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An Updated Systematic Review with Meta-Analysis for the Clinical Evidence of Silymarin

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Key Words
Silymarin · Silibinin · Milk thistle · Phytotherapy · Herbal drug · Liver cirrhosis · *Amanita phalloides* · Alcoholic liver disease · Radical scavenger

Summary
Background: The potential benefit of silymarin (special extract from the fruits of *Silybum marianum*) in the treatment of liver diseases remains a controversial issue. **Methods**: For this systematic review electronic databases identified 65 papers for the search terms silymarin, silibinin, silicristin or milk thistle and clinical trial. Only 19 complied with the criteria 'double-' or 'single-blind'. These publications were analysed from a clinical point of view and meta-analytic calculations were performed. **Results**: The clinical evidence of a therapeutic effect of silymarin in toxic liver diseases is scarce. There is no evidence of a favourable influence on the evolution of viral hepatitis, particularly hepatitis C. In alcoholic liver disease, comparing with placebo, aspartate aminotransferase was reduced in the silymarin-treated groups (p = 0.01) while alkaline phosphatase was not. In liver cirrhosis, mostly alcoholic, total mortality was 16.1% with silymarin vs. 20.5% with placebo (n.s.); liver-related mortality was 10.0% with silymarin vs. 17.3% with placebo (p = 0.01). **Conclusions**: Based on the available clinical evidence it can be concluded – concerning possible risks / probable benefits – that it is reasonable to employ silymarin as a supportive element in the therapy of *Amanita phalloides* poisoning but also (alcoholic and grade Child 'A') liver cirrhosis. A consistent research programme, consolidating existing evidence and exploring new potential uses, would be very welcome.

Schlüsselwörter
Silymarin · Silibinin · Mariendistel · Phytotherapie · Pflanzliches Arzneimittel · Leberzirrhose · *Amanita phalloides* · Alkoholische Hepatopathie · Radikalfänger

Zusammenfassung
Hintergrund: Der potentielle Nutzen von Silymarin (Spezialextrakt aus den Samen der Mariendistel, *Silybum marianum*) in der Behandlung von Lebererkrankungen wird kontrovers diskutiert. **Methoden**: Für diesen systematischen Review fanden sich in elektronischen Datenbanken unter den Suchbegriffen Silymarin, Silibinin, Silicristin oder Mariendistel und klinische Studie 65 Publikationen. Nur 19 Studien erfüllten die Zusatzkriterien 'doppel-' oder 'einfachblind'. Diese Publikationen wurden nach klinisch relevanten Gesichtspunkten analysiert und metaanalytische Berechnungen wurden durchgeführt. **Ergebnisse**: Die klinische Evidenz der therapeutischen Wirkung von Silymarin bei toxischen Leberschäden ist gering. Es gibt keine Daten, die auf eine günstige Beeinflussung der Evolution von viralen Hepatitiden, insbesondere Hepatitis C, hinweisen würden. Bei alkoholischen Leberschäden wurde mit Silymarin, im Vergleich zu Placebo, eine Reduktion der Aspartat-Aminotransferase erzielt (p = 0,01), nicht aber der alkalischen Phosphatase. Bei Patienten mit meistens alkoholischer Leberzirrhose betrug die Gesamtmortalität 16,1% mit Silymarin vs. 20,5% mit Placebo (n.s.); die leberbezogene Mortalität hingegen betrug 10,0% mit Silymarin vs. 17,3% mit Placebo (p = 0,01). **Schlussfolgerungen**: Aufgrund der vorliegenden Evidenz scheint die Verwendung von Silymarin sinnvoll bezüglich wahrscheinlichem Nutzen / möglicher Risiken bei der unterstützenden Behandlung von Vergiftungen mit *Amanita phalloides* und von (alkoholischer und Child 'A') Leberzirrhose. Ein systematisches Forschungsprogramm zur Konsolidierung der bestehenden Evidenzen und zur Erforschung neuer möglicher Anwendungen wäre wünschenswert.

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Introduction

Dating back to the time of the ancient Greeks (Theophrastus, 4th century B.C.) and Romans (Pliny the Elder, 1st century A.D.), the seeds of milk thistle (*Silybum marianum*, also known as St. Mary's thistle and lady's thistle), have been used to protect liver health. During the Middle Ages the seed of the milk thistle was also commonly used to treat liver diseases. Milk thistle grows up to 6 feet tall, particularly well on sunny slopes in Mediterranean countries, particularly Spain and Greece. The plant of the milk thistle blooms from June through August, and the shiny black seeds are harvested after the end of the summer to be used for medicinal purposes.

The potential benefit of silymarin (extracted from the seeds of *Silybum marianum*) in the treatment of liver diseases remains controversial [1]. However, the drug continues to elicit great interest in the scientific community, as reflected by more than 800 publications dealing with it, and it has a good safety record with only rare case reports of gastrointestinal disturbances and allergic skin rashes. The high prevalence of liver diseases such as chronic hepatitis and cirrhosis and the high costs of current effective treatments [2] emphasize the need for efficient and more cost-effective treatments.

The pharmacological data [3] show that silymarin possesses fairly specific effects on cell-regulating mechanisms, beyond the well known reactive oxygen species (ROS) scavenging properties confirmed in new studies indicating a potential to reduce toxic effects of other drugs (e.g. cisplatin, amiodarone). These data could lead to new and/or improved clinical applications of this drug although, in our view, there are not enough dose-response data available. There is consistent evidence that silymarin influences the regulation of cell membrane permeability either by inhibiting uptake, reducing cellular efflux or by stimulating it. Furthermore, silymarin has been shown to inhibit various systems such as leukotriene synthesis, the effects of tumour necrosis factor (TNF) α and of other autacoids. In some models, silymarin was shown to reduce the inducible nitric oxide synthetase-mediated production of nitric oxide and to modulate the inflammatory immune response already at low dose.

Although still unconfirmed, the interactions with P-glycoprotein, multidrug resistance associated protein 1 and breast cancer resistance protein, have attracted some interest since they may be beneficial for the reversal of multi-drug resistance in cancer, increasing the bioavailability and decreasing the clearance and possibly the toxicity of drugs. Less controversial are the dermatologic UV-protecting properties of silymarin which affords strong protection against UV-induced damage including photocarcinogenesis.

Milk thistle's clinical efficacy is not as well studied and established since the evidence is sometimes clouded by poor design and reporting. While possible benefit has been shown most frequently, but inconsistently, for aminotransferases, survival and other clinical outcomes have been studied less and with mixed results [4]. The primary objective of this systematic review is to assess the efficacy and safety of silymarin, mainly from a clinical point of view and taking into account clinically relevant end-points. It does not aim to replace future prospective trials which shall provide 'final' evidence of the efficacy of silymarin, but provide a quantified update due to meta-analysis of the 'status ubi'.

Methods

Search Strategy: Among the data sources consulted to identify trials for this systematic review were bibliographic databases (TOXLINE, MEDLINE, HealthSTAR, AIDSLINE, CANCERLIT, Embase, AMED, Cochrane Col., PubMed, TOXMAP, TOXLINE Special, DART Special, HSDB, IRIS, ITER, GENETOX, CHEMIDplus, Haz-Map), reference lists from pertinent review articles and books, personal contacts with experts active in the area and manufacturers up to February 2007. All papers were screened, and any dealing with prospective clinical trials were retained for classification according to criteria described in an earlier paper [5]. In the case of double publications, the authors retained those that were most recent and/or had appeared in a peer-reviewed journal. All trials rendered eligible were classified by indication and summarized in a tabulated format by one reviewer (R.B.). The standard table included a full reference, a quality rating, the type of pathology, demographic data and treatments, end-points and adverse events (AE). The trials were grouped by indication and by comparator. These tables were discussed and verified with the other authors until consensus was reached. No formal validation process was employed. The guidelines provided by the Cochrane Collaboration Handbook for Reviews [6] have been applied in the analysis of the clinical data. For the meta-analysis significances were calculated using 2-sided tests, the threshold of significance being $p \leq 0.05$ and for non-significance $p > 0.1$; values between $p > 0.05$ and $p \leq 0.1$ were reported as trends.

Results

We found 860 papers of which 53 (n = 16 from the past 5 years since our last review) also complied with the descriptor 'clinical trial', but only 19 were 'double-blind' (n = 11; 4 of them from the past 5 years) or 'single-blind' (n = 8). 12 additional clinical studies were identified through other channels. In the following the results are presented according to clinical features.

Pharmacokinetics of Silymarin

The bioavailability of silibinin (INN), deemed the main active flavonolignan of silymarin (milk thistle extracts from seeds), is low [7] and seems to depend on several factors such as: (a) the content of accompanying substances with a solubilizing character such as other flavonoids, phenolics, aminoacids, proteins, tocopherol, fat, cholesterol and others found in the extract, and (b) the concentration of the extract itself. The systemic bioavailability can be enhanced by adding solubilizing substances or carriers such as phosphatidylcholine and β-cyclodextrin and possibly by the choice of the capsule material [8–12]. The variations in content, dissolution and (oral) bioavailability of silibinin between different commercially available silymarin products are significant [13], in spite of the same declaration of content. Although systemic plasma levels are irrelevant if the site of action of silibinin is the liver, these are usually measured for accessibility reasons, as they should reflect the quantity of the drug being absorbed from the gastrointestinal tract. Adequate bioavailability accounts for dose-related oral activity of silymarin in the liver.

Silibinin was tested 'in vitro' for inhibition of human cytochrome P-450 enzymes in concentrations of 3.7–300 μM. Clear inhibition was only found for denitronifedipine oxidation (CYP3A4; IC_{50} = 29 μM) and S(-)-warfarin 7-hydroxylation (CYP2C9; IC_{50} = 43 μM) [14]. When additional substrate concentrations were tested to assess enzyme kinetics, silibinin was a potent competitive in-

hibitor of dextromethorphan metabolism at the low affinity site, which is not CYP2D6 (Ki,c = 2.3 μM and 2.4 μM). Inhibition was competitive for S(-)-warfarin 7-hydroxylation (Ki,c = 18 μM and 19 μM) and mainly non-competitive for denitronifedipine oxidation (Ki,n = 9 μM and 12 μM). Silibinin and its beta-glycosides did not interfere with the expression of CYP1A2 and CYP3A4 in human hepatocytes [15], and were considered unlikely to produce drug-drug interactions in terms of inducibility of these cytochromes.

With therapeutic silibinin peak plasma concentrations of 0.6 μM and biliary concentrations up to 200 μM it was concluded [14] that metabolic interactions with xenobiotics metabolised by CYP3A4 or CYP2C9 cannot be excluded, a view not shared by others [17]. No interactions have been described in humans so far; silymarin 160 mg tid (3 × daily) had no apparent effect on indinavir plasma concentrations, a potent inhibitor of CYP3A3/4 [18, 19] with the possible exception of a 25% decrease of mean trough levels [20]. In a recent study [21], 200 mg silymarin tid, for 14 consecutive days, did not affect the function of CYP3A4 and UGT1A1 in cancer patients treated with irinotecan. Following milk thistle administration (900 mg daily during 14 days) [22], digoxin AUC_{0-24} showed a small decrease (–9.4%; p = 0.06), thus suggesting that it is not a potent P-glycoprotein-modulator 'in vivo'. The administration of a single dose of silibinin does not modify the kinetics of alcohol [23].

In men, after single oral administration of a standardised dose of 100–360 mg silibinin, peak plasma levels were reached after approximately 2 h and ranged between 200 and 1,400 ng/ml silibinin, of which approximately 75% was presented in the conjugated form [24, 25]. For total silibinin, an elimination half-life of approximately 6 h is estimated [26]. 3–8% of an oral dose is excreted in the urine [27], while 20–40% is recovered from the bile as glucuronide and sulphate conjugates [28, 29]. The remaining part is excreted via faeces (unchanged, not absorbed). Silibinin levels in bile reach approximately 100 × higher concentrations than in serum (10^{-5} to 10^{-4} mol/l of silibinin in bile), with peak concentrations within 2–9 h. Biliary excretion continues for 24 h after a single dose. No accumulation is observed after multiple dosing. In cirrhotic patients, as compared to healthy volunteers, plasma levels (120 ng/ml after a dose of 360 mg silibinin) were somewhat lower and t_{max} (2.6 h) slightly delayed [30, 31]. The AUC suggest that extrahepatic biliary obstruction is associated with a reduced clearance of conjugated silibinin.

Poisoning with Amanita phalloides

The *Amanita phalloides* mushroom ('death cap') has been known and feared for at least 2 millennia and continues to cause serious illness and death. Of the many cytotoxins produced by mushrooms, the most important is the potent amanitin found in some mushrooms belonging to the genera *Amanita* and *Galerina*. Amanitin is a cyclic octapeptide which inhibits RNA polymerase II, thus interfering with protein synthesis. Phalloidin, a cyclic heptapeptide that accompanies amanitin and may interfere with actin polymerization, is probably responsible for the initial gastrointestinal symptoms. *Amanita phalloides* intoxications are not very frequent.

Symptoms of amanitin poisoning appear from 6–24 h after ingestion, starting with abdominal pain, followed by severe vomiting, diarrhoea and fever. After 1–2 days, the gastrointestinal symptoms abate but at this stage, serum levels of aminotransferases may begin to rise [32, 33]. Significant amounts of *Amanita* toxin can be detected in gastroduodenal fluid even at 48 h after ingestion, suggesting an enterohepatic circulation [34].

There have been no reported controlled trials of the various treatments suggested for mushroom poisoning. Toxins should be eliminated from the digestive tract as soon as possible by gastric lavage and subsequent administration of activated charcoal. Penicillin (300,000–1,000,000 U/kg/day) and silibinin (20–50 mg/kg/day) have been reported to be effective against amanitin poisoning. Anecdotal and animal studies also suggest a potential benefit of cimetidine, aucubin (an iridoid glycoside of *Aucuba japonica*), and kutkin. Haemodialysis has been used to treat acute renal failure, but this procedure does not remove the toxin, which is rapidly fixed in tissues [29].

Silibinin is considered to be the main active isomer of silymarin. Consequently, and for the purposes of completeness, the results obtained with intravenous silibinin in the therapy of mushroom poisoning are also briefly presented here. Pharmacologically, the use of silibinin appears to be justified as described in our publication on pharmacology [3]. On some occasions, oral silymarin has been used as a substitute for silibinin or for maintenance treatment [35].

In case series produced before 1970, mortality varied between 30 and 46% (mean 31%); in the period 1974–80, mortality varied between 0 and 26% (mean 11.4%). Detailed case control studies dealing with patients not treated with silibinin / silymarin, show an overall mortality of 18.3%, with a trend towards a decreasing mortality over time [33], and the most recent reports still show a mean mortality of 12.8% [36]. The entire series of case control studies with silibinin / silymarin show an overall mortality of 9.8%, falling to <8% if only the last 15 years are taken into account. An analysis based on 154 cases of intoxication with *Amanita phalloides* reported in Germany from 1983–1992 [37], showed a mortality of 15.2% in 38 not silibinin-treated cases vs. 8.3% in the remaining, silibinin-treated patients. However, the dose of *amanita*, time between ingestion and treatment and other factors might have been different. The daily dose of silibinin was ≤ 20 mg/kg day in 53.2% of the cases and up to 50 mg/kg day in 31.8% of the cases. Treatment duration was 4–5 days in most cases. Silibinin or silymarin, eventually combined with N-acetylcysteine, continue to represent the therapeutic mainstay in the therapy of the intoxications with *Amanita phalloides* [38].

Silymarin in Other Toxic and Iatrogenic Liver Diseases

In spite of the large number of animal pharmacology studies, the study-based clinical evidence of a therapeutic effect of silymarin in toxic liver diseases other than mushroom poisoning is scarce, mostly outdated and frequently of poor quality. More than 20 years ago, 2 open comparative studies examined silymarin in the treatment of liver diseases in workers exposed to hepatotoxic industrial solvents, describing improvement in liver function tests [39, 40].

Several studies dealt with iatrogenic liver diseases. 2 older trials have investigated whether silymarin could inhibit the liver enzyme increase observed after cholecystectomy in patients anaesthetized with either Halothane [41] or with Fentanyl [42]. While

the former was incompletely reported but nonetheless showed some inhibitory effect on the postsurgical aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increases, the latter was entirely excluded from further analysis due to lack of randomisation and incomplete reporting of data.
Other trials dealt with the prevention of hepatic disorders caused by neuroleptics. In 1 early trial [43], silymarin was associated with an improvement of mean AST and ALT values but the comparability of the two groups is questionable. A not significant reduction in the AST and ALT levels was reported [44] in female patients treated with phenothiazines or butirophenones with silymarin, but the malondialdehyde levels decreased by approximately 18% ($p < 0.001$).
Only 1 study [45] complies with modern standards of design and reporting. This 12-week randomised double-blind placebo-controlled study on 222 out-patients suffering from mild-to-moderate dementia of the Alzheimer type, was set up to assess the ability of silymarin to antagonise or prevent the hepatotoxic effects of tacrine. Silymarin was started first (1 week) and tacrine was then added at 40 mg/day for 6 weeks, then increased to 80 mg/day (6 weeks). Although no statistical difference was observed for serum ALT (the main evaluation criterion), unwanted effects and notably gastrointestinal complaints were less frequent in the silymarin group: the incidences of AEs rated as possibly treatment-related were: silymarin + tacrine = 9.5% vs. placebo + tacrine = 23.6% ($p = 0.01$). This study was too short to detect an eventual beneficial outcome on the dementia, as reported for other antioxidants [46].

Viral Hepatitis

All 5 known types of viral hepatitis (A to G) cannot be distinguished reliably by clinical features or routine laboratory tests. The specific aetiology of viral hepatitis is determined by serological testing. Chronic hepatitis does not occur after hepatitis A or E. It ensues in 1–5% of cases of acute hepatitis B virus (HBV) infection and 85% of cases of acute hepatitis C virus (HCV) infection [47].
In patients with chronic hepatitis B, treatment with interferon alpha (IFN-α) and the resulting loss of hepatitis Be antigen (HBeAg) from the blood, leads to a reduction in inflammatory activity and is associated with improved clinical outcome [48]. Recommended therapies include long-term treatment with lamivudine for chronic hepatitis B, treatment of hepatitis C and hepatitis D with IFN [49]. Treatment of HCV with IFN-α has been disappointing, but new therapies (e.g. pegylated IFN-α) and treatment schemes combining with ribavarin have led to an improved suppression of HCV RNA levels [50, 51]. Although silymarin is not known to affect viral replication, from a pharmacological perspective, it might be expected to inhibit the inflammatory and cytotoxic cascade of events triggered by the viral infection.
4 trials were conducted in the 1970s and are of historical interest only [52–55]. Lirussi and Okolicsanyi [56] compared silymarin and ursodeoxycholic acid (UDCA) in a rather heterogeneous population of patients with 'active cirrhosis', the majority of which were HCV-positive. No efficacy was shown in this study with inadequate reporting of statistics. More recently, a new silibinin complex (IdB1016 240 mg of silibinin 2 × daily) was studied in a short-term placebo-controlled pilot study on 20 patients with chronic active hepatitis, showing reduction of the AST concentrations but no consistent differences in the other liver function tests [57].
3 studies have been clearly negative. Huber et al. [58] retrospectively investigated the effects of silymarin on aminotransferase levels in 40 patients with chronic hepatitis C, not eligible for treatment with pegylated IFN and ribavirin, treated with 420 mg, 840 mg or 1,260 mg per day during 125 ± 78 days. Aminotransferase levels were determined before, during and at the end of treatment. ALT, AST and gamma glutamyltransferase (GGT) levels did not change significantly from baseline in any dose-group.
Recently, 2 double-blind trials evaluated silymarin for preventing complications of chronic HCV infection [59, 60]. In the first trial 177 consenting residents of an Egyptian village were included (Egypt has the highest prevalence of HCV infection in the world, averaging 15–25% in rural communities). They were randomly assigned to receive either silymarin (124.5 mg tid) or a low-dose multivitamin supplement deemed to be a placebo. Community nurses visited weekly to ascertain compliance, distribute supplements and record AEs. At 12 and at 24 months, almost all of 141 remaining subjects reported feeling better, although symptoms and quality of life (QoL) scores (Short Form 36 – SF 36) did not differ between the silymarin and the multivitamin group. Serum ALT elevations, serum hepatic fibrosis marker (hyaluronic acid and YKL-40, a growth factor participating in inflammation and remodelling of the extracellular matrix), and abdominal ultrasound results were similar in both groups and the latter may have progressed slightly.
El-Zayadi et al. [61] studied 170 naive hepatitis C patients, also in Egypt, with elevated ALT (>1.5-fold) and detectable HCV which could not afford IFN-based therapy. They were randomly allocated to either group I (n = 87; biopsy proved chronic hepatitis in 62) who were administered a daily combination of ribavirin (600–800 mg) plus amantadine (200 mg) and UDCA (500 mg) for 24 weeks, or to group II (n = 83) who were administered silymarin 450 mg/day for 24 weeks. Normalization of ALT at the end of treatment was achieved in 58.5 and 15.3% of the patients, whereas end-of-treatment virologic response (ETVR) was achieved in 2.4 and 0% of group I and II, respectively. While this study was not placebo-controlled, it confirmed earlier trials in that little or no benefit was afforded by the silymarin treatment in hepatitis C.
In a small double-blind, placebo-controlled, crossover pilot trial reported by Gordon et al. [62], patients (n = 24, completed n = 17) received 12 weeks of *S. marianum* (either 600 mg or 1,200 mg/day) and placebo separated by a 4-week washout interval. *S. marianum* was well tolerated in subjects with chronic hepatitis C, but did not significantly affect serum HCV RNA, ALT levels, QoL or psychological well-being (SF-36, State-Trait Anxiety Inventory) of the patients.

Alcoholic Liver Disease

The available data [63] show the induction of free radicals by ethanol to be a complex interactive process. The classical pathway for ethanol metabolism, catalyzed by alcohol dehydrogenase to form acetaldehyde, leads to the formation of free radicals, resulting from concomitant changes in reduced nicotinamide adenine dinucleotide (NADH) levels and NADH/NAD+ redox ra-

Table 1. Weighted mean differences (WMD) in AST levels at the end of trials (WMD, random model) dealing with alcoholic liver disease or cirrhosis

First author	Disease studied	Treatment		Control		Weight	WMD	95% CI
		n	mean (sd)	n	mean (sd)			
Pares	Cirrhosis	96	58.00 (37.00)	89	50.00 (34.00)	3.69	8	–2.23, 18.23
Bunout	Cirrhosis	34	31.91 (9.20)	37	25.40 (6.60)	27.45	6.51	2.76, 10.26
DiMario	Alcoh. liv. dis.	15	25.40 (15.60)	14	50.60 (24.30)	1.72	–25.2	–40.18, –10.22
Feher	Alcoh. liv. dis.	17	22.80 (5.10)	19	31.30 (4.50)	38.78	–8.5	–11.66, –5.34
Muzes	Alcoh. liv. dis.	10	28.00 (11.00)	20	52.00 (13.00)	4.9	–24	–32.88, –15.12
Salmi	Alcoh. liv. dis.	47	38.20 (15.99)	50	51.20 (24.99)	5.61	–13	–21.30, –4.70
Trinchet	Cirrhosis & Alcoh. liv.dis.	57	57.00 (15.00)	59	53.00 (10.00)	17.84	4	–0.66, 8.66
Total		276		288		100	–2.84	–4.81, –0.87

Test for heterogeneity: $Chi^2 = 84.89$, df = 6 ($p < 0.00001$), $I^2 = 92.9\%$.
Test for overall effect: Z = 2.83 (p = 0.12).
For fixed effect model, test for overall effect: Z = 2.83 (p = 0.005).
After excluding cirrhosis only trials (Bunout and Pares):
Test for heterogeneity: $Chi^2 = 44.04$, df = 4 ($p < 0.00001$), $I^2 = 90.9\%$.
Test for overall effect: Z = 2.52 (p = 0.01).
For fixed effect model, test for overall effect: Z = 5.91 ($p < 0.00001$).

Table 2. AP levels at the end of trials (WMD, random model) dealing with alcoholic liver disease or cirrhosis

First author	Disease studied	Treatment		Control		Weight	WMD	95% CI
		n	mean (sd)	n	mean (sd)			
Pares	Cirrhosis	96	206.00 (116.00)	89	189.00 (124.00)	19.8	17	–17.67, 51.67
Bunout	Cirrhosis	34	207.00 (14.00)	37	196.00 (9.40)	34.53	11	5.40, 16.60
DiMario	Alc. liv. dis.	15	145.00 (58.00)	14	177.00 (78.00)	13.34	–32	–82.31, 18.31
Feher	Alc. liv. dis.	17	144.00 (17.00)	19	164.00 (19.00)	32.33	–20	–31.76, -8.24
Total		165		162		100	–3.57	–26.87, 19.73

Test for heterogeneity: $Chi^2 = 24.31$, df = 3 ($p < 0.0001$), $I^2 = 87.7\ \%$.
Test for overall effect: Z = 0.30 (p = 0.76).

tios, which in turn modulate the activity of the free radical generating enzyme xanthine oxidase. The induction of CYP2E1 in the microsomes leads to the generation of hydroxyethyl radicals, another major route by which ethanol induces free radical formation. In addition to the above, ethanol may also induce free radical formation via the reaction of aldehyde oxidase with acetaldehyde or NADH to generate oxyradicals via disturbance in the metabolism of the pro-oxidant iron, or via increased efflux from mitochondria following altered mitochondrial oxidative metabolism. Likewise, pancreatic stellate cells are activated 'in vitro' on exposure to ethanol with generation of oxidant stress within the cells [64]. However, until consistent evidence of a relation between the induction of free radicals and the prognosis of these patients is available, thus eventually justifying their use as surrogate end-points, the clinician will be forced to continue using clinical predictors of outcome and classical end-points when evaluating the therapeutic value of a treatment. Clinical and laboratory features are powerful prognostic indicators for short-term mortality. Hepatic encephalopathy, derangement in renal function, hyperbilirubinaemia, and prolonged prothrombin time are seen more frequently in patients who succumb to their illness than in those who survive. Long-term survival in patients with alcoholic hepatitis who discontinue alcohol is significantly better than in those who continue to drink, although it remains considerably below that of an age-matched population. 3-year survival approaches 90% in abstainers, whereas it is <70% in active drinkers [65].

In a double-blind study on patients with chronic alcoholic liver disease [66], a 6-month treatment (at a daily dose of 420 mg) with silymarin significantly restored the superoxide dismutase activity of erythrocytes and lymphocytes, the serum level of free-SH groups and the activity of glutathione peroxidase.

Silymarin has been studied most exhaustively in alcohol-induced liver diseases with the largest number of studies and patients. Such diseases have been classified, somewhat arbitrarily, for the purposes of this review, into 2 main chapters: (1) alcohol-induced liver diseases (in principle excluding cirrhosis) with mainly biochemical end-points, (2) liver cirrhosis (mostly alcohol-induced) with clinical end-points as primary criteria.

Alcohol-Induced Liver Diseases

Fintelmann et al. [67] published the first double-blind placebo-controlled study with silymarin in alcohol-induced liver disease. There was no diagnostic work-up and the reporting did not comply with modern criteria. The graphics presented in that paper show significant differences in favour of the active therapy at day 28 on AST and ALT, but not significant on GGT nor alkaline phosphatase (AP). A second study in 1981 reported that the symptoms of dyspepsia, asthenia, anorexia and nausea were all significantly improved with silymarin, while only dyspepsia im-

proved significantly with placebo [68]. In that study, there was a significant difference in favour of the silymarin-treated group at the end of treatment with regard to AST and ALT, but not with regard to total bilirubin, AP, cholesterolaemia and albuminaemia. Prothrombin time was 91.7 ± 8.4% in the silymarin-treated group vs. 80.6 ± 29.2% in the placebo group (not significant, n.s.).

In what is probably the best study in this chapter from a qualitative perspective, Salmi and Sarna [69] studied 106 patients with mild acute and subacute liver disease, of which 90 had histological diagnosis. They were selected on the basis of elevated serum transaminase levels. Alcohol was forbidden during the trial. The patients were randomly allocated into a group treated with silymarin and a group receiving placebo. A significantly larger decrease of ALT and AST was noted in the verum group vs. controls. Serum total and conjugated bilirubin did not show differences. In the sub-populations examined, bromosulphalein (BSP) retention test and histological changes returned to normal more frequently with silymarin ($p = 0.035$ and $p = 0.022$, respectively).

The effects of silymarin therapy on liver function tests, serum procollagen III peptide level and liver histology were studied in 36 patients with chronic alcoholic liver disease in a 6-month double-blind clinical trial [70]. During silymarin treatment, serum bilirubin, AST and ALT values normalised and GGT activity and procollagen III peptid level decreased. The differences between the 2 groups were significant at the end of the observation period. At the end of treatment, no differences on total protein, albumin, or globulin were found between groups; plasmatic prothrombin levels were 95 ± 4% in the silymarin-treated group and 86 ± 4% in the placebo-treated group (n.s.). Regrettably, this publication did not provide adequate reporting of study flow, dropouts or AEs.

The pooled analysis of the studies briefly outlined above can be summarized as follows:

Although there were no clinical end-points, histological findings were reported as improved in 2 out of 2 trials but only a part of the patients had control histology. BSP retention test is reported in 1 trial as significantly better after silymarin.

The improvement of prothrombin time becomes significant if trials are combined (weighted mean at end of treatment, silymarin 92.3 ± 5.2% vs. placebo 84.3 ± 10.7%; 2 trials).

After treatment, the enzyme levels were heterogeneous between trials, AST was lower in the silymarin-treated groups ($p = 0.01$) while AP was not (tables 1, 2).

The evolution of ALT, GGT and of bilirubin values was not assessable due to differences at baseline.

2 major open trials have been reported [71, 72]; they may help to complete the picture from the perspective of daily practice, particularly concerning the rates of disappearance of symptoms such as tiredness (52.1%), epigastric discomfort (59.6%), lack of appetite (67.6%), nausea (78.6%) or pruritus (72.3%). In a small trial [73], 30 patients affected by chronic ethylic hepatopathy were treated with 450 mg/daily of UDCA; after 6 months, a significant decrease of serum hepatic enzymes was noted. The addition of silymarin (400 mg/daily) to UDCA in other 30 patients induced a further improvement of hepatic function.

Liver Cirrhosis

Liver cirrhosis is the relatively frequent final common pathology of non-alcoholic or alcoholic fatty liver disease and chronic hepatitis (HBV, HCV). The treatments for the underlying causes are of limited efficacy. In the case of chronic HBV, IFN is the preferred treatment in patients with well-compensated cirrhosis (Child's A and B) while lamivudine is a safer therapy for patients with more advanced cirrhosis [74].

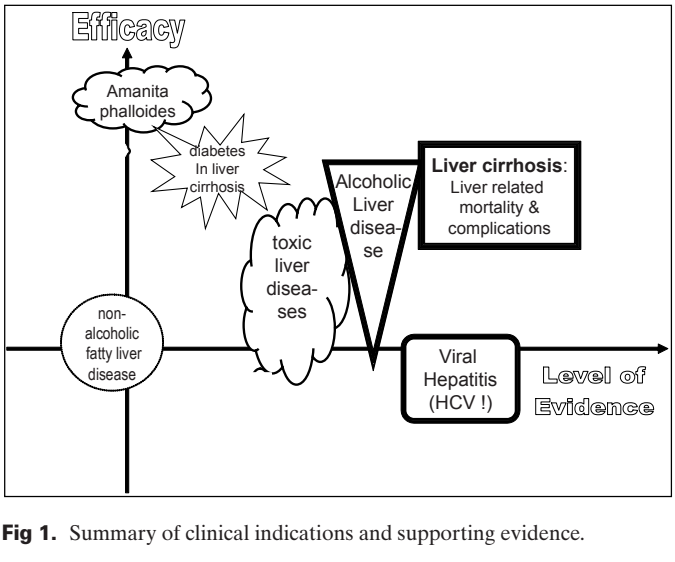

Fig 1. Summary of clinical indications and supporting evidence.

Tools that have been proven useful in the prediction of survival in patients with chronic liver disease include the Child-Pugh classification and the degree of ascites [75]; that is, patients with ascites unresponsive to diuretics have <50% chance of surviving 1 year without transplantation [76].

Clinical trials in liver cirrhosis are complicated by a series of confounding factors which may make it difficult to show an effect. The following factors seem particularly relevant: (1) persistence of 'offending' factors (e.g. alcoholism, viral infection, etc.), (2) concomitant therapies such as supportive treatments or corticosteroids, (3) compliance of the patients (known to be a major problem in alcoholics), etc.

The pharmacological rationale for using silymarin in this indication(s) is fairly broad. The inhibition of the 5-lipoxygenase pathway, particularly leukotriene B4 (LTB_4), the ROS scavenging properties of the compound, the inhibition of activation of NF-κB, kinases and caspases may explain its role in cytoprotection. In 1 placebo-controlled study [77] with patients suffering from alcoholic cirrhosis (n = 60), silymarin (150 mg tid) increased total Glutathione (GSH) at 6 months by 29% ($p < 0.001$) and platelet-derived non-induced malondialdehyde decreased by 33% ($p < 0.015$). A parallel decrease in N-terminal propeptide of type III collagen values (PIIINP, which has been investigated for assessing and monitoring fibrotic processes in liver diseases, thus avoiding the need for liver biopsies) was seen with silymarin, but values were not comparable at baseline. There were no concurrent changes on laboratory indices of the pathology, which makes the assessment of the relevance of these findings somewhat difficult.

The study by Ferenci et al. [78] was performed to determine the effect of silymarin on the outcome of patients with cirrhosis. This randomised controlled trial (RCT) was performed on 170 patients with cirrhosis of which 87 were treated with 140 mg silymarin tid (alcoholic: 46, non-alcoholic: 41) and 83 received a placebo (alcoholic: 45, non-alcoholic: 38). The mean observation period was 41 months. There were 10 dropouts in the placebo group and 14 in the treatment group (mostly non-compliance). In the placebo group, 37 (+2 dropouts) patients had died; 31 of these deaths were

Table 3. Total mortality and liver-related mortality in trials with patients with cirrhosis taking silymarin ((Spalten bitte dezimal ausrichten am Punkt))

Author [ref]	Child A/B/C Distrib. (%)	Silymarin (mg/day)	Continued alcohol (% of patients)		Age, years (mean ± sd)	Duration months	Patients (n total)	Total mortality (%)			Liver-related mortality (%)		
			silymarin	placebo				silymarin	placebo	sign.	silymarin	placebo	sign.
Ferenci [78]	52 / 41 / 7	420	30	40	58 ± 12	24	170	32.20	47.00	0.056	18.40	37.30	0.007
Trinchet [79]	50 / 0 / 0*	420	50	51	51 ± 10	3	116	1.80	5.10	n.s.	1.80	5.10	n.s.
Bunout [80]	No data	280	58	65	49 ± 3	15	71	14.70	13.50	n.s.	13.20	12.20	n.s.
Velussi [81]	100 / 0 / 0	600	0	0	63 ± 4	12	60	0.00	0.00	–	0.00	0.00	–
Pares [82]	33 / 60 / 7	450	38	29	50 ± 10	24	185	15.60	15.70	n.s.	9.40	14.60	n.s.
Total	44 / 30 / 4	280–600	37	38	54 ± 9	3–24	602	16.10	20.50	n.s.	10.00	17.30	0.01
OR silymarin : placebo (95% CI)								0.75 (0.49, 1.13)			0.53 (0.33, 0.86)		

*50% Alcoholic hepatitis without cirrhosis.

related to liver disease. In the treatment group, 24 (+4 dropouts) had died, and 18 of these deaths were related to liver disease. The 4-year survival rate was 58% in silymarin-treated patients and 39% in the placebo group (p = 0.036). Analysis of subgroups indicated that treatment was effective in patients with alcoholic cirrhosis (p = 0.01) and in patients initially rated 'Child A' (p = 0.03).

Trinchet et al. [79] conducted a randomised double-blind trial of silymarin vs. placebo in 116 patients with histologically proven alcoholic hepatitis, 58 of them with cirrhosis. 57 patients received silymarin orally 420 mg/day and 59 received placebo during 3 months. Biological parameters were assessed in the serum and a percutaneous liver biopsy was obtained both at the start of the trial and, in some of the patients, after 3 months. The 2 therapeutic groups were comparable at inclusion. 26% of patients were lost to follow-up at 3 months. Abstinence was obtained in 46% of patients at the end of the trial. 4 patients died during the trial, all of hepatic failure, 1 under silymarin vs. 3 in the placebo group (not specified whether having alcoholic hepatitis or cirrhosis, therefore the entire population was included in the meta-analysis). Significant improvement in the score of alcoholic hepatitis and serum aminotransferase activity was noted in both groups during the trial, irrespective of treatment with silymarin or placebo but clearly correlated with abstention from alcohol. The authors concluded that 'silymarin 420 mg/d is not clinically relevant in the treatment of moderate alcoholic hepatitis'.

Bunout et al. [80] conducted a controlled trial on the use of silymarin in patients with alcoholic cirrhosis. 71 patients were admitted to the trial and randomly assigned to an experimental or control group. The verum group (n = 34) received a relatively low dose of 280 mg/day of silymarin, controls (n = 37) received an equal number of placebo tablets. Both groups did not differ in their initial laboratory assessment and were followed up for an average of 15 months. 10 patients died during the follow up (5 in placebo and 5 in silymarin; liver-related death in 9). No details concerning distribution by therapeutic group are provided. No significant differences were observed between these 2 groups. It was concluded that silymarin did not change the evolution or mortality of alcoholic liver disease in this trial.

The trial by Velussi et al. [81] is a 12-month open, controlled study which was conducted in 2 well-matched groups of insulin-treated diabetics with alcoholic cirrhosis, abstaining from alcohol for at least 2 years prior to the study (randomisation not specified). 1 group received 600 mg silymarin per day plus standard therapy, while the control group received standard therapy alone. Since the authors, in addition to the metabolic variables which shall be discussed in more detail under complications of cirrhosis, also reported about deaths and other clinical outcomes, it was decided to incorporate this trial in the meta-analysis.

The double-blind multi-centre RCT by Pares et al. [82] specifically aimed at determining the effect of silymarin (150 mg tid) in alcoholics with liver cirrhosis with respect to survival and clinical and laboratory changes. They enrolled 200 alcoholics with histologically (n = 191) or laparoscopically (silymarin = 6 cases, placebo = 3 cases) proven liver cirrhosis. The primary outcome was time to death, and the secondary outcome was the progression of liver failure. Additional analyses identified 75 patients with HCV antibodies. 103 patients were assigned to receive silymarin and 97 to receive placebo. The 2 groups were well matched for demographic and baseline clinical and laboratory features. There were 185 assessable patients and 125 (silymarin = 57, placebo = 68) completed a 2-year study period. 42 patients left the trial during the 2-year period (silymarin = 29, placebo = 13; mostly non-compliance, voluntary withdrawal; p < 0.01). 29 patients (silymarin = 15, placebo = 14) died during the trial. Survival was not influenced by sex, the persistence of alcohol intake, the severity of liver dysfunction or by the presence of alcoholic hepatitis in the liver biopsy. The authors also describe the frequency of complications at the end of the observation period which was smaller in the silymarin than in the placebo group (p = 0.06). The frequency of encephalopathy was not significantly lower in the silymarin-treated population.

1 small open trial [83] examined silymarin in patients with primary biliary cirrhosis and a suboptimal response to ursodeoxycholic acid (UDCA). In 27 patients, additional oral silymarin (140 mg tid) was given for 1 year. No significant changes in serum enzymes nor the Mayo risk score were noted with the combination therapy.

From these summaries, it is clear that only 2 of these 5 trials were proper RCTs primarily set up to study mortality rates (Ferenci et al. [78], Pares et al. [82]). The study by Bunout et al. [80] had the same end-point but a low dose was used and it did not state if it was properly blinded. The study by Trinchet [79] is relatively short and half the studied population was not classified as cirrhotic; since the deaths were not allocated to a diagnostic group, the entire group of patients was considered 'at risk'. The study by Velussi et al. [81] was retained in spite of being an open trial because

the 2 groups of diabetic patients were well balanced and, according to the authors, had to abstain from alcohol for at least 2 years prior to the study in order to be eligible. Note that there were no deaths recorded in this study.

Total Mortality

5 placebo RCTs in cirrhosis conducted with silymarin adequately reported about mortality and, to an extent, about other details of clinical interest (total: 602 patients; table 3). The dropout rates were significantly higher in the silymarin-treated population (21.3% vs. 14.3% with placebo, $p < 0.05$) mostly due to withdrawal of study consent in both groups. Total mortality was lower in the silymarin-treated patients in 2 out of 5 trials, similar in 2, with no mortality reported in the 5th trial. The overall difference of –4.2% deaths (all trials pooled as reported, without correction for duration of trial) did not attain the threshold of significance. Other variables, such as percentage of patients abstaining from alcohol may have influenced the outcomes but most of the trials considered did not report results by covariates.

Liver-Related Mortality

In the studies analysed, the usual cause of 'liver-related death' as defined by Khan et al. [84] was upper gastrointestinal bleeding (UGB), hepatic failure or primary liver cell carcinoma. No deaths were specifically attributed to spontaneous bacterial peritonitis (SBP). 3 out of 5 trials reported a lower liver-related mortality with the active treatment. Among the remaining studies, in the trial by Bunout et al. [80], 9 out of 10 deaths were liver-related without attribution to a therapeutic group, while there were no deaths in the trial by Velussi et al. [81]. The pooled liver-related mortality per year was 4.9% with silymarin and 9.3% with placebo. The overall liver-related mortality as reported in the trials (without correction for duration) was 10% with silymarin and 17.3% with placebo, i.e. a reduction of 7.3% ($p = 0.01$). Sensitivity analysis by random elimination of trials showed that the result was not driven by any particular trial, but significance was dependent on the study by Ferenci et al. [75] which contributed the largest number of deaths.

The overall (not corrected for trial duration) non-liver related mortality was somewhat, but not significantly, higher in the silymarin-treated population (6.7%) than in the placebo-treated population (3.4%); this trend is mainly driven by the study of Pares et al. [82].

Rates of Complications During Trials

None of the studies on cirrhosis carried out with silymarin considered clinical outcomes other than death as a primary end-point. Based on the available data, several issues could be partially examined in the above mentioned studies. Different issues were usually addressed in different trials; although these issues are interrelated, they have to be regarded individually and cannot be consolidated into one global picture.

Hospitalisations: Data about the need for hospitalisation during the study were available from 3 studies. The following hospitalisations were rated as cirrhosis-related: (UGB), hepatic failure, hepatic encephalopathy, ascites, SBP or primary liver cell carcinoma. Non-related hospitalisations were not considered; that is, hospitalisations after trauma or for hernial surgery. Most of the hospitalisations were for sclerotherapy due to oesophageal varices. 1 trial [78] reported no hospitalisations, and the 2 remaining ones showed fewer hospitalisations in the silymarin-treated population, i.e. 25.0% of the silymarin-treated patients vs. 31.9% of the placebo patients [80], and 6.3% vs. 13.5% [82], respectively. The overall percentage of patients ever hospitalised was 10.0% for silymarin vs. 16.9% for placebo ($p = 0.086$).

UGB: Bleeding oesophageal varices constitute one of the most serious complications of cirrhosis. Many factors associated with decompensated cirrhosis increase the risk of UGB, including general debility, coagulation defects and hepatic encephalopathy; the size of the varix is also correlated with the risk of bleeding [85]. The total incidence of UGBs, both as a co-factor of death and as 'last data', was reported in the 2 largest trials: 4.6% with silymarin vs. 9.6% with placebo [78], and 6.3% vs. 13.5% [82], respectively, and showed a difference in favour of the active treatment ($p = 0.042$; Odds Ratio (OR) 0.44 (95% CI 0.20, 0.97)). However, the number of patients having one or more episodes of UGB during the study without fatal outcome in [82] was similar in the 2 groups (placebo 7 cases, silymarin 5 cases). This finding suggests that the decreased rate of UGBs reflects an overall improvement in the patients, also evidenced by the lower liver-related mortality rate, rather than being a direct (vascular) effect of silymarin.

Hepatocellular Carcinoma (HCC): HCC has not been investigated systematically in any of the trials, but in 2 trials it has been reported as cause of death, and/or found at biopsy or at autopsy (silymarin = 3.1%, placebo = 4.5% [82]; silymarin = 3.4%, placebo = 7.1% [78]). Since in the latter study a similar number of patients were autopsied in both groups, it seemed reasonable to pool and compare the data with a descriptive purpose. Interestingly, in both studies the incidence of HCC was lower in the silymarin-treated population.

Diabetes Mellitus in Patients with Chronic Liver Disease

The risk of diabetes is increased in patients with liver cirrhosis due to hepatitis C or alcoholic liver disease [86]. Glucose intolerance in cirrhosis results from 2 abnormalities that occur simultaneously: (1) insulin resistance of muscle and (2) an inadequate response of the β-cells to appropriately secrete insulin in order to overcome the defect in insulin action [87]. The prevalence of diabetes mellitus has been reported to be higher in HCV-related cirrhosis (23.6%) than in HBV-related [88] cirrhosis (9.4%). The prevalence of diabetes mellitus is also closely associated with the Child-Pugh score, increasing age and obesity [89]. However, the cardiovascular and retinopathy risk is low in these patients [90].

The aim of the study by Velussi et al. [81] was to ascertain whether long-term treatment with silymarin is effective in reducing lipoperoxidation and insulin resistance in diabetic patients with cirrhosis. The efficacy parameters included fasting blood glucose levels (at admission: 190 ± 14 mg/dl), mean daily blood glucose levels (at admission: 202 ± 19 mg/dl), daily glucosuria levels (at admission: 37 ± 12 g/day), glycosylated haemoglobin (HbA1c; at admission: 7.9 ± 0.3%) and malondialdehyde levels (at admission: 2.2 ± 0.3 μmol/ml). There was a significant decrease in fasting blood glucose levels and in fasting insulin levels (–40%) already after 4 months of treatment in the silymarin group, also seen for mean daily blood glucose levels (–14.6%) and daily glucosuria (–32%). However, the HbA1c levels at the end of the 12 months had diminished by 8.8%. In addition, there was a significant decrease in the mean exogenous insulin requirements in the treated group from 55 ± 5 IU/day to 45 ± 3 IU/day after 6 months and 42 ± 2 IU/day after 12 months, while the untreated group

Table 4. Summary of AEs by seriousness, as reported in the published clinical trials

	Controlled trials		Silymarin, open studies
	silymarin	placebo	
Total number of patients	296	297	3612
Deaths, related to underlying disease, %	7.09	7.41	0.19
Interrupted trial (excluding deaths), %	0.68	0.67	0.17
Total patients with AEs, %	2.36	5.05	1.02

showed a significant increase in fasting insulin levels and a stabilized insulin requirement.
Similar results were reported by Lirussi et al. [91] with silybin-beta-cyclodextrin (135 mg/day silibinin per os) in a placebo-controlled, 6-month trial (admitted 60, finalized 42 patients). Fasting blood glucose levels, which were similar at baseline in the silybin-beta-cyclodextrin and in the placebo group decreased by –14.7% vs. baseline in the silybin-beta-cyclodextrin group ($p = 0.03$ vs. placebo). The same trend was observed in mean daily blood glucose levels, HbA1c and insulin sensitivity (HOMA-IR), although differences were not significant. Insulin secretion was virtually unaffected. Plasma triglycerides concentrations dropped by 40% in the silybin-beta-cyclodextrin group while they increased by 16% in the placebo group, while total and HDL cholesterol as well as liver function tests did not change significantly. However, the weight of this study was limited by the small number of patients participating, the large proportion of dropouts and the absence of an 'intent to treat' analysis.
One small open trial [92] suggests similar effects in non-alcoholic fatty liver disease with a silybin + vitamin E + phospholipids preparation by improving insulin resistance and plasma levels of markers of liver fibrosis.

Planned and Ongoing Trials
Several of the various leads derived from experimental pharmacology also have induced clinical trials, some of which have been mentioned as planned in the literature: (1) pilot study of silymarin in patients receiving hepatotoxic chemotherapy (National Cancer Institute, NCI, USA, http://cancer.gov/search/viewclinicaltrials); (2) study of botanical/drug interactions in HIV (National Center for Complementary and Alternative Medicine, NCCAM; USA), (3) IdB 1016 treatment for hepatitis C disease (NCCAM; USA), (4) prevention of the serological recurrence of ovarian cancer with IdB 1016 (Catholic University of the Sacred Heart; Rome, Italy), (5) silibinin in phase I clinical trial in prostate cancer patients (University of Colorado Cancer Center; USA). Furthermore, in preparation, the silymarin Product Development Program and the silymarin Research Network (NCCAM; and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) and of the Department of Health and Human Services (DHHS); USA).

Safety Data
The safety data of silymarin have been reviewed based on data derived from clinical trials adequately reporting on safety providing a full list of AEs reported by the patients [55, 65, 66, 68, 69, 76–79] or from AEs spontaneously reported in the literature [93]. The safety of silymarin has been analysed 'in extenso' in an earlier paper [3] and will only merit a short chapter here (table 4).
In the clinical trials, neither deaths nor other serious AEs were attributed to the therapy, and the incidence was slightly lower in the silymarin than in the placebo-treated population. The incidence of AEs justifying an interruption of treatment and of AEs in general was very low and within the range of placebo values. In comparative studies the side effects with an incidence of ≥1% were headaches (silymarin 1.01%; placebo 1.35%) and pruritus (silymarin 1.35%; placebo 3.70%). The side effects reported in open silymarin trials showed a predominance of digestive symptoms (diarrhoea 0.20%, irregular stools 0.10%, nausea 0.13%, dyspepsia 0.08%).
The spontaneously reported cases provided little information concerning incidences of AEs but gave a better picture of rare and usually more severe AEs. There were neither deaths nor life-threatening events. Only 1 case deemed serious or potentially serious was probably related to silymarin, i.e. a woman aged 57 years with intermittent episodes of sweating, nausea, colicky pain, diarrhoea, vomiting, weakness and collapse was admitted to hospital during 1 day; clinical examination and laboratory values within normal range; rechallenge a few weeks later reproduced symptoms (product: Aust L 56929; Optimum Healthcare Pty Ltd). The authors considered that some component other than silibinin could have been responsible. In 2 cases of urticaria / pruritus a positive rechallenge was reported.

Discussion and Conclusions

The biopharmaceutical problems related to silymarin-containing products are very important, resulting in a wide range of plasma levels following one same dose of different pharmaceutical products. A large proportion of the studies analyzed in this review were performed with one well-standardised product (Legalon™) but the results cannot be extrapolated to any silymarin-containing preparation without checking the bioavailability data. Neither optimal nor maximal therapeutic doses have been defined so far and, in view of the experimental data, they are likely to be indication dependent. In most of the trials, the reportedly effective daily doses of silymarin were 420–600 mg (range: 280 mg/day, 'not effective' [80]; and 800 mg, 'effective' [44]. The experimental and clinical evidence concerning the hepatologic indications of silymarin are summarised in figure 1 and have also been recently discussed in detail by a group of US experts [94].
In the opinion of the reviewers, the limited available evidence still supports the use of silymarin in *Amanita phalloides* poisoning (silibinin hemisuccinate i.v.). The reviewers were unable to draw a valid conclusion on the use of silymarin in the therapy of other toxic and iatrogenic liver diseases often associated with, or aggravated by, an alcoholic problem. However, 'no evidence of effect' is

not 'evidence of no effect' [3]. The conclusions of the different authors are favourable to the drug and (in view of its excellent safety profile) the reviewers conclude that, for the time being, the treating physician may consider the use of silymarin as part of a treatment scheme in case of poisoning with drugs inducing lipid peroxidation.

The situation is similar concerning its use in the therapy of acute or chronic viral hepatitis, frequently a cause of cirrhosis, although the negative results in hepatitis-C patients in Egypt are not necessarily representative for Western countries (differences in nutrition and absence of alcoholic intake). 'There is no evidence that silymarin affects viral load or improves liver histology in hepatitis B or C. No studies were found that investigated the use of silymarin concomitantly with interferon, nucleoside analogues, or other conventional treatments for hepatitis B or C. It may be worthwhile to determine its effects in conjunction with standard antiviral treatment' [95]. Interestingly, it has been described in the literature that in viral hepatitis, enzymatic improvements (particularly of ALT) which were the main end-points in some of the silymarin trials, tend to under-reflect the degree of histological improvement [96].

Although more data are needed, it seems reasonable to employ silymarin in the therapy of alcoholic liver disease, obviously as a supportive element in the broader context of the management of the alcoholic patient, especially after discontinuation of alcohol intake. In the opinion of the reviewers, the available evidence supports the therapeutic use of silymarin in alcohol-induced liver diseases, including liver cirrhosis. The limited evidence available does not support its use in primary biliary cirrhosis.

The selection of cirrhosis studies retained herein, even if not fully correct from a methodological perspective, probably has the advantage of reflecting different settings, thus rendering it closer to clinical reality; that is, increasing the external validity of the analysis. In the trials dealing with (alcoholic) liver cirrhosis, while the reduction in total mortality does not reach the threshold of significance (reduced –4.2%, OR 0.75 (95% CI 0.5, 1.1)), the reduction of liver-related mortality (i.e. due to hepatic failure and/or upper digestive haemorrhage) is significant (reduced –7%, OR 0.54 (95% CI 0.3, 0.9)). Consistent with this findings, a recent Cochrane review [97] stated that 'liver-related mortality was significantly reduced by milk thistle in all trials (Risk Reduction (RR) 0.50, 95% CI 0.29, 0.88), but not in high-quality trials (RR 0.57, 95% CI 0.28, 1.19)'.

Some secondary end-points also yielded results in favour of the silymarin therapy, such as number of patients requiring hospitalisation during the trial, number of patients with UGB, and possibly number of patients with hepatic carcinomas. Obviously, these secondary variables are interwoven to some extent with the primary variable and may help to explain the reduction in liver-related mortality. Furthermore, they are likely to have an impact on the QoL and use of medical resources by cirrhotic patients. Future prospective trials should address these issues as well as other confounding factors such as persistence of 'offending' factors, doses and characterisation of silymarin preparation administered, concomitant therapies or interventions and, last but not least, the compliance of the patients. The reviewers conclude that, from a pragmatic point of view and with the available evidence of possible risks/probable benefits, it is reasonable to employ silymarin as a supportive element in the therapy of (alcoholic and grade Child 'A') liver cirrhosis.

The improvement in the management of cirrhosis associated diabetes reported by 2 groups (in 2 relatively small trials) was most interesting and deserves to be confirmed in a larger context. It is, however, somewhat disturbing that the improvement of the glycosylated HbA1c levels vs. controls was very modest and did not reach the threshold of significance vs. controls.

Concerning the practical consequences of a meta-analysis, it is clear that 'the clinical application of results from meta-analyses to the individual patient often remains a difficult matter of judgment' [98]. The limited positive and negative predictive value of meta-analysis should be taken into consideration, e.g. in a large analysis of the matter, the agreement between the meta-analyses and large clinical trials was only fair [99]. The positive predictive value of the meta-analyses was 68%, and the negative predictive value 67%. However, the difference in point estimates between the randomised trials and the meta-analyses was statistically significant for only 5 of the 40 comparisons (i.e. 12%).

The analysis of the safety data available on silymarin confirms that this compound is very safe. Rare cases of digestive troubles and of allergic skin rashes have been reported. In the case of treatment with high doses of silymarin in polymedicated patients, the treating physician should be aware of the hypothetical possible interactions.

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