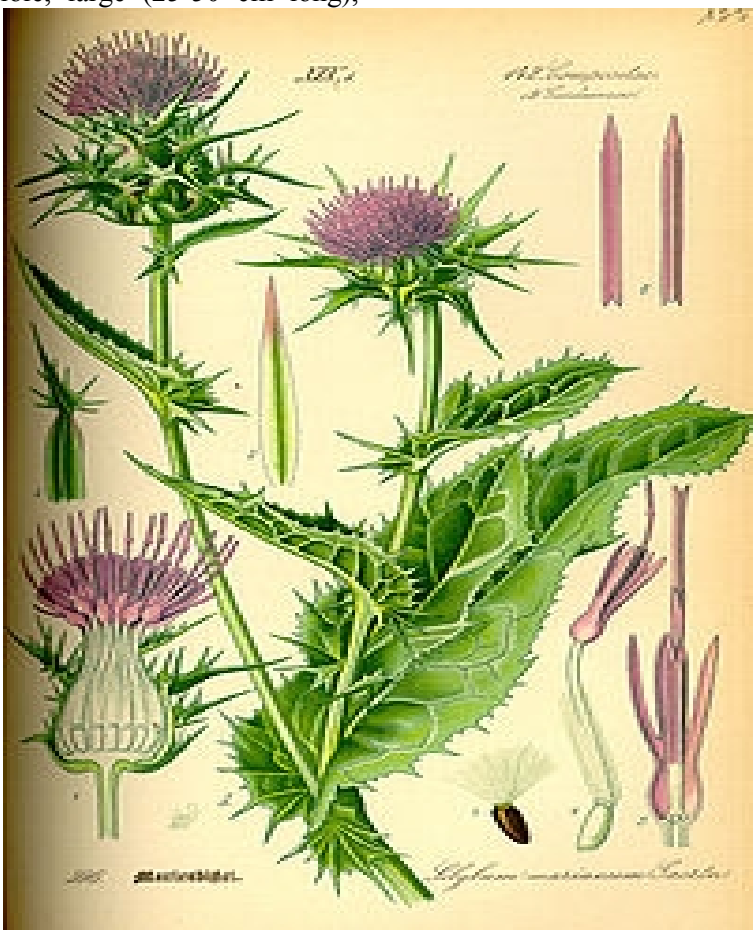


Figure: Plant of Silybum marianum

Figure: Flower and seeds of Silybum marianum

Parts Used: In modern herbal medicine, the active ingredients of Silybum marianum are extracted from the fruit, [13] sometimes called seed [14] but is technically an achene [11]. Traditionally, the whole Silybum marianum plant was used as food-leaves; young shoots, flower heads and stem were baked or boiled. Powdered dried leaves were used as tea. It was used by herbalists dating back to Dioscorides who used a decoction of seeds for snake bites. Herbalist John Evelyn mentioned the seeds as a galactagogue; English herbalist John Gerard mentioned roots

for treating melancholy; and Culpepper recommended infusing fresh roots and seeds for jaundice and oedema (dropsy), and boiling young plants as blood cleansers.[12,15]

Relevant Constituents: The main constituents of Silybum marianum are:[11,13]

- Flavonolignans: 1.5-3% silymarin comprising mainly of:
- 50% silybin or silibinin
- Silychristin
- Silydianin
- 2, 3 –dehydrosilybin
- 2, 3 –dehydrosilychristin Flavonoids:
- 2, 3 –dihydroflavonol
- Quercetin, taxifolin, dehydrokaempferol
- Lipids: 20% - 30%. Linoleic acid, oleic acid, palmitic acid
- Sterols: Cholesterol, campesterol, stigmasterol
- Other Constituents: Mucilages, sugars, amines, saponins

Historical Information: The use of Silybum marianum for ailments relating to the liver, spleen and gall bladder dates back some 2,000 years to Pliny the Elder, and as far back as Dioscorides.[16] Tales behind the white mottling appearance of the leaves of St Mary's thistle, Blessed thistle, and Holy thistle spoke of a drop of Virgin Mary's breast milk falling onto the leaves whilst feeding baby Jesus.[12] Culpeper thought such naming was the sacrilegious deed of 'some that had little holiness'.[17] According to herbal astrology, Silybum marianum is associated with Mars. Its thorns act as barriers, signifying its protective ability. The thorny appearance depicts the combative spirit of Mars-removing body odours (as a deodorant or mouth wash) and the stench of urine (as a diuretic), and regrowing fallen hair (symbolic of a fighting spirit, rising up after having fallen). The fiery red flowers symbolise the power to cleanse blood. Mars, the medieval ruler of choler, who secretes phlegm in anger and irritability, bestows upon Silybum marianum a remedial treatment for jaundice and other liver disorders. [18] Silybum marianum has the energetics of a dry temperament, capable of removing obstructions through sweating and cleansing of the pores. It is a remedy for vertigo, deafness, stomach cramps, brain fog, intestinal worms and inflammation of the liver. Being a hot temperament herb, it is great for 'hot swellings' caused by ulcers, snake bites and being bitten by mad dogs. [17] Holmes described Silybum marianum as pungent, bitter, warm, dry, stimulating, decongesting, astringent, restoring, dissolving, and softening. In contrast, the liver is associated with cold energetics and the tendency to cause stagnancy due to Yang deficiency, indigestion, nausea, headache, jaundice, constipation, and chilliness. Such ailments are remedied by the warm, stimulating energetics of Silybum marianum which restore the integrity of the liver by promoting bile flow, bowel movement, reducing liver congestion, and stopping free radical formation. [19]

Medicinal Actions: The main contemporary medicinal actions of Silybum marianum include: [20]

- Hepatoprotective
- Hepatorestorative
- Toxin blockade
- Chelates iron
- Antioxidant
- Anti-inflammatory
- Antifibrotic

Medicinal Indications: The main contemporary medicinal indications include: [20]

- Toxic liver damage
- Chronic liver diseases
- Alcoholic liver disease
- Acute viral hepatitis
- Hepatitis C infection
- Chemotherapy support
- Hypercholesterolemia

Milk thistle (*Silybum marianum* L. Gaert., Asteraceae) seeds have been used for centuries as a herbal medicine, mainly for the treatment of liver diseases. The common name, milk thistle, is derived from the milky-white veins on the leaves, which, when broken open, yield a milky sap. The active constituents of milk thistle seeds are three isomeric flavonolignans, namely silibinin (silybin), silychristin, and silidianin, collectively known as silymarin, which is extracted from the dried milk thistle seeds. Silibinin is the most biologically active. The seeds also contain other flavonolignans, betaine, apigenin, silybonol, proteins, fixed oil, and free fatty acids, which may contribute to the health-giving effects of milk thistle seeds [21, 22]. Silymarin is the extract of Silybum marianum, or milk thistle, and consists of seven flavonoglignans (silibinin, isosilibinin, silychristin, isosilychristin and silydianin) and a flavonoid (taxifolin) [23]. Molecules 2017, 22, 191 2 of 16 among these substances, silybin is mainly prevalent and has the most important biological effect. It makes up about 70% of the total

composition of silymarin in the form of two diastereoisomeric compounds: silybin A and silybin B [24,25]. With respect to pharmacokinetics, silymarin is a low bioavailability compound if administered, with a lack in solubility in water. This is due to both its inefficient absorption in the intestine and an elevated metabolism of the first liver passage after its absorption; two mechanisms that decrease haematic concentration and consequently the arrival at the target organ [26–28]. However, this limitation has been efficaciously surpassed by the introduction of complexing with phosphatidylcholine that has a better absorption, and new silibinin glyco-conjugates (gluco, manno, galacto, and lacto-conjugates), which have both a high solubility in water and a strong antioxidant power [29,30]. The elevated absorption of these compounds has led to assessing the safety of silymarin in its therapeutic use. Its high tolerability was demonstrated by toxicity studies on animals treated with silymarin for a long time, whereas other studies on humans highlighted, among the most common side effects, its prolonged and high dosage use, headaches and itching [31,32]. No deaths or life-threatening adverse events have been reported [32]. Even if silymarin is a well-tolerated molecule, it is necessary to point out the few cases of scientific evidence in literature that demonstrate potentially harmful effects: in a phase I clinical trial, the use of 13 g per day of silybin in patients affected by prostate cancer was correlated to hyperbilirubinemia and alanine aminotransferase (ALT) increase. Moreover, it should be taken into consideration the possible side effects derived from the influence both on estrogen signaling, a potentially usable function even for therapeutic purposes, and on the aryl hydrocarbon receptor [33,34].

Scientific evidence, achieved so far, allows us to understand the mechanisms of action through which silybin carries out its activity by interacting with various tissues. In this regard, the action of silybin manifests in the modulation of inflammation and apoptosis, which, together with its antioxidant power, represent the key points that led to using it in different pathologies [35–37]. Silybin acts through the turning-off of pro-inflammatory signals, derived from nuclear factor-κB (NFκB) activation, involved in the induction of the synthesis of cytokines such as tumor necrosis factorα (TNF-α), interleukin (IL)-1, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF) [35,36]. Furthermore, silybin induces apoptosis through the modulation of cytoplasmatic levels of bcl-2-like protein 4 (Bax) and B-cell lymphoma 2 (Bcl-2) proteins, cytochrome c release and caspase3 and 9 activation [37]. The anti-oxidant activity is due to its capacity to act as both free radical scavenging and lipid peroxidation inhibitors, as demonstrated in vitro and in vivo [38–44].

Silymarin is also a modulator of estrogen signaling [45], insulin sensitizer [44,46–48], regulator of intracellular transport of drugs [49–52], anticarcinogen [45,53–59], antidiabetic through signal regulation of peroxisome proliferator-activated receptor γ (PPAR-γ) [60], antifibrotic [61–65] and choleretic [28].

The great number of actions carried out by silymarin explains the reason why a lot of scientific studies have been performed in order to understand its efficacy in various pathologies [66]. In rheumatic diseases, such as rheumatoid arthritis, silymarin acts as an anti-inflammatory by inhibiting migration and activation of neutrophil in the articulations [67]. In different oncological diseases, such as prostate cancer, cervical cancer, hepatocellular carcinoma (HCC), bladder cancer and lung cancer, silymarin reduces cell vitality and runaway cell replication [68–72]. Because of its detoxifying power, its hydrosoluble endovenous formulation, it is used as an anti-hepatotoxic drug in poisonings due to acetaminophen, arsenic, carbon tetrachloride, butyrophenones, phenothiazines and Amanita phalloides toxins [73–76]. In hypercholesterolemia, silymarin/silybin inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, reducing cholesterol synthesis [77]. Lastly, in neurological and psychiatric diseases, this molecule acts through the turning-off of inflammatory signals, which underlies the degeneration of dopaminergic neurons in Parkinson's disease, and it improves the clinical picture ascribable to obsessive-compulsive disorder [78,79]. Of note, the role of herbal products in chronic liver disease, which currently represents one of the most important health problems in about 10% of the world population, is the most studied topic in the scientific community [80]. Indeed, in chronic liver diseases, silymarin acts through different mechanisms and complex biological interactions able to produce benefits in various pathologies, some of which are systemic and can involve the liver.

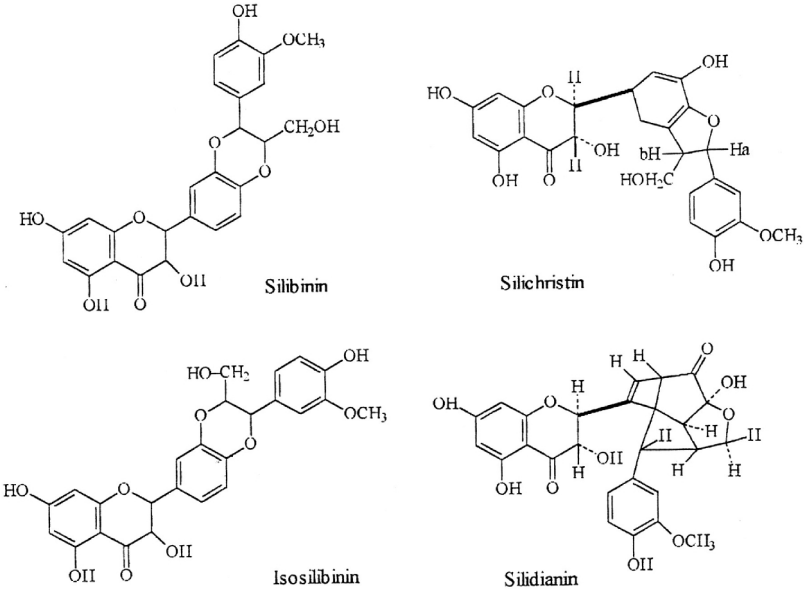
Definition and Characteristics of Silybin: As mentioned, silybin and silymarin are not synonymous [11,13]. Silymarin is a complex of at

least seven flavonolignans that are the most common class of compounds present in milk thistle extract, and one flavonoid, taxifolin. The relative abundance of each compound may vary depending on the source of botanical material, supplier, and extraction processes. Silybin represents about 50% to 70% of the silymarin extract. Silybin can be resolved into two 1:1 diastereoisomers, silybin A and silybin B. In addition, silybin may be present as isosilybin, a 1:1 mixture of two diastereoisomeric compounds, isosilybin A and isosilybin B[81-87]. The concentrations of silybin in the main pharmaceutical products containing silymarin present in the US and other countries range from 20% to 40% [86].

Pharmacokinetics and Pharmacodynamic Aspects: Flavonolignans are known for their poor and erratic bioavailability; for example, silymarin absorption rate levels vary between 20% and 50%. Silybin has been separated commercially as a pure substance [81-86], and the study of silybin pharmacokinetics properties using an HPLC method has shown that the concentration-response relationship is linear over a concentration range of 0.5-100 µg/mL [86]. After administration to rats, the disposition of silybin in the plasma and bile fluid is due to rapid distribution and equilibrium between the blood and hepatobiliary system, and the bile levels of unconjugated and total silybin are greater than those in plasma [88-92].

Similarly to other flavolignans, limiting factors for the use of silybin are its low solubility in water, low bioavailability, and poor intestinal absorption. To counteract this aspect, different more soluble derivatives of silybin have been synthesized, such as silybin bis-hemisuccinate, β-cyclodextrin complex, silybin-N-methyl-glucamine, silybin 11-O-phosphate, and silybin-phosphatidylcholine. Another strategy for improving silybin solubility is represented by the enzymatic synthesis of its β-glycosides, such as silybin β-galactoside, silybin β-glucoside, silybin β-maltoside, and silybin β-lactoside. A soluble silybin prodrug has been finally synthesized with a high aqueous soluble polymeric carrier (polyethylene glycol) [93-100].

Chemistry of silymarin: Silymarin is extracted from the dried seeds of milk thistle plant, where it is present in higher concentrations than in other parts of the plant [101]. The active principle was first isolated and chemically characterized during 1968-1974 [102]. Later the biochemical effects of silymarin on RNA, protein and DNA synthesis was reported by Sonnenbichler and Zetl [103]. Silymarin is a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin with an empirical formula $C_{25}H_{22}O_{10}$. The structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory actions. Among the isomers silybin is the major and most active component and represents about 60-70 per cent, followed by silychristin (20%), silydianin (10%), and isosilybin (5%) [104]. Silipide (IdB 1016) is the silybin - phosphatidylcholine complex which ensures a large increase in the bioavailability of silybin [105].



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Antioxidant Properties: Free radicals, including the superoxide radical, hydroxyl radical (OH), hydrogen peroxide (H_2O_2), and lipid peroxide radicals have been implicated in liver diseases [106]. These reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to xenobiotics [107]. The mechanism of free radical damage include ROS-induced peroxidation of polyunsaturated fatty acid in the cell membrane bilayer, which causes a chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane lipids and proteins. Subsequently cell contents including DNA, RNA, and other cellular components are damaged [108]. The cytoprotective effects of silymarin are mainly attributable to its antioxidant and free radical scavenging properties. Silymarin can also interact directly with cell membrane components to prevent any abnormalities in the content of lipid fraction responsible for maintaining normal fluidity [109].

Stimulation of Protein Synthesis: Silymarin can enter inside the nucleus and act on RNA polymerase enzymes resulting in increased ribosomal formation. This in turn hastens protein and DNA synthesis [103]. This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver.

Anti-inflammatory Actions: The inhibitory effect on 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis is a pivotal pharmacological property of silymarin. Leukotriene (B_4) synthesis was reduced while prostaglandin (E_2) synthesis was not affected at higher concentrations of use of silibinin [104].

Viral Hepatitis: In Present time, even if a change in the etiology of chronic liver diseases is occurring, different strains of viral hepatitis still represent an important cause of chronic liver damage [110]. The anti-oxidant and anti-inflammatory action of silymarin allows us to understand easily its potentially healthy activity oriented towards the reduction of virus-related liver damage through the softening of inflammatory cascade and immune system modulation [111]. However, the relationship between chronic viral hepatitis and silymarin cannot be limited to this simple approximation. From the analysis of literature, it is possible to deduce the poor quality and lack of studies that analyse the interaction between silymarin and hepatitis B virus (HBV) infection. A meta-analysis performed by Wei et al. evaluated the efficacy and safety of silymarin and its therapeutic combination with antivirals (lamivudine and interferon) in the treatment of HBV chronic hepatitis [112]. The research highlighted that, from the analysed studies, it was possible to deduce a similar efficacy of silymarin and antiviral agents in normalizing aspartate aminotransferase (AST) and ALT levels, as well as an equivalent negative conversion rate of serum HBsAg (Relative Risk (RR) = 1.50; 95% Confidence Interval (CI) = 0.18–12.35) and HBeAg (RR = 1.80; 95% CI = 0.43–7.60). Furthermore, they highlighted that silymarin, associated with the use of antivirals, was able to promote a major effect on serum level reduction of transaminases compared to the use of antivirals alone [112]. Nevertheless, the same authors stated that there was no remarkable data in literature for suggesting the use of silymarin associated with antiviral therapy in the treatment of HBV chronic infection, probably due to various criticism in the construction of analysed trials [112]. Similar outcomes were obtained by other researchers, who highlighted the role of silymarin in inducing a reduction of transaminase levels during viral hepatitis. However, with respect to the histology or serum viral content, there were no direct effects due to its use [67]. Virus C chronic hepatitis (HCV) represents the most frequent cause of viral chronic hepathopathy worldwide, especially after the introduction of HBV vaccination in the 1980s [113].

Although, in clinical practice, most of the patients affected by HCV, who undergo or do not undergo antiviral treatment, use herbal products such as silymarin, its use cannot be recommended because it is not supported by significant scientific evidence [114]. As highlighted by analysis of scientific literature, even for the role of silymarin in determining the block of both entry and fusion HCV and viral replication [115–120], in a meta-analysis of Yang et al., a healthy effect on HCV-RNA serum level has been demonstrated (although not statistically significant). This effect was proved only when silybin was administered both per os and through high-dose intravenous injection [114]. Intravenous administration of silybin is able to inhibit viral replication by intervening directly in the HCV

lifecycle. Indeed, it is able to inhibit HCV RNA-dependent RNA polymerase function independently from intracellular interferon (IFN)-induced antiviral pathways [119]. Silymarin is unable to block HCV binding to cells; however, it blocks both HCV entry and fusion of HCV with liposomes [117]. Furthermore, silymarin, but not silybin, inhibits JFH-1 genotype 2a NS5B-dependent RNA polymerase activity, microsomal triglyceride transfer protein activity, apolipoprotein B secretion, and, therefore, the leakage of infectious virion from the cell [117]. The effects on inhibition of viral replication carried out by intravenous administration were also analysed by Ferenci et al., who demonstrated how silybin, by blocking HCV polymerase function at a half maximal inhibitory concentration (IC50) between 75 μM and 100 μM, is able to reduce HCV viral loads from three to four logs within one/four weeks in previous peginterferon nonresponder patients [121]. This fact is confirmed by a case report in which a potential antiviral direct effect carried out by a combined treatment of 238 days with 1200 mg/day of endovenous silybin, 1200 mg/day of ribavirin and 6000 U/day of vitamin D has been highlighted. This therapeutic approach has been demonstrated to be very tolerable, and it allowed the achievement of sustained virologic response (SVR) in a 44 year-old female HCV genotype-1 infected patient with a previous therapeutic failure based on interferon and ribavirin [122]. Moreover, the endovenous administration of silybin is able to reduce the viral load of patients affected by genotype 3 HCV, opening the doors to a possible therapeutic combination with the latest direct-acting antivirals (DAA) therapies, in light of the most recent viral eradication data for the different HCV genotypes, towards difficult-to-treat genotypes [123–125].

Some years ago, before new therapies based on DAAs, the endovenous treatment with silybin was also studied as possible adjuvant, lead-in therapy for 14 days (20 mg/kg/day) followed by a triple therapy with peginterferon-ribavirin and telaprevir for 12 weeks, in order to obtain SVR in difficultto-treat patients: HCV/human immunodeficiency virus (HIV) co-infected patients or with advanced fibrosis, in whom a viral eradication rate of 63% was found, which is higher than deriving data, for the same type of patients, from CUPIC (Compassionate Use of Protease Inhibitors in viral C Cirrhosis) and REALIZE (A Safety and Effectiveness Study of Telaprevir in Chronic, Genotype 1, Hepatitis C Patients That Failed Previous Standard Treatment) (20% and 50%, respectively) studies [126–128]. This interesting data has to be re-assessed in light of the latest therapies for the eradication of HCV infection, in which most DDA therapeutic regimens reach rates of SVR higher than 90% and an onset of side effects lesser than previous therapies based on interferon and ribavirin. Consequently, in clinical practice, the implementation of a daily endovenous therapy based on silymarin is not applicable.

Alcoholic Liver Disease: The excessive ethanol consumption represents one of the most widespread causes of chronic hepatopathy worldwide, with a prevalence that varies depending on the geographical area considered. Sometimes, alcohol abuse can associate itself to other causes of liver damage, including HCV, causing a coexistence of harmful stimuli for the liver, which is able to hugely accelerate the progression of the pathology to more advanced forms, as well as cause acute liver failure in patients with HCV-related chronic hepatopathy [129]. Alcohol liver damage is mainly linked to the alteration of the oxidoreductive potential of cells due to ethanol metabolism. Indeed, the activity of alcohol dehydrogenase at first, and of aldehyde dehydrogenase later, causes a reduction in NAD+/NADH ratio, which underlies the process that causes a reduced mitochondrial capacity to metabolize lipids. The high quantity of lipids, together with the elevated intracellular oxidative stress, due to activation of secondary metabolic pathways for ethanol, such as microsomal ethanol oxidizing system (MEOS), leads to lipid lypoperoxidation, responsible for the loss of cellular and mitochondrial membrane function, with consequent cellular death [129,130].

The protective effect derived from silybin-phosphatidylcholine complex (SilPho) towards oxidative stress was demonstrated in one of our studies: in vitro, the use of SilPho is able to increase cellular vitality, evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT), in conditions of oxidative stress induced by the incubation of HepG2 and MKN28 cells, with xanthine oxidase and its substratum called xanthine [43]. Moreover, we highlighted the effect derived from SilPho treatment, which reduced, in conditions of oxidative stress, both lipid peroxidation, evaluated by the measurement of a stress oxidative marker (Malondialdehyde), and

cellular necrosis [43]. A study on mice, by Song et al. demonstrated that silymarin (200 mg/kg) was able to reduce oxidative stress, due to the gavage of ethanol 5 g/kg body weight every 12 h for a total of three doses, as well as prevent ALT increase, Glutathione (GSH) decrease, lipid peroxidation and TNF-α increase [132]. However, the lack of a pharmacokinetics assessment of silymarin administered per os represents a limitation in this study, given the low bioavailability of the compound, a condition that generates some doubts on the real role of silymarin regarding the results obtained in the aforementioned study. Similarly, in another study on mice, it was shown how administration of 250 mg/kg per os of silybin is able to antagonize the increase in thiobarbituric acid reactive substance (TBARS), GSH reduction and the decrease of the content and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx), which are effects linked to ethanol exposure [133].

The key point for the comprehension of pathogenesis of ethanol-induced damage is the triggering of mitochondrial dysfunction caused by both lipid peroxidation and direct toxic effect due to intracellular accumulation of acetaldehyde. Indeed, in mitochondria, the main cellular metabolic reactions occur, many of which are potentially able to produce reactive oxygen species (ROS), especially in conditions of mitochondrial dysfunction, leading to closure of a vicious circle able to cause cellular death [134, 135]. The use of silymarin and SilPho is able to optimize mitochondrial metabolic processes and the chain of electronic transport, to increase intracellular SOD activity, and to reduce monoamine oxidase (MAO) activity, definitively leading to the reduction of intracellular ROS levels for the improvement of mitochondrial functionality [136–139]. Therefore, the essential therapy in hepatopathy caused by ethanol abuse, is the abstinence from alcohol supported by pharmacological therapy, psychological support and counseling. However, from the pathogenic point of view, in the antagonism of alcohol-related liver damage, silymarin could represent a useful support therapy for the improvement of liver metabolic processes en route to breaking drinking habits, but further studies are necessary.

Non-Alcoholic Fatty Liver Disease Non-alcoholic fatty liver disease (NAFLD) is a potentially evolutive pathology that causes fat accumulation in hepatocytes without other pathological conditions able to generate it, such as viral hepatitis, alcohol consumption, and chronic drug use [140]. With respect to the epidemiology, in the last few years, the incidence of NAFLD has shown an exponential increase in Western countries; on the contrary, a reduction of viral hepatitis has been demonstrated. Therefore, NAFLD will be the most frequent cause of chronic hepatopathy in the near future [140]. Nowadays, NAFLD represents both the second most frequent cause of HCC development and the second most frequent indication for liver transplants [141–144]. NAFLD pathogenesis involves both genetic and environmental factors, which promote the onset of insulin resistance, that play a key role in metabolic syndrome, a complex systemic condition [145–152]. Currently, the problem to be dealt with concerns the lack of specific therapeutic approaches able to antagonize the progression to severe forms or to intervene by breaking the complex network of pathogenetic events that cause its appearance [153,154]. This problem is even more relevant if we consider the elevated distribution of NAFLD in the pediatric population, in which the elevated life expectancy could lead to progression of the pathology from “simple” fat accumulation into the liver to inflammation, cirrhosis and HCC, which manifests itself in youth compared to HCC observed in viral hepatitis [155,156].

The scientific evidence efficaciously gathered in the latest European Association for the Study of the Liver (EASL)-European Association for the Study of Diabetes (EASD)-European Association for the Study of Obesity (EASO) Clinical Practice Guidelines, highlights an improvement of the histological picture and serum liver enzymes derived from a weight loss of about 7%–10% (B1 evidence level). The weight loss is obtained by both healthy diet, specifically the Mediterranean diet without consumption of processed food, without food and beverages high in added fructose, and by regulation of macronutrient composition (B1 evidence level), in addition to an aerobic and resistance exercise (B2 evidence level) [157,158]. Furthermore, the analysis of potentially usable pharmacological approaches has currently generated controversies and doubts about the real hepatic health effect, as well as about the tolerability due to a long-term use [157]. In this context, different studies have attempted to correlate silymarin/silybin use to the biological effects able to antagonize NAFLD progression, by intervening in various therapeutic targets.

Silybin could be an insulin sensitizer: it is able to reduce intrahepatic fat accumulation, lobular inflammation, ballooning and serum fat, as well as to improve homeostasis model assessment-IR index (HOMA-IR) and insulin tolerance test (ITT) [46]. Moreover, silybin has an important role in reducing visceral fat accumulation, in inducing lipolysis through the transcription of the adipose triglyceride lipase (ATGL) gene and inhibiting gluconeogenesis for silencing of some genes involved in the aforementioned metabolic pathway [46]. Nevertheless, in this work, the timing for the administration of pure silybin is not clear, that is without molecules that increase its oral bioavailability, and high-fat diet (HFD) in the group of rats fed with HFD+silybin. Therefore, taking into account the low bioavailability of pure silybin, if administered per os [159], it cannot exclude that the outcome observed in this study could depend on a reduction of absorption of fat contained in HFD mediated by the formation of non absorbable complexes with silybin, rather than depending on its real role in interrupting the pathogenetic mechanisms that are responsible for NAFLD.

The effect of insulin in determining its biological effects is correlated to the activation of pathway insulin receptor substrate 1 (IRS-1)-phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt) able to generate the activation of substrates such as Akt substrate 160 and consequently the expression of glucose transporter type 4 (GLUT4) on the cell surface [160]. The increase of phosphorylation in IRS-1 and PI3K serine, as happens in insulin-resistance, is mediated by the c-Jun N-terminal kinase (JNK) and nuclear factor-κB kinase β (IKK-β) activation, as happens in experimental models of insulin-resistance induced by HFD or palmitic acid [47,161,162]. The treatment with increasing doses of silybin (16, 40 and 100 µg/mL), in vitro, is able to generate an increase in glucose captation, induced by insulin in a model of palmitate-induced insulin-resistance on myoblast C2C12 cells, in which the role of silybin is crucial in the increase of PI3K activity [47].

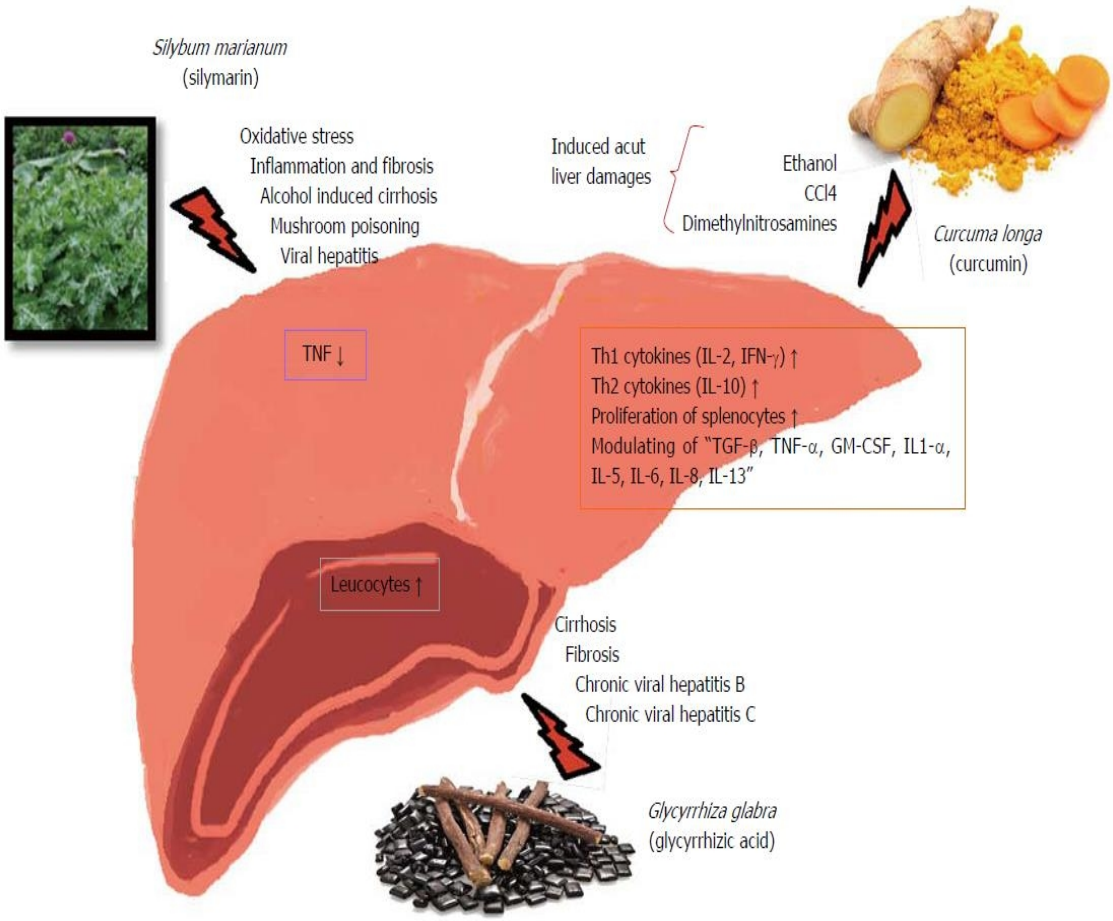

Figure: Liver immunology and herbal treatment

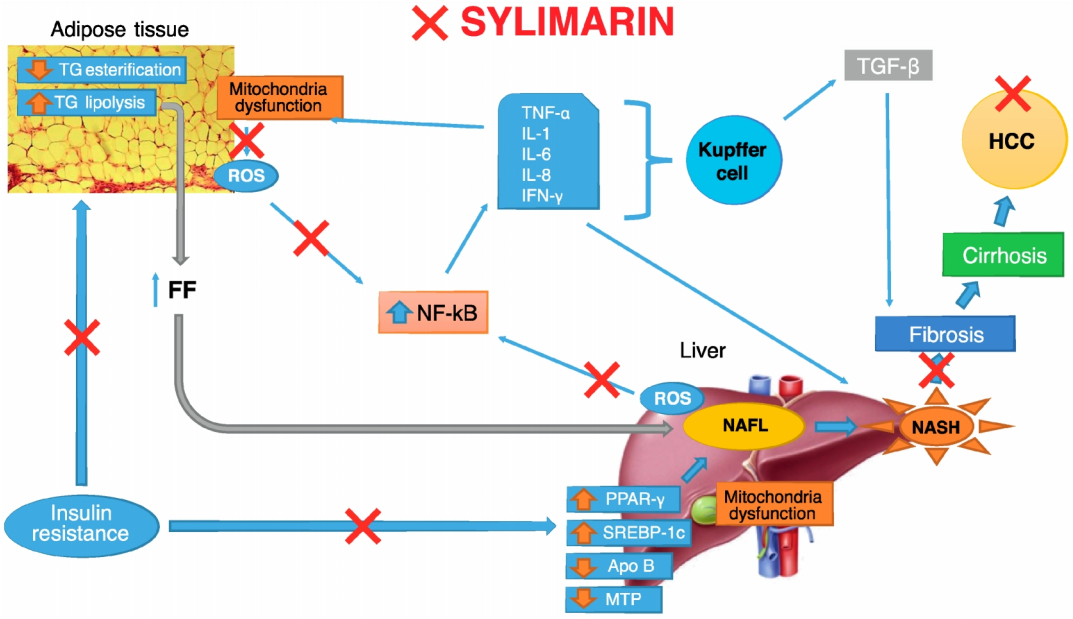

Figure: Therapeutic targets of silymarin in non-alcoholic fatty liver disease.

Conclusion: In this study we found that Silybum marianum that is very useful palnt for the cure of different diseases like cancer, liver disorder, inflammation, immune disorders etc. In this plant chemical compounds of pharmacokinetics and pharmacodynamics on silymarin have improved, in the last few years. For the study of different research papers, it has been demonstrated that silymarin has an effect that allows its use in all of the most frequent causes of liver damage. Indeed, silymarin has three important activities: anti-inflammatory, antioxidant and proapoptotic, which represent the "functional triad" that allows for antagonizing the onset and the progression of mechanisms of damage which are responsible for the progression of hepatitis to cirrhosis and Hepatocellular carcinoma. The treatment with silymarin/silybin in routine clinical practice is strongly limited, since it is necessary to obtain scientific data deriving from well-structured trials based on large populations of patients, and to achieve a standardization of methods used for evaluating the therapeutic efficacy, especially in an NAFLD context, that is particularly promising. Silymarin is a chemical compound which also used in liver cirrhosis and hepatocellular carcinoma that represent common end stages of different hepatopathies by modulating different molecular patterns.

References

1. Simánek, V., Kren, V., Ulrichová, J., Vicar, J., Cvak, L. (2000). Silymarin: What is in the name...? An appeal for a change of editorial policy. *Hepatology*. 32:442–444.
2. Saller, R., Meier, R., Brignoli, R. (2001). The use of silymarin in the treatment of liver diseases. *Drugs*. 61:2035–2063.
3. Gazák, R., Walterová, D., Kren, V. (2007). Silybin and silymarin--new and emerging applications in medicine. *Curr Med Chem.*, 14:315–338.
4. Rambaldi, A., Jacobs, B.P., Iaquinto, G., Gluud, C. (2005). Milk thistle for alcoholic and/or hepatitis B or C liver diseases--a systematic cochrane hepato-biliary group review with meta-analyses of randomized clinical trials. *Am J Gastroenterol.*, 100:2583–2591.
5. Seeff, L.B. (2009). Are herbals as safe as their advocates believe? *J Hepatol.*, 50:13–16.
6. Gazák, R., Walterová, D., Kren, V. (2007). Silybin and silymarin--new and emerging applications in medicine. *Curr Med Chem.*,14:315–338.
7. Tamayo, C., Diamond, S. (2007). Review of clinical trials evaluating safety and efficacy of milk thistle (Silybum marianum [L.] Gaertn.) *Integr Cancer Ther.*, 6:146–157.
8. Stickel, F., Schuppan, D. (2007). Herbal medicine in the treatment of liver diseases. *Dig Liver Dis.*, 39:293–304.
9. Fehér, J., Lengyel, G. (2008). Silymarin in the treatment of chronic liver diseases: past and future. *Orv Hetil.*, 49:2413–2418.
10. Tindle, H.A., Davis, R.B., Phillips, R.S., Eisenberg, D.M. (2005). Trends in use of complementary and alternative medicine by US adults: 1997-2002. *Altern Ther Health Med.*, 11:42–49.
11. World Health Organisation. (2004). Monographs on selected medicinal plants [Internet]. Vol 2, p. 300-15. Available from: http://apps.who.int/medicinedocs/es/d/Js4927e/29.html#Js4927e.29

12. Silybum marianum. (1931). In: Grieve M. A modern herbal. Vol 2, I-Z and indexes. New York: Harcourt, Brace & Company; 1971, p. 796-7.
13. Barnes, J., Anderson, L.A., Phillipson, J.D. (2011). Milk thistle. In: Herbal medicines. 3rd ed. London: Pharmaceutical Press; p. 429-35.
14. Bone, K. (2003). Milk Thistle. In: A clinical guide to blending liquid herbs: Herbal formulations for the individual patient. St. Louis, Missouri: Churchill Livingstone. p. 326-8.
15. Culpeper, N. (2013). The complete herbal [Internet]. London: Thomas Kelly; 1843. Carduus benedictus, p. 41. Available from: http://babel.hathitrust.org/cgi/pt?id=wu.89051196020
16. Post-White, J., Ladas, E.J., Kelly, K.M. (2007). Advances in the use of milk thistle (Silybum marianum). *Integr Cancer Ther.*, 6(2):104-209.
17. Culpeper, N. (1826). Carduus Benedictus. In: Culpeper's complete herbal, and English physician. Barcelona (Spain): Industria grafica sa; 1981. p. 33.
18. Culpeper, N. (2013). The complete herbal [Internet]; London: Thomas Kelly; 1843. *Thistles*, p. 179-80.
19. Holmes, P. (1993). Milk thistle seed. In: The energetics of western herbs: A material medica integrating western and oriental herbal medicine traditions. 2nd ed. Vol 1. Berkley (USA): NatTrop Publishing, p. 195-7.
20. Braun, L., Cohen, M. (2011). St Mary's thistle. In: Herbs & natural supplements: An evidence-based guide. 3rd ed. Sydney: Elsevier, p. 824-36.
21. DerMarderosian, A. (2001). The Reviews of natural products. 1st ed. Facts and Comparisons: St. Louis, Missouri.
22. Trease, G.E., Evans, W.C. (2002). Pharmacognosy. 15th Edn. Saunders, pp. 214-393.
23. Kim, N.C., Graf, T.N., Sparacino, C.M., Wani, M.C., Wall, M.E. (2003). Complete isolation and characterization of silybins and isosilybins from milk thistle (Silybum marianum). *Org. Biomol. Chem.*, 1: 1684–1689.
24. Loguercio, C., Festi, D. (2011). Silybin and the liver: From basic research to clinical practice. *World J. Gastroenterol.*, 17: 2288–2301.
25. Crocenzi, F.A., Roma, M.G. (2006). Silymarin as a new hepatoprotective agent in experimental cholestasis: New possibilities for an ancient medication. *Curr. Med. Chem.*, 13: 1055–1074.
26. Saller, R., Meier, R., Brignoli, R. (2001). The use of silymarin in the treatment of liver diseases. *Drugs*, 61: 2035– 2063.
27. Hawke, R.L., Schrieber, S.J., Soule, T.A., Wen, Z., Smith, P.C., Reddy, K.R., Wahed, A.S., Belle, S.H., Afdhal, N.H., Navarro, V.J., et al. (2010). Silymarin ascending multiple oral dosing phase I study in noncirrhotic patients with chronic hepatitis C. *J. Clin. Pharmacol.*, 50: 434–449.
28. Saller, R., Melzer, J., Reichling, J., Brignoli, R., Meier, R. (2007). An updated systematic review of the pharmacology of silymarin. *Forsch. Komplementmed.*, 14: 70–80.
29. Barzaghi, N., Crema, F., Gatti, G., Pifferi, G., Perucca, E. (1990). Pharmacokinetic studies on IdB 1016, a silybinphosphatidylcholine complex, in healthy human subjects. *Eur. J. Drug Metab. Pharmacokinet.*, 15: 333–338.
30. Zarrelli, A., Romanucci, V., Tuccillo, C., Federico, A., Loguercio, C., Gravante, R., Di Fabio, G. (2014). New silibinin glyco-conjugates: Synthesis and evaluation of antioxidant properties. *Bioorg. Med. Chem. Lett.*, 24: 5147–5149.
31. Dunnick, J.K., Singh, B., Nyska, A., Peckham, J., Kissling, G.E., Sanders, J.M. (2011). Investigating the potential for toxicity from long-term use of the herbal products, goldenseal and milk thistle. *Toxicol. Pathol.*, 39: 398–409.
32. Saller, R., Brignoli, R., Melzer, J., Meier, R. (2008). An updated systematic review with meta-analysis for the clinical evidence of silymarin. *Forsch. Komplementmed.*, 15: 9–20.
33. Flaig, T.W., Gustafson, D.L., Su, L.J., Zirrolli, J.A., Crighton, F., Harrison, G.S., Pierson, A.S., Agarwal, R., Glode, L.M. (2007). A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. *Investig. New Drugs*, 25: 139–146.
34. Pliskova, M., Vondracek, J., Kren, V., Gazak, R., Sedmera, P., Walterova, D., Psotova, J., Simanek, V., Machala, M. (2005). Effects of silymarin flavonolignans and synthetic silybin derivatives on estrogen and aryl hydrocarbon receptor activation. *Toxicology*, 215: 80–89.
35. Saliou, C., Valacchi, G., Rimbach, G. (2001). Assessing bioflavonoids as regulators of NF-κB activity and inflammatory gene expression in mammalian cells. *Meth. Enzymol.*, 335: 380–386.
36. Polyak, S.J., Morishima, C., Lohmann, V., Pal, S., Lee, D.Y., Liu, Y., Graf, T.N., Oberlies, N.H. (2010). Identification of hepatoprotective flavonolignans from silymarin. *Proc. Natl. Acad. Sci.*, USA, 107: 5995–5999.
37. Yoo, H.G., Jung, S.N., Hwang, Y.S., Park, J.S., Kim, M.H., Jeong, M., Ahn, S.J., Ahn, B.W., Shin, B.A., Park, R.K., et al. (2004). Involvement of NF-κB and caspases in silibinin-induced apoptosis of endothelial cells. *Int. J. Mol. Med.*, 13: 81–86.
38. Flora, K., Hahn, M., Rosen, H., Benner, K. (1998). Milk thistle (Silybum marianum) for the therapy of liver disease. *Am. J. Gastroenterol.*, 93: 139–143.
39. Kiruthiga, P.V., Shafreen, R.B., Pandian, S.K., Arun, S., Govindu, S., Devi, K.P. (2007). Protective effect of silymarin on erythrocyte

haemolysate against benzo(a) pyrene and exogenous reactive oxygen species (H2O2) induced oxidative stress. *Chemosphere*, 68: 1511–1518.
40. Valenzuela, A., Guerra, R., Garrido, A. (1987). Silybin dihemisuccinate protects rat erythrocytes against phenylhydrazine-induced lipid peroxidation and hemolysis. *Planta Med.*, 53: 402–405.
41. Nencini, C., Giorgi, G., Micheli, L. (2007). Protective effect of silymarin on oxidative stress in rat brain. *Phytomedicine*, 14: 129–135.
42. Varzi, H.N., Esmailzadeh, S., Morovvati, H., Avizeh, R., Shahriari, A., Givi, M.E. (2007). Effect of silymarin and vitamin E on gentamicin-induced nephrotoxicity in dogs. *J. Vet. Pharmacol. Ther.*, 30: 477–481.
43. Federico, A., Dallio, M., Di Fabio, G., Zarrelli, A., Zappavigna, S., Stiuso, P., Tuccillo, C., Caraglia, M., Loguercio, C. (2015). Silybin-Phosphatidylcholine Complex Protects Human Gastric and Liver Cells from Oxidative Stress. *In Vivo*, 29: 569–575.
44. Loguercio, C., Federico, A., Trappoliere, M., Tuccillo, C., de Sio, I., Di Leva, A., Niosi, M., D'Auria, M.V., Capasso, R., Del Vecchio Blanco, C., et al. (2007). The effect of a silybin-vitamin E-phospholipid complex on nonalcoholic fatty liver disease: A pilot study. *Dig. Dis. Sci.*, 52: 2387–2395.
45. Tyagi, A., Agarwal, C., Agarwal, R. (2002). Inhibition of retinoblastoma protein (Rb) phosphorylation at serine sites and an increase in Rb-E2F complex formation by silibinin in androgen-dependent human prostate carcinoma LNCaP cells: Role in prostate cancer prevention. *Mol. Cancer Ther.*, 1: 525–532.
46. Yao, J., Zhi, M., Gao, X., Hu, P., Li, C., Yang, X. (2013). Effect and the probable mechanisms of silibinin in regulating insulin resistance in the liver of rats with non-alcoholic fatty liver. *Braz. J. Med. Biol. Res.*, 46: 270–277.
47. Li, H.B., Yang, Y.R., Mo, Z.J., Ding, Y., Jiang, W.J. (2015). Silibinin improves palmitate-induced insulin resistance in C2C12 myotubes by attenuating IRS-1/PI3K/Akt pathway inhibition. *Braz. J. Med. Biol. Res.*, 48: 440–446.
48. Federico, A., Trappoliere, M., Tuccillo, C., de Sio, I., Di Leva, A., Del Vecchio Blanco, C., Loguercio, C. (2006). A new silybin-vitamin E-phospholipid complex improves insulin resistance and liver damage in patients with non-alcoholic fatty liver disease: Preliminary observations. *Gut*, 55: 901–902.
49. Krena, V., Walterov, D. (2005). Silybin and silymarin—New effects and applications. *Biomed. Pap.*, 149: 29–41.
50. Zhang, S.H., Morris, M.E. (2003). Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on Pglycoprotein-mediated transport. *J. Pharmacol. Exp. Ther.*, 304: 1258–1267.
51. Zhang, S.Z., Morris, M.E. (2003). Effect of the flavonoids biochanin A and silymarin on the P-glycoproteinmediated transport of digoxin and vinblastine in human intestinal Caco-2 cells. *Pharm. Res.*, 20: 1184–1191.
52. Nguyen, H., Zhang, S.Z., Morris, M.E. (2002). Effect of flavonoids on MRP1-mediated transport in Panc-1 cells. *J. Pharm. Sci.*, 92: 250–257.
53. Sinnberg, T., Menzel, M., Kaesler, S., Biedermann, T., Sauer, B., Nahnsen, S., Schwarz, M., Garbe, C., Schittek, B. (2010) Suppression of casein kinase 1α in melanoma cells induces a switch in β-catenin signaling to promote metastasis. *Cancer Res.*, 70: 6999–7009.
54. Klaus, A., Birchmeier, W. (2008). Wnt signalling and its impact on development and cancer. *Nat. Rev. Cancer*, 8: 387–398.
55. Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G.H., Tan, Y., Zhang, Z., Lin, X., He, X. (2002). Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell*, 108: 837–847.
56. Hart, M., Concordet, J.P., Lassot, I., Albert, I., del los Santos, R., Durand, H., Perret, C., Rubinfeld, B., Margottin, F., Benarous, R., et al. (1999). The F-box protein β-TrCP associates with phosphorylated β-catenin and regulates its activity in the cell. *Curr. Biol.*, 9: 207–210.
57. Agarwal, R. (2000). Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem. *Pharmacol.*, 60: 1051–10591.
58. Wang, J.Y., Chang, C.C., Chiang, C.C., Chen, W.M., Hung, S.C. (2012). Silibinin suppresses the maintenance of colorectal cancer stem-like cells by inhibiting PP2A/AKT/mTOR pathways. *J. Cell. Biochem.*, 113: 1733–1743.
59. Singh, R.P., Agarwal, R. (2004). Prostate cancer prevention by silibinin. *Curr. Cancer Drug Targets*, 4: 1–11.
60. Pferschy-Wenzig, E.M., Atanasov, A.G., Malainer, C., Noha, S.M., Kunert, O., Schuster, D., Heiss, E.H., Oberlies, N.H., Wagner, H., Bauer, R., et al. (2014). Identification of Isosilybin A from milk thistle seeds as an agonist of peroxisome proliferator-activated receptor gamma. *J. Nat. Prod.*, 77: 842–847.
61. Trappoliere, M., Caligiuri, A., Schmid, M., Bertolani, C., Failli, P., Vizzutti, F., Novo, E., di Manzano, C., Marra, F., Loguercio, C., et al. (2009). Silybin, a component of sylimarin, exerts anti-inflammatory and antifibrogenic effects on human hepatic stellate cells. *J. Hepatol.*, 50: 1102–1111.
62. Favari, L., Perez-Alvarez, V. (1997). Comparative effects of colchicine and silymarin on CCl4-chronic liver damage in rats. *Arch. Med. Res.*, 28: 11–17.

63. Boigk, G., Stroedter, L., Herbst, H., Waldschmidt, J., Riecken, E.O., Schuppan, D. (1997). Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. *Hepatology*, 26: 643–649.
64. Jia, J.D., Bauer, M., Cho, J.J., Ruehl, M., Milani, S., Boigk, G., Riecken, E.O., Schuppan, D. (2001). Antifibrotic effect of silymarin in rat secondary biliary fibrosis is mediated by down-regulation of procollagen α1(I) and TIMP-1. *J. Hepatol.*, 35: 392–398.
65. Lieber, C.S., Leo, M.A., Cao, Q., Ren, C., De Carli, L.M. (2003). Silymarin retards the progression of alcohol-induced hepatic fibrosis in baboons. *J. Clin. Gastroenterol.*, 37: 336–339.
66. Neha; Jaggi, A.S., Singh, N. (2016). Silymarin and Its Role in Chronic Diseases. *Adv. Exp. Med. Biol.*, 929: 25–44.
67. Mayer, K.E., Myers, R.P., Lee, S.S. (2005). Silymarin treatment of viral hepatitis: A systematic review. *J. Viral Hepat.*, 12: 559–567.
68. Haddad, Y., Vallerand, D., Brault, A., Haddad, P.S. (2011). Antioxidant and hepatoprotective effects of silibinin in a rat model of nonalcoholic steatohepatitis. Evid.-Based Complement. *Altern. Med.*, 647903, doi:10.1093/ecam/nep164.
69. Colturato, C.P., Constantin, R.P., Maeda, A.S., Jr., Constantin, R.P., Yamamoto, N.S., Bracht, A., IshiiIwamoto, E.L., Constantin, J. (2012). Metabolic effects of silibinin in the rat liver. *Chem. Biol. Interact*, 195: 119–132.
70. Meeran, S.M., Katiyar, S., Elmets, C.A., Katiyar, S.K. (2006). Silymarin inhibits UV radiation induced immunosuppression through augmentation of interleukin-12 in mice. *Mol. Cancer Ther.*, 7: 1660–1668.
71. Bhattacharya, S. (2011). Milk thistle (Silybum marianum L. Gaert.) seeds in health. In Nuts and Seeds in Health and Disease Prevention, 1st ed.; Preedy V.R., Watson R.R., Patel, V., Eds.; Academic Press: London, UK; Burlington, VT, USA; San Diego, CA, USA, Chapter 90, pp. 759–766.
72. Kreeman, V., Skottova, N., Walterova, D. (1998). Silymarin inhibits the development of diet-induced hypercholesterolemia in rats. *Planta Med.*, 64:138–142.
73. Tyagi, A., Agarwal, C., Harrison, G., Glode, L.M., Agarwal, R. (2004). Silibinin causes cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI-CDK-cyclin cascade, and caspase 3 and PARP cleavages. *Carcinogenesis*, 25: 1711–1720.
74. Das, S., Roy, P., Auddy, R.G., Mukherjee, A. (2011). Silymarin nanoparticle prevents paracetamol-induced hepatotoxicity. *Int. J. Nanomed.*, 6: 1291–1301.
75. Jain, A., Yadav, A., Bozhkov, A.I., Padalko, V.I., Flora, S.J. (2011). Therapeutic efficacy of sylimarin and naringenin in reducing arsenic-induced hepatic damage in young rats. *Ecotoxicol. Environ. Saf.*, 74: 607–614.
76. Mohamed, O., Salam, E.A., Saleem, A.A., Shafee, N. (2010). Hepatoprotective effects of the nitric oxide donor isosorbide-5-mononitrate alone and in combination with the natural hepatoprotectant, silymarin on carbon tetrachloride-induced hepatic injury in rats. *Inflammopharmacology*, 18: 87–94.
77. Sharma, Y., Agarwal, C., Singh, A.K., Agarwal, R. (2001). Inhibitory effect of silibinin on ligand binding to erbB1 and associated mitogenic signaling, growth, and DNA synthesis in advanced human prostate carcinoma cells. *Mol. Carcinog.*, 30: 224–236.
78. Wang, M.J., Lin, W.W., Chen, H.L., Chang, Y.H., Ou, H.C., Kuo, J.S., Hong, J.S., Jeng, K.C. (2002). Silymarin protects dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation. *Eur. J. Neurosci.*, 16: 2103–2112.
79. Sayyah, M., Boostani, H., Pakseresht, S., Malayeri, A. (2010). Comparison of Silybum marianum (L.) Gaertn. with fluoxetine in the treatment of obsessive-compulsive disorder. *Prog. Neuro-Psychopharm. Biol. Psychiatry*, 34: 362–365.
80. Wood, N.J. (2010). Liver: Nonobese individuals in the developing world are at risk of nonalcoholic fatty liver and liver disease. *Nat. Rev. Gastroenterol. Hepatol.*, 7: 357.
81. Kim, N.C., Graf, T.N., Sparacino, C.M., Wani, M.C., Wall, M.E. (2003). Complete isolation and characterization of silybins and isosilybins from milk thistle (Silybum marianum) Org Biomol *Chem.*,1:1684–1689.
82. Kvasnicka, F., Bíba, B., Sevcík, R., Voldrich, M., Krátká, J. (2003). Analysis of the active components of silymarin. *J Chromatogr A.*, 990:239–245.
83. Lee, D.Y., Liu, Y. (2003). Molecular structure and stereochemistry of silybin A, silybin B, isosilybin A, and isosilybin B, Isolated from Silybum marianum (milk thistle) *J Nat Prod.*, 66:1171–1174.
84. Lee, J.I., Hsu, B.H., Wu, D., Barrett, J.S. (2006). Separation and characterization of silybin, isosilybin, silydianin and silychristin in milk thistle extract by liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr A.*, 1116:57–68.
85. Li, W., Han, J., Li, Z., Li, X., Zhou, S., Liu, C. (2008). Preparative chromatographic purification of diastereomers of silybin and their quantification in human plasma by liquid chromatography-tandem mass spectrometry. *J*

Chromatogr B Analyt Technol Biomed Life Sci., 862:51–57.
86. Lee, J.I., Narayan, M., Barrett, J.S. (2007). Analysis and comparison of active constituents in commercial standardized silymarin extracts by liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.*, 845:95–103.
87. Wen, Z., Dumas, T.E., Schrieber, S.J., Hawke, R.L., Fried, M.W., Smith, P.C. (2008). Pharmacokinetics and metabolic profile of free, conjugated, and total silymarin flavonolignans in human plasma after oral administration of milk thistle extract. *Drug Metab Dispos.*, 36:65–72.
88. Han, Y.H., Lou, H.X., Ren, D.M., Sun, L.R., Ma, B., Ji, M. (2004). Stereoselective metabolism of silybin diastereoisomers in the glucuronidation process. *J Pharm Biomed Anal.*, 34:1071–1078.
89. Barnes, S., Prasain, J.K., Wang, C.C., Moore, D.R. (2006). 2nd. Applications of LC-MS in the study of the uptake, distribution, metabolism and excretion of bioactive polyphenols from dietary supplements. *Life Sci.*, 78:2054–2059.
90. Yanyu, X., Yunmei, S., Zhipeng, C., Qineng, P. (2006). The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. *Int J Pharm.*, 307:77–82.
91. Wu, J.W., Lin, L.C., Hung, S.C., Chi, C.W., Tsai, T.H. (2007). Analysis of silibinin in rat plasma and bile for hepatobiliary excretion and oral bioavailability application. *J Pharm Biomed Anal.*, 45:635–641.
92. Tang, N., Wu, D., Lu, Y., Chen, J., Zhang, B., Wu, W. (2009). A comparative study on the stability of silybin and that in silymarin in buffers and biological fluids. *Drug Metab Lett.*, 3:115–119.
93. Yanyu, X., Yunmei, S., Zhipeng, C., Qineng, P. (2006). The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. *Int J Pharm.*, 307:77–82.
94. Song, Y., Zhuang, J., Guo, J., Xiao, Y., Ping, Q. (2008). Preparation and properties of a silybin-phospholipid complex. *Pharmazie*, 63:35–42.
95. Jia, L.J., Zhang, D.R., Li, Z.Y., Feng, F.F., Wang, Y.C., Dai, W.T., Duan, C.X., Zhang, Q. (2009). Preparation and characterization of silybin-loaded nanostructured lipid carriers. *Drug Deliv.*, Epub ahead of print
96. Kidd, P., Head, K. (2005). A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex (Siliphos). *Altern Med Rev.*, 10:193–203.
97. Flaig, T.W., Gustafson, D.L., Su, L.J., Zirrolli, J.A., Crighton, F., Harrison, G.S., Pierson, A.S., Agarwal, R., Glodé, L.M. (2007). A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. *Invest New Drugs*, 25:139–146.
98. Voinovich, D., Perissutti, B., Magarotto, L., Ceschia, D., Guiotto, P., Bilia, A.R. (2009). Solid state mechanochemical simultaneous activation of the constituents of the Silybum marianum phytocomplex with crosslinked polymers. *J Pharm Sci.*, 98:215–228.
99. Kidd, P.M. (2009). Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. *Altern Med Rev.*, 14:226–246.
100. Bai, T.C., Yan, G.B., Hu, J., Zhang, H.L., Huang, C.G. (2006). Solubility of silybin in aqueous poly(ethylene glycol) solution. *Int J Pharm.*, 308:100–106.
101. Luper, S. (1998). A review of plants used in the treatment of liver diseases: Part 1. *Altern Med Rev.*, 3: 410-21.
102. Wagner, H., Diesel, P., Seitz, M. (1974). The chemistry and analysis of silymarin from Silybum marianum Gaertn. *Arzneimittelforschung*, 24: 466-71.
103. Sonnenbichler, J., Zetl, I. (1986). Biochemical effects of the flavonolignan silibinin on RNA, protein, and DNA synthesis in rat liver. *Progr Clin Biol Res.*, 213 : 319-31.
104. Saller, R., Meier, R., Brignoli, R. (2001). The use of silymarin in the treatment of liver diseases. *Drugs*, 61: 2035-63.
105. Vailati, A., Aristia, L., Sozze, E., Milani, F., Inglese, V., Galenda, P., et al. (1993). Randomized open study of the dose-effect relationship of a short course of IdB 1016 in patients with viral or alcoholic hepatitis. *Fitoterapia*, 64: 219-31.
106. Miguez, M.P., Anundi, I., Sainz-Pardo, L.A., Lindros, K.O. (1994). Hepatoprotective mechanism of silymarin: no evidence for involvement of cytochrome P4502E1. *Chem Biol Interact,* 91 : 51-63.
107. Miller, A.L. (1996). Antioxidant flavonoids: Structure, function and clinical usage. *Altern Med Rev.*, 1 : 103-11.
108. Wiseman, H. (1996). Dietary influences on membrane function: Importance in protection against oxidative damage and disease. *J Nutr Biochem.,* 7 : 2-5.
109. Muriel, P., Mourelle, M. (1990). Prevention by silymarin of membrane alterations in acute CCl4 liver damage. *J Appl Toxicol.*, 10: 275-9.
110. Charlton, M. (2004). Nonalcoholic fatty liver disease: A review of current understanding and future impact. Clin. Gastroenterol. *Hepatol.*, 2: 1048–1058.
111. Lozano-Sepulveda, S.A., Bryan-Marrugo, O.L., Cordova-Fletes, C., Gutierrez-Ruiz, M.C., Rivas-Estilla, A.M. (2015). Oxidative stress modulation in hepatitis C virus infected cells. *World J. Hepatol.*, 7: 2880–2889.

112. Wei, F., Liu, S.K., Liu, X.Y., Li, Z.J., Li, B., Zhou, Y.L., Zhang, H.Y., Li, Y.W. (2013). Meta-analysis: Silymarin and its combination therapy for the treatment of chronic hepatitis B. *Eur. J. Clin. Microbiol. Infect. Dis.*, 32: 657–669.
113. Petruzziello, A., Marigliano, S., Loquercio, G., Cacciapuoti, C. (2016). Hepatitis C virus (HCV) genotypes distribution: An epidemiological up-date in Europe. Infect. *Agents Cancer*, 11: 53.
114. Ghany, M.G., Strader, D.B., Thomas, D.L., Seeff, L.B. (2009). American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: An update. *Hepatology*, 49: 1335–1374.
115. Yang, Z., Zhuang, L., Lu, Y., Xu, Q., Chen, X. (2014). Effects and tolerance of silymarin (milk thistle) in chronic hepatitis C virus infection patients: A meta-analysis of randomized controlled trials. Biomed. *Res. Int.,* 2014: 941085.
116. Blaising, J., Lévy, P.L., Gondeau, C., Phelip, C., Varbanov, M., Teissier, E., Ruggiero, F., Polyak, S.J., Oberlies, N.H., Ivanovic, T., et al. (2013). Silibinin inhibits hepatitis C virus entry into hepatocytes by hindering clathrin-dependent trafficking. *Cell. Microbiol.*, 15: 1866–1882.
117. Wagoner, J., Negash, A., Kane, O.J., Martinez, L.E., Nahmias, Y., Bourne, N., Owen, D.M., Grove, J., Brimacombe, C., McKeating, J.A., et al. (2010). Multiple effects of silymarin on the hepatitis C virus lifecycle. *Hepatology*, 51: 1912–1921.
118. Wagoner, J., Morishima, C., Graf, T.N., Oberlies, N.H., Teissier, E., Pécheur, E.I., Tavis, J.E., Polyak, S.J. (2011). Differential in vitro effects of intravenous versus oral formulations of silibinin on the HCV life cycle and inflammation. *PLoS ONE*, 6: e16464.
119. Ahmed-Belkacem, A., Ahnou, N., Barbotte, L., Wychowski, C., Pallier, C., Brillet, R., Pohl, R.T., Pawlotsky, J.M. (2010). Silibinin and related compounds are direct inhibitors of hepatitis C virus RNA-dependent RNA polymerase. *Gastroenterology*, 138: 1112–1122.
120. Guedj, J., Dahari, H., Pohl, R.T., Ferenci, P., Perelson, A.S. (2012). Understanding silibinin's modes of action against HCV using viral kinetic modeling. *J. Hepatol.*, 56: 1019–1024.
121. Ferenci, P., Scherzer, T.M., Kerschner, H., Rutter, K., Beinhardt, S., Hofer, H., Schöniger-Hekele, M., Holzmann, H., Steindl-Munda, P. (2008). Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated interferon/ribavirin therapy. *Gastroenterology*, 135: 1561–1567.
122. Dahari, H., Shteingart, S., Gafanovich, I., Cotler, S.J., D'Amato, M., Pohl, R.T., Weiss, G., Ashkenazi, Y.J., Tichler, T., Goldin, E., et al. (2015). Sustained virological response with intravenous silibinin: Individualized IFNfree therapy via real-time modelling of HCV kinetics. *Liver Int.*, 35: 289–294.
123. Rutter, K., Scherzer, T.M., Beinhardt, S., Kerschner, H., Stattermayer, A.F., Hofer, H., Popow-Kraupp, T., Steindl-Munda, P., Ferenci, P. (2011). Intravenous silibinin as "rescue treatment" for on-treatment non-responders to pegylated interferon/ribavirin combination therapy. *Antivir. Ther.*, 16: 1327–1333.
124. Neumann, U.P., Biermer, M., Eurich, D., Neuhaus, P., Berg, T. (2010). Successful prevention of hepatitis C virus (HCV) liver graft reinfection by silibinin monotherapy. *J. Hepatol.*, 52: 951–952.
125. Goossens, N., Negro, F. (2014). Is genotype 3 of the hepatitis C virus the new villain? *Hepatology*, 59: 2403–2412.
126. Braun, D.L., Rauch, A., Aouri, M., Durisch, N., Eberhard, N., Anagnostopoulos, A., Ledergerber B., Müllhaupt, B., Metzner, K.J., Decosterd, L., et al. (2015). A Lead-In with Silibinin Prior to Triple-Therapy Translates into Favorable Treatment Outcomes in Difficult-To-Treat HIV/Hepatitis C Coinfected Patients. *PLoS ONE*, 10: e0133028.
127. Hezode, C., Fontaine, H., Dorival, C., Zoulim, F., Larrey, D., Canva, V., de Ledinghen, V., Poynard, T., Samuel, D., Bourliere, M., et al. (2014). Effectiveness of telaprevir or boceprevir in treatment-experienced patients with HCV genotype 1 infection and cirrhosis. *Gastroenterology,* 147: 132–142.e4.
128. De Meyer, S., Dierynck, I., Ghys, A., Beumont, M., Daems, B., Van Baelen, B., Sullivan, J.C., Bartels, D.J., Kieffer, T.L., Zeuzem, S., et al. (2012). Characterization of telaprevir treatment outcomes and resistance in patients with prior treatment failure: Results from the REALIZE trial. *Hepatology*, 56: 2106–2115.
129. Federico, A., Dallio, M., Ormando, V.M., Abenavoli, L., Masarone, M., Persico, M., Loguercio, C. (2016). Alcoholic Liver Disease and Hepatitis C Chronic Infection. *Rev. Recent Clin. Trials*, 11, 201–207.
130. Galicia-Moreno, M., Gutiérrez-Reyes, G. (2014). The role of oxidative stress in the development of alcoholic liver disease. *Rev. Gastroenterol. Mex.*, 79: 135–144.
131. Yeh, M.M., Brunt, E.M. (2014). Pathological features of fatty liver disease. *Gastroenterology*, 147: 754–764.
132. Song, Z., Deaciuc, I., Song, M., Lee, D.Y., Liu, Y., Ji, X., McClain, C. (2006). Silymarin protects against acute ethanolinduced hepatotoxicity in mice. *Alcohol. Clin. Exp. Res.*, 30: 407–413.
133. Das, S.K., Mukherjee, S. (2012). Biochemical and immunological basis of silymarin effect, a milk thistle (Silybum marianum) against ethanol-induced oxidative damage. *Toxicol. Mech. Methods*, 22: 409–413.

134. Lin, M.T., Beal, M.F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, 443: 787–795.
135. Sekine, S., Ichijo, H. (2015). Mitochondrial proteolysis: Its emerging roles in stress responses. *Biochim. Biophys. Acta*, 1850: 274–280.
136. Rolo, A.P., Oliveira, P.J., Moreno, A.J., Palmeira, C.M. (2003). Protection against post-ischemic mitochondrial injury in rat liver by silymarin or TUDC. *Hepatol. Res.*, 26: 217–224.
137. Detaille, D., Sanchez, C., Sanz, N., Lopez-Novoa, J.M., Leverve, X., El-Mir, M.Y. (2008). Interrelation between the inhibition of glycolytic flux by silibinin and the lowering of mitochondrial ROS production in perifused rat hepatocytes. *Life Sci.*, 82: 1070–1076.
138. Grattagliano, I., Diogo, C.V., Mastrodonato, M., de Bari, O., Persichella, M., Wang, D.Q., Liquori, A., Ferri, D., Carratù, M.R., Oliveira, P.J., et al. (2013). A silybin-phospholipids complex counteracts rat fatty liver degeneration and mitochondrial oxidative changes. *World J. Gastroenterol.*, 19: 3007–3017.
139. Mazzio, E.A., Harris, N., Soliman, K.F. (1998). Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells. *Plant Med.*, 64: 603–606.
140. Chalasani, N., Younossi, Z., Lavine, J.E., Diehl, A.M., Brunt, E.M., Cusi, K., Charlton, M., Sanyal, A.J. (2012). The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology*, 55: 2005–2023.
141. Charlton, M.R., Burns, J.M., Pedersen, R.A., Watt, K.D., Heimbach, J.K., Dierkhising, R.A. (2011). Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology*, 141: 1249–1253.
142. Younossi, Z.M., Otgonsuren, M., Henry, L., Venkatesan, C., Mishra, A., Erario, M., Hunt, S. (2015). Association of non-alcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology*, 62: 1723–1730.
143. Starley, B.Q., Calcagno, C.J., Harrison, S.A. (2010). Non-alcoholic fatty liver disease and hepatocellular carcinoma: A weighty connection. *Hepatology*, 51: 1820–1832.
144. Yasui, K., Hashimoto, E., Komorizono, Y., Koike, K., Arii, S., Imai, Y., Shima, T., Kanbara, Y., Saibara, T., Mori, T., et al. (2011). Characteristics of patients with non-alcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin. Gastroenterol. Hepatol.*, 9: 428–433.
145. Buzzetti, E., Pinzani, M., Tsochatzis, E.A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism*, 65: 1038–1048.
146. Stefan, N., Kantartzis, K., Haring, H.U. (2008). Causes and metabolic consequences of fatty liver. *Endocr. Rev.*, 29: 939–960.
147. Cai, D., Yuan, M., Frantz, D.F., Melendez, P.A., Hansen, L., Lee, J., Shoelson, S.E. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-β and NF-κB. *Nat. Med.*, 11: 183–190.
148. Younossi, Z.M., Otgonsuren, M., Venkatesan, C., Mishra, A. (2013). In patients with non-alcoholic fatty liver disease, metabolically abnormal individuals are at a higher risk for mortality while metabolically normal individuals are not. *Metabolism*, 62: 352–360.
149. Hossain, N., Afendy, A., Stepanova, M., Nader, F., Srishord, M., Rafiq, N., Goodman, Z., Younossi, Z. (2009). Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. Clin. Gastroenterol. *Hepatol.*, 7: 1224–1229.
150. Reddy, S.K., Steel, J.L., Chen, H.W., Demateo, D.J., Cardinal, J., Behari, J., Humar, A., Marsh, J.W., Geller, D.A., Tsung, A. (2012). Outcomes of curative treatment for hepatocellular cancer in nonalcoholic steatohepatitis versus hepatitis C and alcoholic liver disease. *Hepatology*, 55: 1809–1819.
151. Federico, A., Dallio, M., Godos, J., Loguercio, C., Salomone, F. (2016). Targeting gut-liver axis for the treatment of nonalcoholic steatohepatitis: Translational and clinical evidence. *Transl. Res.*, 167: 116–124.
152. Miele, L., Forgione, A., Hernandez, A.P., Gabrieli, M.L., Vero, V., Di Rocco, P., Greco, A.V., Gasbarrini, G., Gasbarrini, A., Grieco, A. (2005). The natural history and risk factors for progression of non-alcoholic fatty liver disease and steatohepatitis. *Eur. Rev. Med. Pharmacol. Sci.*, 9: 273–277.
153. Anderson, N., Borlak, J. (2008). Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. *Pharmacol. Rev.*, 60: 311–357.
154. Trappoliere, M., Tuccillo, C., Federico, A., Di Leva, A., Niosi, M., D'Alessio, C., Capasso, R., Coppola, F., Dauria, M., Loguercio, C. (2005). The treatment of NAFLD. *Eur. Rev. Med. Pharmacol. Sci.*, 9: 299–304.
155. Masarone, M., Federico, A., Abenavoli, L., Loguercio, C., Persico, M. (2014). Non alcoholic fatty liver: Epidemiology and natural history. *Rev. Recent Clin. Trials*, 9: 126–133.
156. Nobili, V., Svegliati-Baroni, G., Alisi, A., Miele, L., Valenti, L., Vajro, P. (2013). A 360-degree overview of paediatric NAFLD: Recent insights. *J. Hepatol.*, 58: 1218–1229.

157. Byrne, C.D., Targher, G. (2016). EASL-EASD-EASO Clinical Practice Guidelines for the management of nonalcoholic fatty liver disease. *J. Hepatol.*, 64: 1388–1402.
158. Godos, J., Federico, A., Dallio, M., Scazzina, F. (2016). Mediterranean diet and nonalcoholic fatty liver disease: Molecular mechanisms of protection. *Int. J. Food Sci. Nutr.*, 2: 1–10.
159. Christodoulou, E., Kechagia, I.A., Tzimas, S., Balafas, E., Kostomitsopoulos, N., Archontaki, H., Dokoumetzidis, A., Valsami, G. (2015). Serum and tissue pharmacokinetics of silibinin after per os and i.v. administration to mice as a HP-β-CD lyophilized product. *Int. J. Pharm.*, 493: 366–373.
160. Huang, S., Czech, M.P. (2007). The GLUT4 glucose transporter. *Cell Metab.*, 5: 237–252.
161. Hotamisligil, G.S. (2008). Inflammation and endoplasmic reticulum stress in obesity and diabetes. *Int. J. Obes.*, 32: S52–S54.
162. Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C.Z., Uysal, K.T., Maeda, K., Karin, M., Hotamisligil, G.S. (2002). A central role for JNK in obesity and insulin resistance. *Nature*, 420: 333–336.