# Liver Disease in Diabetes Mellitus: Potential Therapeutic Value of Vitamin E-Silybin Phytosomal Complex

Elena Matteucci*, Maria Chiara Masoni, Cristina Consani and Ottavio Giampietro

*Department of Internal Medicine, University of Pisa, Pisa, Italy*

**Abstract:** The liver has a central role in glucose homeostasis. In turn, glucose-induced signals modulate the transcriptional regulation of genes involved in the glycolysis and lipogenesis pathways and could favour fatty acid storage in the liver. The prevalence of hepatobiliary diseases is increased in patients with diabetes mellitus. Type 1 diabetes is associated with a hepatic form of microvascular disease (diabetic hepatosclerosis), hemochromatosis and autoimmune hepatitis. Type 2 diabetes is associated with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) whose prevalence increases with multiple components of metabolic syndrome. Hepatitis C virus infection has been reported to be associated with and predispose to diabetes mellitus. Silibinin or silybin is a flavonoid derived from *Silybum marianum* and known to have hepatoprotective, anti-carcinogenic and anti-inflammatory effects. Silymarin is a standardised extract of four polyphenolic flavanolignans (silybin, isosilybin, silydianin and silychristin) from the seeds of *Silybum marianum*; it possesses a potent scavenging capacity of oxidizing free radicals whose mechanistic aspects have been extensively evaluated. This single herbal drug formulation is used for hepatoprotection although recent evidence in carbon tetrachloride-induced cirrhotic rats suggests that chronic silymarin treatment might compromise the hemodynamic endothelial nitric oxide synthase activity. In order to enhance silymarin bioavailability flavonoid molecules have been converted into lipid-compatible molecular complexes, phytosomes. A new silybin-phosphatidylcholine-Vitamin E complex, characterised by elevated oral bioavailability and lipophilicity, was effective on rat hepatic fibrosis induced by dimethylnitrosamine administration and by bile duct ligation. The complex has been suggested as a complementary approach to the treatment of patients with chronic liver damage. The manuscript provides a review of literature on this topic and discusses the potential usefulness of the complex to prevent/treats liver disease in diabetes as well as contraindications.

**Keywords:** Diabetes mellitus, liver diseases, silybin-phytosome.

## INTRODUCTION

The liver has a central role in the regulation of metabolism and energy homeostasis. This organ buffers the excursions of plasma glucose concentration in the fasting/feeding cycle through a coordinated modulation of both the disposal of glucose from plasma and the entry into the circulation of endogenous glucose [1]. This constant metabolic adjustment is achieved by a complex interplay of regulatory networks. The circadian clock, located in the suprachiasmatic nuclei of the anterior hypothalamus, regulates metabolism and energy homeostasis in the liver and other peripheral tissues by mediating the expression and/or activity of metabolic enzymes and transport systems involved in glycogen and glucose metabolism, the citric acid cycle, lipid metabolism and amino acid regulation [2]. The response to dietary glucose combines both effects related to glucose metabolism itself and effects secondary to nutrient-dependent hormonal modifications. The mechanisms by which food supply (and thus glucose, insulin and glucagon fluctuations) regulates gene transcription and expression are under intensive investigation [3, 4].

*Address correspondence to this author at the Dipartimento di Medicina Interna, *Via* Roma 67, 56126 Pisa, Italy; Tel: 0039 050 993246;
Fax: 0039 050 553414; E-mail: ematteuc@int.med.unipi.it

## LIVER AND DIABETES

The increase in diabetes prevalence constitutes a worldwide health concern related to the chronic nature of the disease that can lead to life-threatening short- and long-term complications (http://www.idf.org/Facts_and_Figures). Liver disease may occur as a consequence of diabetes mellitus or may occur coincidentally with diabetes mellitus. Alternatively, diabetes mellitus may complicate liver disease.

The most common disease of the liver in patients with type 2 diabetes and metabolic syndrome is non-alcoholic fatty liver disease (NAFLD) whose feature-based scoring system identifies a core group of histological features for evaluation: steatosis (score 0-3), lobular inflammation (score 0-3), hepatocellular ballooning (score 0-2), and fibrosis (score 0-4) [5, 6]. Population-based studies estimated a prevalence ranging from 3 to 46% among different ethnic groups depending on the screening test used [7]. Overall prevalence of NAFLD increased up to 40-87% of patients with type 2 diabetes and increased with multiple components of metabolic syndrome [7, 8]. Excessive accumulation of triglycerides in hepatocytes, ranging from simple fatty liver to the progressive non-alcoholic steatohepatitis (NASH), could result from various combination of 1) increased fat delivery, 2) increased liponeogenesis, 3) reduced fat oxidation, and 4) reduced fat export (very low-density lipopro-

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teins) [9, 10]. In patients with long-standing poorly controlled type 1 diabetes mellitus additional liver diseases have been described. Glycogenic hepatopathy is a generally under-recognised hepatic complication of diabetes mellitus characterised by glycogen accumulation within hepatocytes, hepatomegaly and/or elevated liver enzymes. Glycogenic hepatopathy can be distinguished from NAFLD only by liver biopsy and could be reversed by improving glycemic control [11]. Mauriac's syndrome is a rare condition of infancy characterized by growth failure, delayed puberty, hepatomegaly and Cushingoid features [12]. A biopsy survey revealed that not in all cases hepatomegaly was reflected by corresponding histologic findings, but behaviour and extent of fat and glycogen deposits showed marked variations [13].

The term diabetic hepatosclerosis (DHS) was proposed to describe a noncirrhotic form of hepatic sinusoidal fibrosis, not associated with NASH or other forms of NAFLD, in patients with long-standing diabetes mellitus. DHS had a prevalence varying from 2 to 12% in autopsy series and could represent a hepatic form of diabetic microangiopathy because it was usually accompanied by other end-organ damage [14, 15]. Furthermore, type 1 diabetes mellitus may coexist with primary biliary cirrhosis; familial associations of type 1 diabetes with several autoimmune and related diseases have suggested genetic sharing [16].

Cholelithiasis is more common in patients with type 1 or type 2 diabetes mellitus than in the general population, partly contributed by the presence of autonomic neuropathy [17]. Diabetes has also been regarded as a risk factor for hepatocellular carcinoma; however, a systematic review of the published studies concluded that, up until now, it has not been proven to be a "true" independent risk factor [18].

On the other side, the so-called hepatogenous diabetes mellitus may develop as a complication of liver diseases [19]. NAFLD, alcoholic cirrhosis, hepatitis C virus (HCV) infection and haemochromatosis are more frequently associated with diabetes mellitus. Glucose intolerance is contributed by multiple factors: insulin resistance in muscular cells and hepatic tissue, reduction in insulin clearance, and impairment in insulin secretion. In patients with haemocromatosis iron loading of Langerhans islets with damage to the β-cells can accelerate their functional deterioration. In an eleven-year follow-up study, subjects with NAFLD and elevated aminotransaminase levels were at an increased risk of developing diabetes and the metabolic syndrome seemingly because of the presence of associated metabolic risk factors. Indeed, in multivariate logistic regression models, NAFLD was no longer a significant independent predictor of the development of diabetes after adjusting for baseline waist circumference, hypertension, and insulin resistance [20].

Evidence suggests that the chronic HCV infection is associated with an increased risk of developing insulin resistance and type 2 diabetes. HCV impairs directly and/or indirectly glucose metabolism *via* mechanisms that involve the insulin signalling cascade and cytokine production. In turn, insulin resistance and diabetes accelerate fibrogenesis and reduce the virological response to interferon-α-based therapy [21].

## PREVENTION AND CLINICAL MANAGEMENT OF LIVER DISEASE IN DIABETES MELLITUS

As is evidenced from the previous brief excursus of the literature, diabetes related complications include damage to liver tissue as well as insulin resistance has been described among the complications of chronic liver diseases. Recognition of the problem should encourage solutions. However, the optimal intervention to prevent and treat NAFLD/NASH remains controversial [22].

Although prospective data are scant, generic recommendations pertain to nutrient intake and lifestyle: diet, modest wine drinking, weight reduction and exercise [23, 24]. Tight glycaemic control, rather than weight reduction, seemed able to prevent histological progression in Japanese patients with NAFLD [25]. Insulin and atorvastatin combination improved glycaemic and lipid profiles and gave a superior level of liver protection in a model of type 2 diabetes with hyperlipidemia [26]. Indeed, transgenic, knockout and knockdown animal models are helping to clarify the molecular determinants of hepatic steatosis that could be potential therapeutic targets in the future [10].

Following 4 Cochrane reviews dated 2007 [27-30], the next systematic review dated 2009 found a limited number (15) of randomised controlled trials on pharmacological and dietary supplement interventions were available [22]. One of main limitations was the methodology used to follow NAFLD progression. Although liver biopsy is still regarded as the gold standard to assess liver disease, non-invasive methods are more convenient. Several pharmacologic agents had been used either alone or in various combinations:

- insulin sensitisers, such as metformin and pioglitazone,
- antioxidants, such as vitamin E, vitamin C, N-acetylcysteine, and the herbal compound Yo Jyo Hen Shi Ko,
- lipid-lowering drugs, such as probucol (which possesses also antioxidant properties),
- carnitine (in attempt to improve fatty acid oxidation),
- choleretic drugs, such as ursodeoxycholic acid,
- orlistat, an oral inhibitor of gastric lipases that blocks the absorption of approximately 30% of dietary triglycerides.

A significant effect on normalisation of alanine transaminase was obtained in patients treated with metformin and high-dose carnitine. Aspartate aminotransferase normalisation was higher in those treated with metformin and in those treated with ursodeoxycholic acid combined with vitamin E. Pioglitazone improved liver histology in one study.

The most recent meta-analysis assessed the efficacy of proposed treatments for NAFLD/NASH by reviewing reports of randomized controlled trials on online databases and national and international meeting abstracts through January 2010. It included 49 trials of which only 23 (22 in NASH, 1 in NAFLD) had post-treatment histology. Most trials were small and did not exceed 1-year duration. Weight loss, thiazolidinediones, and antioxidants were most extensively evaluated. Weight loss was safe and dose-dependently improved histological disease activity in NASH, but more than 50% of patients failed to achieve target weight loss. The ef-

fect of regular physical activity, that seemed able to enhance prolonged weight loss, needs further evaluation. Thiazolidinediones improved steatosis and inflammation but yielded significant weight gain. Trials with antioxidants yielded conflicting results and were heterogeneous with respect to type and dose of drug, duration, implementation of lifestyle intervention. Pentoxifylline, telmisartan and L-carnitine improved liver histology in at least 1 trial in NASH; polyunsaturated fatty acid ameliorated biochemical and radiological markers of NAFLD. Other approaches yielded negative results. Only a few trials evaluated clinical predictors of response to pharmacological treatment [31].

Intracellular oxidative stress due to mitochondrial failure to convert energy from oxidized fatty acids to adenosine triphosphate and overexpression of the prooxidant enzyme cytochrome P450 2E1 has been implicated in the pathogenesis of liver cell injury. Reactive oxygen species and the lipid peroxidation product 4-hydroxynonenal cause synergistic activation of the c-Jun N-terminal kinase signalling pathway and its downstream target c-Jun in hepatocytes thus promoting liver injury [32]. On the basis of this pathogenetic mechanism, antioxidant therapies have been attempted. The study PIVENS (Pioglitazone vs Vitamin E vs Placebo for Treatment of Non-Diabetic Patients with Nonalcoholic Steatohepatitis) has been recently completed. Adults with nonalcoholic steatohepatitis and without diabetes were randomly assigned to receive pioglitazone at a dose of 30 mg daily (80 subjects), vitamin E at a dose of 800 IU daily (84 subjects), or placebo (83 subjects), for 96 weeks. The primary outcome was an improvement in histologic features of nonalcoholic steatohepatitis, as assessed with the use of a composite of standardized scores for steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis. Vitamin E was superior to placebo for the treatment of nonalcoholic steatohepatitis in adults without diabetes. There was no benefit of pioglitazone over placebo for the primary outcome; however, significant benefits of pioglitazone were observed for some of the secondary outcomes (reduction in steatosis, inflammation, and hepatocellular ballooning as well as improvements in insulin resistance and liver-enzyme levels) [33]. Treatment of NAFLD in Children (TONIC) is an ongoing multicenter, randomized, placebo-controlled therapeutic clinical trial of NAFLD in children whose final results are expected by the end of 2010. Three treatment groups include metformin 500 mg twice a day, vitamin E 400 IU twice a day and placebo administered for 96 weeks. The primary outcome measure is a reduction in serum alanine aminotransferase levels; histological changes are the major secondary outcome measures [34].

## HEPATOPROTECTIVE HERBAL DRUG (*SILYBUM MARIANUM*)

*Silybum marianum*, commonly known as 'milk thistle' (Family: Asteraceae/Compositae) is one of the oldest and thoroughly researched plants in the treatment of liver diseases. The plant itself grows as a stout thistle in rocky soils with large purple flowering heads. The leaves are characterized by milky veins, from which the plant derives its name [35]. The extract of milk thistle is being used as a general medicinal herb from as early as 4$^{th}$ century B.C. and first reported by Theophrastus [36]. In the 1st century A.D., Dioskurides used this plant as emetic as well as a general medicinal herb [36]. In 16th century it became a favoured medicine for hepatobiliary diseases and the drug was revived again in 1960 in central Europe [35, 36].

The active constituents of the plant are obtained from the dried seeds where they are present in higher concentrations than in other parts of the plant, and primarily consist of an isomeric mixture of active flavonolignans, which are collectively known as silymarin. The active principle was first isolated and chemically characterized during 1968-1974 [37-39].

Silymarin primarily consists of a complex of eight major compounds, including seven flavolignans: silybin A (Sb A), silybin B (Sb B), isosilybin A (ISb A), isosilybin B (ISb B), silychristin (Sc), isosilychristin (ISc), silydianin (Sd), and one flavonoid, taxifolin [40]. The different isomers of silymarin have been reported to have different biological activities [41, 42]. The chemical structures of five of the main active constituents of Silybum marianum are shown in Fig. (**1**). 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%) [39, 41].

Silymarin has been used widely for centuries for the protection of the liver from toxic substances but also for the treatment of toxic liver damage and for the therapy of hepatitis and cirrhosis [39]. Hepatoprotective activity of silymarin has been demonstrated against partial hepatectomy models: rats with 70 per cent of liver removed, when subjected to silymarin pretreatment showed increased synthesis of DNA, RNA, protein and cholesterol, suggesting the regeneration of liver [43]. Silymarin can enter inside the nucleus and fits in to a specific binding site on the RNA polymerase enzymes, owing to its structural similarity to steroids, resulting in increased ribosomal formation; this in turn hastens protein and DNA synthesis [43]. This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver.

Silymarin has other multiple actions as a hepatoprotective agent, as demonstrated against toxic models in experimental animals by using substances like carbon tetrachloride, acetaminophen, ethanol, D-galactosamine, and *Amanita phalloides* toxin. Silymarin has anti-inflammatory [44] and antifibrotic [45] actions by inhibiting cytokines synthesis and retarding hepatic stellate cells activation, it also inhibits protein kinases and other kinases involved in signal transduction and may interact with intracellular signaling pathways [45]. Silymarin shows also antioxidant properties and cell-regenerating functions that are considered as most important. In liver diseases [46] reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes and as a result of increased exposure to xenobiotics [46]. 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. Subse-

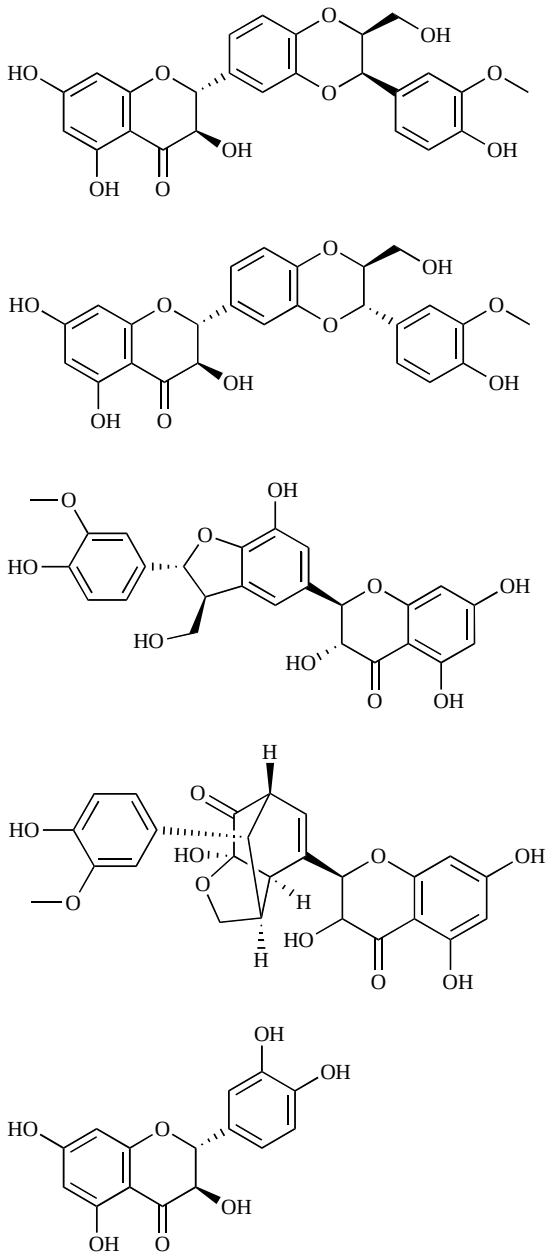

**Fig. (1).** The structures of four of the seven flavonolignan diasteroisomers (silybin A, silybin B, silychristin, and silydianin) and the flavonoid taxifolin are shown (from top to bottom).

quently cell contents including DNA, RNA, and other cellular components are damaged [47]. Silymarin has a regulatory action on cellular and mitochondrial membrane permeability in association with an increase in membrane stability against xenobiotic injury [48]. 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 [48]. These actions along with antiperoxidative property make silymarin a suitable candidate for the treatment of iatrogenic and toxic liver diseases, although recent evidence in carbon tetrachloride-induced cirrhotic rats suggest that chronic silymarin treatment might compromise the hemodynamic endothelial nitric oxide synthase activity. Despite the experimental evidence provided by *in vitro* and *in vivo* animal studies, the hepatoprotective efficacy of silymarin remains unproven because of the paucity of well-designed randomized clinical trials [49, 50].

## PHARMACOKINETICS

Silymarin has a limited solubility in water and is typically administered as a sugar coated tablet or as an encapsulated standardized extract. In pharmacokinetic studies, the maximum serum concentrations of silibinin were low in human subjects. Renal excretion amounted to only 1-2% of the silibinin dose over 24 hours [51]. Silibinin undergoes phase I and phase II metabolism, especially multiple conjugation reactions in humans: most of the silibinin present in the systemic circulation was in conjugation with sulfates and glucuronides. About 20-40 per cent of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates in human beings [52]. The peak plasma levels after an oral dose are achieved in 4-6 h in experimental animals and in human beings [41] and elimination half-life is approximately 6 h [51, 52].

In order to enhance silymarin bioavailability, antioxidant and antifibrotic activity, flavonoid molecules have been converted into lipid-compatible molecular complexes, phytosomes. Pharmacokinetic studies with silybinphosphatidylcholine complex have shown an increase in the oral bioavailability of silybin in healthy human subjects, probably by a facilitatory role of drug complex on the passage of the drug across the gastrointestinal tract [42]. Silybin is absorbed 4.6-times better from its phytosome than its conventional form and the human liver receives about a four-fold higher exposure. It has a hematic peak of 2 hr and is widely distributed in plasma and tissues, which include liver, lung, stomach, skin, and prostate [53]. In this new complex silybin reduces collagen accumulation and lipid peroxidation in rats with induced fibrosis [54]. The silybin-phosphatidylcholine-Vitamin E complex, characterised by elevated oral bioavailability and lipophilicity, was effective on rat hepatic fibrosis induced by dimethylnitrosamine administration and by bile duct ligation [54]. These results suggest that this new silybin-phosphatidylcholine-Vitamin E complex could be an interesting drug to be tested in patients with chronic liver disease.

Silymarin has a good safety profile, but little is known regarding its potential for drug interaction. Silymarin seems to have limited effect on the pharmacokinetics of several drugs *in vivo*, although it decreases the activity of cytochrome P-450 enzymes, UDP-glucuronosyltransferase enzyme, and reduces P-glycoprotein transport. Thus, health-care practitioners are invited to caution patients against co-administration of silymarin and pharmaceutical drugs [55].

## FINAL REMARKS AND FUTURE WORK

A literature survey highlights that the assessment of therapeutic agents for NASH is a complex process. Since there are no validated biomarkers of response to treatment, percutaneous liver biopsy is considered as the gold standard to diagnose NASH and assess treatment efficacy. It is an invasive procedure, however, that includes expense and

medical risk to patients. Furthermore, concerns exist related to the sampling variability of liver biopsy samples and histological features [56]. Candidate non-invasive surrogates (multiple serum markers, liver elastography, etc.) have been proposed but their diagnostic accuracy is still debated. Error in the liver biopsy result itself makes it impossible to effectively assess the accuracy of tests used to stage liver disease [57]. A position statement based on the 2009 European Association for the Study of the Liver (EASL) Special Conference on NAFLD/NASH stated that 1) there is a significant need for the non-invasive quantification of fibrosis in order to facilitate screening of the large number of patients at risk and 2) the association of serum markers with an imaging method (elastometry) is recommended in order to restrict biopsy to indeterminate or discordant results or those predicted to have advanced fibrosis [58]. Long-term evaluation of efficacy and safety of proposed treatments may encounter additional drawbacks and problems. Indeed, liver enzymes and even steatosis spontaneously fluctuate over time in NAFLD, and their improvement may not reflect treatment efficacy. Furthermore, aminotransferases and hepatic steatosis do not parallel the course of necroinflammation and fibrosis in NASH [31]. Musso *et al*. conclude that well-designed, rigorously performed, randomized, controlled trials of adequate size and duration, with histological endpoints, are needed to assess long-term efficacy, durability, and safety of proposed treatments on patient-oriented clinical outcomes including liver-related (for example, cirrhosis, liver failure, hepatocellular carcinoma) but also cardiovascular, and metabolic morbidity. Long-term safety of the available therapies must be evaluated because they will need to be taken indefinitely due to the certainty of relapse after discontinuation.

The assessment of therapeutic agents for NASH in people with diabetes is an even more complex process due to the intra-individual variability of the long-term metabolic control. Furthermore, drug interactions must be properly considered in diabetic people often receiving multi-drug therapy.

Diabetes-related complications include damage to liver tissue as well as insulin resistance has been described among the complications of chronic liver diseases, so there is a very strong relationship between diabetes mellitus and/or insulin resistance and NAFLD, HCV infection, haemochromatosis. Diabetes mellitus in insulin resistant patients with cirrhosis leads to a progressive impairment in insulin secretion together with the development of hepatic insulin resistance leading to fasting hyperglycaemia and a diabetic glucose tolerance profile [59]. Velussi *et al.* [60] conducted a trial in 60 cirrhotic diabetic patients who were being treated with silymarin. Patients were randomly assigned to receive silymarin 600 mg/day or no silymarin for 12 months, with both the groups receiving standard therapy. The baseline features were similar in both the groups. Silymarin treatment produced significant reduction in mean daily and fasting blood glucose, daily glycosuria, glycosylated haemoglobin values, malondialdehyde values and a drop in insulin requirement and fasting insulinaemia. In contrast, the status of untreated patients declined during the trial. The authors concluded that silymarin may reduce the lipoperoxidation of cell membrane and insulin resistance, by significantly decreasing endogenous insulin overproduction and the need for exogenous insulin administration.

It has been suggested that in the future a therapeutic approach to chronic liver disease would consist of a number of complementary approaches considering the multitude of pathogenic mechanisms, like showed by a recent study [61], performed on two different groups of outpatients, primitive NAFLD patients and HCV related chronic hepatitis C patients. All subjects were divided into two subgroups using a systematic random sampling procedure: one subgroup (39 NAFLD and 14 HCV) was treated with the complex silybin-vitamin E-phospholipids for six months followed by another six months of follow up, while the other (20 NAFLD and 12 HCV) served as a control group (no treatment). Treatment with silybin-vitamin E-phospholipids complex significantly reduced all indices of liver fibrosis in both treated groups, improved liver enzyme levels and reduced hyperinsulinaemia.

These data suggest that this new complex of silybin-vitamin E-phopsholipids should be tested in a well controlled larger trial to further confirm its possible therapeutic effect on insulin resistance and liver damage, particularly when other drugs are not indicated or have failed, or as a complementary treatment associated with other therapeutic programmes.

## CONFLICT OF INTEREST

None.

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