7 July 2015
EMA/HMPC/294188/2013
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Silybum marianum* (L.) Gaertn., fructus

Draft

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Silybum marianum* (L.) Gaertn., fructus
Herbal preparation(s)	Dry extract (DER 36-44:1), (extraction solvent: ethyl acetate) standardised to contain 40-65% silymarin, calculated as silibinin Comminuted herbal substance Powdered herbal substance Dry extract (DER 20-70:1), extraction solvent acetone 95% (V/V) Dry extract (DER 30-40:1), extraction solvent ethanol 96% (V/V) Dry extract (DER 20-35:1), extraction solvent ethyl acetate Dry extract (DER 26-45:1), extraction solvent ethyl acetate Liquid extract (DER 10-17:1), extraction solvent ethanol 60% (V/V)
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use Herbal preparation in solid or liquid forms for oral use
Rapporteur(s)	Olga Palomino
Peer-reviewer	Werner Knöss

Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monographon *Silybum marianum* (L.) Gaertner, fructus. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of contents

1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Silybum marianum (L.) Gaertner, fruchtus, is included in the European Pharmacopeia and some national pharmacopeias. Currently the following monographs exist:

- Milk-thistle fruit published in the European Pharmacopoeia (Ph.Eur. 7th Edition 2012 (7.5); Ref. 01/2008:1860): Mature fruit, devoid of the pappus, of *Silybum marianum* L. Gartner (Asteraceae). Content: minimum 1.5 per cent of silymarin, expressed as silibinin ($C_{25}H_{22}O_{10}$; M_T 482.4) (dried drug)
- Cardui mariae (o mariani) fructus (BP 1996): Dried ripe fruits of *Silybum marianum* (L.) Gaertn. (No chemical composition or minimum content is included).
- Cardo mariano, fruto de (Spanish Pharmacopoeia, 5th Edition 2015; Ref 01/2008, 1860)

The fruit is an achene strongly compressed, elongate-obovate, about 6-8mm long. 3mm broad and 1.5mm thick; the outer surface is smooth and shiny with a grey or pale brown ground colour variably streaked dark brown longitudinally to give an overall pale greyish or brown colour: the fruit is tapering at the base and crowned at the apex with a glistening, pale yellow extension froming a collar about 1mm high surrounding the remains of the style. Cut transversel, the fruit shows a narrow, brown outer area and 2 large, dense, white oily cotyledons (Bruneton, 1999).

Synonyms: Blessed Milk Thistle, Milk Thistle, Marian Thistle, St. Mary's Thistle, Mediterranean Milk Thistle, *Carduus marianus* L.

Constituents: (**Kim et al., 2003; Sweetman, 2002; Wagner, 1973; Wagner et al., 1978**)

Flavonolignans (1.3-3%)

- Flavonol derivatives, silibinin and isosilibinin (A and B), silicristin and silidianin (Figure 1).

Flavonoids

- Flavones: Apigenin, chrysoeriol, eriodictyol
- Flavonols: Taxifolin, quercetin, dihydrokaempferol, kaempferol

Fatty oil (20-30%)

- Linoleic (35-55%) and oleic (24-30%) acids, together with palmitic (8-12%), linolenic (3-7%), behenic (3-9%) and other fatty oils (British Herbal Compendium, 2006)

Phytosterols (0.2-0.6%)

- β-sitosterol

Others: dehydrodiconiferyl alcohol, 5,7-dihydroxychromone; essential oil (mostly monoterpenes)

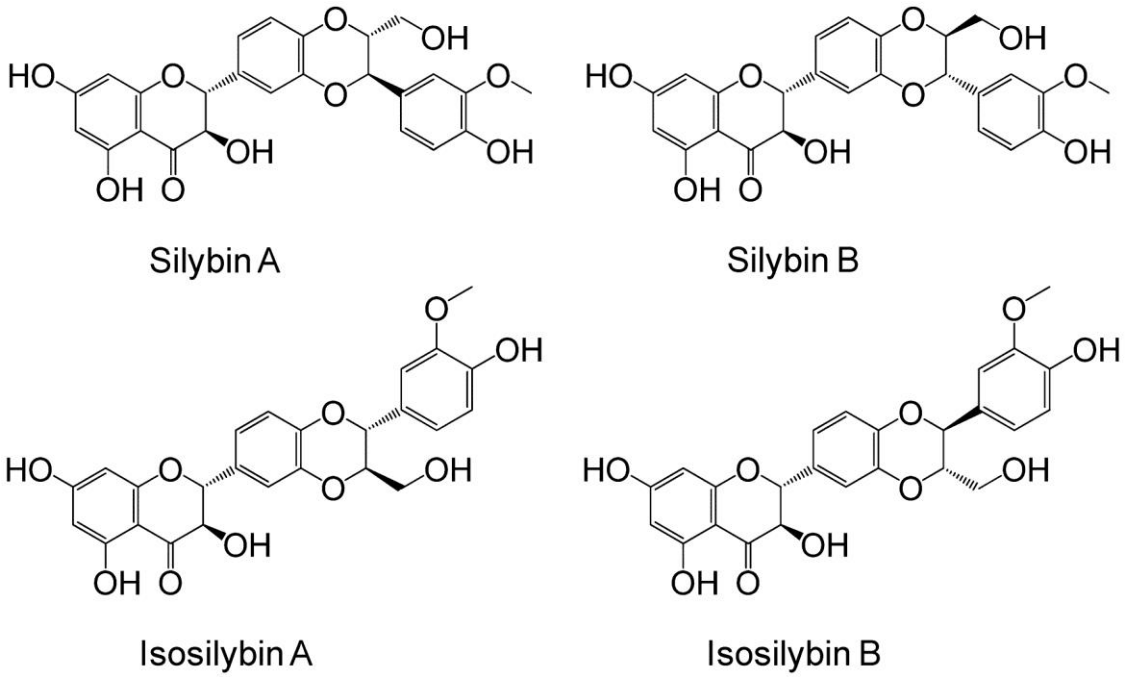

Figure 1. Main flavonolignans from *Silybum marianum* fruit (Brantley et al., 2010)

- Herbal preparation(s)

Silybum marianum dry extract is included in the European Pharmacopeia and some national pharmacopeias.Currently the following monographs exist:

- Milk thistle dry extract, refined and quantified (Ph.Eur. 7th Edition 2012 (7.5); Ref. 01/2008:2071)
- Mariendistelfrüchtetrockenextrakt – Cardui mariae fructus extractum siccum (DAB 2003)
- Cardo mariano, extracto seco refinado y normalizado de - Silybi mariani extractum siccum raffinatum et normatum (Spanish Pharmacopoeia, 5th Edition 2015; Ref 01/2008, 2071)

According to the European Pharmacopoeia monograph, this extract should contain 90 per cent to 110 per cent of the normal content of silymarin, expressed as silibinin ($C_{25}H_{22}O_{10}$; M_T 482.4), stated on the label. The nominal content of silymarin is within the range 30 per cent m/m to 65 per cent m/m (dried extract).

The content of silymarin corresponds to:

- *sum of the contents of silicristin and silidianin* (both $C_{25}H_{22}O_{10}$; M_T 482.4): 20 per cent to 45 per cent, calculated with reference to total silymarin;
- *sum of the contents of silibinin A and silibinin B* (both $C_{25}H_{22}O_{10}$; M_T 482.4): 40 per cent to 65 per cent, calculated with reference to total silymarin;
- *sum of the contens of isosilibinin A and isosilibinin B* (both $C_{25}H_{22}O_{10}$; M_T 482.4): 10 per cent to 20 percent, calculated with reference to total silymarin.

The extract is produced from the herbal substance using one or more of the following solvents: ethyl acetate, acetone or mixture of acetone and water, ethanol or mixture of ethanol and water, methanol or mixture of methanol and water.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

  Not applicable

1.2. Search and assessment methodology

Available literature on *Silybum marianum* at the "Bundesinstitut für Arzneimittel und Medizinprodukte" (BfArM) and the incoming, on the "call for scientific data for use in HMPC assessment work on *Silybum marianum* (L.) Gaertner, fruit", was used for a literature search. For most current publications a literature search in the DIMDI-database XMEDALL, Derwent Drug File (DD83), AMED (CB85), IPA (IA70), Biosis Previews (BA70), Medline (ME00), Embase (EM00) was performed twice on October 2011 and January 2014. Articles were filtered by using the following terms: *Silybum marianum*, milk thistle, Language in English, German, French or Spanish and concerning humans and pharmacological *in vitro* and *in vivo* studies.

Only articles found to be relevant for assessment are included in the list of references.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

(Information was collected when drafting of the European Union monograph was started.)

Austria: Well-established use

1. Standardised dry extract, DER 35-40:1 corresp. silymarin 70 mg (spectrophotometry) = 60 mg (HPLC). Extraction solvent acetone
2. Standardised dry extract, DER 36-44: 1, corresp. silymarin 70 mg (spectrophotometry). Extraction solvent ethyl acetate
3. Standardised dry extract, DER 36-44: 1, corresp. silymarin 140 mg (spectrophotometry). Extraction solvent ethyl acetate
4. Standardised dry extract, DER 36-44: 1, corresp. silymarin 105 mg (spectrophotometry). Extraction solvent ethyl acetate
5. Standardised dry extract, corresp. silymarin 140 mg (spectrophotometry), = 108.2 mg (HPLC). Extraction solvent acetone 95% V/V g
6. Standardised dry extract, corresp. silymarin 83 mg (HPLC). Extraction solvent acetone 95% V/V

Since when on the market?	Pharmaceutical form	Posology/daily dosage
1. 1979	Capsules	Adults and adolescents: 3 x daily 1(-2) capsules
2. 1977	Capsules	Adults and adolescents: Initially and in severe cases 3 x daily 2 capsules, otherwise 3 x daily 1 capsule
3. 1989	Capsules	Adults and adolescents: 3 x daily 1 capsule

4. 2002	Coated tablets	Adults and adolescents: 2 x daily 1-2 coated tablets
5. 2012	Capsules	Adults and adolescents: 3 x daily 1 capsule
6. 2012	Film-coated tablets	Adults and adolescents: 3-4- x daily 1 film coated tablet

Indications:

1.-4. Toxic liver damage, e.g. due to alcohol, medicines, or due to metabolic dysfunctions like diabetes; supportive treatment of chronic inflammatory liver diseases and cirrhosis of the liver.

5.-6. As an adjuvant for liver complaints and for the improvement of liver function

Combination products:

Combination products with artichoke, or fumatory; Iberogast as multicomponent product.

Belgium: Well-established use

1. *Silybum marianum*, fruit, dry extract, 36-44:1, extraction solvent ethyl acetate, 173.0-186.7 mg per capsule, corresponding with 140 mg silymarin (DNPH) or 108.2 mg silymarin (HPLC)

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 1996	Capsules, hard	Oral administration Initial dose: 1 capsule, three times daily, with a glass of water, preferably during meals (= also maximal dose) Maintenance dose: 1 capsule, twice daily, with a glass of water Not for children below 12y

Indications:

1. Used to facilitate hepatic functioning after serious pathologies have been excluded.

Croatia: Well-established use

1. 150 mg of refined and normated dried extract of *Silybum marianum*, fructus (26-45:1) which corresponds to 100 mg of silymarin expressed as silibininin; extraction solvent: acetone.

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 1999	Capsules, hard	For oral use. Administrate after meal with some fluid. In the beginning of the treatment and with severe conditions – 2 capsules twice daily; later continue with 1 capsule twice daily. In less severe conditions it is recommended to take 1 capsule twice daily

Indications:

1. To ease disorders of liver function

Risks:

When used, capsules could have laxative effect.

Czech Republic: Well-established use

1. Silybi mariani extractum siccum 36–44:1, extraction solvent ethyl acetate 173.0–186.7 mg/cps corresponding to Silymarinum expressed as silibininum 140 mg (spectrophotometry) or 108.2 mg (HPLC)
2. Silybi mariani extractum siccum 36–44:1, extraction solvent ethyl acetate 86.5–94.5 mg/cps corresponding to silymarinum expressed as silibininum 70 mg (spectrophotometry)
3. Silybi mariani extractum siccum 35–40:1, extraction solvent methanol 204 mg/tbl, corresponding to 150 mg of silymarin (spectrophotometry)
4. Silybi mariani extractum siccum 20–33.1:1, extraction solvent acetone 68.03–75.19 mg/tbl corresponding to 50 mg of silymarinum expressed as silibininum
5. Silybi mariani extractum siccum raffinatum et normatum 87.5–116.7 mg/tbl corresponding to 70 of silymarin expressed as silibinin (HPLC) (Silygal) – the active substance has CEP (R0-CEP 2008-237-Rev 01)
6. Silymarinum 70 mg/tbl

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 1999	Capsules	Start with 1 capsule 3 times daily, later 1 capsule 2 times daily, children over 5 years of age 5 – 6 mg/kg/day
2. 1995	Capsules	Start with 2 capsules 3 times daily, later 1 capsule 3 times daily
3. 1997	Tablets	3 to 6 mg/kg/day, usually 1 tablet 2 – 3 times daily (1 – 6 weeks) than the dosage should be reduced in half Duration of use 3 months to 1 year, regular controls at the doctor
4. 1999	Tablets	2 – 3 tablets 3 times daily (300 – 450 mg of silymarin) later 4 tablets daily (200 mg of silymarinum)
5. 1998	Tablets	Adults sub acute and chronic liver diseases: 1 tablet 3 times daily Acute liver diseases: 2 tablets 3 times daily Children over 5 years of age: 5 – 6 mg of silymarin/kg/day
6. 1982	Tablets	Adults: in minor impairments 1 tablet 3 times daily, in severe cases of impairment 2 tablets 3 times daily Children over 5 years of age: 5 – 6 mg of silymarin/kg/day

Indications:

1. Supportive treatment in toxic hepatic impairment, chronic persistent and acive hepatitis, cirrhosis, toxico-metabolic hepatic impairment (steatosis, drug-caused hepatic impairment, hepatotoxic substances intoxication).
2. Supportive therapy in chronic hepatitis or hepatic cirrhosis.
3. Toxico-metabolic hepatic impairment such as steatosis, impairment alcohol-, toxic substances- or drug-caused hepatic impairment, mushrooms intoxication etc. Supportive therapy in chronic inflammatory liver diseases (persistent and active chronic hepatitis), hepatic cirrhosis.
4. Supportive therapy in chronic inflammatory liver diseases, hepatic cirrhosis, toxic hepatic impairment.
5. Adjuvant therapy in patients with chronic inflammatory liver diseases or cirrhosis. Toxic hepatic impairment.
6. Adjuvant therapy in patients with chronic persistent and active hepatitis, hepatic cirrhosis, toxico-metabolic hepatic lesions (steatosis, drug-caused hepatic impairment, hepatotoxic substances intoxication).

Risks:

1. *Contraindications*: use in children below 5 years of age, hypersensitivity to the active substance
Adverse effects: rarely laxative effect

2. *Contraindications*: hypersensitivity to the active substance, use in children bellow 12 years of age
Adverse effects: rarely laxative effect

3. *Contraindications*: hypersensitivity to the active substance, use in children below 5 years of age
Adverse effects: laxative effect, skin reactions, frequency is not known

4. *Contraindications*: hypersensitivity to the active substance
Special warning: use in children below 12 years of age is not recommended due to lack of adequate data
Adverse effects: one case of asthmatic attack (very rare)
Gastrointestinal disorders such as nausea and mild laxative effect (uncommon)
Hypersensitive reactions such as skin reaction, itching, dyspnoea (uncommon)

5. *Contraindications*: hypersensitivity to the active substance, use in children below 5 years of age
Adverse effects: dyspeptic disorders, mild laxative effect, allergic skin reactions

6. *Contraindications*: hypersensitivity to the active substance
Adverse effects: diarrhoea, dyspeptic disorders, allergic skin reactions, the frequency is not known

Combination products:

Combination products with artichoke, or fumatory; Iberogast as multicomponent product.

Estonia: Well-established use

1. 1 coated tablet contains 40.9-56.3 mg Silybi mariani fructus extractum siccum (35-50:1), equivalent to 22.5 mg silymarin, as silibinin (HPLC) (extraction reagent: methanol)
2. 1 hard capsule contains 167.69-206.44 mg dry native extract from milk-thistle (Silybi mariani fructus), corresponding to 140 mg silymarin expressed as silibinin; DER: 36-44:1, extraction solvent: ethyl acetate

3. 1 hard capsule contains 239.57-294.92 mg dry native extract from milk-thistle (Silybi mariani fructus), corresponding to 200 mg silymarin expressed as silibinin. DER: 36-44:1, extraction solvent: ethyl acetate
4. 1 coated tablet contains 162.5-250 mg Silybi mariani fructus extractum siccum (20-33.3:1), equivalent to 105 mg silymarin, as silibinin (HPLC). Extraction reagent: ethyl acetate.

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 2004	Coated tablet	1-2 coated tablets 3 times per day.
2. 2009	Capsule, hard	1 hard capsule 2-3 times daily.
3. 2009	Capsule, hard	1 hard capsule 2 times daily.
4. 2001	Coated tablet	1-2 coated tablets 2 times per day.

Indications:

1.-4. Supportive treatment of liver disorders.

Risks:

1. Enhancement of existing vestibular disorder, nausea, dyspepsia, diarrhoea, itching, rashes.

2. Lightly laxative effects. Rash or dyspnoea.

3. Lightly laxative effects. Rash or dyspnoea.

4. Lightly laxative effects.

Combination products:

Combination products with artichoke, or fumatory; Iberogast as multicomponent product

France: Well-established use

1. Quantified extract
2. Quantified extract

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 1993	Coated tablet	1 tablet 2-3 times daily. 1 tablet contains 140 mg of silymarin
2. 1972	Coated tablet	2 tablets 2-3 times daily. 1 tablet contains 100 mg of extract with 70 mg of silymarin

Indications:

1.-2. Functional digestive disorders observed with hepathopathy.

Risks:

Undesirable effects: very rare cases of gastralgia or diarrhoea. Extremely rare: allergic reactions.

France: Traditional use

1. Powdered dry fruit

Since when on the market?	Pharmaceutical form	Posology/daily dosage
1. 1982	Hard capsules	4 capsules (300 mg/each) daily

Indication:

Dyspeptic complaints

Germany: Well-established use

1) Dry extract (35-40:1), extraction solvent: acetone

2) Dry extract (50-60:1), extraction solvent: acetone

3, 19) Dry extract (35-45:1), extraction solvent: acetone

4, 5, 33, 34, 35) Dry extract (36-44:1), extraction solvent: ethyl acetate

6) Dry extract (20-35:1), extraction solvent: ethyl acetate

7) Dry extract (40-70:1), extraction solvent: acetone

8) Dry extract (26-45:1), extraction solvent: ethyl acetate

9, 36, 37) Dry extract (25-40:1), extraction solvent: acetone

10) Dry extract (20-35:1), extraction solvent: acetone

11, 15) Dry extract (60-70:1), extraction solvent: ethanol 96% V/V

12) Dry extract (20-33.3:1), extraction solvent: ethyl acetate

13, 23, 24, 28, 29, 30, 31, 32) Dry extract (36-44:1), extraction solvent: ethyl acetate

14) Dry extract (60-70:1), extraction solvent: ethanol 96% V/V

16, 17, 18, 25, 26, 27) Dry extract (50-70:1), extraction solvent: acetone

20, 21) Dry extract (20-50:1), extraction solvent: ethanol 96% V/V

22) Dry extract (DER variable), extraction solvent: acetone

38, 39, 40, 41, 42, 43) Dry extract (36-44:1), extraction solvent: ethyl acetate

44) Dry extract (32-48:1), extraction solvent: ethyl acetate

45, 46, 47, 48) Dry extract, extraction solvent: acetone 95% V/V

Since when on the market?	pharmaceutical form	Posology/daily dosage
1, 2, 3, 4, 5, 6, 7, 8, 9, 10) at least since 1976	2, 3, 10, 19, 22 film-coated tablet 7 capsule, soft 6, 8, 12 coated tablet 1, 4, 5, 9, 11, 13, 14, 15, 16, 17,	1) > 12 y: 3 x daily 2 each containing 82-111 mg dry extract corresponding to 60 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 360 mg Silymarin)

11, 13, 14, 15) 1994	18, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41,42, 43, 44, 45, 46, 47, 48 capsule, hard	2, 22) > 12 y: 2 x daily 2 each containing 131.6-161.0 mg dry extract corresponding to 84 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 336 mg Silymarin) or 3 x daily 1 containing 131.6-161.0 mg dry extract corresponding to 84 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 252 mg Silymarin)
12) 1995		3, 19) > 12 y: 2-3 x daily 1 containing 176-200 mg dry extract corresponding to 105 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 210 mg-315 mg Silymarin)
16, 17, 18) 1996		4) > 12 y: 3 x daily 1 containing 173-186.7 mg dry extract corresponding to 108.2 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 324.6 mg Silymarin)
19, 20, 21, 22) 1998		5, 33, 34) > 12 y: 3 x daily 2 each containing 86.5-93.3 mg dry extract corresponding to 54.1 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 324.6 mg Silymarin)
23, 24, 28, 29, 30, 31) 2000		6) > 12 y: 2 x daily 1-2 each containing 162.5-250 mg dry extract corresponding to 105 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 210-420 mg Silymarin)
25, 26, 27) 1997		7) > 12 y: 2 x daily 1 containing 170-239 mg dry extract corresponding to 140 mg Silymarin calculated as Silibinin (HPLC)
32, 33, 34, 35, 36, 37) 2006		8) > 12 y: 3-4 x daily 1 containing 123-208.3 mg dry extract corresponding to 83.3 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 250-333 mg Silymarin)
38, 39, 40, 41, 42, 43, 44) 2007		9, 36, 37) > 12 y: 2 x daily 2 each containing 150-163 mg dry extract corresponding to 83 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus

		corresponding to 332 mg Silymarin)
45, 46, 47, 48) 2009		10) > 12 y: 3-4 x daily 1 containing 135-152 mg dry extract corresponding to 83 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 249-332 mg Silymarin)
		11) > 12 y: 2-3 x daily 1 containing 170-200 mg dry extract corresponding to 117 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 234-351 mg Silymarin)
		12) > 12 y: 2 x daily 1-2 each containing 162.5-250 mg dry extract corresponding to 105 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 105-210 mg Silymarin)
		13, 23, 24, 28, 29, 30, 31, 34) > 12 y: at the beginning of the treatment: 3 x daily 1 containing 173.0-186.7 mg dry extract corresponding to 140 mg Silymarin calculated as Silibinin extract from Cardui mariae fructus corresponding to 420 mg Silymarin) maintenance dose: 2 x daily 1 containing 173.0-186.7 mg dry extract corresponding to 140 mg Silymarin calculated as Silibinin (extract from Cardui mariae fructus corresponding to 280 mg Silymarin)
		14) > 12 y: 2 x daily 1 containing 243-286 mg dry extract corresponding to 172 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 344 mg Silymarin)
		15) > 12 y: 2 x daily 1 containing 242.8-285.7 mg dry extract corresponding to 167 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 334 mg Silymarin)
		16) > 12 y: 3 x daily 1 containing 136.0-160.0 mg dry extract corresponding to 85 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 255 mg Silymarin)
		17) > 12 y: 3 x daily 1 containing 136-160 mg dry

		extract corresponding to 86.5 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to approx. 260 mg Silymarin)
		18) > 12 y: 3 x daily 1 containing 136-160 mg dry extract corresponding to 86.6 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 250 mg Silymarin)
		20) > 12 y: 2-3 x daily 1 containing 194.45-218.75 mg dry extract corresponding to 117 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 234-351 mg Silymarin)
		21) > 12 y: 2 x daily 1 containing 277.8-312.5 mg dry extract corresponding to 167 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 334 mg Silymarin)
		25) > 12 y: 3 x daily 1 containing 136.0-160.0 mg dry extract corresponding to 86 mg Silymarin calculated as Silibinin (HPLC) resp. 110 mg Silymarin calculated via photopmetric determination (extract from Cardui mariae fructus corresponding to 258 mg Silymarin (HPCL)
		26, 27) > 12 y: 3 x daily 1 containing 136.0-160.0 mg dry extract corresponding to 86.5 mg Silymarin calculated as Silibinin (HPLC)
		32) > 12 y: <u>at the beginning of the treatment:</u> 3 x daily 2 each containing 86.5-93.3 mg dry extract corresponding to 70 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 420 mg Silymarin) <u>maintenance dose:</u> 3 x daily 1 containing 86.5-93.3 mg dry extract corresponding to 70 mg Silymarin calculated as Silibinin (HPLC) (extract from Cardui mariae fructus corresponding to 210 mg Silymarin)
		38, 40, 42) > 18 y: 3 x daily 1 containing 167.69-206.44 mg dry extract corresponding to 109 mg Silymarin calculated as Silibinin (HPLC)

		39, 41, 43) > 18 y: 2 x daily 1 containing 239.57-294.32 mg dry extract corresponding to 156 mg Silymarin calculated as Silibinin (HPLC)
		44) > 12 y: 2 x daily 1 containing 228.9-238.2 mg dry extract corresponding to 140 mg Silymarin calculated as Silibinin (HPLC)
		45, 46, 47, 48) > 18 y: 3 x daily 1 containing 177.4-240.4 mg dry extract corresponding to 108.2 mg Silymarin calculated as Silibinin (HPLC)

Indications:

1-48: For the supportive treatment of chronic inflammatory liver diseases, hepatic cirrhosis and toxic liver damage

Risks:

Undesirable effects:

1-48: Common: gastrointestinal complaints like nausea and a mild laxative effect; common: hypersensitivity reactions like exanthema, pruritus and dyspnoea

Interactions:

The metabolisation of concomitant used medicinal products can be affected caused by improvement of the hepatic function; the antiarrhythmic effect of amiodarone can be increased **(Vereckei *et al.* 2003)**

Traditional use

1. Dry extract (30-40:1), extraction solvent: ethanol 96% V/V

2. Soft extract (10-17:1), extraction solvent: ethanol 60% V/V

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 1976	Capsule, soft	Oral use in adults and adolescents over 12 years
2. 1976	Oral liquid	2 x 15 ml daily (2 x 392mg soft extract)

Indications

1, 2. Traditionally used to support digestive function

Risks

Undesirable effects:

1, 2. Gastrointestinal complaints like nausea and a mild laxative effect; hypersensitivity reactions like exanthema, pruritus and dyspnoea (frequency not known)

Interactions:

1, 2. The antiarrhythmic effect of amiodarone can be increased

Latvia: Well Stablished Use

1. Dry extract from milk thistle fruit (36-44:1), extraction solvent 98% ethylacetate
2. Dry extract from milk thistle fruit (36-44:1), extraction solvent 98% ethylacetate
3. Dry extract from milk thistle fruit (36-44:1), extraction solvent 98% ethylacetate
4. Dry extract from milk thistle fruit (36-44:1), extraction solvent 98% ethylacetate
5. Dry extract from milk thistle fruit (20-33:1), extraction solvent 98% ethylacetate
6. Silymarin

Since when on the market?	Pharmaceutical form	Posology/daily dosage
1. 1999	capsules, each capsule contains 86.5-93.3 mg dry extract (36-44:1), corresponding to 70 mg silymarin, expressed as silibinin	In the beginning of treatment and in severe conditions – 2 capsules 3 times per day, corresponds to 420 mg silymarin per day. Maintenance dose – 1 capsule 3 times per day, corresponds to 210 mg silymarin per day
2. 1999	capsules, each capsule contains 173.0–186.7 mg dry extract (36-44:1), corresponding to 140 mg silymarin, expressed as silibinin	In the beginning of treatment and in severe conditions – 1capsules 3 times per day, corresponds to 420 mg silymarin per day. Maintenance dose – 1 capsule 2 times per day, corresponds to 280 mg silymarin per day
3. 2009	capsules, each capsule contains 167.69–206.44 mg dry extract (36-44:1), corresponding to 140 mg silymarin, expressed as silibinin	1 capsule 3 times per day
4. 2009	capsules, each capsule contains 239.57–294.92 mg dry extract (36-44:1), corresponding to 200 mg silymarin, expressed as silibinin	1 capsule 2 times per day
5. 1997	film-coated tablets, each tablet contains 162.5–250 mg dry extract (20-33:1), corresponding to 105 mg silymarin, expressed as silibinin.	1 - 2 film-coated tablets 2 times per day
6. 1996	coated tablets, each tablet contains 35 mg silymarin.	Mild and moderate cases in adults – 1-2 tablets 3 times per day; in severe cases the dose can be doubled; prophylactic dose – 2-3 tablets daily

Indications:

1, 2. Toxic liver damage: additional treatment in patients with chronic inflammatory liver conditions and hepatic cirrhosis.

3, 4. Additional therapy in patients with toxic liver damage, chronic inflammatory liver conditions and hepatic cirrhosis.

5. Herbal medicinal product for additional therapy in patients with toxic liver damage, chronic inflammatory liver conditions and hepatic cirrhosis.

6. As an additional treatment in patients with chronic inflammatory liver diseases, cirrhosis, liver dystrophy, fatty degeneration after hepatitis and toxic functional liver disorders. Prophylaxis of liver impairment during prolonged administration of medicinal products, alcohol consumption, in chronic intoxication (including occupational).

Combination products:

The herbal substance is also available in combination product. Combination with vitamins.

Lithuania: Well Stablished Use

1. 35 mg film-coated tablets (35 mg silymarin in each)

Since when on the market?	Pharmaceutical form	Posology/daily dosage
1. 2002	Film-coated tablets	Mild or moderate liver disease: 1 - 2 film-coated tablets are taken orally 3 times a day

Indications:

1. Toxic liver injury. As complementary medicine in patients with chronic hepatitis, liver cirrhosis.

Spain: Well Established Use

1. Standardised dry extract, DER 36-44:1, corresp. silymarin 150 mg (spectrophotometry). Extraction solvent ethyl acetate
2. 80 mg silymarin

Since when on the market?	Pharmaceutical form	Posology/daily dosage
1. 1974	Hard capsules	1 capsule (150 mg) x three times a day
2. 1974	Hard capsules	1 capsule (80 mg) x three times a day up to 6 capsules/day

Indications:

1, 2. Toxico-metabolic hepatic impairment, especially those with a high peroxidation levels such as the ones derived from chronic alcoholic intake, drug-caused hepatic impairment, steatosis, alcoholic hepatitis and cirrhosis

Spain: Traditional use

1.Powdered dry fruit

2. Powdered dry fruit

Since when on the market?	Pharmaceutical form	Posology/daily dosage
1. 1990	hard capsules	1 or 2 capsules (300 mg/each) x three times a day
2. 1990	hard capsules	2 capsules (400 mg/each) x three times a day

Indications:

For all products: Dyspeptic complaints

Combination products:

The herbal substance is also available in combination products (herbal teas).

Slovakia: Well-established use

1. Cardui marianae fructus extractum siccum (20-33:1) 162.5-250 mg with 105 mg silimarin calculated as silibinin in 1 coated tablet, extraction solvent: ethylacetate
2. Cardui mariae fructus extractum siccum 240.0 mg with150 mg silimarin calculated as silibinin in 1 coated tablet
3. Cardui mariae fructus extractum siccum 86.5–93.3 mg with silimarin 70 mg calculated as silibinin in 1 capsule,extraction solvent: ethylacetate 96.7%.

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 1996	Coated tablet	Adults and children above 12 yrs: 2 times a day 1 - 2 tablets
2. 1996	Coated tablet	Adults: 2 times a day 1 tablet
3. 1994	Capsule, hard	Adults: 3 times a day 2 capsules

Indications:

For all the products: Toxic injury of liver, for supportive treatment in chronic inflamamtory conditions of liver and liver cirrhosis.

Risks:

For all the products: diarrhea.

United Kingdom: Traditional Use

1. 450 mg powdered Milk thistle fruit
2. 150 mg standardised dry extract, equivalent to 3.600-4.050 g of Milk thistle fruits corresponding to 87 mg of silymarin, calculated as silibinin, extraction acetone 95% v/v
3. 125 mg standardised dry extract, equivalent to 2.5-5.0 g of Milk thistle fruits corresponding to 62.5-75 mg of silymarin, calculated as silibinin,extraction solvent ethyl acetate
4. 125 mg standardised dry extract, equivalent to 2.5-5.0 g of Milk thistle fruits corresponding to 62.5-75 mg of silymarin, calculated as silibinin, extraction solvent ethyl acetate
5. 125 mg standardised dry extract, equivalent to 2.5-5.0 g of Milk thistle fruits corresponding to 62.5-75 mg of silymarin, calculated as silibinin, extraction solvent ethyl acetate
6. 137.5-165 mg of standardised dry extract, equivalent to 2,750-6,600 mg of Milk thistle fruits corresponding to 82.5 mg of silymarin calculated as silibinin, extraction solvent ethyl acetate

7. 193-261 mg of standardised dry extract (21-45:1) equivalent to 3.725-10.818 g of Milk thistle fruits corresponding to 108 mg of silymarin calculated as silibinin, extraction solvent acetone 95% V/V
8. 193-261 mg of standardised dry extract (21-45:1) equivalent to 3.725-10.818 g of Milk thistle fruits corresponding to 108mg of silymarin calculated as silibinin, extraction solvent acetone 95% V/V
9. 193-261 mg of standardised dry extract (21-45:1) equivalent to 3.725-10.818 g of Milk thistle fruits corresponding to 108 mg of silymarin calculated as silibinin, extraction solvent acetone 95% V/V
10. 89-121 mg of standardised dry extract (21-45:1) equivalent to 1.721-5.0 g of Milk thistle fruits corresponding to 50 mg of silymarin calculated as silibinin, extraction solvent acetone 95% V/V
11. 500 mg of standardised dry extract equivalent to 10.0-20.0 g of Milk thistle fruits corresponding to 250-300 mg of silymarin calculated as silibinin, extraction solvent ethyl acetate

12, 13, 14, 15. 300 mg of standardised dry extract equivalent to 7200-8100 mg of Milk thistle fruits corresponding to 174 mg of silymarin calculated as silibinin, extraction solvent acetone 95% V/V

Since when on the market?	**Pharmaceutical form**	**Posology/daily dosage**
1. 2011	Capsules containing 450 mg powdered fruits	Adults and adolescents >18 years: 3 x daily 1-2 capsules
2. 2012	Coated tablets containing 150 mg extract	Adults and adolescents >18 years: daily 1-2 tablets
3. 2012	Coated tablets containing 125 mg extract	Adults and adolescents >18 years: 2 x daily 1-2 tablets
4. 2012	Coated tablets containing 125 mg extract	Adults and adolescents >18 years: 2 x daily 1-2 tablets
5. 2012	Coated tablets containing 125 mg extract	Adults and adolescents >18 years: 2 x daily 1-2 tablets
6. 2012	Tablets containing 137.5-165 mg extract	Adults and adolescents > 18 years: 2 x daily 1-2 tablets
7. 2011	Capsules containing 193-261 mg extract	Adults and adolescents > 18 years: 2 x daily 1 capsule
8. 2010	Capsules containing 193-261 mg extract	Adults and adolescents > 18 years: 2 x daily 1 capsule
9. 2010	Capsules containing 193-261 mg extract	Adults and adolescents > 18 years: 2 x daily 1 capsule
10. 2010	Capsules containing 89-121 mg extract	Adults and adolescents > 18 years: 2 x daily 1 capsule
11. 2012	Tablets containing 125 mg extract	Adults and adolescents > 18 years: 2 x daily 1-2 tablets

12, 13, 14, 15. 2011	Coated tablets Containing 300 mg extract	Adults and adolescents > 18 years: daily 1-2 tablets

Indications:

1-15. Relief of symptoms associated with occasional over indulgence of food and drink such as indigestion and upset stomach.

Risks:

Undesirable effects:

Gastrointestinal reactions (nausea, upset stomach, diarrhoea), headache, allergic reactions (urticaria, skin rash, pruritis, anaphylaxis).

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable.

Information on other products marketed in the EU/EEA (where relevant)

Not applicable.

2.1.2. Information on products on the market outside the EU/EEA

Not applicable.

2.2. Information on documented medicinal use and historical data from literature

The **monographs on the Commission E (Blumenthal 1986, 2000)** include several forms to use Milk thistle fruit for digestive disorders: decoction (3.0g seed in 150ml water); infusion 3.5g seed in 150ml water); tincture (15-25 drops, four to five times daily or 1-2ml, three times daily). Also the use of the standardized extract (70 per cent silymarin) in toxic liver damage and for supportive treatment in chronic inflammatory liver disease and hepatic cirrhosis is included.

The latter monograph describes that leaves from *Silybum marianum* have been used since Greco-Roman times as an herbal remedy for a variety of ailments, particularly liver problems. Eclectic physicians in the United States in the late nineteenth and early twentieth centuries acknowledged the clinical benefits of the preparations of the milk thistle seeds (technically the fruits) for congestion of the liver, spleen, and kidneys. It is cited as *widely used in Germany for "chronic hepatitis of all types" and especially for fatty liver (cirrhosis) associated with alcoholics* **(Weiss, 1988 in Blumenthal, 2000).**

The **ESCOP monograph (2009)** describes the use of Milk Thistle fruit for the treatment of: toxic liver damage; supportive treatment in patients with chronic inflammatory liver conditions and hepatic cirrhosis.

The **British Herbal Compendium (Bradley 2006)** listed the following indications for Silybi mariani fructus: Inflammatory conditions and cirrhosis of the liver, particularly as supportive treatment in patients with alcoholic liver disease with cirrhosis and /or abnormal liver function; liver damage following exposure to chemical toxins. Dosage is included for the extract (corresponding to 165-330mg silymarin), dried fruits (4-9g) and liquid extract 1:1 (4-9ml). Other uses, based on experience or tradition are: liver and gall bladder problems, including jaundice and gall bladder colic; dyspeptic complaints such as flatulence and bloating; loss of appetite. It also describes the use of milk thistle

fruit in France for the symptomatic treatment of functional digestive disorders attributed to a hepatic origin in Germany with the uses included in the Comission E monograph.

WHO monograph (2002) listed the following medicinal uses: *Uses supported by clinical data*: supportive treatment of acute or chronic hepatitis and cirrhosis induced by alcohol, drugs or toxins. *Uses described in pharmacopoeias and in traditional systems of medicine*: treatment of dyspeptic complaints and gallstones. *Uses described in folk medicine, not supported by experimental or clinical data*: treatment of amenorrhoea, constipation, diabetes, hay fever, uterine haemorrhages and varicose veins.

Mills & Bone (2000) included different preparations such as dried seed as a decoction or liquid extract to be traditionally used for liver and gallbladder problems.

The monograph included in the **PDR for Herbal Medicines (1998)** describes the use of *preparations of milk thistle herb* as a tonic, stimulant, for functional disorders of the liver and gallbladder as well as for jaundice, gallbladder colic, and dieases of the spleen. Formerly used as malaria treatment, emmenagogue, and for uterine complaints. *Cardui mariae fructus* is used for dyspeptic complaints, toxic liver damage, supportive treatment in chronic inflammatory liver disease and hepatic cirrhosis; it is also used as an antidote to Death-cap (*Amanita phalloides*) poisoning.

The monograph in the **Potter's New Cyclopaedia of Botanical Drugs and Preparations (1988, 2003)** listed the following medicinal uses: hepatoprotective. Milk Thistle was formerly used in the United Kingdom for nursing mothers, as a bitter tonic, demulcent, as an antidepressant, for liver complaints. In Germany and other parts of Europe it was used extensively for liver diseases and jaundice.

Barnes et al (2007) described the traditional use of milk thistle fruits for disorders of the liver, spleen and gall bladder such as jaundice and gall bladder colic.

The Merk Index (1976) includes the therapeutical use of silymarin group for liver dysfunction.

The **Homeopathic Pharmacopoeia of India (Vol. I, pp. 94, 1971)** recognized the use of Silybum marianum seeds for jaundice, cirrhosisof the liver, dropsical conditions and pathological conditions related to liver and gall stone **(Varma et al., 1980).**

The reference in the **Materia Medica Vegetabilis** (Steinmetz, 1997) for Herba Cardui Mariae (number 295) lists its use as an infusion: febrifugue and good against chest complaints, jaundice, diseases of the spleen, dropsy and leucorrhoea; blood purifier and to assist the circulation.

The monograph in the **Martindale (2009)** includes its use for gastrointestinal and hepatobiliary disorders. Silymarin is claimed to be a free radical scavenger and to have hepatoprotectant properties; it has been used in various liver disorders, as well as to prevent hepatotoxicity associated with poisoning.

Rambaldi et al (2005) and Navarro et Montilla (2012) reported the internal use of the extracts of milk thistle since the time of ancient Greece, Early Middle Ages and Modern ages.

The **French Pharmacopoeia (1989)** also includes a monograph for Carduus marianus for homeopathic preparations.

2.3. Overall conclusions on medicinal use

The standardized dry extract (DER 36-44:1) is marketed in the European Union at least since 1974, with different strength and posologies, under the WEU (see Table 1a) with similar indications related to liver diseases such as toxic liver damage, chronic inflammatory liver diseases or cirrhosis.

Several preparations fulfil the 30 years period of medicinal use (for the treatment of dyspeptic complaints and to support digestive function) in the European Union and can be considered under Traditional use: powdered fruit, dry extract (DER 30-40:1; extraction solvent ethanol 96% V/V); soft extract (DER 10-17:, extraction solvent ethanol 60%)(Table 1b). Some preparations (mainly standardized extracts) can be found which do not fulfil the WEU criteria but are in the EU market for more than 30 years, so they can be included under Traditional Use: Dry extract (DER 20-35:1), extraction solvent: ethyl acetate; Dry extract (DER 26-45:1), extraction solvent: ethyl acetate; Dry extract (20-70:1), extraction solvent acetone 95% (V/V). Data from literature allows the inclusion of the comminuted herbal substance.

Table 1: Overview of evidence on period of medicinal use for a) WEU and b) TU

a) Well Established Use

Herbal preparation Pharmaceutical form	**Indication**	**Period of medicinal use**
Standardised dry extract, DER 35-40:1 corresp. silymarin 70 mg (spectrophotometry) = 60 mg (HPLC), extraction solvent acetone	Toxic liver damage, e.g. due to alcohol, medicines, or due to metabolic dysfunctions like diabetes. Supportive treatment of chronic inflammatory liver diseases and cirrhosis of the liver. Supportive therapy in chronic hepatitis or hepatic cirrhosis.	1979 1995 1999
Standardised dry extract, DER 36-44:1, corresp. silymarin 70 mg (spectrophotometry), extraction solvent ethyl acetate	Toxic liver damage, e.g. due to alcohol, medicines, or due to metabolic dysfunctions like diabetes; supportive treatment of chronic inflammatory liver diseases and cirrhosis of the liver. Supportive therapy in chronic hepatitis or hepatic cirrhosis. Supportive treatment in toxic hepatic impairment, chronic persistent and acive hepatitis, cirrhosis, toxico-metabolic hepatic impairment (steatosis, drug-caused hepatic impairment, hepatotoxic substances intoxication). Supportive treatment of liver disorders. Supportive treatment of chronic inflammatory liver diseases, hepatic cirrhosis and toxic liver damage.	1977 1999 2006 2009
Standardised dry extract, DER 36-44:1, corresp. silymarin 140 mg (spectrophotometry), extraction solvent ethyl acetate	Toxic liver damage, e.g. due to alcohol, medicines, or due to metabolic dysfunctions like diabetes; supportive treatment of chronic inflammatory liver diseases and cirrhosis of the liver. Supportive treatment in toxic hepatic impairment, chronic persistent and acive hepatitis, cirrhosis, toxico-metabolic hepatic impairment (steatosis, drug-caused hepatic impairment, hepatotoxic substances intoxication). Toxic liver damage: additional treatment in patients with chronic inflammatory liver conditions and hepatic cirrhosis. Used to facilitate hepatic functioning after serious pathologies have been excluded. Supportive treatment of liver disorders.	1989 1996 1999 2009
Standardised dry extract, DER 36-44:1, corresp.	Toxico-metabolic hepatic impairment, especially those with a high peroxidation levels such as the ones derived from chronic alcoholic intake, drug-	1974

Herbal preparation Pharmaceutical form	Indication	Period of medicinal use
silymarin 150 mg (spectrophotometry), extraction solvent ethyl acetate	caused hepatic impairment, steatosis, alcoholic hepatitis and cirrhosis.	
Standardised dry extract, DER 36-44:1, corresp. silymarin 105 mg (spectrophotometry), extraction solvent ethyl acetate	Toxic liver damage, e.g. due to alcohol, medicines, or due to metabolic dysfunctions like diabetes; supportive treatment of chronic inflammatory liver diseases and cirrhosis of the liver.	2002
Standardised dry extract, corresp. silymarin 140 mg (spectrophotometry), = 108.2 mg (HPLC), extraction solvent acetone 95% V/V	As an adjuvant for liver complaints and for the improvement of liver function.	2012
Standardised dry extract, corresp. silymarin 83 mg (HPLC), extraction solvent acetone 95% V/V	As an adjuvant for liver complaints and for the improvement of liver function.	2012
150 mg of refined and normated dried extract of *Silybum marianum*, fructus (26-45:1) which corresponds to 100 mg of silymarin expressed as silibinin; extraction solvent: acetone	To ease disorders of liver function	1999
Silybi mariani extractum siccum 35–40:1, extraction solvent methanol 204 mg/tbl, corresponding to 150 mg of silymarin (spectrophotometry)	Toxico-metabolic hepatic impairment such as steatosis, impairment alcohol-, toxic substances- or drug-caused hepatic impairment, mushrooms intoxication etc. Supportive therapy in chronic inflammatory liver diseases (persistent and active chronic hepatitis), hepatic cirrhosis	1997
Silybi mariani extractum siccum 20–33.1:1, extraction solvent acetone 68.03–75.19 mg/tbl corresponding to 50 mg of silymarinum expressed as silibininum	Supportive therapy in chronic inflammatory liver diseases, hepatic cirrhosis, toxic hepatic impairment	1999
Silybi mariani extractum siccum raffinatum et normatum 87.5–116.7 mg/tbl corresponding to 70 mg of silymarin expressed as silibinin (HPLC)	Adjuvant therapy in patients with chronic inflammatory liver diseases or cirrhosis. Toxic hepatic impairment	1998

Herbal preparation Pharmaceutical form	Indication	Period of medicinal use
Silymarinum 70 mg/tbl	Adjuvant therapy in patients with chronic persistent and active hepatitis, hepatic cirrhosis, toxico-metabolic hepatic lesions (steatosis, drug-caused hepatic impairment, hepatotoxic substances intoxication).	1982
40.9-56.3 mg Silybi mariani fructus extractum siccum (35-50:1), equivalent to 22.5 mg silymarin, as silibinin (HPLC); extraction solvent: methanol	Supportive treatment of liver disorders	2004
239.57-294.92 mg native dry extract from milk-thistle (Silybi mariani fructus), corresponding to 200 mg silymarin expressed as silibinin. DER: 36-44:1, extraction solvent: ethyl acetate	Supportive treatment of liver disorders	2009
82-111 mg dry extract (35-40:1), extraction solvent: acetone, corresponding to 60 mg silymarin calculated as Silibinin (HPLC)	Supportive treatment of chronic inflammatory liver diseases, hepatic cirrhosis and toxic liver damage.	1976
131.6-161.0 mg dry extract (50-60:1), extraction solvent: acetone, corresponding to 84 mg silymarin calculated as silibinin (HPLC)		
173-186.7 mg dry extract (36-44:1), extraction solvent: ethyl acetate, corresponding to 108.2 mg silymarin calculated as silibinin (HPLC)		
170-239 mg dry extract (40-70:1), extraction solvent: acetone, corresponding to 140 mg silymarin calculated as silibinin (HPLC)		
123-208.3 mg dry extract (26-45:1), extraction solvent: ethyl acetate, corresponding to 83.3 mg silymarin calculated as silibinin (HPLC)		

Herbal preparation Pharmaceutical form	Indication	Period of medicinal use
135-152 mg dry extract (20-35:1), extraction solvent: acetone, corresponding to 83 mg silymarin calculated as silibinin (HPLC)		
176-200 mg dry extract (35-45:1), extraction solvent: acetone, corresponding to 105 mg silymarin calculated as silibinin (HPLC)		1976 1998
86.5-93.3 mg dry extract (36-44:1), extraction solvent: ethyl acetate, corresponding to 54.1 mg silymarin calculated as silibinin (HPLC)		1976 2006
162.5-250 mg dry extract (20-35:1), extraction solvent: ethyl acetate, corresponding to 105 mg silymarin calculated as silibinin (HPLC)	Supportive treatment of chronic inflammatory liver diseases, hepatic cirrhosis and toxic liver damage. Supportive treatment of liver disorders.	1976 2001
150-163 mg dry extract (25-40:1), extraction solvent: acetone, corresponding to 83 mg silymarin calculated as silibinin (HPLC) (3 marketed products)	Supportive treatment of chronic inflammatory liver diseases, hepatic cirrhosis and toxic liver damage.	1976 2006
170-200 mg dry extract (60-70:1), extraction solvent: ethanol 96% V/V, corresponding to 117 mg silymarin calculated as silibinin (HPLC)		1994
242.8-285.7 mg dry extract (60-70:1), extraction solvent: ethanol 96% V/V, corresponding to 167 mg silymarin calculated as silibinin (HPLC)		1994
162.5-250 mg dry extract (20-33.3:1), extraction solvent: ethyl acetate, corresponding to 105 mg silymarin calculated as silibinin (HPLC)		1995

Herbal preparation Pharmaceutical form	Indication	Period of medicinal use
173.0-186.7 mg dry extract (36-44:1), extraction solvent: ethyl acetate, corresponding to 140 mg silymarin calculated as silibinin (8 marketed products)	Supportive treatment of chronic inflammatory liver diseases, hepatic cirrhosis and toxic liver damage.	1994 2000 2006
243-286 mg dry extract (60-70:1), extraction solvent: ethanol 96% V/V, corresponding to 172 mg silymarin calculated as silibinin (HPLC)		1994
136.0-160.0 mg dry extract (50-70:1), extraction solvent: acetone, corresponding to 85, 86, 86.5 or 86.6mg silymarin calculated as silibinin (HPLC)		1996 1997
194.45-218.75 mg dry extract (20-50:1), extraction solvent: ethanol 96% V/V, corresponding to 117 mg silymarin calculated as silibinin (HPLC)		1998
277.8-312.5 mg dry extract (20-50:1), extraction solvent: ethanol 96% V/V, corresponding to 167 mg silymarin calculated as silibinin (HPLC)		1998
131.6-161.0 mg dry extract (DER variable), extraction solvent: acetone, corresponding to 84 mg silymarin calculated as silibinin (HPLC)		1998
167.69-206.44 mg dry extract (36-44:1), extraction solvent: ethyl acetate, corresponding to 109 mg silymarin calculated as silibinin (HPLC) (3 marketed products)		2007

Herbal preparation Pharmaceutical form	Indication	Period of medicinal use
239.57-294.32 mg dry extract (36-44:1), extraction solvent: ethyl acetate, corresponding to 156 mg silymarin calculated as silibinin (HPLC) (3 marketed products)	Supportive treatment of chronic inflammatory liver diseases, hepatic cirrhosis and toxic liver damage.	2007
228.9-238.2 mg dry extract (32-48:1), extraction solvent: ethyl acetate, corresponding to 140 mg silymarin calculated as silibinin (HPLC)		2007
177.4-240.4 mg dry extract extraction solvent: acetone 95% V/V, corresponding to 108.2 mg silymarin calculated as silibinin (HPLC) (4 marketed products)		2009
239.57 – 294.92 mg of dry extract from milk thistle fruit (36-44:1), corresponding to 200 mg silymarin, expressed as silibinin	Additional therapy in patients with toxic liver damage, chronic inflammatory liver conditions and hepatic cirrhosis	2009
162.5 – 250 mg of dry extract from milk thistle fruit (20-33:1), corresponding to 105 mg silymarin, expressed as silibinin	Herbal medicinal product for additional therapy in patients with toxic liver damage, chronic inflammatory liver conditions and hepatic cirrhosis	1997
35 mg silymarin	As an additional treatment in patients with chronic inflammatory liver diseases, cirrhosis, liver dystrophy, fatty degeneration after hepatitis and toxic functional liver disorders. Prophylaxis of liver impairment during prolonged administration of medicinal products, alcohol consumption, in chronic intoxication (including occupational).	1996 2002
80mg silymarin	Toxico-metabolic hepatic impairment, especially those with a high peroxidation levels such as the ones derived from chronic alcoholic intake, drug-caused hepatic impairment, steatosis, alcoholic hepatitis and cirrhosis.	1974
162.5 - 250 mg Cardui marianae fructus extractum siccum (20-33:1), with 105 mg silimarin calculated as silibinin in 1 coated tablet, extraction solvent: ethylacetate	Toxic injury of the liver, for supportive treatment in chronic inflammatory conditions od liver and liver cirrhosis.	1996

Herbal preparation Pharmaceutical form	Indication	Period of medicinal use
240.0 mg Cardui mariae fructus extractum siccum with 150 mg silimarin calculated as silibinin in 1 coated tablet		1996
86.5–93.3 mg Cardui mariae fructus extractum siccum with silimarin 70 mg calculated as silibinin in 1 capsule, extraction solvent: ethylacetate 96.7%		1994

b) Traditional Use

Herbal preparation	Indication	Period of medicinal use
300 mg powdered dry fruit	Dyspeptic complaints	1982 1990
400 mg powdered dry fruit	Dyspeptic complaints	1990
Dry extract, DER 30-40:1 Extraction solvent ethanol 96% V/V	Traditionally used to support digestive function	1976
Soft extract, DER 10-17: 1 Extraction solvent ethanol 60% V/V		
450 mg powdered fruit	Relief of symptoms associated with occasional over indulgence of food and drink such as indigestion and upset stomach	2011
150 mg standardised dry extract, equivalent to 3.60-4.05 g of Milk thistle fruits corresponding to 87mg of silymarin, calculated as silibinin, extraction acetone 95% v/v		2012
125 mg standardised dry extract, equivalent to 2.5-5.0 g of Milk thistle fruits corresponding to 62.5-75 mg of silymarin, calculated as silibinin, extraction solvent ethyl acetate (3 marketed products)		2012
137.5-165 mg of standardised dry extract, equivalent to 2,750-6,600 mg of Milk thistle fruits corresponding to 82.5 mg of silymarin calculated as silibinin, extraction solvent ethyl acetate	Relief of symptoms associated with occasional over indulgence of food and drink such as indigestion and upset stomach	2012

193-261 mg of standardised dry extract (21-45:1) equivalent to 3.725-10.818 g of Milk thistle fruits corresponding to 108 mg of silymarin calculated as silibinin, extraction solvent acetone 95% V/V (3 marketed products)		2010 2011
89-121 mg of standardised dry extract (21-45:1) equivalent to 1.721-5.0 g of Milk thistle fruits corresponding to 50 mg of silymarin calculated as silibinin, extraction solvent acetone 95% V/V		2010
500 mg of standardised dry extract equivalent to 10.0-20.0 g of Milk thistle fruits corresponding to 250-300 mg of silymarin calculated as silibinin, extraction solvent ethyl acetate		2012

3. Non-Clinical Data

Several pharmacological studies have demonstrated that *Silybum marianum* and its isolated constituents display many properties *in vivo* and *in vitro*. A systematic review of all these studies will not be attempted here, rather a selection of studies with emphasis on studies with relevance for the clinical efficacy will be reviewed (for a more extensive review, see **Saller *et al*. 2007, 2008)**. Further findings from pharmacological and pharmacokinetic studies on humans are available and will be discussed below (see section 4.1.).

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

The pharmacological activity of *Silybum marianum* is due to a mixture of flavonoids known as silymarin (1.5 to 3% of dry weight), which is made up mostly by flavonolignan isomers: silibinin (or silybin) A and B (50-60%), isosilibinin (or isosilybin) A and B (5%), silichristin A and B (20%) and silidianin (10%) **(Lee & Liu, 2003; Morelli, 1978; Quercia *et al.*, 1980; Smith *et al.*, 2005; Sziági *et al.*, 1981)**. Most pharmacological studies have been performed using a standardized extract of *Silybum marianum* fruit. Investigations conducted with other *Silybum marianum* fruit preparations are stated explicitly.

More than 700 publications have been found concerning pharmacological data on silymarin, silibinin, silicristin or milk thistle. Results show that silymarin exerts a multifunctional and multitarget activity. A summary of these findings is included here. **Saller *et al*. (2001)** reported the following pharmacological activities for *Silybum marianum*:

- Regulation of cell membrane permeability
- Leukotriene inhibition
- Reactive oxygen species scavenging (antioxidant effect)
- Action on DNA expression: anticarcinogenic and anti-inflammatory effects; cytoprotection

- Other effects (*in vitro/ in vivo* experiments): inhibition of reactive collagen formation, reduction of lectin-dependent and natural killer cell-mediated cytotoxicity, inhibition of glucose-stimulated insulin release *in vitro* but not *in vivo*, increase in pancreatic and blood glutathione (GSH) and inhibition of the alloxan-induced hyperglycaemia.

Saller *et al*. (2007):

- Regulation of cell membrane functions
- Cytoplasmatic effects: antioxidant, prevention of relief of drug-induced hepatic injury
- Inhibition of inflammation pathway
- Effects mediated by nuclear DNA/RNA
- Immunomodulation
- Potential antitumoral effect
- Skin protective properties against UV-induced damage in experimental models (topical and/or systemic use)

Navarro & Montilla (2012):

- On the liver:
  - Antioxidant activity
  - Cytoprotection: enhancement of cell membrane stability
  - Enhancement of protein biosynthesis
  - Antifibrotic activity
  - Anti-inflammatory and immunomodulatory activities
  - Antiviral activity
  - Anticholestatic activity
- Antitumoral activity
- Prevention of bone loss
- Neuroprotection
- Antiaterogenesis

3.1.1. Primary pharmacodynamics

In vitro studies

Extracts

Milk thistle extract (not further specified) induced a concentration-dependent (0.025-0.2 mM) reduction in the sluicing out [^{14}C]adenine nucleotides and lactate dehydrogenase triggered by microcrystine LR in rat liver cells, as well as a reduction in morphological deformation of the cells **(Mereish & Solow, 1990; Münter *et al*., 1986).**

Milk thistle extract (not further specified) at 0.5 mM diminished the lesions caused by paracetamol in both a human cell line and human hepatoblastoma cells due to an increase in glutathione concentration which is responsible for detoxifying reactive metabolites of paracetamol **(Shear *et al*., 1995).**

Isolated substances

Sonnenbicher & Zeti (1992) elucidated the molecular mechanism of action of silibinin. It binds to a regulative subunit of the DNA-dependent RNA polymerase 1 at a specific site and thus activates this enzyme; this action provokes a stimulation of the synthetic rate of ribosomal RNAs and an increased formation of intact ribosomes which finally causes a propagation of the protein biosynthesis.

Silibinin does not induce new genes but can stimulate DNA replication and mitosis, if the signal for cell proliferation and mitosis is given (i.e. in partial hepatectomy).

Silibinin changes the physical properties of the liver cell membrane by decreasing the turnover rate of phospholipids and by inhibiting the incorporation rate of cholesterol precursors [1-^{14}C] acetate and [2-^{14}C] mevalonate in the postmitochondrial supernatant of rat liver homogenates **(Nassuato *et al*., 1991).** Intraperitoneal administration of silymarin to rats at 200mg/kg/body weight raised the *in vitro* uptake of [^{3}H]uridine in bone marrow cells due to enhances membrane permeability (no increase in the activity of RNA polymerase was detected) **(Garrido *et al*., 1989).**

This effect on cell membrane permeability is also responsible of preventing the transport of toxins of the death cap (*Amanita phalloides*) and so their uptake in the liver cell **(Kröncke *et al*., 1986).**

At high concentrations (32-61 µM), silibinin induced a reduction of anti-IgE and f-met-peptide-induced histamine release from human basophilic leucocytes, this effect being attributed to membrane stabilization **(Miadonna *et al*., 1987).**

All the previous assays, together with the fact that silibinin concentration-dependently (0.5-25µg/ml) inhibited the activation of T-lymphocytes by lectins and anti-CD3 monoclonal antibodies (as activation takes place via membrane structures), allows some authors to assume the "membrane effect" of silibinin **(Meroni *et al.*, 1988).**

Silibinin (as a water-soluble hemisuccinate at 10^{-3} M for 12 h) is capable of preventing the damage induced by various hepatotoxins (release of LDH, ASAT and ALAT, development of vacuoles, deformation cellular necrosis) **(Davila *et al*., 1989).**

Silibinin is able to prevent the bile salt secretory failure (cholestasis) associated with taurolithocholate (TLC)-induced and oestradiol-β-D-glucuronide (E,17G)-induced retrival of bile salt export pump (Bsep) in rat hepatocytes. These toxins are responsible of inducing the endocytic internalization of Bsep and so block the transport of tauro- and glycoconjugated bile salts trough the canalicular membrane. Silibinin (2.5µM) prevented that effect by improving the capacity of hepatocytes to transport and accumulate into their canalicular vacuoles the fluorescent Bsep substrate cholyl-lysylfluorescein probably involving cAMP as a second messenger **(Crocenzi *et al.*, 2005).**

Silibinin antagonizes the release of leukotriene B_4 in isolated Kupffer's cells of the rat and in human liver macrophagues (IC_{50}: 15µM) **(Dehmlow *et al*., 1996).**

Chronic liver diseases, such as cirrhosis, induce fibrotic nodules. Fibrosis provokes hepatic functional remodelations (mainly conversion of hepatic starry cells- HSC – to miofibroblasts) that lead to hepatic insufficiency, portal hypertension and hepatic encephalopathy. Silymarin and silibinin delay HSC activation probably due to inhibition of the NF-κB activation (**Gebhardt, 2002)** and the α-SMA (actine muscular alpha) expression after CCl_4 intoxication in rats **(Tsai *et al.,* 2008).** α-SMA decrease is associated with a decrease in activated HSC; the loss in these cells by apoptosis leads to a decrease in

liver fibrosis so the beneficial effect of high doses of silymarin (200mg/kg) on fibrosis may be due to promotion of HSC apoptosis **(Tsai *et al.,* 2008).**

Silibinin also showed a relevant antifibrotic effect when incubated with fat-storing cells of the liver, which are considered as precursors of myofibroblasts (75% inhibition); it lowered their transformation into myofibroblasts, reduced the genetic expression of extracellular matrix constituents and the profibrigenic transforming growth factor β (TGF-β) **(Fuchs *et al*., 1995, 1997).**

Silibinin (as the water-soluble hemisuccinate: 2µg/ml) inhibited extracellular accumulation of fibronectin probably due to its radical scavenging activity **(Wenzel *et al*., 1996).**

More than 40 studies have been published during the last 10 years concerning the radical scavenging properties of silymarin and/or its individual components. A summary of the results is included.

Free radicals induce peroxidation of lipidic components in cell membrane, proteins and nucleic acids. Silymarin exerts radical scavenging activity against different radicals such as 1,1-diphenyl-pycryl-hydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenztiazoline-6-sulphonic acid)(ABTS) and antioxidant activity in other systems due to its ability to scavenge ROS and to quelate Fe^{2+} anions. Moreover, it strongly inhibits peroxidation of linoleic acid (82.7%) **(Köksal *et al*., 2009, *in* Navarro & Montilla, 2012).**

Silymarin and silibinin rapidly react with the radical hydroxyl and decrease lipidic peroxidation although they are not efficient in scavenging hydrogen peroxide radical (6, en Navarro y Montilla). They are capable of restoring and promoting the activity of the endogenous antioxidant enzymatic system (CAT, GSHPx, SOD), when oxidative stress is induced, and so an increase in intracellular GSH concentration **(Connelli *et al*., 2007, *in* Navarro & Montilla, 2012).**

Antioxidant ability of silibinin has been shown in many cellular models (Kupffer cells, hepatocytes, HepG2, isolated mictochondria and models of ischemia-reperfusion). Silibinin inhibitis free radicals formation; it binds to different radical species and acts as a strong scavenger, it interferes with the peroxidation of lipids in cell membrane and thus modulates membrane permeability; it increases the intracellular content of radical scavengers. Under oxidative and nitrosative stress conditions, silibinin inhibitis the synthesis of superoxide radicals and nitric oxid (NO); it increases ADP phosphorilation and so, ATP levels; it decreases malondialdehyde (MDA) levels and counteracts the decrease in several antioxidant enzymes (CAT, GSH, GSHPx, SOD and glutathione reductase) dose-dependently. It is also capable of inhibitihg hydrogen peroxide production in human monocytes that had been treated with phorbol myristate **(Bannwart *et al*., 2010, *in* Navarro & Montilla, 2012).**

While silymarin is able to protect hepatocytes from lipid peroxidation induced by differente cytotoxic agents. Silicristin and silidianin were the most effective between the silymarin components in protecting against the toxic effects of allyl alcohol and CCl_4 in primary human hepatocytes **(Dvorak *et al*., 2003,).** Silibinin (as water-soluble hemisuccinate) at 20 µM completely blocked ethanol-induced release of LD in primary human hepatocytes **(van Pelt *et al*., 2003).**

Moreover, silymarin and isolated silibinin, dehydrosilibinin, silychristin and silydianin are capable of protecting cardiomyocytes against doxorubicin-induced oxidative strees, mainly due to their cell membrane stabilization effect, radical scavenging and iron chelating potency, silydianin being the best protector **(Chlopcíková *et al*., 2004).**

An oxidated derivative from silibinin (dehydrosilibinin-DHS) shows a stronger antioxidant activity than silibinin, probably due to the insaturated groups in the molecula which allow hydrogen donations; moreover, it is more liposoluble and so its interaction with the cell membrane is more favourable **(Katiyar *et al*., 2011 *in* Navarro & Montilla, 2012).**

Silibinin (50 µM) also protected blood constituents from oxidative damage and so exerted an antiatherogenic effect (25-47%); it inhibitis Cu^{2+}/H_2O_2 fluorescence generation by 54%. 2.5 µM silibinin inhibited EDTA-Fe^{2+}/ H_2O_2- induced fluorescence by 31% **(Filipe *et al.,* 1997).** The differences in the antioxidative activities of individual flavonolignans from silymarin were shown by **Boisio *et al*., (1992):** silymarin > silicristin = silidianin > isosilibinin = silibinin. Results are included in Table 2.

Table 2. IC_{50} values (µg/ml) for the formation of malondialdehyde of isolated flavolignans

Compound	IC_{50}
Silymarin (standardized 60% silymarin)	24.2 (23.3 -25.2)
Silibinin	16.3 (13.1 – 20.3)
Isosilibinin	15.4 (14.2 – 16.9)
Silicristin	11.8 (10.4 – 13.4)
Silidianin	102.0 (80.3 – 129.7)

<u>In vivo studies</u>

Extracts:

Oral administration.

The study by **Zhang *et al.* (2013)** evaluated the protective effects of one marketed extract of milk thistle on alcoholic fatty liver model of female Sprague-Dawley rats. The control group received a normal diet; model group was treated with ethanol for 6 weeks; silymarin groups received the standardised dry extract of *S. marianum* at 100, 150 and 200 mg/Kg for 6 weeks (dry extract was previously dissolved into water by 20, 30 and 40mg/ml), together with ethanol by gavage twice a day.

After 6 weeks of treatment, blood was collected and liver tissue was taken for histological examination. In the milk thistle groups, less degree and less extensive of fatty liver were observed than in the model group which were statistically significant for doses of 150 and 200mg/kg ($p<0.05$, $p<0.01$, respectively). Treatment with milk thistle (200mg/kg) showed a greater decrease in serum activities of ALT and ASt and the level of total bilirubin (TBIL) than in model group ($p<0.05$), with no difference in the level of total cholesterol (TC).

Also malondialdehyde (MDA) levels, as a marker of lipid peroxidation, were decreased after milk thistle treatment ($p<0.01$). Ezymatic antioxidant activity was increased for glutathione peroxidase (GPx) at every dose ($p<0.05$) and for superoxide dismutase (SOD) at 150 and 200mg/kg ($p<0.01$). Doses of 150 and 200mg/kg significantly decreased hepatic triglyceride content ($p<0.01$). Lower levels of NF-κB and IL-6 were found in treated groups (150 and 200mg/kg).

Authors concluded that milk thistle extract attenuate the liver injury caused by alcohol; the effect may be related to the alleviation in lipid peroxidation and inhibition of the expression of NF-κB **Zhang *et al.* (2013)**.

The radical scavenging activity of milk thistle and/or its flavonolignans has been tested in *in vivo* systems. Ethyl acetate (100 mg/kg bw) and ethanol seed dried extracts from *S. marianum* (100 mg/kg bw) were tested in rats during 10 days after *i.p.* injection of carbon tetrachloride (2 ml/kg bw). Ethanolic extract showed the most significant decrease in liver enzymes. For the oxidative

experiments, ethyl acetate showed the highest ans significant increase in glutathione levels and the risk factor HDL/LDL (**Shaker *et al.*, 2010**). Also oral silymarin at 50 mg/kg) **(Favari & Perez-Alvarez, 1997)** was capable of significantly decrease liver enzymatic systems levels up to normal values while glutathione levels were increased; lipid peroxidation was prevented; Na^+, K^+, and Ca^{2+}-ATPase activities of liver cell membranes were reduced; glycogen content of the liver was reducted and bilirubin concentration was increased, respectively. Chronic treatment of rats with CCl_4 induced an increase in serum lipoperoxidation and the cholesterol-phospholipid ratio in erythrocyte membranes, together with a decrease in Na^+, K^+, and Ca^{2+}-ATPase activities; simultaneous treatment with milk thistle extract (not further specified at 50 mg/kg/day) improved ATPase activities and maintained the normal cholesterol-phospholipid ratio. These results may indicate that measurement of ATPase activites could act as a useful marker of liver damage **(Mourelle & Franco, 1991; Muriel & Mourelle, 1990)**.

Antiinflammatory and antifibrotic effects of silymarin (oral sylimarin- not further specified- 50mg/kg, 5 times per week) are associated with activation of hepatic stellate cells through the expression of TGF-β1 and stabilization of mast cells **(Jeong *et al*., 2005).** Authors suggested that milk thistle extract may prevent hepatic fibrosis through suppression of inflammation and hypoxia in the hepatic fibrogenesis. With the aim of exploring the antifibrotic mechanism of silymarin (standardized extract with 60% silibinin),

Silymarin (50 mg/kg, oral administration for several weeks) is capable of reducing accumulation of collagen in the liver by 30-35% ($p< 0.01$) and prevent weight increase in liver and spleen when secondary fibrosis is induced **(Boigk *et al*., 1997).** In this study. a rat model of biliary fibrosis secondary to bile duct obstruction (BDO) which can induce progressive portal fibrosis and finally cirrhosis has been applied to the study of the antifibrotic effect of a standardized extract of *S. marianum* (containing 60% silibinin). BDO model is almost devoid of the generation of toxic radicals, massive hepatocyte necrosis or major inflammation and so, it resembles human (biliary) liver fibrosis and allows the detection of antifibrotic effect regardless of the radical scavenging or anti-inflammatory properties which had been previously reported for milk thistle.
Adult female Wistar rats were subjected to abdominal incision and were located to one of the following groups: 1) sham-operated control (n=10); 2) BDO (n=100): midline abdominal incision, dissection of the common bile duct and retrograde injection of Na-amidotrizoate at 0.02ml/100g body weight. Rats with BDO received no treatment (n=10 for 3 weeks and n=20 for 6 weeks) or one of the following doses of oral milk thistle water into the chow: 25mg/kg/day from week 1 to 6 (n=20), 50mg/kg/day from week 1 to 6 (n=20), or 50mg/kg/day from week 4 to 6 (n=20). The sham-operated controls received a dose of 50mg/kg/day. After 6 weeks (or 3 weeks for rats with BDO alone), body weight and abdominal circumference were determined and rats were killed. Heart, liver, spleen and kidneys were weighted and 1- to 2g pieces of the left and right liver lobes were fixed for histological staining and hydroxyproline (HYP) determinations. Also the aminoterminal propeptide of procollagen type III (PIIINP), as another marker of liver fibrogenesis, was assessed.
23 among 100 rats died shortly after operation due to complications of local infection, so survival analysis was not performed. All rats with BDO became icteric within 2-4 days after surgery. BDO induced an active bile duct proliferation accompanied by a progressive and reproducible fibrotic enlargement of portal fields, followed by the formation of incomplete and complete *porto-portal septa* in the absence of significant inflammation or necrosis.
Ascites was absent in sham-operated animals but was found in 60% of the rats with BDO which had received no milk thistle extract; the percentage of ascites was significantly diminished in rats receiving 50mg/kg/day ($p<0.01$). In relation to biochemical parameters, all rats with BDO displayed moderately increased transaminases and highly elevated serum parameters of cholestasis ($p<0.001$); no differences were found among BDO groups, except for a significant decrease in alkaline phosphatase for the group receiving 50mg/kg/day. Also, a significant decrease in the histological grading of fibrosis

was found for the same group, with a less pronounced distortion of the liver architecture. Treatment with milk thistle extract reduced but did not prevent bile duct proliferation and formation of *fibrous septae*. Pharmacokinetic data from the same study showed that cholestasis increased milk thistle extract concentration in the liver by 2,3-fold. PIIINP was significantly reduced in the rats with lowered hepatic collagen, i.e. those animals receiving 50mg/kg/day of milk thistle extract, but not in the control or 25mg/kg/day groups.
Authors concluded that at the tested dose of 50mg/kg/day, milk thistle extract is capable of reducing the accumulation of total liver collagen by 30-35%, even when started at an advanced stage of biliary fibrosis (week 6). Also the relative amount of collagen per gram of liver tissue was reduced by 15% ($p<0.01$), this suggesting a true antifibrotic effect of the drug. The PIIINP model may be relevant to fibrosis in man due to its predictive power: PIIINP is mainly released from newly formed collagen fibrils during fibril growth and should reflect the dynamic process of the rate of collagen deposition in certain time, thus making it necessary to be measured regularly (every 2-3 months) in patients with chronic liver disease (**Boigk *et al*., 1997)**.

Taking in account the previous results, **Jia *et al.,* (2001)** designed an experiment in female adult Wistar rats which received a) milk thistle extract (50mg/kg/day) by oral route after sham operation; b) bile duct occlusion (BDO) with the same milk thistle treatment and posology and c) BDO without milk thistle treatment. After 6 weeks of treatment, a statistically significant suppression in the expression of profibrogenic procollagen α1(I) and TIMP-1 in those animals which had received milk thistle treatment was observed.

This antifibrotic effect has also been proved in baboons that were fed alcohol with or without silymarin (not further specified) (39.8mg/kg day, oral use) for 3 years. Milk thistle extract counteracted the alcohol-induced oxidative stress and the increase of liver lipids and circulating ALT. Alcohol also increased hepatic collagen type I by 50% with a statistically significant increase of mRNA for α1(I) procollagen; both effects and morphological changes (cirrhosis, fibrosis) were prevented by silymarin. Authors concluded that milk thistle extract retards the development of alcohol-induced hepatic fibrosis **(Lieber *et al*., 2003).**

The efficacy of milk thistle in the treatment of acute intoxication of the liver has been proved in several experimental models. *I.p.* injection of silymarin (150 m/kg) after oral administration of CCl_4 (4 g/kg) to rats induced distinct improvement in the studied parameters 24h after injection compared to untreated controls: peroxidation of liver lipids, phosphatidylethanolamine and phosphatidylinositol contents and gamm-glutamyl transpepetidase (GGTP) and alkaline phosphatase (AP) activities of plasma membranes **(Muriel & Mourelle, 1990)**. Silymarin and silibinin (as water-soluble hemisuccinate) at less extent, may reduce metabolic activation of reactive CCl_4 metabolites and then scavenge them as demonstrated in a combined *in vivo* and *in vitro* study **(Letteron *et al*., 1990)**: reduction in covalent binding of CCl_4 metabolites to hepatic lipids; reduction in expiration of ethane (product from CCl_4 metabolism) and in liver cell necroses (*in vivo*); reduction in the activity of various monooxygenases and in the covalent binding of CCl_4 metabolites to microsomal proteins and peroxidation of lipids (*in vitro*).Treatment with silymarin (not further specified) partially alleviated the toxic damage induced by gamma-irradiation (2 x 70 mg/kg/day for 14 days) by *Plasmodium berghei* (2 x 70 mg/kg/day for 14 days) **(Haková & Misurová, 1996).**

Strubelt *et al*. (1980) evaluated the influence of silibinin on the hepatotoxic and hypoglycaemic effects induced by praseodymium, cerium and lanthanum in rats. The toxics produced a dose-dependent increase in the serum activities of GOT, GPT and SDH that were shifted to the right by simultaneous treatment with silibinin (75 mg/kg, *i.p.*). Silibinin did also attenuate the increase of bromosulphthaleine retention and prevented the accumulation of liver triglycerides induced by praseodyum; it also reduced the mortality rate of rats treated with high doses of the lanthanides.

The same tendency was found after intragastric administration of silibinin (dosage not stated), which was capable or restoring abnormal values of lipidic peroxidation, mitochondrial respiration and permeability and cell death. The best results were obtained with silibinin conjugated to phosphatidylcholine; this complex showed scavenging properties towards several radicals, such as hydroxyl, hydroxyethyl, lipodienyl, methyl and tricholoromethyl (**Navarro & Montilla, 2012**).

Several publications demonstrate the efficacy of milk thistle in the prevention of liver damage: Silymarin (100 mg/kg, *i.p.* administration) antagonized tallium-induced liver damage to a large extent **(Mourelle et al., 1988)**. Administration of 100 mg/kg/day silymarin (*i.p.*) for 5 days to rats partially prevented the taurolithocholate-induced cholestasis; it increased biliary excretion of the toxin (+104%, p< 0.05) and accelerated the inactive metabolite formation (+70%, p< 0.05) **(Crocenzi *et al*., 2003)**.

Silymarin (2 x 50 mg/kg/day, not further specified) failed to prevent liver damage induced by bile duct ligation **(Muriel and Moreno, 2004).**

The study by **Strubelt *et al*. (1980)** also showed that silibinin completely prevented hypoglycaemia and corrected partially the changes in the biochemical parameters of liver function except for the decrease of liver GSH, which demonstrated that glutathione is not implicated in the interaction between silibinin and praseodyum.

Isolated substances

Not oral administration

In healthy rats, *i.p.* administration of silymarin (200 mg/kg) increased total glutathione content and redox state of the liver, intestine and stomach of rats, but not of the kidney, lungs and spleen; this selective effect may be due to pharmacokinetic reasons, maintained by enterohepatic circulation **(Valenzuela *et al*., 1989).**

Silibinin (as hemisuccinate, *i.p.* 100 mg/kg/day) administrated for 7 days to healthy rats reducted biliary cholesterol and phospholipid concentrations to 61% and 73% of control values, respectively, probably due to inhibition of HMG-CoA reductase activity. No changes in bile flow, total bile salt concentration and cholesterol content of the liver were found **(Nassuato et al., 1991).**

I.v. administration of silibinin (as the water-soluble hemisuccinate) (25 or 50 mg/kg) to rats was capable of modulating the decrease in GSH and the increase in MDA induced by paracetamol (*i.p.* administration); it also reduced serum transaminase levels almost to the control value **(Campos *et al*., 1989)**. Silibinin (*i.v.* 100 mg/kg in rats) and silymarin (2 mg/kg) are capable of reducing serum ALAT and ASAT levels and the necrotic areas of the liver on ischaemic liver injury **(Wu *et al.,* 1993)**. After intravenously administration to rats (50 mg/kg), silibinin potentiated the protective effect of ethanol on paracetamol induced liver damage (increase in GSH content of the liver, decrease in GPT and GOT in the serum) through inhibition of reactive paracetamol metabolites **(Garrido *et al.,* 1989)**. These results were similar to those obtained in rats which were pre-treated with oral silymarin (200 mg/kg) and then a liver damage was provoked with 3-methylcholanthrene and paracetamol **(Muriel *et al.,* 1992)**.

3.1.2. Secondary pharmacodynamics

Immunomodulating activity

Several studies have been conducted in order to investigate the immunomodulatory activity of silymarin.

Using CD4+ splenocytes from C57/B16 mice, silymarin (ethanolic extract SIGMA) at 50μM concentration, significantly inhibited cells proliferation and IL-2 and IFN-γ production, together with P65/NF-κB phosphorylation. These results indicate that milk thistle extract is able to inhibit T cells activation and proliferation, notably acting on pathways of NF-κB activation/translocation (**Gharagozloo *et al*. 2010).** The same effect was proven when silymarin (ethanolic extract SIGMA) at 100μM concentration was tested in human CD4+ cells obtained from Peripheral Blood Mononuclear Cells (PBMC) from healthy individuals. Milk thistle extract inhibited T cell proliferation and pro-inflammatory cytokine secretion *in vitro*, as well as ERK1/2 and P38 pathway activation (**Gharagozloo et al., 2013a).** Following this line of research, another study demonstrated that the same milk thistle preparation at 100μM concentration induced a significant G1 arrest in cell cycle of activated T lymphocytes and inhibition of the level of phosphor-S6 ribosomal protein and mTOR activity (**Gharagozloo et al., 2013b).**

Nonetheless, immunomodulating effect of silymarin is strongly dose-dependent and results in immunostimulation at low doses and immunosuppression at high doses (**Gharagozloo et al., 2013c).**

Anti-carcinogenic effects

Milk thistle extract (not further specified) inhibited in a dose-dependent manner the phorbol ester-induced tumour promotion in mammary tumour celss of the mouse; a 50% inhibition was observed at 1.0 μM **(Mehta and Moon, 1991).** Another milk thistle extract (not further specified) inhibited the transformation of rat tracheo-epithelial cells after treatment with benzo(a)pyrene **(Steele *et al.,* 1990)**.

Both diastereomers of silibinin at 0.1-20 μM inhibited proliferation of human ovarian and mammary cancer cells and increased the sensitivity of multi-drug-resistant tumour cells to cytostatics such as doxorubicin or cisplatin. Silibinin also inhibited the clonogenic efficiency of human ovarian tumour cells (IC_{50}: 6.4-7.4 μM) **(Scambia *et al.,* 1996).**

Mechanisms of the cancer chemopreventive action of silymarin include the inhibition of activation of epidermal growth factor receptor (EGFR) probably due to antioxidative action; inhibition of EGFR-mediated tyrosine kinase, followed by impairment on the entire cell cycle progression; activation of a cyclin-dependent kinase inhibitor. These effects were observed with several human cancer lines and differente silymarin concentrations: prostate cells (silymarin: 75-150 μg/ml); mammary cells (50-75 μg/ml); skin cells (10-100 μg/ml) **(Ahmad *et al*., 1998; Zi *et al.,* 1999a; 1999b).**

On the other hand, silibinin acts by suppressing antiapoptotic genes through inhibition of NF-κB signalling **(Singh et al, 2002)**.

Both silibinin (100 μM) and silymarin (24-72 μg/ml) exerted antiproliferative and antiapoptotic effects dose-dependent on several rat prostate cancer cell lines, although at concentrations that are usually considered as not relevant for the biological situation **(Singh et al, 2002 &; Tyagi *et al*., 2002)**.

Silibinin inhibits the growth of human prostate tumour xenografts in nude mice **(Singh et al., 2002)**, inhibits telomerase activity and secretion of prostate specific antigen (PSA). Silibinin down-regulates PSA mRNA expression and so PSA secretion, which indicates the interaction between silibinin and the expression of genes regulated trough the androgen receptor **(Thelen *et al*., 2004)**.

Antiatherogenic effects

Silymarin (ethanolic extract) inhibited monocyte adhesion to human umbilical vein endothelial cells; it also suppressed the TNF-K-induced protein and mRNA expression of adhesion molecules, such as

VCAM-1, ICAM-1 and E-selectin. So milk thistle extract exerts an anti-atherosclerotic activity that is due, at least in part, to the inhibition of the expression of adhesion molecules **(Kang *et al.,* 2003).**

Antiviral activity

Antiviral activity of silibinin A and B and their dihydrosuccinates has been assayed against hepatitis C virus- RNA polymerase dependent and on NS3/4A protease. It has also been tested its ability to inhibit replication of the 1b genotype. Both assays demonstrated that the tested compounds inhibited the RNA-polymerase dependent hepatitis C virus, with IC_{50} 75-100 µM, while none of them inhibited NS3/4A protease.

Silymarin isolated compounds inhibit the union and entrance of the virus inside the host cell, probably due to the hydrophobic structure of the flavonolignans that allows their incorporation to the membrane lipids with the following stabilization. Silibinin A, silibinin B and their water-soluble dihydrogen succinate forms inhibited HCV RNA-dependent RNA polymerase function, with inhibitory concentrations 50% of the order of 75-100 microM **(Ahmed-Belkacem *et al.*, 2010; Polyak *et al.,* 2010; Wagoner *et al.*, 2010 *in* Navarro & Montilla, 2012).**

Other activities

In a recent review by **Navarro and Montilla (2012)** the preventive effect of silymarin (dosage not stated) on bone loss when administered to ovariectomized rats is included; this antiosteoporotic activity is mediated trough a selective estrogen receptor modulator activity **(El-Shitany *et al*., 2010, *in* Navarro and Montilla, 2012)** and the inhibition of the osteoclast differentiation mediated by TNF family members **(Kim et al., 2009, *in* Navarro and Montilla, 2012)**.

Oral treatment of partially hepatectomized rats with silymarin (not further specified) at 20 mg/kg for 7 days significantly increased the mitotic index and the rate of synthesis of DNA (thymidine incorporation) and RNA (uridine incorporation) **(Srivastava *et al*., 1994).**

A recent study shows that silymarin potentiates the antinociceptive effect of morphine in mice, probably due to its interaction with the opioid system and its antioxidant capacity. In this study, silymarin (from Sigma-Aldrich) was dissolved in normal saline and injected (s.c.) as a single injection of different doses (5, 10, and 25 mg silymarin/kg, b.w.) **(Malekinejad *et al.,* 2011).**
Silymarin may contribute to the prevention of age-related and degenerative processes in brain due to the antioxidant effect exerted by silymarin: rats treated with doses of 200 and 400 mg/kg/day showed a decrease in lipid peroxidation and on oxidized proteins in aged brain **(Galhardi *et al.,* 2009)**.

Other beneficial effects of milk thistle such as skin protection, prostate, lungs, CNS, kidneys or pancreas diseases are based on its antioxidant and radical scavenging properties, as well as on specific receptor interactions **(**Gažák **et al., 2004, 2009).**

Potential antiatherogenic activity of silibinin (CBS 22888-70-6) is shown from its inhibitory effect on Low density lipoprotein (LDL) oxidation *in vitro*, taking in account that LDL oxidation and smooth muscle cell growth represent key events in atherogenesis. Silibinin (50-200 mu mol/l) prolonged the lag times of both LDL autooxidation and oxidation by copper by > 50 %, as assessed by recordings of diene formation **(Locher *et al.,* 1998).**

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

Vereckei *et al* (2003) studied the effect of the simultaneous administration of one milk thistle extract (silymarin, standardized extract) and the antiarrhythmic drug amiodarone on the electrophysiologic (EP) action of the latter. Sixtytwo mongrel dogs with electrically induced atrial flutter were divided into four groups for different treatments: 1) oral amiodarone (600mg/day, 8 weeks); 2) oral amiodarone and silymarin (70mg bid, 8 weeks); 3) silymarine (70mg bid, 8 weeks); 4) untreated control group.

After the 8 weeks treatment, milk thistle extract prevented the amiodarone-induced increase in postsurgical atrial flutter mean cycle ($AFCL_m$), although its mechanism of action is unknown. Treatment with milk thistle alone does not have any significant EP or antiarrhythmic effect but can modulate the EP action of amiodarone by reuding the conduction velocidity decreasing effect of amiodarone and thereby preventing the amiodarone-induced increase in post-surgical $AFCL_m$ (**Vereckei *et al* 2003).**

3.1.5. Conclusions

Apart from the radical scavenging properties shown by milk thistle extract and isolated components, several specific studies confirm their beneficial effects on liver injury. *S. marianum* flavonolignans exert *in vitro* hepatoprotective activity after the induction of liver damage with different toxic substances**.** The results obtained by *in vivo* studies with milk thistle extract on different liver injuries, with a reduction in collagen content in liver, regulation of gluconeogenesis and significant antiinflamatory and antifibrotic effect, could contribute to the plausibility of the positive effect of *S. marianum*.

Isolated silibinin, the major component of milk thistle extract, stimulates the protein biosynthesis and induces a membrane stabilization of hepatic cells that may prevent toxins transport and so explain its beneficial effect on liver recovery. It also exerts antifibrotic effect; moreover, silibinin is able to prevent cellular damage and secretory failure associated with various hepatotoxins. After *i.p.* administration, it reduces biliary cholesterol and phospholipids concentration in plasma.

All the above cited findings have to be considered when testing the hepatoprotective effects by clinical studies. Also pharmacokinetic data should be taken in account to explain the results obtained after treating healthy subjects or patients with different liver diseases (see section 4.1).

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Silibinin is the main compound, also deemed to be the active one, so pharmacokinetic parameters of silymarin and the active principle of any silymarin-containing product is most frequently referred to silibinin.

3.2.1. Absorption

Milk thistle extract

Milk thistle extract is water insoluble and the absorption after oral administration is about 2-3% of the silibinin recovered from rat bile after 24h. About 20-40 per cent of the administered dose of milk thistle extract is excreted in bile as sulphates and glucuronide conjugates. Maximum plasma levels are obtained 4-6h after administration, while elimination half-life is approximately 6h **(Pradhan & Girish, 2006).**

Woo *et al.,* 2007 reported the value of $AUC_{0-\infty}$ as 22.75 ± 3.19 µg h/ml after the administration of one milk thistle extract capsule to rats at a dose of 140 mg/Kg.

Isolated compounds

Silibinin is partially absorbed from the gastrointestinal tract (23-47%) and it seems to depend on several factors such as its poor hydrosolubility, the content of accompanying substances with a solubilising character (other flavonoids, phenolderivatives, aminoacids, proteins, tocopherol, fat, cholesterol and others found in the extract), degradation by gastric fluid or poor enteral absorption and the extract concentration itself **(Saller *et al.*, 2001; Usman *et al.*, 2009)**; this lead to a low oral bioavailability. After oral administration of single doses of silibinin (102, 153, 203 and 254 mg), a linear correlation is found between the doses and the total area under the plasma concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$) and maximum plasma concentration for both unconjugated and total silibinin isomers.

The unconjugated form of silibinin on plasma reached only 10% of the oral dose **(Saller *et al.,* 2001)**.

3.2.2. Distribution

(Saller *et al.,* 2001).Silymarin appears to undergo extensive enterohepatic circulation, achieving biliary levels several times greater than those in serum.

3.2.3. Metabolism and Elimination

Milk thistle extract

Some 20-40% of the total oral dose of milk thistle extract is excreted in bile as glucuronide and sulphate conjugates and continues for 24h after a single dose, with only 2-5% being excreted in urine.The remaining percentage is excreted in faeces (unchanged, not absorbed) (**Saller *et al.,* 2001).**

Isolated compounds

The elimination half-life of total silibinin is nearly 6h and about 5% of the oral dose is excreted into urine as total silibinin with renal clearance of approximately 30 ml/min **(Saller *et al.,* 2001)**.

3.2.4. Pharmacokinetic interactions with other medicinal products

Silibinin inhibits phase I and phase II enzymes and inactivates cytochromes P450 3A4 and 2C9. It is also a potent inhibitor of UGT1A1, an enzyme responsible for glucuronidation of several drugs such as naltrexone, buprenorphine, estradiol and irinotecan (concentration not stated). Nonetheless, the clinical relevance of these interactions is not well defined or unknown (**The Review of Natural Products: Milk Thistle, 2005).**

3.2.5. Conclusion

Pharmacokinetic studies of *Silybum marianum* have been published for the plant extract, silymarin, and also for the most abundant component in this flavonolignans mixture, silibinin.

In both cases, the low hydrosolubility explains the low absorption after oral administration. Most of the plasmatic silibinin is in the conjugated form and a 20-40 percentage of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates, then being excreted in faeces. Only a low percentage is found in urine.

Silibinin has shown inhibitory activity on several enzymes and cytochromes. This activity is not correlated to pharmacokinetic interacions in humans (see section 4.1.2.5).

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

(DerMarderosian, 2001) Silymarin has proved non toxic in rats and mice after oral doses of 2,500 or 5,000mg/Kg.

In a 12-month study, rats receiving silymarin 50, 500 and 2500mg/kg showed no evidence of toxicity after urine analysis and post-mortem studies. Similar results were obtained for dogs.

No evidence of ante-postnatal toxicity in animals was reported. Silymarin did not affect fertility in rats.

3.3.1. Single dose toxicity

(Pandey *et al.*, 1990). The oral administration of different extracts from *Silybum marianum* fruits (extraction solvents: petroleum ether, alcohol, water) in mice (4 male, 4 female) at 500, 1000, 1500 and 2000 mg/kg body weight induced no deaths within 48 h.

(Hahn *et al*., 1968). Single dose toxicity studies with milk thistle extract showed an acute oral dose superior to 20 g/kg body weight and 1g/Kg in mice and dogs, respectively. No death or adverse effects were observed.

3.3.2. Repeat dose toxicity

(Hahn *et al.*, 1968). No adverse effects or differences from controls were obtained in rats after daily oral doses of silymarin at 1 g/kg body weight for 15 days (n=40) or 100 mg/kg for 16 weeks (n=20) or 22 weeks (n=20).

The NTP in the USA has conducted extensive studies on chronic toxicity, carcinogenicity and genotoxicity of milk thistle extract. The results have been published by **Dunnick et al (2011a; 2011b; 2013)**. In these studies, male and female F344/N rats and B6C3F1 mice were exposed to an ethanol/water extract of milk thistle fruit (milk thistle extract) containing approximately 65% silymarin in feed for 3 months or 2 years.

3-MONTH STUDY IN RATS: Groups of 10 male and 10 female F344/N rats were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm milk thistle extract (equivalent to average daily doses of approximately 260, 525, 1,050, 2,180, or 4,500 mg milk thistle extract/kilogram body weight to males and 260, 510, 1,050, 2,150, or 4,550 mg/kg to females) for 14 weeks. All rats survived to the end of the study. Mean body weights of exposed groups were within 10% of those of the controls. Feed consumption by exposed and control groups was similar. The sperm motility in 12,500, 25,000, and 50,000 ppm males was decreased by 5%, 11%, and 9%, respectively, relative to that of the controls; the total number of spermatid heads per testis decreased by 11%, 21%, and 9% in 12,500, 25,000, and 50,000 ppm males. No significant differences in estrous cyclicity were observed between exposed and control groups of female rats. No exposure-related histopathologic lesions were observed.

3-MONTH STUDY IN MICE: Groups of 10 male and 10 female B6C3F1 mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm milk thistle extract (equivalent to average daily doses of approximately 640, 1,340, 2,500, 5,280, or 11,620 mg/kg to males and 580, 1,180, 2,335, 4,800, or 9,680 mg/kg to females) for 14 weeks. All mice survived to the end of the study. Mean body weights and feed consumption of all exposed groups were similar to those of the controls. Absolute and relative thymus weights were significantly decreased in 25,000 and 50,000 ppm males. No significant differences were observed between exposed and control groups, for sperm parameters of male mice, for estrous cyclicity of female mice, or for reproductive organ weights of male or female mice, when

mice were administered milk thistle extract in feed at 12,500, 25,000, or 50,000 ppm. No exposure-related histopathologic lesions were observed.

3.3.3. Genotoxicity

Dunnick *et al.,* 2011.Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli* and mouse peripheral blood erythrocytes. Milk thistle extracts were tested independently in bacterial mutagenicity studies using various combinations of *S. typhimurium* tester strains and one *E. coli* strain. Results were negative in three of the five studies, with and without exogenous metabolic activation. In two studies, milk thistle extract was mutagenic in *S. typhimurium* strain TA98 in the presence of exogenous metabolic activation enzymes.

Silymarin, a major constituent of milk thistle extract, was positive in *S. typhimurium* strains TA98 and TA100 with exogenous metabolic activation enzymes.

Silibinin, another component of milk thistle extract, was negative in a *S. typhimurium* gene mutation assay, with and without liver S9 activation enzymes.

The extract- and strain-specific results are tabulated below in Table 3.

Table 3. Bacterial genotoxicity results of milk thistle extracts and constituents.

Preparation	TA97	TA98	TA100	TA102	TA104	TA1535	E.coli
Batch of 3-mo study (ethanol/water)		-	-	-			
Batch of the 2-yr study (ethanol/water)		+ (w/o; w)	-				-
Methanol		+ (1/4 w/o; 4/4 w)	-				
Water	-	-	-	-	-	-	
Silymarin		+	+				
Silibinin	-	-	-			-	

Administration of milk thistle extract in feed for 3 months (doses stated above) did not increase the frequencies of micronucleated normochromatic erythrocytes, an indication of chromosomal abnormalities, in the peripheral blood of male or female B6C3F1 mice.

(Anderson *et al.*, 1997a; Anderson *et al.*, 1997b). The Ames test for a milk thistle extract (provided by Sigma-Aldrich and specified as "*Mixture of anti-hepatotoxic flavonolignans from the fruit of Silybum marianum*") showed no mutagenic activity at 1-1000 µg/plate but gave positive responses in the Comet assay with human lymphocytes. This extract reduced the mutagenic/genotoxic activity of two food mutagens in both test systems but had no influence on the mutagenic activity of doxorubicin; it also gaves a weak positive response in a chromosome aberration assay.

Duthie *et al.*, 1997 tested the milk thistle extract (provided by Sigma-Aldrich and specified as "*Mixture of anti-hepatotoxic flavonolignans from the fruit of Silybum marianum*") which gave positive responses in the Comet assay with HeLa (epithelial cells) at concentrations of 450 µM and higher, but no genotoxicity was found with human Caco-2 (colon), HepG2 (liver) and normal human lymphocytes at concentrations of up to 1000 µM.

Concentrations of 0.1-0.5 mmol/plate of one milk thistle extract (not further specified) reduced the mutagenic activity of nitrosamines in the Ames test under metabolic activation (S9 mix) **(Teel, 1997)**, but not without S9 mix **(Miller *et al.,* 1994)**

Another milk thistle extract (not further specified)reduced the DNA-damaging activity of hydroquinone, benzoquinone, benzotriol, hydrogen peroxide and vitamin C but not that of bleomycin and doxorubicin **(ESCOP monograph, 2009).**

In conclusion, some milk thistle extracts have demonstrated mutagenicity principally with metabolic activation in some Salmonella strains, whereas most extracts and strains have been negative. The extract used for the NTP 2-year feed studies was positive in the Ames test in strain TA98, but the extract used for the 3-month study was negative in both the Ames test and the mouse micronucleus test. Other studies employing various cultured cells with the Comet assay gave variable results. Silymarin seems to demonstrate some mutagenicity in the Ames assay. It seems that a compound (or compounds) of the extract, which is (are) not regularly present in sufficient amounts, causes some mutagenicity. Only the water extract which was not used for toxicity studies, was adequately tested according to the OECD guidelines, although this aqueous extract is not part of the Milk thistle monograph.

3.3.4. Carcinogenicity

Dunnick *et al*. (2011). In connection with the NTP repeated dose toxicity 2-year feed studies, groups of 50 male and 50 female F344/N rats were fed diets containing 0, 12,500, 25,000, or 50,000 ppm milk thistle extract (equivalent to average daily doses of approximately 570, 1,180, or 2,520 mg/kg to males and 630, 1,300, or 2,750 mg/kg to females) for 105 to 106 weeks. Exposure to milk thistle extract had no effect on survival of male or female rats. Mean body weights of all exposed groups were similar to those of the controls throughout the study. Feed consumption by exposed groups of males and females was generally similar to that by the controls throughout the study. Significantly decreased incidences of mammary gland fibroadenoma, adenoma, or carcinoma (combined) occurred in females exposed to 25,000 or 50,000 ppm. Significantly increased incidences of clear cell and mixed cell focus of the liver occurred in 25,000 and 50,000 ppm females. The incidences of bile duct hyperplasia were significantly decreased in 50,000 ppm males and in all exposed groups of females while the incidence of mixed inflammatory cell infiltration was significantly decreased in 50,000 ppm males.

In the 2-year feed study in mice, groups of 50 male and 50 female B6C3F1 mice were fed diets containing 0, 12,500, 5,000, or 50,000 ppm milk thistle extract (equivalent to average daily doses of approximately 1,610, 3,530, or 7,770 mg/kg to males and 1,500, 3,175, or 7,180 mg/kg to females) for 105 to 106 weeks. Exposure to milk thistle extract had no effect on survival of male or female mice. The mean body weights of the 25,000 ppm groups were less than those of controls after week 25; mean body weights of 50,000 ppm groups were less than those of controls after week 12. Feed consumption by exposed groups of males and females was generally similar to that by the controls throughout the study. Significantly decreased incidences of hepatocellular adenoma and hepatocellular carcinoma occurred in 50,000 ppm males, and decreased incidences of hepatocellular adenoma or carcinoma (combined) occurred in 25,000 and 50,000 ppm males.

In conclusion, there is no evidence of carcinogenic activity of milk thistle extract in male or female F344/N rats or B6C3F1 mice exposed to 12,500, 25,000, or 50,000 ppm. The original NTP study with Milk thistle extract - M990059 **(NTP, 2011)** was further summarized and discussed together with similar studies conducted on several other herbal preparations by **Dunnick & Nyska (2013)**.

Malewicz *et al.,* (2006) conducted one study in a transgenic mouse model (MMTV-neu/HER2) with 0.3% silymarin in the diet and application from d28-d300 or d120-d300. Sylimarin treatment

increased the incidence and multiplicity of mammary tumors and no-mammary tumours. In a second model of female rats with MNU induced mammary tumors, sylilmarin increased the number of mammary tumors when applied with the diet from d21 to d110 after MNU injection, The mechanism of induction however remains unclear.

3.3.5. Reproductive and developmental toxicity

No data on developmental toxicity are available from the literature.

In the 3-month NTP study referred to above, a slight decrease in sperm motility and number was observed in F344/N male rats. No effect on estrous cyclicity in female rats was observed. No significant differences were observed between exposed and control groups, for sperm parameters of male mice, for estrous cyclicity of female mice, or for reproductive organ weights of male or female mice.

3.3.6. Local tolerance

No data are available from the literature.

3.3.7. Other special studies

Not available.

3.3.8. Conclusions

Studies conducted with milk thistle extract in the NTP long-term feed studies do not show any carcinogenicity and insignificant chronic toxicity. There is some evidence about genotoxicity, but the findings are not consistent and they are sporadic with respect to extract, test system and study and the relevance of these findings as to the risk to humans is in doubt.

3.4. Overall conclusions on non-clinical data

Many pharmacological, pharmacokinetic and toxicological studies conducted with preparations of *Silybum marianum* fruit extract and its isolated constituents have been published in the scientific literature showing their potentially beneficial effects in various models of acute and chronic liver damage.

For one standardised extract of milk thistle silymarin, and for silibinin, the main active ingredient of silymarin, antioxidative, antifibrotic, anti-inflammatory, protein synthesis stimulating and membrane protecting mechanisms have been demonstrated in experimental pharmacology**.** In particular, the antifibrotic potential of silibinin has been confirmed by several in vivo studies conducted in human hepatic stellate cells (HSC) or different animal models such as rats or baboons **(Trappoliere *et al.* 2009; Boigk et at., 1997; Lieber *et al*., 2003).**

These above described mechanisms of actions are relevant for the clinical application in chronic liver disease by reducing the intracellular oxidative stress and by decreasing new collagen deposition, at slowing down the progression to fibrosis and cirrhosis in the liver. In this context, recent studies have provided insights even about the molecular mechanisms by which milk thistle extract exerts its anti-inflammatory action: in particular, it has been shown to reduce inflammatory cytokine (TNF-α) release from activated human T cells, and this reduction is specifically associated (as shown in a human hepatoma cell line) with inhibition in the transcription of NF-κB gene complex **(Polyak *et al.* 2007).**

Pharmacokinetic studies of *Silybum marianum* constituents are available and give an evidence for numerical quantity values and bioavailability.

Milk thistle extract does not demonstrate any carcinogenicity and insignificant chronic toxicity in the NTP long-term feed studies. There is some evidence about genotoxicity, but the findings are not consistent and they are sporadic with respect to extract, test system and study and the relevance of these findings as to the risk to humans is in doubt. Overall, it seems that exposure to milk thistle extract does not cause unacceptable risks to humans. However, because of insufficient genotoxicity studies for the herbal preparations defined in the monograph, the list entry cannot be recommended.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

4. Clinical Data

4.1.1. Clinical pharmacology

4.1.2. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Primary pharmacodynamics

Several clinical studies on the efficacy of *Silybum marianum* in liver diseases have been performed that evaluate the effect of the herbal administration on different liver parameters (ALT, albumin, bilirrubin); as stated in the previous section, when potential antifibrotic agents are evaluated, it is important to include end points relevant to fibrosis **(Schuppan & Harn, 2001)**.

(Realini *et al.,* 1975). An early clinical study was performed on the effect of milk thistle extract (standardized extract containing 70mg silymarin, 3 times daily) on different chronic liver diseases (persistent chronic hepatitis, aggressive chronic hepatitis, liver steatosis and cirrhosis). Only 23 patients were recruited who received 210 mg/day of silymarin (in three doses) for 3, 6 or 12 months in an open trial. Appetite, fatigue, nausea, alcohol consumption, jaundice, hepatomegalia, ascitis, biochemical parameters such as VS, GOT, GPT, alkaline phosphatase, bilirrubin, prothrombin, BSP, and Australian antigen were controlled. A histological control was performed on some patients at the end of treatment. Results were not positive for every patient; the best outcomes are obtained for steatosis, cirrhosis and persistent chronic hepatitis. No dermatological, haematological or gastrointestinal side-effects were observed.

(Saba *et al.,* 1976). A controlled study was performed with 19 patients with liver diseases who were also diagnosed with schizophrenia. They received psychodrugs (chlorpromazine alone or combined with benzodiazepine). Treatment with one standardized extract (70mg silymarin) for 180 days (210 mg/ day, 3 times daily) singificanlty improved biochemical parameters referred to liver cell function (SGOT, SGPT levels, BSF, albumin, bilirrubin, alkaline phosphatise). No adverse effects were reported. Authors concluded that treatment with silymarin is beneficial in the treatment of yatrogenic hepatopathy and that the therapeutic effect of silymarin is based on its action at the cell membrane level.

A more recent study was carried out to determine the effect of silymarin in alcoholics with liver cirrhosis with respect to survival and clinical and laboratory changes **(Parés *et al*., 1998)**. 200 alcoholic patients (daily ethanol intake over 80 g in men and 60 g in women for a period longer that 5 years) were enrolled in a randomized, double-blind multicenter trial comparing 450 mg of silymarin (150 mg/three times per day) with placebo for 2 years. The first parameter to assess was time to death, while the secondary one was the progression of liver failure (serum levels of bilirrubin, AST,

ALT, γ-GT, alkaline phosphatise, albumin, γ-globulins and prothrombin index). 125 patients completed the study period; survival was similar in patients receiving silymarin or placebo; silymarin did not have any significant effect of the course of the disease. So authors concluded that silymarin had no effect on survival and the clinical course in alcoholic with liver diseases.

Also patients with alcoholic cirrhosis were the target population to assess the effects of a new non-standardized silymarin MZ80 (70-80% of the silymarin complex; silibinin 35.07%) **(Lucena *et al.,* 2002)**. Sixty patients with alcoholic liver cirrhosis were randomized to receive either silymarin MZ80 (150 mg, three times per day, orally) or placebo for 6 months. Erythrocyte total glutathione (GSH) content, platelet malondialdehyde (MDA) and serum aminoterminal propeptide of procollagen Type III (PIIINP) were determined at baseline and at the end of treatment. Forty nine patients completed the study; silymarin significantly increased total GSH at 6 months *versus* placebo ($p< 0.001$) whereas platelet-derived non-induced MDA significantly decreased ($p< 0.015$). A parallel decrease in PIIINP values was seen with silymarin ($p< 0.033$). Authors concluded that the tested milk thistle extract was well-tolerated and produced a small increase in glutathione and a decrease in lipid peroxidation in peripheral blood cells in patients with liver cirrhosis, inducing no changes in routine liver tests.

Secondary pharmacodynamics

No data available

Safety pharmacology

No data available.

Pharmacodynamic interactions

Piscitelli *et al.* (2002) *in* The Review of Natural Products, Evidence-Based Herb-drug interactions (2004) reports the potential interaction between milk thistle and indinavir documented from a clinical trial. Pharmacologic effects of indinavir may be decreased although a clinically important interaction is unlikely. The author conclusion hasn't been proved by clinical results.

A recent study was carried out to evaluate the safety and feasibility of milk thistle for the treatment of hepatotoxicity in children with acute lymphoblastic leukemia (ALL) who were receiving maintenance-phase chemotherapy **(Ladas *et al.*, 2010)**, as in this group of patients, the administration of chemotherapy agents is frequently interrupted because of liver toxicity, especially during the maintenance phase of treatment. 50 children with ALL were included in a randomized, double-blind, placebo controlled trial for 28 days; patients were randomized to receive milk thistle extract (capsules containing 240 mg of milk thistle, standardized to 80 mg of silibinin (Silybin A and B) with a target dose of silibinin of 5.1 mg/kg/day) or placebo orally, the day after *i.v.* chemotheraphy. The following dose ranges were prescribed for milk thistle: patients weighing 15-20 kg received 80 mg/day; 21-40 kg patients received 160 mg/day; 41-60 kg patients received 240 mg/day; and 61-70 kg patients received 320 mg/day. After the treatment period, no significant differences in frequency of side effects, incidence and severity of toxicity or infections were observed between groups. No significant changes in mean ALT, AST or total bilirrubin at day 28 were found; at day 56, the group receiving the milk thistle extract had a significant lower ALT ($p= 0.05$) and a trend towards a significant lower ALT ($p=0.07$).

No antagonistic interactions between milk thistle and vincristine or L-asparaginase in cell-culture were observed, while a modest synergistic effect with vincristine was shown. Authors concluded that treatment with milk thistle in children with ALL and liver toxicity is associated to a significant reduction

in liver toxicity, although further studies are needed to determine the most effective dose and duration of milk thistle treatment.

4.1.3. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Absorption

An early pharmacokinetic study with milk thistle extract was carried out with healthy subjects who received 560 mg of standardized milk extract containing 240 mg silibinin **(Lorenz *et al*., 1984)** and patients who had undergone cholecystectomy and received 140 mg of the same extract (60 mg silibinin). Results are presented in Tables 4 and 5.

Table 4. Pharmacokinetic parameters for silibinin in the serum after single oral administration of 560 mg of standardized milk thistle extract containing 240 mg silibinin **(Lorenz *et al.*, 1984)**

	Silibinin
Absorption half life (h)	0.17 ± 0.009
$t_{1/2}$ (h)	6.32 ± 3.94
t_{max} (h)	1.32 ± 0.45
C_{max} (µg/ml)	0.34 ± 0.16
AUC_{0-24} (µg × h/ml)	1.14 ± 0.26
$t_{½}$ (h)	4.86 ± 1.35
Data are mean values ± SD	

Table 5. Pharmacokinetic parameters for silibinin in the bile after single oral administration of two different formulations of standardized milk thistle extract to cholecystectomized patients with T-tube drainage **(Lorenz *et al.*, 1984)**

	Standardized extract (35 mg) (n=8)	**Standardized extract (70 mg) (n=6)**
Elimination half life (h)	4.06 ± 1.05	4.84 ± 1.51
C_{max} (µg/ml)	30.26 ± 12.72	25.75 ± 15.24
AUC_{0-24} (mg x h)	87.40 ± 20.78	83.07 ± 50.64
Mean time (h)	8.12 ± 2.24	8.35 ± 2.35
Relative bioavailability (%)	105	100
Data are mean values ± SD		

Authors conclude that the difference in the mean time for serum (4.86h) and for bile (8.12 or 8.35h) is probably due to a more prolonged stay of silibinin in the liver, or to enterohepatic circulation in part maintained by the T-tube drainage, or to both factors together which might be desirable in a substance intended to treat liver diseases.

According to **Schandalik *et al.* (1992)** the bioavailabililty of silibinin is several fold higher when administered as a lipophilic complex (silibinin-phosphatidylcholine complex) than that of silymarin, this indicating an increased delivery of the compound to the liver. Table 6 shows the obtained results.

Table 6. Pharmacokinetic parameters (mean ± SD) of silibinin in bile after single oral dose (120 mg silibinin) administration of a lipophilic complex or milk thistle extract (200mg extract, equyivalent to 120mg silibinin) in 9 cholecystectomy patients **(Schandalik *et al.*, 1992)**

	Lipophilic complex	**Silymarin**
Amount recovered between 0 and 48h (% of dose)	11.2 ± 1.3*	3.0 ± 0.3
C_{max} (µg/ml)	116 ± 24*	29 ± 5
AUC_{0-24} (mg x h)	512 ± 66*	136 ± 14
$t_{½}$ (h)	10.3 ± 1.4	10.6 ± 1.4
Relative bioavailability (%)	420 ± 60	100
* p < 0.01 (*vs*. slilymarin)		

It is also interesting to note that levels of silydianin and silycristin were very low and even non-detectable in plasma and bile, so they are supposed not to contribute to the therapeutic effect of silibinin in silymarin-treated patients.

After 28 days of administration of milk thistle extracts (175-mg three times daily), the C_{max} for silibinin A, silibinin B, and isosilibinin B was 134.7 ± 72.0, 42.1 ± 27.3, and 26.8 ± 19.9 ng/ml, respectively. The mean T_{max} values were 2, 1, and 1 hour, respectively **(Zhu *et al.,* 2013).**

A more recent study was carried out on 24 healthy vounteers to compare the bioavailability of silibinin from three different preparations: Product A, in capsules (equivalent to 30 mg of silibinin), Product B, in capsules (equivalent to 60 mg of silibinin) and Product C, in tablets (equivalent to 30 mg of silibinin) **(Kim *et al*., 2003)**. Each volunteer received and oral dose of 120 mg of silibinin in a standard 3 x 3 crossover model (single dose, randomized, 3-treatment, 3-period, 6-sequence crossover design) with a 1-week washout period among the doses. The pharmacokinetic results are shown in Table 7.

Table 7. Pharmacokinetic parameters (mean ± SD) of silibinin after oral administration of 2 Legalon capsules, 4 Silymarin tablets, and 4 Liverman capsules, as 120 mg of silibinin, to 24 healthy volunteers **(Kim *et al*., 2003)**

	Product B	**Product C**	**Product A**
C_{max} (µg/ml)	1.33 ± 0.546	1.13 ± 0.516	6.04 ± 1.90
t_{max} (h)	1.83 ± 0.940	2.10 ± 1.07	0.875 ± 0.369[b]
AUC_{inf} (µg/ml x h)	6.00 ± 2.20[a]	4.63 ± 1.96	15.1 ± 3.68
AUC_{0-12} (µg/ml x h)	5.59 ± 1.85[a]	4.24 ± 1.79	13.9 ± 3.38
$t_{½}$ (h)	3.42 ± 0.698	3.20 ± 0.654	3.91 ± 0.631[b]
Ke (h^{-1})	0.220 ± 0.0504	0.226 ± 0.0509	0.82 ± 0.0362[b]
[a] = each group was significantly different (p < 0.05) [b] = Product A was significantly different from products B and C (p < 0.05)			

The highest concentration of silibinin was achieved with the preparation containg 30mg of silibinin, reaching values up to 354 and 435% higher than the other tested preparations; also time to reach the maximum plasma concentration was significantly faster for the first product. These data indicate faster silibinin absorption and an improvement in bioavailability (about 252 and 326%) when compared to products B and C, respectively.

To compare the kinetics of two different formulations of milk thistle was again the aim of the study performed on healthy male volunteers **(Usman *et al.*, 2009)**. Milk thistle extract was tested versus Product D as the reference product. Each volunteer received a single oral dose of 200 mg of both

products according to a randomized cross-over design. Table 8 shows the obtained pharmacokinetic parameters.

Table 8. Comparative bioavailability and pharmacokinetic parameters of silymarin Silliver and milk thistle standardized extract **(Usman *et al*., 2009)**

	Product D	**Milk thistle extract**
C_{max} (µg/ml)	2.9 ± 0.3	1.9 ± 0.1*
t_{max} (h)	1.9 ± 0.1	1.8 ± 0.1
AUC (µg x h/ml)	10.8 ± 0.4	11.2 ± 0.7
$t_{½}$ (h)	2.8 ± 0.1	3.5 ± 0.4
Vd (L/Kg)	1.2 ± 0.1	1.5 ± 0.2
Mean Residence Time (h)	4.6 ± 0.2	6.1 ± 0.4*
* $p < 0.05$		

There was non-significant difference in the controlled parameters except for C_{max} and MRT values; the former was higher for the reference formulation whereas the latter was superior for the tested product.

The difference found in C_{max} values may be due to the difference in the nature and source of *Silybum marianum* seed, extraction procedures used in the manufacture or formulation and processing variables. Both products can be considered similar on the basis of the values obtained for bioavailability coefficient and AUC (80-125%) and can be used equally in chronic conditions of liver disease except in those situations where a maximum plasma concentration must be achieved (i.e. acute conditions such as acute pain).

In summary, after oral administration, absorption is low and maximum plasma concentrations are reached after 4-6 hours.

Several studies have been conducted in healthy volunteers and patients with different liver diseases in order to determine whether the flavonolignanes from *S. marianum* show a different disposition and so, potential efficacy, between liver disease populations.

Schrieber *et al.* (2008). A single-dose, open-label, nonrandomized study enrolled five subjects into each of the four cohorts (n= 20, both sex) with the primary objective of determining preliminary and variance information for the pharmacokinetic parameters AUC, C_{max}, T_{max}, $C_{L/F}$, and $t_{1/2}$ in healthy volunteers and in patients diagnosed with either hepatitis C virus (HCV) and minimal liver disease or cirrhosis, or nonalcoholic fatty liver disease (NAFLD) receiving a single 600-mg oral dose of milk thistle extract (300mg of milk thistle extract standardized as 80%- 240mg- silymarin. Flavanolignans proportion was 37.7mg silibinin A; 58.8mg silibinin B; 14.8mg isosilibinin A; 6.3mg isosilibinin B; 39.2mg silychristin; 15.3mg silydianin, these six flavonoliganes accounting for 57% of the milk thistle extract contained in each capsule). After 24h, blood analysis showed that silibinin A and B accounted for 43% of the exposure to the sum of total flavonolignans in healthy volunteers and only 31 to 38% in liver disease cohorts as a result of accumulation of silychristin (20–36%). $AUC_{0–24h}$ for the sum of total silymarin flavonolignans was 2.4-, 3.3-, and 4.7-fold higher for HCV, noncirrhosis, NAFLD ($p < 0.03$), and HCV cirrhosis cohorts ($p < 0.03$), respectively, compared with healthy volunteers ($AUC_{0–24h}$= 2021 ng.h/ml) **(Tables 9 and 10).** Caspase-3/7 activity correlated with the $AUC_{0–24h}$ for the sum of all silymarin conjugates among all subjects (R^2= 0.52) and was 5-fold higher in the HCV cirrhosis cohort ($p < 0.005$ versus healthy). No correlation was observed with other measures of disease activity, including plasma alanine aminotransferase, interleukin 6, and 8-isoprostane F2α, as a measure of oxidative stress.

These findings suggest that the pharmacokinetics of silymarin is altered in patients with liver disease. Patients with cirrhosis had the highest plasma caspase-3/7 activity and also achieved the highest exposures for the major silymarin flavonolignans.

Table 9. Pharmacokinetics of parent silymarin flavonolignans (from Schrieber *et al.,* 2008).

TABLE 2

Pharmacokinetics of parent silymarin flavonolignans

Data are presented as geometric means (95% CI).

		SA	SB	ISA	ISB	SC	SD
Healthy	C_{max} (ng/ml)	12 (2, 67)	9 (2, 43)	3 (1, 14)	N.D.	N.D.	N.D.
	AUC_{0-24h} (ng · h/ml)	33 (2, 488)	23 (2, 302)	3 (1, 16)	N.D.	N.D.	N.D.
	CL/F (l/h)	970 (168, 5590)[a]	2354 (308, 18,034)[a]	2835[b]	N.D.	N.D.	N.D.
Noncirrhotic	C_{max} (ng/ml)	13 (1, 269)	12 (1, 252)	5 (0.3, 86)	5 (0.3, 85)	9 (1, 119)	4 (0.4, 39)
	AUC_{0-24h} (ng · h/ml)	13 (1,156)	16 (1, 397)	5 (0.4, 57)	5 (0.3, 63)	13 (1, 244)	5 (0.3, 90)
	CL/F (l/h)	713 (344, 1488)[c]	1581 (584, 4316)[c]	720[b]	281[b]	1095 (508, 2357)[c]	484[b]
Cirrhotic	C_{max} (ng/ml)	69 (45, 107)	33 (3, 385)	11 (1, 156)	15 (2, 104)	5 (0.3, 91)	N.D.
	AUC_{0-24h} (ng · h/ml)	41 (3, 549)	149 (115, 195)	12 (1, 191)	16 (2, 115)	7 (0.2, 251)	N.D.
	CL/F (l/h)	509 (389, 667)	1156 (614, 2165)[a]	505 (208, 1222)[c]	402 (240, 675)[a]	541[b]	N.D.
NAFLD	C_{max} (ng/ml)	61 (24, 157)	40 (14, 110)	N.D.	5 (0.3, 62)	N.D.	N.D.
	AUC_{0-24h} (ng · h/ml)	40 (19, 43)	84 (43, 166)	N.D.	4 (0.4, 51)	N.D.	N.D.
	CL/F (l/h)	904 (454, 1800)	3019 (1422, 6438)	N.D.	349[a]	N.D.	N.D.

Not determined (N.D.) indicates concentrations below limit of quantitation. Geometric means for CL/F reflect $n = 5$ unless otherwise specified: [a] $n = 4$, [b] the average of only two values, [c] $n =$ 2

Table 10. Pharmacokinetics of total (parent + conjugated) silymarin flavonolignans (from Schrieber *et al.,* 2008).

Pharmacokinetics of total (parent + conjugated) silymarin flavonolignans

Data are presented as geometric means (95% CI).

		SA	SB	ISA	ISB	SC	SD
Healthy	C_{max} (ng/ml)	37 (23, 61)	106 (63, 177)	71 (32, 157)	41 (21, 80)	37 (18, 76)	12 (2, 64)
	AUC_{0-24h} (ng · h/ml)	256 (118, 557)	617 (327, 1164)	491 (243, 993)	251 (145, 436)	355 (169, 745)	51 (3, 807)
Noncirrhotic	C_{max} (ng/ml)	73 (19, 282)	269 (73, 995)	131 (40, 422)	76 (21, 274)	144 (49, 423)	39 (12, 131)
	AUC_{0-24h} (ng · h/ml)	301 (100, 901)	1195 (374, 3823)	858 (282, 2610)	494 (154, 1588)	1699 (633, 4564)*	228 (82, 634)
Cirrhotic	C_{max} (ng/ml)	151 (71, 319)*	551 (309, 982)*	339 (160, 720)*	193 (110, 338)*	147 (68, 318)*	75 (33, 171)*
	AUC_{0-24h} (ng · h/ml)	685 (265, 1776)*	2899 (1082, 7767)*	2231 (641, 7760)*	1254 (478, 3287)*	1841 (802, 4226)*	507 (167, 1542)*
NAFLD	C_{max} (ng/ml)	124 (56, 278)*	430 (215, 860)*	216 (96, 485)	137 (55, 343)*	190 (100, 361)*	68 (43, 108)*
	AUC_{0-24h} (ng · h/ml)	521 (342, 795)	1720 (943, 3137)	1279 (690, 2371)	706 (406, 1230)	2043 (1170, 3566)*	352 (252, 493)

* $p \leq 0.02$, comparisons with the healthy cohort.

The study by **Schrieber *et al.* (2011)** examined the single and multiple dose pharmacokinetics of silymarin flavonolignans in patients with NAFLD or HCV. Patients received oral silymarin (standardized milk thistle extract: 280 or 560mg) or placebo every 8 hours for 7 days.

The flavonolignan content of each single dose was as follows: 23.2mg silibinin A; 32.0mg silibinin B; 11.8mg isosilibinin A; 6.6mg isosilibinin B; 24.9mg silychristin; 29.0mg silydianin, these six flavonoligannes accounting for 70.8% of the milk thistle extract contained in each capsule (**Schrieber *et al.* 2011)**.

Table 11 presents the pharmacokinetic parameters of silibinin A and silibinin B for NAFLD and HCV cohorts following single oral doses of either 280 or 560 mg milk thistle extract. Silibinin A is the predominant flavonolignan in plasma for both cohorts and is characterized by a 2.7 to 3.3-fold greater C_{max} and a 2 to 4.5-fold greater AUC_{0-48} compared to silibinin B.

Table 11. Single-dose pharmacokinetics of silibinin A and B (from Schrieber *et al.* ,2011)

Single-dose pharmacokinetics of parent silybin A and silybin B

Results are shown as geometric mean (95% confidence interval), except for T_{max}, which is shown as median (minimum, maximum). Data are for $n = 6$ subjects.

Cohort	PK Parameter	SA		SB	
		HCV	NAFLD	HCV	NAFLD
280 mg	$AUC_{0-8\,h}$ (ng · h/ml)	201 (115–338)	228 (75–469)[a]	93 (15–188)[b]	80 (70–91)[a]
	C_{max} (ng/ml)	78 (32–147)	82 (35–153)[a]	27 (8–50)[b]	30 (16–53)[a]
	T_{max} (h)	2.0 (1.0, 4.0)[b]	2.0 (1.0, 6.0)[a]	3.0 (1.0, 4.0)[b]	2.0 (2.0, 6.0)[a]
	$t_{1/2}$ (h)	1.3 (0.7–2.0)	1.8 (−2.2–7.6)	0.9 (−0.9–6.3)[b]	1.8 (−1.6–6.6)
560 mg	$AUC_{0-8\,h}$ (ng · h/ml)	557 (470–657)	859 (508–1397)	125 (94–160)*,[a]	261 (164–395)
	C_{max} (ng/ml)	192 (147–250)	275 (127–491)	58 (31–92)[a]	93 (55–145)
	T_{max} (h)	1.5 (1.0, 4.0)	2.7 (1.5, 4.0)	1.5 (0.5, 4.0)[a]	2.7 (1.0, 4.0)
	$t_{1/2}$ (h)	1.4 (0.9–2.1)	1.4 (0.8–2.3)	1.1 (0.7–1.7)[a]	1.5 (0.6–2.9)

SA, silymarin A; SB, silymarin B.
* $p < 0.05$.
[a] $n = 5$.
[b] $n = 4$.

At the lower dose, no differences were observed in the pharmacokinetics of silibinin A or B between HCV and NAFLD patients. Short elimination half-lives were observed for both compounds (0.9-1.8h).

Nonetheless, at the 560mg dose, pharmacokinetic differences were observed between HCV and NAFLD subjects: AUC_{0-48} for both silibinin A and B were 1.5 and 2.1-fold greater, respectively, for NAFLD subjects (both $p<0.05$). A similar trend was obtained for C_{max} for silibinin A and B, with no statistical significance. Elimination half-lives were similar between groups (1.1-1.5h), while T_{max} was delayed by 1h in NAFLD patients.

The steady-state pharmacokinetics of silibinin A and B following chronic oral administration of either 280 or 560mg milk thistle extract every 8h for 7days also shows silibinin A as the predominant flavonolignan in plasma for both HCV and NAFLD cohorts (Table 12), with 2.1 to 3.6-fold greater C_{max} and a 2.6 to 4.9-fold greater AUC_{0-48} than silibinin A.

Table 12. Steady-state pharmacokinetics of silibinin A and B (from Schrieber *et al.* ,2011)

Results are shown as geometric mean (95% confidence interval), except for T_{max}, which is shown as median (minimum, maximum). Data are for $n = 6$ subjects, except for the HCV 560-mg steady-state cohort where $n = 5$; one subject was dropped from the pharmacokinetic analysis because of incorrect dosing for pharmacokinetic sampling at steady-state on day 8.

Cohort	PK Parameter	SA		SB	
		HCV	NAFLD	HCV	NAFLD
280 mg	$AUC_{0-8\,h}$ (ng · h/ml)	370 (279–480)	317 (191–499)	86 (66–109)	123 (53–219)[a]
	C_{max} (ng/ml)	143 (78–242)	133 (60–262)	45 (28–72)	64 (30–111)[a]
	T_{max} (h)	1.8 (1.0, 4.0)	1.3 (0.5, 4.4)	1.5 (0.5, 4.0)	1.2 (0.5, 2.0)[a]
	$t_{1/2}$ (h)	1.1 (0.7–1.6)	1.1 (0.3–2.6)	0.7 (0.4–1.2)[a]	0.9 (0.2–2.1)[a]
560 mg	$AUC_{0-8\,h}$ (ng · h/ml)	729 (371–1195)	1166 (589–2128)	149 (40–310)	376 (161–759)
	C_{max} (ng/ml)	308 (104–620)	448 (255–724)	86 (26–186)	187 (93–332)
	T_{max} (h)	1.5 (1.5, 2.0)	3.0 (0.5, 4.0)	1.5 (1.5, 2.0)	3 (0.5, 4.0)
	$t_{1/2}$ (h)	1.3 (0.9–1.9)	1.0 (0.6–1.6)	1.1 (0.3–2.2)	0.7 (0.4–1.0)

SA, silymarin A; SB, silymarin B.
[a] $n = 5$.

No evidence of accumulation for either flavonolignan is shown, with elimination half-lives between 0.7 and 1.3h. Also similar to the single dose data, pharmacokinetic differences are only observed at the highest dose: the AUC_{0-8} for silibinin A and B is 1.6-fold and 2.5-fold greater, respectively, in NAFLD patients, while differences in the C_{max} are ranged between 1.5 to 2.2-fold.

The higher silibinin B exposures in NAFLD subjects suggest the metabolism or hepatic uptake of this compound may be reduced in NAFLD when compared to HCV.

Also the differences in plasma concentration of silibinin A and silibinin B conjugates have been estimated by the same authors after single dose or chronic administration **(Schrieber *et al.* (2011)** (Tables 13 and 14). Plasma concentrations are greater for silibinin A than for silibinin B and the converse happens with their conjugates: C_{max} and AUC_{0-8} for silibinin B conjugates is 3 to 4-fold greater than for silibinin A conjugates across both dose levels and disease cohorts.

The obtained data show that conjugation of silibinin B in NAFLD subjects is inferior to HCV at a milk thistle extract dose of 560mg.

Plasma concentrations of silibinin A and B are generally higher and the concentrations of their conjugates are lower in NAFLD subjects compared to HCV subjects irrespective of the dose frequency of oral milk thistle extract administration **(Schrieber *et al.,* 2011)**.

Table 13. Single-dose pharmacokinetics of silibinin A conjugates and silibinin B conjugates (from Schrieber *et al.* ,2011)

Results are shown as geometric means (95% confidence interval), except for T_{max}, which is shown as median (minimum, maximum). Data are for $n = 6$ subjects.

Cohort	PK Parameter	$SA_{conjugates}$		$SB_{conjugates}$	
		HCV	NAFLD	HCV	NAFLD
280 mg	$AUC_{0-48\,h}$ (ng · h/ml)	1327 (860–1925)	1003 (672–1456)	4094 (2465–6308)	3120 (2247–4233)
	C_{max} (ng/ml)	144 (96–205)	80 (45–125)	586 (441–762)	388 (266–544)
	T_{max} (h)	2 (1.5, 4.0)	4.0 (2.0, 12.0)	2.0 (1.5, 4.0)	3.0 (2.0, 8.0)
	$t_{1/2}$ (h)	5.7 (4.0–7.7)	6.4 (4.5–9.0)	4.3 (3.1–5.6)	4.5 (2.5–7.2)
560 mg	$AUC_{0-48\,h}$ (ng · h/ml)	3468 (1747–6024)	2844 (1493–4779)	12,760 (6505–22,432)	7850 (4501–12,664)
	C_{max} (ng/ml)	339 (169–621)	278 (126–488)	1691 (1003–2774)	1125 (805–1570)
	T_{max} (h)	3.0 (2.0, 4.0)	4.0 (4.0, 6.2)	4.0 (1.0, 4.0)	3.0 (1.5, 6.0)
	$t_{1/2}$ (h)	6.6 (4.5–9.2)	7.0 (5.0–9.7)	4.7 (3.7–6.0)	6.2 (3.3–10.3)

SA, silymarin A; SB, silymarin B.

Table 14. Steady-state pharmacokinetics of silibinin A conjugates and silibinin B conjugates (from Schrieber *et al.* ,2011)

Results are shown as geometric means (95% confidence interval), except for T_{max}, which is shown as median (minimum, maximum). Data are for $n = 6$ subjects, except for the HCV 560-mg steady-state cohort where $n = 5$; one subject was dropped from the pharmacokinetic analysis because of incorrect dosing for pharmacokinetic sampling at steady state on day 8.

Cohort	PK Parameter	$SA_{conjugates}$		$SB_{conjugates}$	
		HCV	NAFLD	HCV	NAFLD
280 mg	$AUC_{0\text{-}8\ h}$ (ng · h/ml)	2048 (1465–2815)	1297 (652–2271)	7278 (5633–9287)*	3962 (2338–6292)
	C_{max} (ng/ml)	369 (294–459)*	233 (146–352)	1294 (1040–1589)*	750 (563–981)
	T_{max} (h)	2 (1, 4)	2 (0, 4)	2 (1, 4)	1.8 (0, 2)
	$t_{1/2}$ (h)	5 (3–8.3)	6.8 (2.5–15.5)	4.3 (2.8–6.3)	5.1 (1.2–12.7)
560 mg	$AUC_{0\text{-}8\ h}$ (ng · h/ml)	3229 (1618–5348)	2902 (1403–5163)	11003 (3244–21,930)	7745 (4312–12,547)
	C_{max} (ng/ml)	543 (182–1050)	529 (287–894)	2074 (700–4103)	1592 (927–2600)
	T_{max} (h)	2 (0, 4)	4 (0, 6)	2 (2, 4)	3 (0, 4)
	$t_{1/2}$ (h)	6.2 (3.6–9.3)	3.6 (2.7–4.7)	4.1 (2.9–5.6)	2.8 (1.9–3.9)

SA, silymarin A; SB, silymarin B.
* $p < 0.05$.

Consistently with the short half-life of silibinin A and B, no accumulation is obtained in either cohort with repeated dosing. The overall amount of parent flavonolignanes in plasma is significantly higher in NAFLD patients at 560mg dose due to the appearance of additional parent flavolignanes such as silychristin and silydianin, which are not observed in HCV subjects **(Figure 2)**. In contrast with HCV subjects, significant enterohepatic cycling of the six flavonolignans is observed in NAFLD subjects

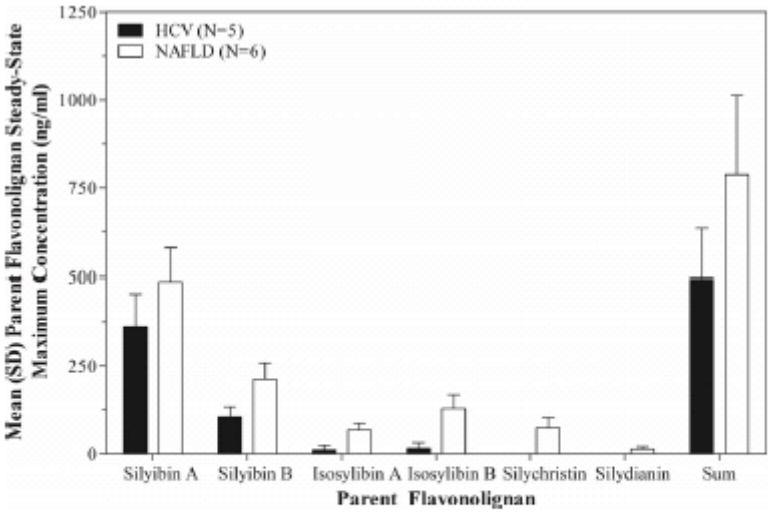

Figure 2. Maximum steady-state plasma concentrations for silymarin flavonolignans at 560 mg of silymarin in HCV (■) and NAFLD ( )□ubjects. Plasma concentrations of isosilybin A, isolsylibin B, silychristin, and silydianin were significantly greater in NAFLD subjects compared with HCV subjects. Silychristin and and silydianin were not detected in the plasma of HCV subjects.

As a conclusion, the above shown results demonstrate that differences exist in the disposition of flavonolignans between NAFLD and HCV patients which may reflect different disease-specific alterations in the function of hepatobiliary transport proteins. The antioxidant activity and potential anti-inflammatory and antifibrotic effects of milk thistle on disease progression are dependent on its hepatic disposition and so it may exert greater benefits in patients with NAFLD due to higher plasma concentrations and more extensive enterohepatic cycling when compared to patients with HCV.

Distribution

No data available.

Metabolism

Silymarin extracts undergo extensive metabolism, the majority of which are subject to phase II metabolic processes. Mono-, di-, and sulpho-glucuronides are known to be formed, then reaching plasma and bile **(Zhu et al., 2013).**

Elimination

Silymarin half life is between 6 and 8 hours: Drug is excreted mainly in bile (80%) and in a much lower extent, in urine **(Wen *et al.,* 2008).**

Pharmacokinetic interactions with other medicinal products

Mills *et al.*, 2005: Randomized controlled pharmacokinetics study in 16 healthy volunteers to determine the influence of milk thistle extract in indinavir pharmacokinetics.

Indinavir is used by patients with Human Immunodeficiency Virus (HIV); milk thistle is commonly taken by these patients in order to relief the hepatotoxicity related to the anti-retroviral drug therapy **(Mills *et al*, 2005).** Patients in the study received 456mg of *Silybum marianum* capsules (Kare and Hope Inc., Toronto, ON, USA), three times per day. Although previous *in vitro* studies had shown that milk thistle extract inhibited CYP 3A4 activity, results from this clinical trial indicate that indinavir levels were not significantly reduced in the presence of this herbal: less than 10% differences in AUC_{0-8}, C_{max} and $t_{1/2}$ values, and less than 25% differences in C_8 ($p > 0.31$).

These results are in accordance with those obtained in the three previous open studies using before-and-after designs to determine milk thistle/indinavir interactions.

Budha *et al*., 2007: A clinical trial on 12 healthy volunteers was carried out to study the effect of one milk thistle preparation on the oral bioavailability of ranitidine, a histamine (H_2) receptor antagonist which mainly blocks H_2 receptors in the stomach and is mainly metabolized by oxidation by CYP 3A4 and then eliminated mainly through urine and biliary system. *In vitro* studies had shown that silymarin may influence drug metabolism by decreasind the metabolic activity of CYP 3A4 and may also alter absorption, distribution and elimination through inhibition of P-glycoprotein (P-gp). Patients received one tablet of 150mg of ranitidine alone; then, after one week, they were administered silymarin 140mg capsules three times per day for 7 days. On day 8, a tablet of ranitidine 150mg and a capsule of silymarin 140mg were administered simultaneously.

Results from this study showed no effect of milk thistle on ranitidine pharmacokinetics: it failed to influence either ranitidine AUC_{0-8} or C_{max}, urinary excretion pattern (cumulative amount of drug excreted in urine) or plasma levels at those doses of silymarin recommended for hepatoprotectant effect (420mg/day).

4.2. Clinical efficacy

Liver diseases are of great relevance as the number of patients affected by alcohol-induced or other toxic liver damage and viral infections is continuously increasing. Elevated serum transaminase levels for over 6 months and/or other pathological biochemical parameters, together with clinical symptoms such as fatigue, exhaustion, abdominal discomfort and loss of appetite are signs that characterize chronic liver disease. Manifestations may vary from steatosis to hepatic cirrhosis or even hepatocellular carcinoma, this indicating that early intervention in liver diseases is of great importance.

Liver cirrhosis exerts a high impact on mortality and implies a large consumption of medical resources without any effective standard therapy. None of the hepatotherapeutic agents developed to date meets

all the therapeutic demands and so a selective causal drug treatment for chronic liver diseases is not available yet.

As a first measure for the treatment of chronic liver diseases, a general approach such as the elimination of noxae (abstinence from alcohol, discontinuation of hepatotoxic drugs, diet etc.) is considered. They are often not fully feasible and they cannot substitute or compensate a pharmacological therapy which is needed when the damage has already occurred.

Several studies of glucocorticoid treatment in decompensated alcoholic liver disease did not show convincing and consistent proof of efficacy, except in the subgroup of patients with severe alcoholic hepatitis and hepatic encephalopathy. Moreover, due the frequent contraindications, only a few such patients qualify for glucocorticoid treatment (Imperiale *et al*., 1990). Although propylthiouracil and colchicine have both been used in patients with alcoholic liver disease, the evidence warranting their use is not conclusive.

Therapy to halt or recert the progression of liver cirrhosis is not available yet due to several factors such as the slow evolution of fibrosis in man or the difficulty in quantifying the fibrosis degree- Ttherapy is often limited to symptomatic treatment for the common complications ascites, variceal bleeding, portosystemic encephalopathy and disturbances in the metabolism of glucose, vitamins and trace elements. In alcoholic cirrhosis, therapy is generally supportive and aimed at improving nutrition, encouraging abstinence and treating complications.

As explained above, there is no fully accepted therapeutic alternative for the treatment of chronic liver disease in the non- decompensated state (especially alcohol induced) **(Grattagliano *et al*., 2011).**

Clinical trials designed to prove efficacy in liver diseases do not follow clinical endpoints as a standard for clinical trials in chronic liver disease of non-viral origin. The number of accepted surrogate endpoints in liver disease is limited, but the following ones are commonly accepted: 1) rate of survival or survival time; 2) progression of fibrosis to cirrhosis which is a primary determinant of morbidity and mortality in chronic liver diseases; 3) non invasive indicators such as serum transaminases which are considered as the most reliable biochemical parameter of hepatocellular injury; 4) changes in clinical symptoms and laboratory parameters.

4.2.1. Dose response studies

None reported

4.2.2. Clinical studies (case studies and clinical trials)

Clinical studies with different milk thistle preparations for the treatment of liver diseases of different aethiology are summarized in Table 15.

Table 15: Clinical studies on humans, in liver diseases (gastroenterology)

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
Alcoholic fatty liver disease							
Standardised dry extract (DER 36-44:1) corresponding to the expressed silymarin content							
Lucena *et al*., 2002 Study of the Clinical outcome, biochemical profile and antiperoxidative effects	6 months Randomized *versus* placebo	3 x 150 mg silymarin (70-80% of the silymarin complex; silibinin 35.07%) 6 months	49: 25/24 Mean age 49±13y (both groups) Drop outs 20%/16.6%	Alcoholic cirrhosis	Clinical outcome Biochemical profile Antiperoxidative effect	Performed Significant increase in GSH ($p< 0.001$) and in PIIINP ($p< 0.033$) Significant decrease in MDA ($p< 0.015$).	A significant difference in biochemical markers of endogenouse antioxidant defence and liver protection was found for silymarin
Effect on survival and clinical laboratory changes Pares *et al*., 1998	2 years Randomized Double-blind *versus* placebo	3 x 150 mg silymarin + alcohol abstinence 2 years	125: 57/68 Mean age 50.5 79% Male Drop outs 45%/30%	Alcoholic cirrhosis. Daily alcohol consumption > 80g in men and > 60g in women for more than 5 years	1.Time to death 2.Progression of liver failure	Performed No differences between groups	Survival was found to be similar in both groups. Laboratory findings and intention-to-treat survival analysis did not show differences between groups
Bunout *et al*., 1992 Study on the Biochemical profile	15 months Randomized Double-blind versus placebo	2 x 140 mg silymarin 15 months	59: 25/34	Alcoholism, liver insufficiency (jaundice, ascites, edema, encephalopahy or bilirubin > 2mg/dl; prothrombin time < 75%, albumin < 3mg/dl)	Hematocrite, AST, ALT, GGT, AP, albumin, creatinine, urea nitrogen, bilirubin, prothrombine time, glycemia	Performed Decrease in GGT No differences between groups	Results on liver parameters show a decrease in GGT with no differences between placebo and

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
							silymarin groups
Deak *et al*., 1990 Immunomodulatory effect	6 months Randomized double blind *versus* placebo	Silymarin (dose not available) 6 months	59	Alcoholic liver disease	Lectin-induced proliferative activity of lymphocytes, T-, CD8+ cells, antibody-dependent and natural cytotoxicity of lymphocytes	Performed Enhancement of lectin-induced proliferative activity of lymphocytes, normalization of T-, CD8+ cell percentage, decrease in antibody-dependent and natural cytotoxicity of lymphocytes in silymarin group	Milk thistle exerted a hepatoprotective effect on patients suffering from alcoholic liver cirrhosis
Müzes *et al*., 1990 Effect on the Antioxidant defense mechanism and lipid peroxidation	6 months Randomized Double-blind *versus* placebo	420 mg silymarin/day 6 months	12	Alcoholic liver disease	SOD, glutathione activity, MDA	Performed Significant increase in SOD expression on lymphocytes and on glutathione activity and decrease in MDA in silymarin group	Results indicative of a positive effect of milk thistle extract on those indicators of liver status
Lang *et al*., 1990	1 month Randomized	3 x 140 mg silymarin 1 month	60	Alcoholic cirrhosis with daily alcohol	AST, ALT, GGT, AP, bilirubin, lectin-induced	Performed Improvemen	Positive effects of

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
Study on the effect on Immunomodulation and hepatoprotection	Double-blind versus placebo and versus Alca-P			consumption > 60 g (men) or >30 g (women) for 6-11 years	lymphoblast transformation, antibody-dependent cell-mediated cytotoxicity (ADCC), spontaneous NK lymphotoxicity, percentage of peripheral T-, B-, CD4+, CD8+ cells	t after one month: significant reduction in bilirubin and GGT ($p< 0.05$), AST ($p< 0.01$), ALT ($p< 0.02$) in silymarin group. Only ASt ($p< 0.01$) and GGT ($p< 0.0$) were reduced n the Alca-P group. Increase in lectin-induced lymphoblast transformation in both groups ($p< 0.01$); decrease in the percentage of suppressor cells (siymarin $p< 0.05$; Alca-P $p< 0.01$). Antibody-dependent	milk thistle extract on histological parameters indicators of liver function

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						cell mediated cytotoxicity significantly decreased by silymarin; significant reduction in NK cell activity in both groups.	
Feher *et al*., 1989 Liver protection of silymarin in chronic alcoholic liver diseases	6 months Randomized Double-blind versus placebo	3 x 140 mg silymarin 6 months	36	Alcoholic liver disease. Daily alcohol intake > 60g (men) or > 30g (women)	AST, ALT, GGT, AP and bilirubin, serum levels. Procollagen III peptide levels	Performed AST, ALT levels were normalized. GGT and procollagen III levels decreased in silymarin group	Improvement in liver function related to milk thistle treatment
Ferenci *et al*., 1989 Outcome and biochemical profile effect	2-6 years (mean= 41 months) Randomized Double-blind versus placebo	3 x 140 mg/ day (420 mg silymarin) 2 years + alcohol abstinence	170: 87/83 37 male 26 female Mean age 57y	Cirrhosis: -alcoholic (n= 46) - non alcoholic (n= 41)	Survival rate Biochemical parameters	Performed Increased survival rate in silymarin group versus placebo group . Treatment effective in liver cirrhosis	Treatment with milk thistle was effective, especially with more effectiveness in patients with alcoholic liver diseases and patients rated Child A. No differences observed

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
							between placebo and silymarin group for non-alcoholic cirrhosis
Trinchet *et al*., 1989 Biological and histological evolution	3 months Randomized Double-blind versus placebo	3 x 140 mg silymarin 3 months	116: 57/59 Drop outs: 35	Alcoholic hepatitis with or without cirrhosis	Bilirubin, AST, ALT, GGT, PT, mean corpuscular volume (MCV), albumin, percutaneous liver biopsy	Performed Significant improvement in both groups associate to alcohol abstinence	Not significant improvement in patients diagnosed with alcoholic hepatitis (with or without cirrhosis)
Salmi & Sama, 1982 Effect of silymarin on chemical, functional, and morphological alterations of the liver.	1 month Randomized Double-blind versus placebo	420 mg daily 1 month	97: 50/47 8 female in treated group, 6 female in placebo group Mean age: 35.2y (female) 38.8y (male)	Increased AST and Alt levels for at least one month. Slight cute and subacute liver disease, mostly by alcohol abuse	Bilirubin, AP, bromosulphalein (BSP), serum immunoglobulins, blind liver biospy	Performed Significant decrease in AST, ALT. Normalizatio n of BSP and histological changes	Results were clinically relevant for patients with acute or subacute alcohol-induced liver disease for milk thistle group
Cirrhosis, acute or chronic hepatitis, non-alcoholic fatty liver disease							
Standardised dry extract (DER 36-44:1) corresponding to the expressed silymarin content							
El-Kamary et al., 2009 Randomized controlled trial to assess the safety and	4 weeks Randomized Double-blind versus placebo (vitamin)	3 x 140 mg silymarin Oral route	105 patients Treated group n=55; mean age: 31 years; 42% men	Acute hepatitis ALT > 2.5 times upper normal limit	Improvement in symptomatology. Liver function tests	Performed Statistically significant difference (intention to treat	The positive effects of milk thistle extract were observed on subjective symptoms and not

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
efficacy of silymarin on symptoms, signs and biomarkers of acute hepatitis			Placebo group n=50; mean age: 29 years: 44% men			paradigm) was found for the milk thistle group	captured by laboratory markers
Schuppan *et al*., 1999 Effect of milk thistle extract on fibrosis in chronic liver disease	3 months Open label	3 x 140 mg silymarin	874 (men and women) mean age 55y	Fibrosis in chronic liver disease	Liver fibrosis Subjective symptoms	Performed Drop to normal range of amino terminal procollagen-III peptide (PIIINP) in 19% patients together with a significant decrease in subjective symptoms (lack of appetite, nausea, upper abdominal pressure)	Total score of relevant decrease in subjective symptoms during treatment with milk thistle extract (e.g., lack of appetite, nausea, upper abdominal pressure).symptom scale showed definite and a clinically
DiMario *et al*., 1981 Effects of silymarin on	1-2 months Randomized placebo controlled	420 mg silymarin/day	43	Alcohol-induced liver disease (fatty liver, hepatitis, cirrhosis)	Biochemical markers. Clinical symptoms	Performed Significant improvement in AST ($p< 0.05$), ALT ($p< 0.01$),	Positive effect in symptoms (weakness, anorexia, and nausea)

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
the liver function parameters						bilirubin (p< 0.05), prothrombin (p< 0.05) and in clinical symptoms of weakeness, anorexia, nausea	and markers of liver function (AST (p<0.05);ALT (p<0.01); bilirubin (p<0.05, and prothrombin (p<0.05) in patients treated with milk thistle extract
Benda *et al*., 1980	4 years Randomized Double-blind versus placebo	2 x 70 mg silymarin + multivitamins	138	Hepatic cirrhosis	Survival rate	Performed The proportion of survivors in the treated group was consistenly above the placebo group	Positive effect of milk thistle in survival rate
Cirrhosis, acute or chronic hepatitis, alcohol or non-alcoholic fatty liver disease							
Other milk thistle preparations							
Tanasescu *et al*., 1988	40 days Randomized Double-blind versus placebo	3 x 2 tablets (700 mg extract with 35 mg silibinin: standardized extract DER: 36-44:1))	177	Chronic persistent hepatitis, chronic active hepatitis or hepatic cirrhosis	Symptoms characteristic for the disease, ALT,GGT, zinc sulfate, bilirubin, AP, serum proteins, IgG, prothrombin concentration	Performed Improvement in clinical aspect of liver disease. No changes in laboratory tests	Positive effects of milk thistle extract in clinical symptoms

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
Viral hepatitis							
Standardised dry extract (DER 36-44:1) corresponding to the expressed silymarin content							
Strickland *et al*., 2005	2 years Randomised versus placebo (multivitamin)	420 mg silymarin	141	Chronic hepatitis C	Patient compliance, safety and effectiveness	Performed No significant differences in serum ALT, hepatic fibrosis markers. No detectable progression of the disease	No efficacy on the viral charge was obtained
Magliulo *et al*., 1978	21-28 days Randomized double blind versus placebo	3 x 140 mg silymarin	57	Acute viral hepatitis A and B	Biochemical markers Immunity Viral charge markers	Performed Significant decrease in AST, ALT, bilirubin. No influence on immune reaction in hepatitis B surface antigen. Regression of pathological markers	No efficacy on the viral charge was obtained
Hammeri *et al.,* 1971	> 1year Open, uncontrolled	First 3 weeks: 3 x 3x35 mg silymarin After 3 weeks: 3 x 2x 35 mg silymarin	43	Chronic hepatitis, cirrhosis and toxic damage to the liver	Biochemical parameters	Performed Significant changes in bromsulphalein test,	Although some positive tendency in the histological

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						albumin/globulin quotient, serum, bilirubin, trasaminase, serum triglycerides	parameters, no efficacy on the viral charge was obtained
Poser, 1971	3 months Open, uncontrolled	3 x 3-5 tablets 35 mg silymarin	67	Chronic liver diseases (toxic metabolic liver damage, chronic hepatitis, cholangitis)	Biochemical parameters	Performed Significant improvement in SGOT, SGPT and glutamate-dehydrogenase (GLDH). No effect on bilirubin. Improvement in conditions associated with bile duct inflammation. After 3 months, chronic-persisting hepatitis bioptically cured	Although some positive tendency in the histological parameters, no efficacy on the viral charge was obtained
Sarre, 1971	3 months Epidemiological	3 x 3-5 tablets 35 mg silymarin	67	Chronic persisting hepatitis and other hepatopathies	Biochemical parameters	Performed Significant decrease in SGOT, SGPT and GLDH.	No efficacy on the viral charge was obtained

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						No effect on bilirubin. Chronic-persisting hepatitis bioptically cured. Toxic-metabolic and cholangitic hepatopathies responded well to silymarin. Effective in both young (4 years) and old (64 years) patients	
Viral hepatitis							
Other milk thistle preparations							
Kalantari *et al*., 2011	24 weeks Prospective auto-controlled	630 mg silymarin	55	Chronic hepatitis C	Biochemical parameters Fibrosis Viral charge	Performed Significant reduction in serum transaminases. Fibrosis decrease. Decrease in serum viral RNA in 9 patients. Improvement in quality of life	Positive effects on clinical symptoms but not relevant for viral charge

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
Bares *et al*., 2008	12 weeks Randomized for 3 silibinin doses	3 x 120 mg silibinin 3 x 240 mg silibinin 3 x 360 mg silibinin	37	Chronic hepatitis C	Serum ferritine	Performed Significant reduction in serum ferritine, mainly for III or IV fibrosis stage	Positive outcomes in biochemical parameters or fibrosis decrease, but no significant effects on viral indicators
Gordon *et al*., 2006	12 weeks Randomized Double blind, crossover versus placebo	600 and 1200 mg milk thistle extract with 80% silymarin	24	Chronic hepatitis C	Serum viral RNA	Performed No significant effects on serum viral RNA, ALT or quality of life and psychological status	Positive outcomes in biochemical parameters or fibrosis decrease, but no significant effects on viral indicators
Psychotropic Drug-induced liver damage							
Standardised dry extract (DER 36-44:1) corresponding to the expressed silymarin content							
Kurz-Dimitrowa, 1971	61 days	3 x 1-2 tablets 35 mg silymarin	66	Psychoactive or anticonvulsivant drug therapy	Index of hepatocellular function	Performed Normalized bromsulphalein levels in 54% patients. Normalized or improved GOT levels in 68% patients. Improved	Improved vitality and mood, together with positive effects on biochemical parameters in patients in the treated group

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						vitality and mood	
Psychotropic Drug-induced liver damage							
Other milk thistle preparations							
Palasciano *et al*., 1994	3 months Randomized Double-blind d versus placebo	2 x 400 mg silymarin (generic Milk thistle) Group 1A & B: psychotropic drugs with either 400 mg silymarin, 2x/day or placebo Group 2A & B: suspension of psychotropic drugs with either 400 mg silymarin, 2x/day or placebo	60, female 40–60 y	Patients receiving chronic psychotropic drug therapy such as phenotiazines and/or butyrophenones for at least 5 years, with AST or ALT activities more than twice the normal value	Serum MDA, index of hepatocellular function	Performed Significant improvement after 3 months	Treatment with milk thistle in lipoperoxidative hepatic damage may be beneficial in cases of long-term psychotropic drug treatment
Saba *et al*., 1976	180 days	3 x 210 mg silymarin	19	Liver diseases caused by psychodrugs (chlorpromazine alone or combined with benzodiazepine)	Biochemical parameters	Performed Significant improvement in SGOT, SGPT, BSF, albumin, bilirubin, AP	Positive effects on biochemical markers of liver function
Toxic and iatrogenic liver diseases (including Amanita Mushroom poisoning)							
Standardised dry extract (DER 36-44:1) corresponding to the expressed silymarin content							
Szilard *et al*., 1988	1 month Open study	3 x 140 mg silymarin	49	Liver disease attributable to toluene and/or xylene exposure	Blood cell count, AST, ALT, GCT, cholesterol, urine, urobilinogen	Performed Improvement in appetite,	Possitive effects of milk thistle extract on

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						reduction in headaches. Significant decrease in AST, ALT and a trend towards decrease in GGT	biochemical parameters indicators of liver function in treated group
Boari *et al*., 1981	1 month Open study	3 x 140 mg	24	Chronic exposition to organophosphorate pesticide (malathion) (n=14) and healthy volunteers (n=10)	AP, GGT, leucine aminopeptidase (LAP), pseudocholinesterase, total lipemia, triglyceridemia	Performed Significant increase in GGT, cholinesterase and LAP (p< 0.01) in silymarin-treated group.	Possitive effects of milk thistle extract on biochemical parameters indicators of liver function in treated group
Other studies							
Standardised dry extract (DER 36-44:1) corresponding to the expressed silymarin content							
Velussi *et al*., 1993	1 year Randomized Open study	3 x 200 mg silymarin	60	Non-insulin-dependent diabetics with alcohol liver cirrhosis	Fasting and mean daily blood glucose levels, glucosuria, mean daily insulin requirements (every 30 days). Glycosylated hemoglobin (HbA1C) (every 60 days). Fasting insulin, basal and stimulated C-peptide, MDA, AST, ALT, GGT, AP, bilirubin, triglycerides, total HDL cholesterol	Performed Significant decrease (p< 0.01) in fasting blood glucose levels, mean daily glucose levels, daily glucosuria and HbA1C after 4 months. Significant decrease (p< 0.01) in	Improvement in liver status as proved by a reduction in lipoperoxidation of cell membranes and insulin resistance

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						fasting insulin levels and mean exogenous inulin requirements. Significant decrease ($p< 0.01$) in basal and glucagon-stimulated C-peptide, as well as in MDA levels	
Nassuato *et al*., 1991	30 days Placebo controlled	420 mg silymarin + low lipid diet	19	Gallstone and cholecystectomized patients	Biliary lipid composition	Performed Significant decrease in cholesterol concentration ($p< 0.02$). Slight increase in phospholipids and total bile salts. Significant decrease in cholesterol saturation index	Treatment lowers biliary cholesterol concentration in humans, thus inducing a significant reduction on the bile saturation index
Fintelmann, 1973	12 days (2-4 days preoperative; 1-8 days postoperative)	3 x 2 x 35 mg silymarin	83	Narcotic use in cholecystectomy surgery	Toxicity of narcotics in cholecystectomy surgery	Performed Decrease in toxicity of narcotics. No significant effect on	Not significant effect of milk thiste extract

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						enzyme parameters	
Other studies							
Other milk thistle preparations							
Di Pierro *et al*., 2008	63 days Randomized Double blind versus placebo	420 mg silymarin	50	Breast feeding	Milk production	Performed Increase in milk production (85% silymarin group verus 32.09% for placebo). Quality of milk unchanged	Not significant effect of milk thiste extract
Allain *et al*., 1999	12 weeks Randomized Double blind versus placebo	420 mg silymarin and 40 mg tacrine for 6 weeks, then increase to 80 mg tacrine or tacrine and placebo	217	Aminotransferase levels in tacrine-induced liver transaminase elevation (patients with Alzheimer's dementia)	Biochemical parameters. Impact on cognitive status	Performed Not prevention of tacrine-induced ALAT elevation. Reduction in the rate of gastrointestinal and cholinergic side effects without impact on cognitive status	Milk thistle extract can be used to improve tolerability of tacrine in initial phases of Alzheimer's treatment
Petronelli *et*	2 months Randomized	3 x 300 mg silymarin	20	Hypertriglyceridemic or	Lipid levels	Performed Reduction of	No significant

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
al., 1980	Placebo controlled			hypercholesterolemic patients		triglycerides and pre-β-lipoproteins without parallel increase in β-lipoproteins	changes in the treated group
Reviews/ Metanalysis							
Saller *et al*., 2008	-	Meta-analysis of 19 clinical trials (double blind: 11; single blind: 8)	-	Chronic hepatitis C Cirrhosis	-		Rational evidence for the use of silymarin for Amanita phalloides intoxication and for alcoholic liver cirrhosis. Scarce clinical evidence of therapeutical effect of silymarin on chronic hepatitis C or cirrhosis.
Tamayo *et al*., 2007	-	Review of 8 clinical trials	-	Chronic hepatitis C Alcoholic cirrhosis	-	In chronic hepatitis C, 420mg silymarin/ day improve	Although positive effects are observed in symptoms

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
						symptoms and general health. No effect on viral charge. In cirrhotic patients, antioxidant activity of silymarin; when associated to diabetes type II, silymarin improves glycemic profile, decreases fast glucose and serum triglycerides.	and general health in patients with viral hepatitis, no effect on viral charge was obtained. Positive effects on diabetic patients in those parameters related to glycemic profile
Rambaldi *et al*., 2005	6 months (mean value)	Meta-analysis of 13 randomized clinical trials	915	Alcoholic disorders related to alcoholism or viral infections (B,C)	Survival rate. Bilirubin, GGT	No significant differences for milk thistle groups	Reduction in liver-related mortality by milk thistle in all trials

FD: Fatty dysthrophy

NASH: Non-alcoholic steatohepatitis

Several clinical trials have been conducted with milk thistle standardized extract (DER 36-44:1) on patients with alcoholic liver disease. Most of them showed and improvement in clinical aspects of liver sidease, with no changes in laboratory parameters.

The main objectives of the study by **Lucena *et al.* (1992)** were the Clinical outcome, biochemical profile and antiperoxidative effects of the treatment with 450 mg silymarin (3 x 150 mg) during 6 months in patients diagnosed with alcoholic cirrhosis. The patients were randomized to receive milk thistle treatment or placebo during 6 months. 49 patients were included (25 in silymarin group/24 in placebo group) with a mean age of 49±13y (in both groups). Drop outs were 20% in the treated group and 16.6% in the placebo group. A significant increase in biochemical markers of endogenous antioxidant defense and liver protection was found (GSH ($p< 0.001$) and PIIINP ($p< 0.033$)), together with a significant decrease in the lipoperoxidation marker, MDA ($p< 0.015$).

The study by **Pares *et al.* (1998)** aimed to assess the survival and clinical laboratory changes after a 2 years treatment with 450mg silymarin (3 x 150 mg) and alcohol abstinence in patients diagnosed with alcoholic cirrhosis who took daily alcohol consumption > 80 g in men and > 60 g in women for more than 5 years. This randomized, double-blind *versus* placebo study lasted 2 years and included 125 patients (57 in silymarin group/68 in placebo group) with a mean age of 50.5 years. 79% of patients were men; drop outs were 45% and 30% in treated and placebo group, respectively. The primary and secondary endpoints were time to death and progression of liver failure. No serious adverse events were reported. Survival was found to be similar in both groups; laboratory indices and intention-to-treat survival analysis did not show differences between groups.

Bunout *et al.*, (1992) randomly assigned patients suffering from alcoholism, liver insufficiency (jaundice, ascites, edema, encephalopathy or bilirubin > 2mg/dl; prothrombin time < 75%, albumin < 3 mg/dl) to treatment with 280mg/d of silymarin (n=25) or placebo (n=34) for a 15 months period. Follow-up controls indicated that 65% in the placebo group and 58% in the silymarin group had alcohol intake during the study. Results on hematocrite, AST, ALT, GGT, AP, albumin, creatinine, urea nitrogen, bilirubin, prothrombine time and glycemia showed a decrease in GGT with no differences between the placebo and silymarin groups. Also the study by **Trinchet et al., (1989)** indicated not significant improvement in patients diagnosed with alcoholic hepatitis (with or without cirrhosis) and receiving 420mg silymarin for 3 months, as a significant improvement in both groups (silymarin and placebo groups) was associated to alcohol abstinence.

Positive results were obtained after several controlled studies in which the hepatoprotective effects of silymarin in alcoholic liver disease could be demonstrated as accelerating normalization of impaired liver function.

The study by **Deak *et al*., (1990)** analysed the immunomodulatory effect of silymarin (dose not available) after a 6 months treatment in 59 patients with alcoholic liver disease. Different parameters such as lectin-induced proliferative activity of lymphocytes, T-, CD8+ cells, antibody-dependent and natural cytotoxicity of lymphocytes were studied. An enhancement of lectin-induced proliferative activity of lymphocytes, normalization of T-, CD8+ cell percentage, and a decrease in antibody-dependent and natural cytotoxicity of lymphocytes in silymarin group were assessed. Authors concluded that milk thistle exerted a hepatoprotective effect in patients suffering from alcoholic liver cirrhosis.

The study by **Feher *et al.,* (1989)** included 36 patients with alcoholic liver disease and daily alcohol intake > 60g (men) or > 30g (women). Patients were randomized to receive 420mg of milk thistle extract (3 x 140 mg silymarin) or placebo during 6 months. Biochemical markers such as AST, ALT,

GGT, AP and bilirubin, serum levels, and procollagen III peptide levels were analyzed. Results showed that AST and ALT levels were normalized and GGT and procollagen III levels decreased in silymarin treated patients, this indication an improvement in liver function.

Results from the study by **Ferenci *et al.* (1989)** indicate that milk thistle treatment was more effective in patients with alcoholic liver diseases and patients rated Child A than in non-alcoholic patients. This study lasted a mean of 41 months of treatment (3 x 2 capsules/ day = 420 mg silymarin or placebo) plus alcohol abstinence, and included 170 patients (87 in the silymarin group/ 83 in the placebo group) with diagnosed cirrhosis, alcoholic or not (n=46, n=41, respectively). The 4-years survival rate was 58% in the treatment group and 39% in the placebo group (p= 0.036). Analysis of subgroups indicated that treatment was effective in patients with alcoholic cirrhosis, while significant group differences were not observed between the silymarin and placebo groups for non-alcoholic cirrhosis.

Positive results were also obtained by **Salmi & Sama (1982)** after a 1 month randomized, double-blind *versus* placebo study conducted on 97 patients (n=50 silymarin group/n=47 placebo group), with a mean age of 35.2 and 38.8 years for women and men, respectively. Patients received 420 mg silymarin daily or placebo. A significant decrease in AST, ALT, normalization of BSP and histological changes were observed that resulted to be clinically relevant in patients with acute or subacute alcohol-induced liver disease

In the study by **Muzes *et al*. (1990a)** on the antioxidant defense mechanism and lipid peroxidation effect of milk thistle extract, 12 patients with alcoholic liver disease received 420mg silymarin/day or placebo for 6 months. Biochemical analysis of SOD, glutathione activity and MDA levels showed a significant increase in SOD expression on lymphocytes and on glutathione activity and decrease in MDA in silymarin group, this indicating a positive effect of milk thistle extract on those indicators of liver status.

Similar outcome was obtained by **Lang *et al.,* (1990)** when assessing the hepatoprotective effect of 420mg silymarin on 60 patients diagnosed with alcoholic cirrhosis through a randomized, double-blind *versus* placebo and *versus* Alca-P lasting 1 month. A statistically significant improvement was obtained after one month: significant reduction in bilirubin and GGT ($p< 0.05$), AST ($p< 0.01$), ALT ($p< 0.02$) in silymarin group. Only AST ($p< 0.01$) and GGT ($p< 0.0$) were reduced in the Alca-P group. Increase in lectin-induced lymphoblast transformation in both groups ($p< 0.01$); decrease in the percentage of suppressor cells (siymarin $p< 0.05$; Alca-P $p< 0.01$). Antibody-dependent cell mediated cytotoxicity significantly decreased by silymarin; significant reduction in NK cell activity in both groups. These values indicate a positive effect of milk thistle extract on those biochemical markers of liver function.

Also the she study by **DiMario *et al*., (1981)** included only 43 patients with alcohol-induced liver disease (fatty liver, hepatitis, cirrhosis) receiving 420 mg silymarin/day or placebo. A significant improvement in AST ($p< 0.05$), ALT ($p< 0.01$), bilirubin ($p< 0.05$), prothrombin ($p< 0.05$) and in clinical symptoms of weakeness, anorexia and nausea were obtained for patients receiving milk thistle.

Clinical trials on alcoholic liver disease with other milk thistle preparations are scarce and do not show conclusive positive results. I.e., the study by **Tanasescu *et al*., (1988)** lasted 40 days and included 177 patients receiving 700 mg extract with 35 mg silibinin (3 x 2 tablets) or placebo. Patients with chronic persistent hepatitis, chronic active hepatitis or hepatic cirrhosis experienced an improvement in clinical aspect of liver disease with no changes in laboratory tests.

Liver diseases of different aetiology have also been the objective of milk thistle treatment. The study by **El-Kamary *et al.,* (2009)** was designed to assess the safety and efficacy of silymarin (milk thistle standardized extract) on symptoms, signs and biomarkers of acute hepatitis. 105 patients were included in a 4 weeks study and received 420mg silymarin (3 x 140mg, n= 55) or placebo (n=50). A

statistically significant difference (intention to treat paradigm) was found for the silymarin group, with a quicker resolution of symptoms related to biliary retention: dark urine, jaundice and scleral ictus and reduction in indirect bilirubin. Authors concluded that the effects of silymarin were noted mainly on subjective symptoms, this suggesting that the beneficial effect of silymarin is not captured by traditional laboratory biomarkers used at that moment. The same conclusions were achieved by **Schuppen *et al.,* (1999)** after a 3 months' study including 874 patients suffering from fibrosis in chronic liver disease. Patients received 420mg silymarin (3 x 140mg) in an open label study; at the end of the treatment period, a drop to normal range of amino terminal procollagen-III peptide (PIIINP) in 19% patients was seen, together with a significant decrease in subjective symptoms (lack of appetite, nausea, upper abdominal pressure).

In the study by **Benda *et al*., (1980),** 138 patients diagnosed with hepatic cirrhosis were randomly assigned to receive silymarin (2 x 70mg) or placebo for 4 years. By the end of the study, the proportion of survivors in the treated group was consistenly above the placebo group.

Several studies have been conducted in patients with viral hepatitis (acute or chronic A,B or C), with a duration between 21 days and 2 years **(Strickland *et al*., 2005; Magliulo *et al*., 1978; Hammeri *et al.,* 1971; Poser, 1971; Sarre, 1971)** receiving different doses of milk thistle standardised dry extract (DER 36-44:1). Although some positive tendency was found in the analysed biochemical parameters, no efficacy on the viral charge was obtained.

More recent studies performed with different milk thistle preparations led to positive outcomes in biochemical parameters or fibrosis decrease, but again, no significant effects on serum viral RNA, ALT or quality of life and psychological status of patients was achieved (**Kalantari *et al*., 2011; Bares *et al*., 2008; Gordon *et al*., 2006**).

Taking in account all the basic laboratory and clinical data obtained after several clinical trials performed with milk thistle preparations, milk thistle is thought to stabilize the cell membrane and stimulate protein synthesis while accelerating the process of regeneration in damaged liver tissue, and that these effects are important in its therapeutic efficacy on liver diseases, mainly alcohol-related.

Other clinical studies have been conducted to evaluate the effect of milk thistle preparations in different liver-related paramenters.**Petronelli *et al*., 1980).** One randomized placebo-controlled study was conducted to evaluate the effect of silymarin on serum lipid levels. 20 patients suffering from hypertriglyceridemy (n=12) or hypertriglyceridemy (n=8) were included. Treated group received 300mg/day (3 times per day) silymarin for 2 months. The following biochemical parameters were controlled: triglyceride and cholesterol levels, Lipoproteic fraction and primary conjugated biliary acids. After two months' treatment, a reduction of triglycerides and pre-β-lipoproteins in the silymarin-treated subjects without a parallel increase in β-lipoproteins was observed. Again, no adverse events were reported.

A clinical trial studied the effects of 1 month silymarin treatment on biliary lipid composition *versus* placebo in gallstone and cholecystectomized patients **(Nassuato et al., 1991).** Four gallstone patients and six cholecystectomized patients received 420mg of silymarin per day for 30 days; nine cholecystectomized patients received placebo for the same period. All individuals received a standard diet containing 60g of lipids per day. After one month, cholecystectomized patients receiving silymarin showed a significantly decreased cholesterol concentration ($p < 0.02$ before and after silymarin treatment);phospholipids an total bile salts were slightly, but not significantly increased. The cholesterol saturation index (SI) which is the expression of the biliary lipid composition, was significantly decreased after silymarin treatment, reaching the normal range. According to the authors, the reduced cholesterol excretion might be related to a reduction in cholesterol neosynthesis as silibin exerts a dose-dependent inhibitory activity that affect the HMG-CoA reductase activity. Moreover, the reduced biliary cholesterol concentration is probably not related to a dilution of bile following drug

administration, as in treated patients bile flow was not significantly modified. Changes in biliary cholesterol excretion are not due to silymarin-induced modification of the detergent power of bile acid pool. So silibinin lowers biliary cholesterol concentration in humans, thus inducing a significant reduction on the bile saturation index.

The liver is the first and most important tissue involved in insulin utilization as it captures almost 50% of the insulin produced by the pancreas **(Velussi *et al.*, 1993).** Liver is responsible of maintaining blood glucose levels via the combined mechanisms of gluconeogenesis and glycogen synthesis. Secondary diabetes due to liver damage provokes an elevated insulin resistance, possibly related to the reduced hepatic glucose uptake and consequent hyperglycemia. The hyperglycemia might then stimulate hyperinsulinemia and insulin resistance on the target tissues, which is also partly the result of the reduced liver degradation of the insulin molecule. Secondary diabetes caused by alcoholic liver damage is often treated with elevated doses of insulin due to insulin resistance. Alcoholic damage of the liver is related to lipoperoxidations of liver cell membranes and to the alteration of the balance between lipid peroxidation and antioxidant defence of the cells.

In order to study the effect of silymarin in cirrhotic diabetic patients, 60 subjects receiving insulin were randomly assigned to receive 600 mg of silymarin daily or no silymarin for 6 months **(Velussi *et al.*, 1993).** After treatment period, silymarin-treated patients showed significantly lower levels of fasting blood glucose, daily blood glucose, daily glycosuria, glycosilated haemoglobin, daily insulin need, fasting insulinemia, blood malondialdehyde and basal and glucagon stimulated C peptide ($p< 0.01$; $p< 0.05$). The liver enzyme levels decreased only in the silymarin-treated patients which proved the ability of silymarin to restore normal liver membrane permeability and to reduce enzyme dispersion in the extracellular medium.

Authors concluded that silymarin can reduce lipoperoxidation of liver cell membranes in cirrhotic diabetic patients, and decrease endogenous production of insulin and the need for exogenous insulin, probably by restoring the plasma membrane of liver cells and increasing the sensitivity of the insulin receptors.

Lirussi *et al*, 2002, carried out a clinical trial to study the effect of a different oral formulation of silybin-β-cyclodextrin (IBI/S) in patients with chronic alcoholic liver disease and concomitant non-insulin-dependent diabetes mellitus (T2DM). 42 patients out of the 60 enrolled (21 in the group IBI/S-135mg/ day silibinin, per os, and 21 in the placebo group) concluded the 6 month treatment period. The efficacy parameters included fasting and mean daily plasma glucose levels, glycosilated haemoglobin (HbA_{1C}), basal, stimulated C-peptide and insulin levels, total, HDL-cholesterol and triglyceride levels as well as malondialdehyde (MDA) and conventional liver function tests. Fasting blood glucose and glucose levels significantly decreased in the IBI/S group. It was also observed the same trend in mean daily blood glucose levels, HbA_{1C} and insulin sensitivity, although with no significant differences. Results indicate that insulin secretion was unaffected. A significant reduction on plasma trycglicerides was also found ($p< 0.01$): no clinically relevant side effects were observed. Authors concluded that oral administration of silibinin-β-cylcodextrin in patients with T2DM and compensated chronic alcoholic liver disease caused a significant decrease in both glucose and trycglicerides plasma levels probably due to the recovery of energy substrates, consistent with a reduction in lipid peroxidation and an increase in insulin activity.

4.3. Clinical studies in special populations (e.g. elderly and children)

None reported

4.4. Overall conclusions on clinical pharmacology and efficacy

44 clinical trials have been performed with *Silybum marianum* fruit preparations in the last years: sixteen studies for testing the efficacy of the herbal in liver cirrhosis, 11 for viral hepatitis, 4 clinical trials on toxic liver diseases and 3 clinical trials on the efficacy on patients already being treated with psychotropic drugs. Another category of studies includes those related to other therapeutical indications such as breast feeding, gallstone and cholecystectomized patients or hypercholesterolemic patients. Most of these studies demonstrate positive effects for indications including cirrhosis and alcoholic liver disease, hepatitis, and psychotropic drug-induced liver damage.

These large quantity of clinical trials included more than 4000 patients, mainly in randomized double-blind *versus* placebo studies; 1298 of them where included in studies testing efficacy of milk thistle in cirrhosis, chronic hepatitis and alcohol liver disease, together with 612 patients diagnosed with viral hepatitis. One observational study on 998 patients showed that milk thistle extract reduced collagen fibrogenesis in patients with toxic liver (**Schuppan *et al*., 1998**). Treatment of psychotropic drug-induced hepatic damage with purified silymarin was the subject of another R, DB, PC study (**Palasciano *et al*., 1994**). A recent systematic review and meta-analysis (**Mulrow *et al.*, 2000**) concluded that (1) the available evidence suggests that milk thistle extract is safe, associated with few minor adverse effects, (2) despite substancial *in vitro* and animal research, the mechanism of action is not fully defined and may be multifactorial, and (3) clinical efficacy is not clearly stablished because interpretation of the evidence is hampered by poor study methods and/or poor quality reporting in publications. Other problems include small sample sizes and variation in formulations, dosing and duration of therapy.

14 clinical trials have been conducted with the dried and refined extract (36-44:1) corresponding to 70 or 140 mg silymarin for liver diseases such as cirrhosis, acute or chronic hepatitis and alcohol liver disease. Twelve of these studies are well designed (randomized double-blind versus placebo) with duration between 1 month and 6 years. The main endpoints were time to death (patients' survival) and liver function (biochemical markers and fibrosis). Five of the published studies showed a significant improvement in the biochemical markers. No statistically significant differences were observed for survival rate. So the available data fulfil the recomendation set at the "EMEA/HMPC/104613/2005 Guideline on the assessment of clinical safety and efficacy in the preparation of community herbal monographs for well-established and of community herbal monographs" of at least one randomised controlled trial as part of the body for evidence level Ib for this indication.

5 other clinical studies have been published with the dried and refined extract (36-44:1) corresponding to 70 or 140 mg silymarin for viral hepatitis (B o C) with a duration of 28 days to 2 years. The most recent in 2005 didn't show any significant improvement in biochemical profile. The other four studies were performed during the 70's in patients suffering from hepatitis B or chronic hepatitis. All of them showed a significant decrease in liver markers. Nonetheless, further investigations on products on the market and their efficacy on this indication is needed according to the actual legislation.

Studies performed with different preparations of Milk thistle (600-960mg silymarin or 360-1080mg silibinin) on viral hepatitis demonstrated a significant decrease in fibrosis, biochemical markers and serum ferritine, with no effect on viral charge.

5 randomized clinical trials lasting 1 week to 6 month with different preparations of Milk thistle (35mg silibinin or 450mg silymarin) have been performed on patients with liver disorders (not viral or toxic hepatitis). The main endpoints were biochemical markers and symptomatology. All of them except for one (with the lowest dose) showed a significant decrease in enzymatic levels, together with a symptomatology improvement.

High doses of Milk thistle preparations showed positive results in the biochemical profile of patients suffering from psychotropic drug-induced liver damage. Nonetheless, the duration of the studies was short with a low number of patients, so further well-designed studies are needed for this indication.

Studies conducted on patients suffering from toxic and iatrogenic liver diseases proved the hepatoprotective effect of the dried and refined extract (36-44:1) corresponding to 70 or 140mg silymarin, as a significant decrease in biochemical hepatic markers was observed.

Thirteen randomised clinical trials assessed milk thistle in 915 patients with alcoholic and/or hepatitis B or C virus liver diseases. Milk thistle versus placebo or no intervention had no significant effect on mortality, but **liver-related mortality *was* significantly reduced by milk thistle in all trials (Rambaldi *et al.*, 2005**: The Cochrane Collaboration).

There are several clinical studies on the efficacy of IDB 1016 (Silipide) on different liver diseases. Most of them are well designed and positive results are reported. Based on the information on manufacturing process, the Rapporteur is of the opinion that Silipide cannot be classified as an herbal preparation due to the manufacturing steps and composition of such product.

Nonetheless, the information about these studies is included in the AR, as it is considered to be part of the demonstration of the beneficial effects of Silybum marianum on liver diseases.

Silybum marianum has traditionally been used for the treatment of liver diseases. Silymarin has proved to exert antioxidant, antifibrotic and anti-inflammatory effects in different pharmacological models; also protein synthesis stimulating and membrane protecting mechanisms have been demonstrated **(Abenavoli *et al.,* 2010; Saller *et al.,* 2007).** The antifibrotic potential of silibinin has been proved in human hepatic stellate cells (HSC), the activation and proliferation of which is known to be conductive to hepatic fibrogenesis: a dose-dependent inhibition of such fibrogenetic process is observed when HSC are exposed to silibinin **(Trappoliere *et al.,* 2009).**

These effects are relevant for the clinical application in chronic liver disease by reducing the intracellular oxidative stress and by decreasing new collagen deposition, at slowing down the progression to fibrosis and cirrhosis in the liver.

Most of the published studies have been performed with one standardized extract from *Silybum marianum* fruit with 70-80% silymarin (extraction solvent Ethyl acetate), which is marketed as a medicinal product and used since 1968 in the EU (Germany). Its efficacy has been investigated in patients with chronic liver disease of various aetiology and degrees of severity. The majority of the trials were conducted before the principles of Good Clinical Practice (GCP) were introduced, and so only few studies comply with GCP standards. In the light of today‘s standards of clinical practice, most of the older trials are somewhat deficient in reporting with respect to patient populations (heterogeneous), patient numbers (limited, lack of sample-size calculations), inclusion criteria (insufficiently defined) and reasons for dropouts, compliance assessment, and monitoring of concomitant intake of alcohol, particularly in alcohol-induced liver disease. Other shortcomings of the older trials occasionally include the absence of an a priori hypothesis. These aspects had been generally less important in clinical studies at that time - not just in studies involving herbal drugs - and the results of these studies should be considered valid as long as the trials had been conducted in accordance with the regulations operational at that time. Information on methods and applied units of biochemical parameters as well as normal ranges is often limited in the publication of the studies. Consequently, the reported findings can often be interpreted only in the context of the conclusions drawn by the authors themselves.

The long-term studies conducted by **Ferenci *et al*., 1989** and **Pares *et al*., 1998** considered survival rate in patients with liver cirrhosis as primary variable. In other studies in liver cirrhosis, survival rate was not determined as a primary variable. Instead, the variables measured (mainly biochemical

endpoints) were those usually applied in the monitoring of treatment effects in liver disease, supported by variables characterizing complications and by parameters of fibrosis, antioxidant status, and lipidperoxidation.

For efficacy analysis, several studies have been conducted with the standardized extract of *Silybum marianum* fruit with 70-80% silymarin in patients with acute and chronic liver diseases of various aetiology and progression. They include 18 randomized, placebo-controlled, double-blind trials, 3 reference-controlled trials and 10 open, case-controlled trials comprising a total of more than 2,000 patients. Additional evidence for efficacy is derived from 7 open studies (including four post-marketing surveillance-studies with approximately 6,350 patients and one observational long-term study in over 1,000 patients) **(Saller *et al*., 2008).** Recently, a non-interventional study was conducted with the aim to investigate the impact of silymarin treatment on quality of life and liver function in patients under therapy with potentially hepatotoxic drugs. A total of 190 patients with increased serum transaminases were treated with 420 mg silymarin per day over a period of up to 3 months. As a result, liver related symptoms, laboratory parameters (ALT, AST, GGT, alkaline phosphatase and total bilirubin) as well as quality of life significantly improved during therapy (LE13K0.55. 2013).

Clinical trials were conducted in more than 600 patients with liver cirrhosis of the liver, mainly alcohol-related. In the most important trial, `survival` was the primary clinical endpoint. Cumulative 4-year survival rate was significantly higher in silymarin-treated group compared to placebo (p = 0.036). Analysis of subgroups indicated that treatment was effective in patients with alcoholic cirrhosis, independent of severity (p = 0.01) and in patients initially rated `Child A` independent of aetiology (p = 0.03). Overall, more patients died in the placebo group **(Ferenci *et al.,* 1989).** A further trial, with 2-year treatment in patients with alcoholic cirrhosis of the liver did not reveal a difference in total mortality between the treatment groups. However, subgroup analysis of patients with HCV positive analysis (29 of 75) resulted in no death in the silymarin group (0/13) while in the placebo group 4 patients died (4 / 16) **(Pares *et al.,* 1998).**

When analysing the liver-related mortality, however, three out of five trials reported a lower mortality with silymarin treatment, this being 9.7% versus 16.7% in placebo group **(Saller *et al.,* 2008).** In addition, there was a trend for upper intestinal bleeding, and encephalopathy to occur less frequently in the treated group, together with a lower need for hospitalization for liver-related reasons.

The results of one 12-month trial in patients with cirrhosis who had developed diabetes (hepatogenic diabetes) could show that silymarin treatment reduced the lipoperoxidation of cell membranes, fasting blood glycaemia and even insulin resistance, thus reducing the daily insulin dose required by almost 25% **(Velussi *et al.,* 1997).**

19 clinical trials have been published including more than 800 patients suffering from liver diseases derived from alcohol intake with the standardized milk thistle extract, with a duration between 1 month and 6 years fulfilling the criteria or "single" or "double blind". When compared to placebo, aspartate aminotransferase is reduced in the silymarin-treated groups (p = 0.01) while alkaline phosphatase is not. In liver cirrhosis, mostly alcoholic, total mortality is 16.1% with silymarin vs. 20.5% with placebo (n.s.); liver-related mortality is 10.0% with silymarin vs. 17.3% with placebo (p = 0.01). 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 alcoholic and grade Child 'A' liver cirrhosis **(Saller *et al.,* 2008).**

The treatment of toxic - drug-induced - liver damage with silymarin was evaluated within five studies. Although silymarin did not prevent the increase in serum ALT caused by the cholinesterase-inhibitor tacrine, it did reduce the rate of gastrointestinal and cholinergic side effects of tacrine **(Allain *et al*., 1999)**. Results from three open controlled trials with 160 patients clearly showed a marked reduction of elevated transaminase levels induced by potentially hepatotoxic neuroleptics or anaesthetics. In

summary, silymarin is able to improve liver conditions after damage caused by intoxication with industrial chemicals or by certain drugs **(Saller *et al.,* 2008).**

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of conditions ranging from simple fatty infiltration of the liver to steato-hepatitis, fibrosis, and cirrhosis. Non-alcoholic steato-hepatitis (NASH) is histologically characterized by significant accumulation of hepatic lipid and predominantly lobular necroinflammation, with or without centrilobular fibrosis **(Hashemi *et al*., 2009).** In a randomized placebo-controlled trial in patients with NAFLD, silymarin 280 mg/day was given orally over a period of 24 weeks and results indicated that silymarin treatment was significantly effective in biochemical improvement and decreasing transaminases levels in patients with NAFLD **(Hashemi *et al*. 2009).** Another open, randomised, comparative, parallel-group trial in 70 patients with NAFLD was conducted to assess the efficacy of sylimarin (daily oral doses of 420 mg over 2 months). Silymarin treatment decreased or normalised transaminases, and significantly improved the ultrasound picture in treated patients when compared to the control group, with no side effects **(Butorova *et al*., 2010).** The study by **Hajiaghamohammadi *et al.* (2012)** included patients suffering from NAFLD who were assigned to three different groups (n=22 each) and treated with either metformin, pioglitazone or silymarin (140 mg/day). After 8 weeks, all drugs showed beneficial effects. For silymarin, there was a significant reduction in ALT, AST, serum insulin and HOMA index when comparing baseline and follow-up values.

Clinical studies failed to prove silymarin efficacy in viral hepatitis, as no effect on viraemia, serum ALT and ecographic parameters of hepatic fibrosis was shown **(El-Kamary *et al*., 2009; Freedman *et al*., 2011; Fried *et al.,* 2012; Reddy *et al*., 2012; Strickland *et al*., 2005; Tanamly *et al*., 2004).** A two-year randomised double-blinded clinical trial evaluating silymarin (429 mg/day) for chronic hepatitis C showed no objective evidence of improvement in physical and hepatic ultrasound examinations, or serum ALT and hepatic fibrosis marker levels **(Strickland *et al*., 2005)**: most of the patients receiving silymarin still had antibodies to HCV; HCV RNA persisted in almost them; a similar situation was observed in the placebo group, receiving a multivitamin product, p= 0.56 and 0.61, respectively. Every patient had fewer symptoms and reported they felt better that prior to participation in the study. They also had no detectable progression of their liver disease over the 24 months period of the study.The most frequent reason for patients to consult a physician when suffering from liver diseases are the subjective symptoms such as continuous fatigue, anorexia, epigastric pressure, flatulence and nausea. Two clinical trials and three post-marketing surveillance studies showed a beneficial effect of silymarin treatment on these symptoms. The majority of more than 6,000 patients with mainly alcohol-induced liver disease treated in several post-marketing surveillance studies complained of various subjective symptoms at baseline (up to 70%). After treatment with 140mg b.i.d or t.i.d. for 12-16 weeks, subjective complaints like fatigue, tiredness, nausea, anorexia, upper abdominal pain and flatulence as well as pruritus which were monitored from the perspective of every day medical practice had improved in most patients. While chronic illness has a major impact on the patient`s wellbeing and his ability to fulfil daily activities, it finally influences his quality of life. Therefore, any improvement in these clinical symptoms is likely to enhance the quality of life.

Studies conducted in patients with other diseases involving liver pathology such as primary sclerosing cholangitis, thalassemia or diabetes also showed beneficial results **(Angulo et al., 2008; Gharagozloo *et al*., 2009, 2013; Huseini *et al*., 2006; Hussain, 2007; Hutchinson *et al.,* 2010; Moayedi *et al*., 2013).**

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

See section 4.1. and 4.2.

No case of overdose has been reported. Data from clinical trials show a low toxicity for *Silybum marianum* fruit. Data obtained from more than 4000 patients, tested for safety during clinical trials, showed the following results (Table 14).

Several clinical trials have been conducted in patients treated with milk thistle together with other drugs, such as indinavir or ranitidine **(Mills *et al*., 2005; Nageshwar *et al*., 2007).** As milk thistle extract has shown an inhibitory activity of CYP3A4, potential pharmacokinetic interactions may be observed. Nonetheless, no influence of milk thistle on pharmacokinetics of the other drugs was observed and so, the risk of clinically relevant drug-drug interactions is considered to be low.

Table 16. Main adverse events observed in clinical trials with milk thistle preparations

Study	**Herbal preparation**	**Treatment duration**	**Adverse event**
El-Kamary *et al.*, 2009	Standardised dry extract (36-44:1): 3 x 140mg	4 weeks	Abdominal pain, diarrhoea, nausea, vomiting, constipation, increased fatigue, insomnia (similar in frequency in both groups)
Schuppen *et al.,* 1999	Standardised dry extract (36-44:1): 3 x 140mg	3 months	Diarrhoea, flatulence, gastrointestinal fullness, gastrointestinal pain (2% patients)
Pares *et al.,* 1998	Standardised dry extract (36-44:1): 3 x 150mg	2 years	Arthragials, prutitis, headache, urticarial
Bunout *et al.,* 1992	Standardised dry extract (36-44:1): 2 x 140mg	15 months	Pruritu, cephalic, constipation, dryness of the mouth, abdominal pain (no differences between groups)
Ferenci *et al.,* 1989	Standardised dry extract (36-44:1): 3 x 140mg	41 months	Epigastric discomfort and nausea
Boari *et al.,* 1981	Standardised dry extract (36-44:1): 3 x 140mg	1 month	Mild laxative effect

5.2. Patient exposure

The clinical trials performed with milk thistle preparations include more than 4,000 patients who were also taken in account for safety analysis. Most of them are represented by placebo-controlled trials with a treatment duration ranging from 1 up to 41 months.

Data from PSUR for the *Standardised dry extract (DER 36-44:1)* indicate that the patient months-exposure (based on daily standard dosages, 30 days/month) for all oral formulation (different strengths of capsules, granules and suspensions) is 6,664,480 (01.07.2011-30.06.2014) and 23,530,938 (01.01.2004-30.06.2014).

The preparation containing 170-239 mg dry extract (24-27:1) (corresponding to 140 mg silymarin (calculated as silibinine, HPLC); extraction solvent: acetone, defined daily dosages worldwide: 1,509,200 (01.07.2011-30.06.2014) and 18,179,665 (31.01.1995-30.06.2014).

5.3. Adverse events, serious adverse events and deaths

The frequency for the most reported adverse events from the above-mentioned clinical trials (see section 4.2.) can be considered as low. The following adverse events are included: mild gastrointestinal symptoms such as dry mouth, nausea, upset stomach, gastric irritation and diarrhoea; headache; allergic reactions (dermatitis, urticaria, skin rash, pruritus, anaphylaxis, asthma). Nonetheless, most of the clinical trials reported no adverse events or even no differences between placebo and treated groups.

Also the safety assessment during pharmacokinetics studies is positive **(Schrieber *et al.* 2011).** Adverse events frequency was low (3 subjects within 28 patients) and were classified as neurological (e.g. headache) or gastrointestinal, but only one adverse event (dizziness) was considered possibly related to milk thistle administration and resolved in less than one day. For NAFLD patients, 2 of 12 subjects (16.7%) of the milk thistle group reported at least one adverse event compared to 1 out of 4 subjects (25%) in placebo group; the former included upper respiratory infection and abdominal pain (group receiving 560mg) which were considered unrelated to treatment.

As no data are available for the traditional use part, the possible adverse events regarding the use of traditional products are the same than those reflected for the WEU part.

According to the pharmacovigilance database, no adverse events, serious adverse events or deaths related to milk thistle have been reported.

5.4. Laboratory findings

The pharmacokinetics study conducted by **Schrieber et al. (2011)** in patients with NAFLD and HCV carried on a safety assessment before dosing on study days 1 (baseline), 6, 8 and 10, including laboratory tests. No reduction in serum transaminases for either HCV or NAFLD subjects, or reductions in HCV RNA titer for HCV subjects were observed at the end of the 7 day treatment period with placebo or milk thistle extract at 280 or 560mg (3 doses daily).

5.5. Safety in special populations and situations

No data available.

5.5.1. Use in children and adolescents

Not applicable

5.5.2. Contraindications

Not applicable

5.5.3. Special Warnings and precautions for use

Not applicable

5.5.4. Drug interactions and other forms of interaction

Not applicable

5.5.5. Fertility, pregnancy and lactation

Not applicable

5.5.6. Overdose

Not applicable

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

Not applicable

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

Information on clinical safety comes from several clinical trials which included more than 4000 patients, mainly in randomized double-blind *versus* placebo studies; more than 800 of them where included in studies testing efficacy of milk thistle in alcohol liver disease, together with 612 patients diagnosed with viral hepatitis.

One observational study on 998 patients showed that milk thistle extract reduced collagen fibrogenesis in patients with toxic liver (**Schuppan *et al*., 1998**). Treatment of psychotropic drug-induced hepatic damage with purified silymarin was the subject of another R, DB, PC study (**Palasciano *et al*., 1994**) and one multi-center study involving 220 patients over four years found *i.v.* purified silibinin complemented standard treatment, lowering mortality rates in cases of acute Amanita mushroom poisoning (**Hruby *et al.*, 1984**).

The safety analysis of the adverse events reported after *Silybum marianum* treatment when compared to placebo shows a low incidence, with no statistically significant differences in the rate of serious adverse events in the mentioned studies and confirms the excellent tolerability and safety of milk thistle in hepatic disorders. Additional safety data is derived from post-marketing surveillance-studies with more than 6,000 patients **(Saller *et al.*, 2008).**

The incidence of adverse drug reactions is low, mainly affecting the gastro-intestinal tract and they are generally mild: mild laxative effect, abdominal pain or discomfort, nausea, flatulence, dyspepsia. Also slight allergic skin reactions (itching and rash), headache and insomnia have been reported in a few cases **(Bunout *et al*., 1992; El-Kamary *et al.,* 2009; Pares *et al.,* 1998).**

Moreover, the traditional use of different milk thistle preparations such as Comminuted or Powdered herbal substance, Dry extract (DER 20-70:1), (extraction solvent acetone 95%), Dry extract (DER 30-40: 1), (extraction solvent ethanol 96%) and Liquid extract (DER 10-17:1), (extraction solvent ethanol

60%) for more than 30 years in the European Union proves that Silybum marianum, fruit is not harmful in the specified conditions of use.

6. Overall conclusions (benefit-risk assessment)

Based on the results obtained from several clinical trials, a positive benefit-risk ratio for silymarin standardized extract is assessed for supportive treatment of alcoholic liver disease. Silymarin treatment induces a statistically significantly and clinically relevant reduction of elevated serum parameters which are reflecting hepatocellular injury in patients with chronic liver disease of various aetiologies and progression. In addition, biochemical parameters known to characterize antioxidative action were also significantly improved. Moreover, the values of P-III-P as a marker of fibrogenesis could be reduced in different stages of chronic liver disease. In patients with cirrhosis of the liver, the total mortality reported was lower in the silymarin treated patients in two out of five trials, while the overall liver- related mortality was lower in three out of five trials. Concerning clinical complications in cirrhosis there was a trend for fewer occurrences with silymarin treated patients. Subjective and objective symptoms and signs improvement was also observed in patients with acute hepatitis.

The results of these clinical trials are convincing and justify the treatment with one specific standardized milk thistle extract for patients suffering from alcoholic liver disease. Other preparations which are nowadays marketed in different member States do not fulfil the WEU criteria, as no efficacy is proven through clinical trials.

The data also confirm the tolerability and safety of silymarin in the various hepatic disorders. The incidence of adverse drug reaction is low; they are mainly affecting the gastrointestinal tract and are generally mild. Isolated instances of a mild laxative effect are listed as side-effects.

In summary, on the basis of the pharmacological and clinical evidences, silymarin is safe and beneficial and can be recommended for the supportive treatment of alcoholic liver disease. Early intervention is of particular importance to prevent from further progression of the disease and favour regression of signs and symptoms with the aim to improve quality of life.

Possible benefits have been shown most frequently for improvement in aminotransferases and liver function tests in alcoholic liver cirrhosis and as a supportive element in the therapy of *Amanita phaloides* poisoning, although this therapy is related to controlled intravenous administration. There is no evidence of a favourable influence in the evolution of viral hepatitis. The results are supported by several clinical trials and meta-analysis which reported statistically significant benefits with silymarin, remarkably the Cochrane review which concluded that milk thistle exerted a significant beneficial effect on liver-related mortality (relative risk 0.50; 95% CI 0.29 to 0.88, p= 0.02).

There exist some preparations from *S. marianum* which improve the bioavailabililty of silibinin (i.e., as a silibinin-phosphatidylcholine complex), but they haven't been taken in account as they cannot be classified as an herbal preparation due to the manufacturing steps and composition of such product and so don't fulfil the requirements of well-established use.

All the above indicate that *Silybum marianum* fruit refined and standardized extract fulfil the requirements of well-established use in the following preparation: Dry extract (DER 26-48:1), (extraction solvent: ethyl acetate) standardised to contain 40-65% silymarin.

In conclusion, the following indication is proposed for well established use:

Herbal medicinal product for supportive treatment of alcoholic liver disease

Based on the data on its long-standing use in the European Union and on the available bibliographic references, traditional use can be granted for *Silybum marianum* fruit: powdered or comminuted herbal substance for infusion or decoction and tincture. Dry extract (30-40:1), extraction solvent ethanol 96% V/V; soft extract (10-17:1), extraction solvent ethanol 60% V/V; dry extract (DER 20-35:1), extraction solvent: ethyl acetate; dry extract (DER 26-45:1), extraction solvent: ethyl acetate; dry extract (20-70:1), extraction solvent acetone 95% (V/V).

In conclusion, Milk thistle preparations can be accepted as traditional herbal medicinal products in the following indication:

Traditional herbal medicinal product for the relief of symptoms of digestive disorders with a sensation of fullness, bloating and flatulence.

In the absence of data in children and adolescents under the age of 18 years, Milk thistle should not be used in this target population and should be limited to adults and elderly.

There is a positive safety profile for the following *Silybum marianum* preparations; Dry extract (DER 26-48:1), (extraction solvent: ethyl acetate) standardised to contain 40-65% silymarin, Comminuted or Powdered herbal substance, Dry extract (DER 20-70:1), (extraction solvent acetone 95%), Dry extract (DER 30-40: 1), (extraction solvent ethanol 96%), dry extract (DER 20-35:1), extraction solvent: ethyl acetate; dry extract (DER 26-45:1), extraction solvent: ethyl acetate; and Liquid extract (DER 10-17:1), (extraction solvent ethanol 60%) With respect to side effects gastrointentinal events have been observed. This signal can be adequately addressed by appropriate labelling.

Pharmacotherapeutic group: Liver therapy, lipotropics

Proposed ATC code: A05B

A European Union list entry is not supported due to lack of adequate data on genotoxicity. Only one water extract was adequately tested according to the OECD guidelines, although this aqueous extract is not part of the Milk thistle monograph.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Annex

List of references