

Antioxidant and hepatoprotective effects of silymarin phytosomes compared to milk thistle extract in CCl₄ induced hepatotoxicity in rats

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Abstract

Milk thistle extract is a well-known hepatoprotectant with low bioavailability (20–50%). The objective of the present study is to prepare and characterize silymarin phytosomes and to test the hepatoprotective effect of the phytosomes in CCl₄ induced liver injury in rats compared to milk thistle extract. Phytosomes were prepared using lecithin from soybeans and from egg yolk. The prepared phytosomes were examined using scanning electron microscopy, transmission electron microscopy, differential scanning calorimetry, Fourier transform infrared spectroscopy and proton nuclear magnetic resonance spectroscopy (¹H-NMR). The loading efficiency was >85% in all phytosomal formulations. Formula P2 (with the molar ratio of soybean lecithin to silybin 1:1) and P4 (with the molar ratio of egg-yolk lecithin to silybin 0.25:1) exhibited significantly ($p < 0.05$) faster release than milk thistle extract. The *in vivo* study revealed that phytosomes significantly ($p < 0.05$) decreased glutamic pyruvic transaminase and super oxide dismutase activities compared to milk thistle extract.

Keywords

Liver enzymes, phosphatidylcholine, silybin,

History

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Introduction

Milk thistle (*Silybum marianum* L. Gaertn, Asteraceae) is hepatoprotectant used in the treatment of hepatitis C, hepatocarcinoma, nonalcoholic fatty liver disease and gall bladder disorders (Bares et al., 2008; Freedman et al., 2011). Silymarin flavonolignans of milk thistle have other interesting properties, e.g. canceroprotective, neuroprotective, use in the treatment and prevention of gastrointestinal problems and cardio-pulmonary problems, and in skin protection against harmful UV irradiation. These effects are due to the antioxidative and free radical-scavenging properties of milk thistle (Křen and Walterová, 2005). There are four isomers within silymarin consisting of: silybin, isosilybin, silicristin, and silidianin. The main active compound is silybin, which is concentrated in the bile where concentrations can be 60 times greater than in the serum. Other flavonoids in *S. marianum* include dehydrosilybin, deoxysilycistin, deoxysilydianin, silandrin, silybinome, silyhermin and neosilyhermin (Jacobs et al., 2002).

Silymarin suffers from poor bioavailability that is attributed to either degradation by gastric fluid, poor absorption or low water solubility. Absorption is significantly enhanced if silybin is administered in a complex with phosphatidylcholine (Giacomelli et al., 2002).

Phytosomes are complexes of natural products and natural phospholipids like soy phospholipids. This complex is obtained by the stoichiometric reaction of phospholipids and the substrate in an appropriate solvent. Phytosomes are better absorbed and utilized and produce better results than conventional herbal extracts (Sharma and Sikarwar, 2005). The increased

bioavailability of phytosomes over the non-complexed botanical derivatives has been demonstrated by pharmacokinetics studies and by pharmacodynamic tests in experimental animals and in human subjects (Semalty et al., 2010).

When treated with water, phytosomes take a micellar shape forming liposome-like structures, but whereas in liposomes the active portion is dissolved in the internal core or is floating in the membrane layer, in phytosomes the active portion is bound to the polar head of the phospholipids and acts as an integral part of the membrane (Sharma and Sikarwar, 2005).

The silymarin phytosomes were predicted to exhibit superior antioxidant, hepatoprotective and renal protective effects compared to milk thistle extract due to their improved bioavailability. The enhanced absorption of phytosomal formulations might be due to the solubilizing effect of insoluble drugs, such as silymarin flavonoids, by the surface active properties of phosphatidylcholine (Bernhard et al., 2001). In addition, phosphatidylcholine molecules themselves are absorption enhancers (Vermehren et al., 1997). However, phosphatidylcholine is an integral part of the cell membrane, and it is the most common phospholipid in animal and plant tissue, including milk thistle.

Accordingly, the objective of this work was to study silymarin phytosomes that were prepared using soybean and egg yolk lecithin in different molar ratios. The antioxidant and hepatoprotective effects of the prepared phytosomes are compared to milk thistle extract in carbon tetrachloride induced hepatotoxicity in rats.

Materials

Milk thistle extract containing 80% silymarin and silybin standard were purchased from Sigma-Aldrich (Buenos Aires, Argentina); phosphatidylcholine from soybeans was obtained from Scharlau Chemie (Barcelona, Spain); phosphatidylcholine from egg yolk was purchased from Sigma-Aldrich Chemie

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(Munich, Germany); Silipex[®] capsules were a gift from EmaPharm (Cairo, Egypt). All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

Methodology

Determination of silybin content in milk thistle extract

An HPLC assay of silybin in methanol was performed according to European Pharmacopeia (2005) with some modifications. The HPLC instrument consisted of an LC-10 AS isocratic pump and an SPD-10A UV/VIS detector connected to a C-R7A Integrator; Shimadzu, Kyoto, Japan. The analytical column was a Ponapak C18 HPLC column, 4.8 × 250 I.D mm, particle size 125 Å; Waters Associates, Wexford, Ireland. The mobile phase consisted of solution A; phosphoric acid, methanol and water, in a ratio of 0.5:35:65 V/V/V and solution B; phosphoric acid, methanol and water, in a ratio of 0.5:50:50 V/V/V. A gradient mobile phase with a flow rate of 0.8 ml/min was used. A silybin standard solution was also used. The system was operated at ambient temperature, injection volume was 20 µl and the detection wavelength was 288 nm.

Preparation of silymarin phytosomes

Phytosomes were prepared using soybean lecithin and egg yolk lecithin as follows: specified weights of milk thistle extract and lecithin were dissolved in methanol (100 ml) at room temperature while stirring. When completely dissolved, the mixture was concentrated under vacuum to 30 ml and poured while stirring into 300 ml petroleum ether. The precipitate was left to settle overnight, collected by filtration, washed with petroleum ether and dried under vacuum at 40 °C. The composition of the prepared phytosomes is given in Table 1.

Scanning electron microscopy

Phytosomes (formula P2) were coated with gold in a sputter coater (JFC-1100, Jeol, Tokyo, Japan), and the surface morphology was viewed and photographed by a scanning electron microscope (JSM-5400, Jeol, Tokyo, Japan) using Orion software version 6.60.5.

Transmission electron microscopy

Samples were prepared by dropping distilled water onto the phytosomes (formula P2) and swirling for 3 min. A drop of the resultant phytosome dispersions was placed onto a carbon-coated copper grid, leaving a thin liquid film. The films on the grid were stained with osmium tetroxide (0.4% in distilled water), the excess staining solution was removed with a filter paper, and the grids were air dried. The stained films were then photographed using a transmission electron microscope (JEM-100CX, Jeol, Tokyo, Japan) operating at 100 kV, and the diameter of phytosomes were determined from the images using the Image J software (National Institutes of Health, Bethesda, MD).

Study of the interaction between silymarin phytosome components

Milk thistle extract, lecithin from soybean, lecithin from egg yolk, and formula P2 and P4 were subjected to the following tests:

(1) Differential scanning calorimetry (DSC)

Samples (3–4 mg) were placed in flat-bottomed aluminum pan and heated at a constant rate of 10 °C/min, in an atmosphere of nitrogen and a temperature range of 20–400 °C (DSC7, Perkin-Elmer, Waltham, MA).

(2) Fourier transform infrared spectroscopy (FTIR)

Infrared spectra of the test samples were determined by the KBr disc technique. The FTIR measurements were performed in the scanning range from 4000 to 400 cm⁻¹ (Bruker 22, Coventry, UK).

(3) Proton nuclear magnetic resonance spectroscopy (H¹NMR)

The H¹NMR spectra were recorded on a Brüker Avance 300 MHz Nuclear Magnetic Resonance, Coventry, UK. The lecithin from soybeans and from egg yolk was dissolved in CDCl₃, whereas the milk thistle extract and formula P2 and P4 were dissolved in dimethyl sulphoxide.

Drug content and loading efficiency

A specified weight of phytosomes (0.1 g) was dissolved in methanol, and the concentration of silybin was determined by the aforementioned HPLC assay. All experiments were run in triplicate. The drug content and loading efficiency were calculated from the following equations:

$$\text{Drug content} = \frac{\text{amount of silybin in phytosomes} \times 100}{\text{weight of phytosomes}}$$

$$\text{Loading efficiency} = \frac{\text{calculated amount of silybin in phytosomes} \times 100}{\text{theoretical silybin content}}$$

Release study

The experiment was conducted using the USP Dissolution Tester Apparatus 2 (PharmaTest, Hainburg, Germany). The paddle speed was set at 100 rpm and the temperature was maintained at 37 ± 5 °C. The release medium was 900 ml phosphate buffer (pH 7.5) containing 2% sodium lauryl sulphate. The release study was performed using Silipex[®] capsules (112 mg silybin) containing soybean lecithin and silybin (0.5:1 molar ratio) and a transparent hard gelatin capsule containing 50 mg silybin in the form of either milk thistle extract or phytosomes. Aliquots were withdrawn from the release medium at specific time intervals (10 min) over a period of 2 h and immediately replaced by an equal volume of the release medium. Samples were analyzed by HPLC, and the percentage silybin released was calculated. All of the release experiments were run in triplicate.

Table 1. Composition of silymarin phytosomes, mean silybin content and loading efficiency (*n* = 3).

Formula name	lecithin:silybin	Calculated silybin content (Mean ± SD)	Theoretical silybin content	Loading efficiency (Mean ± SD)	
Soybean lecithin	P ₁	0.5:1	48.63 ± 1.43%	54.50%	89.61 ± 1.13%
	P ₂	1:1	37.53 ± 1.30%	37.50%	97.46 ± 1.16%
	P ₃	2:1	23.47 ± 1.07%	25.00%	89.37 ± 1.28%
Egg yolk lecithin	P ₄	0.25:1	75.90 ± 0.26%	76.10%	99.34 ± 0.33%
	P ₅	0.5:1	49.66 ± 1.14%	54.70%	87.78 ± 0.94%
	P ₆	1:1	37.53 ± 0.75%	37.50%	98.31 ± 0.71%
	P ₇	2:1	23.13 ± 0.81%	24.47%	94.52 ± 1.17%

In vivo study in carbon tetrachloride induced liver injury in rats

Experimental animals and basal diet

Thirty-two male albino rats (150–200 g) were obtained from the farm of the National Organization for Drug Control and Research, Giza, Egypt. The rats were housed at ambient temperature, $22 \pm 3^\circ\text{C}$, in a 12-h light/dark cycle and a minimum relative humidity of 40%. Rats were maintained on a basal diet formula composed of: 10% protein, 10% corn oil, 4% salt mixture, 1% vitamin mixture, 5% cellulose, 70% starch and water *ad libitum* (Khiralla and Salem, 2012).

Experimental design

The experimental protocol was approved by the Ethical Committee for the Scientific Research, Faculty of Pharmacy, Cairo University, approval number ECSRFPCU3221. The study was conducted over 10 days after 1-week acclimatization to laboratory conditions. Rats were divided into four groups of eight rats as follows:

Group I “normal control”: received olive oil (1 ml/kg, i.p.) daily for 7 days from day 4 to day 10.

Group II “toxicant control”: received 1:1 (v/v) mixture of CCl_4 and olive oil (1 ml/kg, i.p.) daily for 7 days from day 4 to day 10.

Group III “milk thistle extract group”: received milk thistle extract (equivalent to 200 mg silybin/kg, orally) daily for 10 days and a 1:1 (v/v) mixture of CCl_4 and olive oil (1 ml/kg, i.p.) daily for 7 days from day 4 to day 10.

Group IV “silymarin phytosomes group”: received phytosomes (formula P2) equivalent to 200 mg/kg silybin, orally, daily for 10 days and a 1:1 (v/v) mixture of CCl_4 and olive oil (1 ml/kg, i.p.) daily for 7 days from day 4 to day 10.

Evaluation parameters

The following procedures were performed 24 h after the last injection:

- (1) Blood samples were collected from the eye sac of the rats (retro-orbital venous plexus bleeding) into heparinized capillary tubes and centrifuged at 300 g for 10 min. Clear plasma was collected in polyethylene tubes and deep frozen at -20°C until assayed for the following marker enzymes: serum glutamic-oxaloacetic transaminase (SGOT) or aspartate aminotransferase (AST), serum glutamic-pyruvic transaminase (SGPT) or alanine aminotransferase (ALT), serum alkaline phosphatase (SALP), albumin (Alb), globulin (Glob) and total protein (TP).
- (2) Animals were sacrificed by cervical decapitation to avoid stress, and the livers were dissected out immediately, washed with ice-cold saline and 10% homogenates were centrifuged at 7000 g for 10 min. The supernatant was used for assays of super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR).

Biochemical assays

The activities of SGOT, SGPT, SALP, SOD, CAT Alb, and TP concentration were determined according to standard procedures using commercially available diagnostic kits obtained from STCO (Scientific Trade institution), Giza, Egypt, as follows:

Colorimetric determination of SGOT and SGPT activity

SGOT and SGPT activity was determined according to Reitman and Frankel (1957). The principle of the assay is as follows: SGOT catalyzes the transfer of the amino group of aspartic acid to

α -ketoglutarate to form oxaloacetate and glutamate. The ketoacid oxaloacetate that is formed reacts with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form the corresponding hydrazine which is a brownish-red-coloured complex in an alkaline medium. The colour intensity is directly proportional to SGOT activity and is measured spectrophotometrically at 505 nm. Similarly, SGPT catalyzes the transfer of the amino group of alanine to α -ketoglutarate to form pyruvate and glutamate. The pyruvate reacts with 2,4-DNPH to form a brownish-red-coloured complex in an alkaline medium.

Colorimetric determination of SALP activity

SALP activity was determined according to Belfield and Goldberg (1971). Phenyl phosphate is hydrolyzed by SALP to liberate free phenol, which reacts with 4-aminophenazone in the presence of alkali ferricyanide to give a red-coloured complex. The absorbance measured at 510 nm is directly proportional to enzyme activity.

Colorimetric determination of SOD activity

SOD activity was determined according to Nishikimi et al. (1972). The assay is based on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. The absorbance of the solution at the end of the reaction is measured at 560 nm.

Colorimetric determination of CAT activity

CAT activity was determined according to Aebi (1984). CAT reacts with a known quantity of H_2O_2 , and the reaction is stopped after exactly 1 min with CAT inhibitor. The remaining H_2O_2 reacts with a colouring agent to form a chromophore with absorbance at 510 nm that is exactly proportional to the amount of catalase in the original sample.

Ultra violet determination of GR and GPR activity

GR and GPR activity was determined according to Goldberg and Spooner (1983) and Paglia and Valentine (1967), respectively. The activity of GR and GPR was measured by the decrease in the absorbance at 340 nm caused by the oxidation of NADPH by GR or GPR.

Colorimetric determination of Alb concentration

The determination of Alb concentration was conducted according to Doumas et al. (1971). Alb in the presence of bromocresol green at a slightly acidic pH produces a colour change from yellow-green to green-blue. The colour intensity, measured at 630 nm, is directly proportional to Alb concentration.

Colorimetric determination of TP concentration

The determination of TP concentration was conducted according to Gornal et al. (1949). The assay is based on the polypeptide chelation of cupric ion in a strong alkaline medium to form a deep purple complex with maximum absorbance at 550 nm.

The globulin concentration (g/dl) was calculated by subtracting the albumin concentration from total protein.

Statistical analysis

Two-way analysis of variance (two-way ANOVA) and Tukey Highest significant difference (HSD) were performed using the statistical product for the social sciences 10.0 (SPSS Inc., Chicago, IL).

Results and discussion

Determination of silybin content in milk thistle extract

The percentage of silybin in milk thistle extract was calculated as 76.3%. Given that silymarin formed 80% of the milk thistle extract product used in this study, the other isomers of silymarin, such as isosilybin, silicristin and silidianin, represent <4% of the flavonolignans of milk thistle extract.

Surface morphology of silymarin phytosomes

The surface morphology of phytosomes as examined by scanning electron microscopy appeared spongy and highly porous (Figure 1). This could contribute to the solubilizing effect of phytosomal formulations and the consequent increase in bioavailability and therapeutic effects of such formulations.

The transmission electron micrographs of the phytosomes (figures not shown) after slight swirling in distilled water showed suspended particles with particle sizes ranging from 40 to 150 nm. On shaking with water, the silymarin phospholipid complex molecules were arranged in micelle-like vesicles resembling liposomes, but the drug became an integral part of the vesicular membrane instead of being entrapped in the core of the vesicles.

Study of the interaction between phytosome components

Differential scanning calorimetry

Figure 2(a) exhibits the DSC thermograms of the milk thistle extract, lecithin from soybeans, lecithin from egg yolk, and formula P2 and P4. The thermogram of milk thistle extract showed an endothermic peak at 136.1 °C, denoting the melting point of silybin (Yanyu et al., 2006). The thermograms of lecithin from soybeans and egg yolk showed a different appearance. This difference in the thermograms of the two types of phospholipids is mainly due to the difference in their structure. Egg yolk lecithin contains long chain polyunsaturated fatty acids (LC-PUFAs), mainly arachidonic acid and docosahexaenoic acid. The former occurs exclusively in cell membranes, whereas the latter is found in high quantities in optic nerve membranes. Lecithin from soybeans contains fatty acids, with roughly the same proportion as that of soy oil, which is 50–57% linoleic acid and 5% linolenic acid (Kent, 2005). The thermograms of formula P2 and P4 showed the absence of the characteristic peaks of their individual components. This could be attributed to the presence of physical interaction between silymarin and both types of lecithin in

phytosomes, most likely by hydrogen bonding between silymarin flavonolignans and the polar head of the lecithin. Similar observations were recorded by El-Samaligy et al. (2006) and Yanyu et al. (2006) who studied the DSC of silymarin liposomes and phytosomes, respectively.

Fourier transform infrared spectroscopy

The spectrum of milk thistle extract (Figure 2b) exhibited the characteristic bands of silymarin flavonolignans: at 1082 cm^{-1} for the benzopyran ring, at 1736 cm^{-1} corresponding to carbonyl ester stretching and 1640 cm^{-1} for the flavonolignan ketone (Sonai et al., 2010; Das et al., 2011). The spectra of soybean and egg yolk lecithin showed similar bands with slight shifts at 1240 cm^{-1} assigned to the P=O group, 2850 cm^{-1} for the C–H aliphatic stretching and 1740 cm^{-1} corresponding to the carbonyl ester group (Popova and Hinch, 2003). The finger print regions in the spectra of soybean and egg yolk lecithin were different between the two types of lecithin due to the difference in the substitution on the phosphatidylcholine molecule. The spectra of formula P2 and P4 maintained the characteristic bands of silymarin and lecithin, suggesting no chemical interaction between phosphatidylcholine and silymarin flavonolignans.

^1H NMR spectroscopy

^1H NMR Spectra of lecithin from soybean and egg yolk (Figure 2c) showed a signal at 3.2 ppm, corresponding to the N–(CH₃)₃ group of the choline moiety, among the other characteristic signals of phosphatidylcholine (Everts and Davis, 2007). The spectra of formula P2 and P4 exhibited a markedly broadened N–(CH₃)₃ group signal with a small upfield shift, indicating that this moiety was involved in complex formation, because the spectra of phospholipids in the same solvent appeared as a sharp singlet. Additionally, signals from the hydroxyflavone moiety of silybin at 3.813, 3.803, 3.614 and 3.552 ppm (Syn-Cordero et al., 2010) were broadened in the spectra of formula P2 and P4 denoting the formation of hydrogen bonds between the polar heads of the phospholipids and the polar functional groups of the silymarin flavonolignans (Pang et al., 2012).

Characterization of silymarin phytosomes

Drug content and loading efficiency

Table 1 shows the calculated silybin content and the loading efficiency in the prepared phytosomes. The calculated drug contents in the phytosomes were very close to the theoretical values and the loading efficiency was >85% in all formulations, indicating that the preparation method was efficient and that drug loss during preparation was minimal.

Release study

Figure 3 shows the release profiles of the phytosomal formulations compared to milk thistle extract and Silipex[®] capsules. The release of silybin from formula P2 was faster than that from formula P3 and Silipex[®], but the difference between the three formulations was not significant ($p > 0.05$). The release of silybin from formula P3 was significantly ($p < 0.05$) faster than that from milk thistle extract and formula P1. Formula P4 exhibited significantly faster release ($p < 0.05$) than formula P5, milk thistle extract and formula P6. Tukey HSD test also found that the difference between formula P2, P4 and the Silipex[®] capsule was not significant ($p > 0.05$). Although there was no significant difference between formula P2 and P4, formula P2 was selected for the *in vivo* study because the structure of soybean lecithin contains a minimal quantity of hydratable components (1.8%)

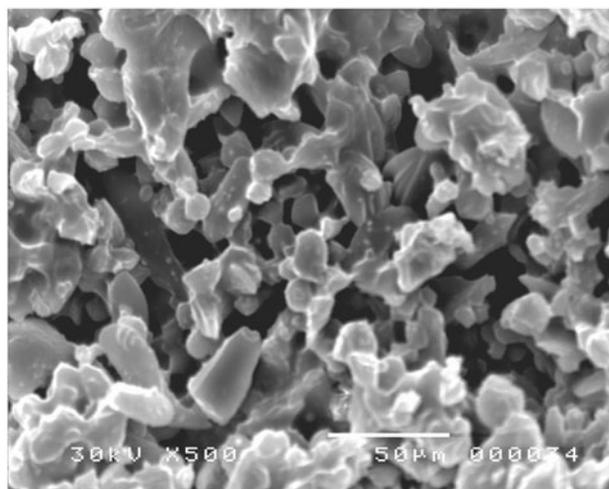


Figure 1. Scanning electron micrographs of silymarin phytosomes (formula P2) at $\times 500$ magnification.

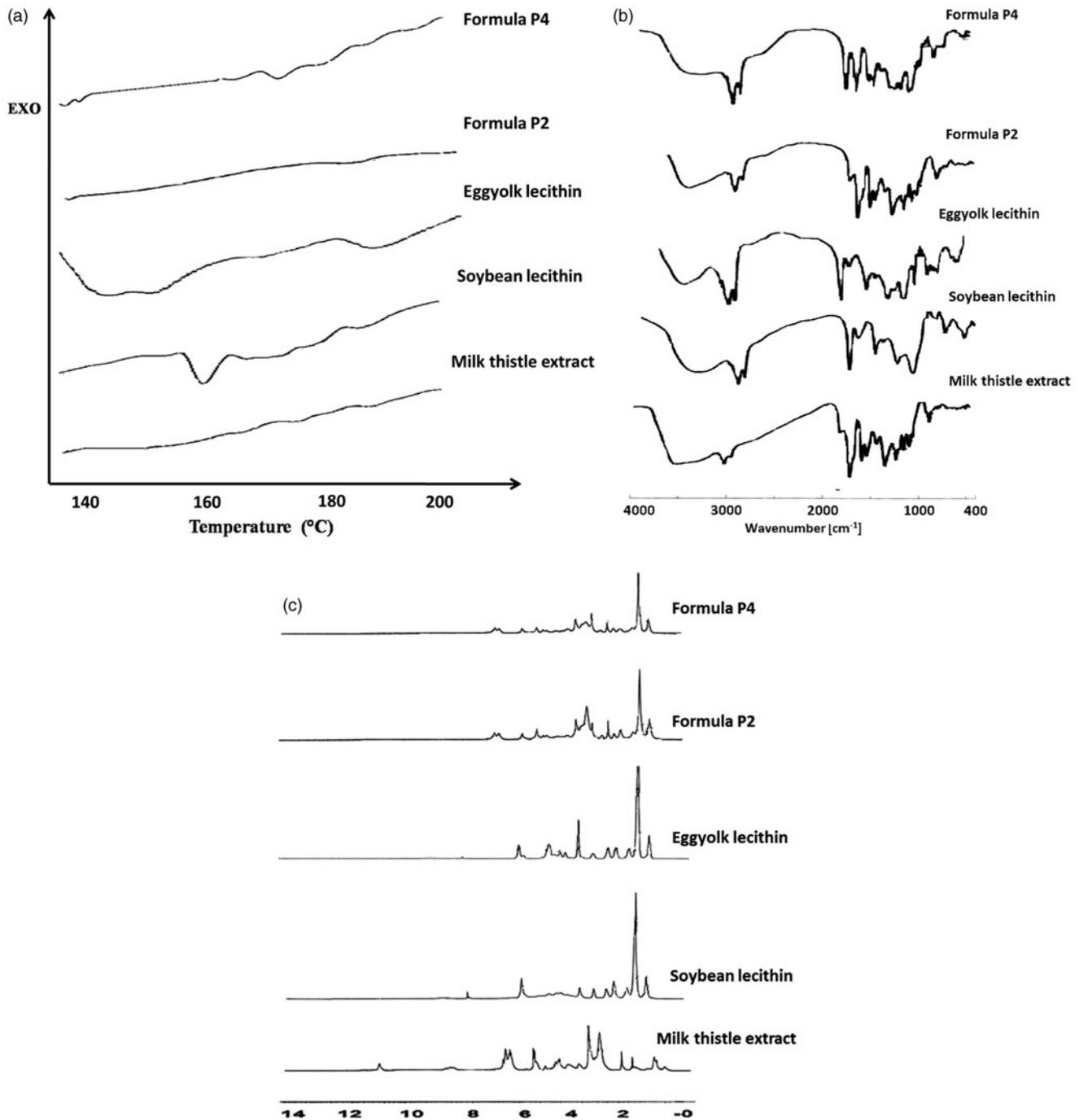


Figure 2. DSC thermograms (a), FTIR spectra (b) and ¹H-NMR spectra (c) of milk thistle extract, lecithin from egg yolk, lecithin from soybeans and formula P2, P4.

compared to egg yolk lecithin (Bush et al., 1985; Paschke et al., 2001). In addition, egg yolk lecithin may cause allergic reactions, whereas the allergens in soybean lecithin reside in the protein fraction of the bean, which is removed during the extraction process of soybean lecithin.

In vivo study in carbon tetrachloride induced liver injury in rats

Liver injury induced by CCl₄ is the best-characterized model used for screening the hepatoprotective activity of drugs (Tiwari et al., 2011). The extent of hepatic damage is assessed by the increase in the serum levels of cytoplasmic enzymes (SGOT, SGPT, and SALP) (Zangar et al., 2000; Södergren et al., 2001). In our study, there was a marked increase in SGPT and SGOT in the CCl₄

treated group compared to the normal control group, which was reversed in the milk thistle extract and the silymarin phytosomes treated groups. Phytosomes were more efficient than milk thistle extract ($p < 0.05$) at reversing the increase in SGPT induced by CCl₄. Regarding SALP, there was a non-significant difference between the four groups (Table 2).

The normal detoxification pathways involve protective physiological molecules such as glutathione (GSH) and α -tocopherol. Endogenous antioxidants, such as GSH, play a major role in detoxifying the reactive toxic metabolites of CCl₄, whereas liver necrosis begins when antioxidant levels markedly decrease. Silymarin prevents glutathione depletion in human hepatocytes, protecting liver cells from damage (Masini et al., 2000; Malekinejad et al., 2012). GPX catalyzes the reduction of H₂O₂ to water and of organic peroxides to the corresponding stable

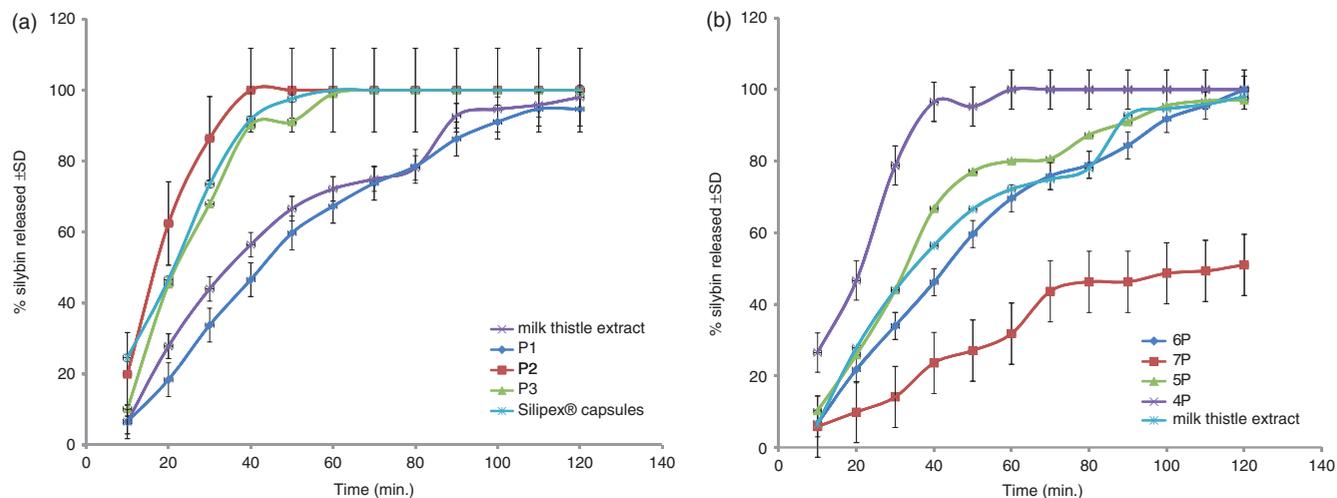


Figure 3. Release profiles of silymarin phytosomes prepared from soybean lecithin (a) and from egg yolk lecithin (b) compared to milk thistle extract and Silipex[®] capsules in phosphate buffer (pH 7.5) containing 2% sodium lauryl sulphate.

Table 2. Biochemical parameters in plasma and liver (liver enzyme markers), antioxidant enzymes and kidney function tests after 10 days ($n = 6$).

		Treatments			
		Group I** normal control Mean \pm SD	Group II** toxicant control Mean \pm SD	Group III** milk thistle extract Mean \pm SD	Group IV** silymarin phytosomes Mean \pm SD
Biochemical parameters in plasma and liver	SGOT* (μ /ml)	45.98 \pm 7.29	103.50 \pm 13.21	81.60 \pm 11.52	67.00 \pm 22.93
	SGPT* (μ /ml)	25.27 \pm 3.90	95.16 \pm 11.50	74.83 \pm 12.38	51.60 \pm 7.23
	SALP* (IU/L)	214 \pm 1.72	212 \pm 2.46	208 \pm 4.03	204 \pm 13.05
Antioxidant enzymes	CAT* (U/g)	7.79 \pm 0.82	4.48 \pm 0.72	6.90 \pm 1.21	7.18 \pm 1.33
	SOD* (U/g)	438.80 \pm 90.71	331.90 \pm 60.71	408 \pm 11.01	501.67 \pm 11.69
	GR* (U/L)	208 \pm 67.47	43.50 \pm 14.89	181.67 \pm 10.33	189.16 \pm 7.36
	GPX* (mU/ml)	17.50 \pm 7.71	7.10 \pm 0.59	19.267 \pm 10.63	25.00 \pm 4.47
Kidney function tests	TP* (g/dl)	7.01 \pm 1.35	6.86 \pm 1.67	7.25 \pm 1.63	7.90 \pm 1.23
	Alb* (g/dl)	5.07 \pm 0.33	4.04 \pm 0.33	3.85 \pm 0.33	4.01 \pm 0.42
	Glob* (g/dl)	1.94 \pm 1.44	2.82 \pm 1.49	3.39 \pm 1.69	3.96 \pm 0.96

*SGOT or AST: glutamic oxaloacetic transaminase or aspartate amino transferase, SGPT or ALT: glutamic pyruvic transaminase or alanine amino transferase, SALP: alkaline phosphatase, CAT: catalase, SOD: super oxide dismutase, GR: glutathione reductase, GPX: glutathione peroxidase, TP: total protein, Alb: albumin, Glob.: globulin. **Group I: received olive oil daily for 7 days from day 4 to day 10, Group II: received 1:1 (v/v) mixture of CCl₄ and olive oil daily for 7 days from day 4 to day 10, Group III: received milk thistle extract, daily for 10 days and a 1:1 (v/v) mixture of CCl₄ and olive oil daily for 7 days from day 4 to day 10, Group IV: received formula P2 daily for 10 days and a 1:1 (v/v) mixture of CCl₄ and olive oil daily for 7 days from day 4 to day 10.

alcohols using glutathione as a source of reducing equivalents. GR is required for the glutathione redox cycle, which maintains adequate levels of reduced GSH (Singh et al., 2008). In the present experiment, GR and GPX activity decreased in the toxicant group and increased in the phytosomes and milk thistle extract treated groups, but there was no significant difference between the two groups ($p > 0.05$).

SODs are metalloenzymes that catalyze the conversion of the superoxide anion to molecular oxygen and hydrogen peroxide, and thus form a vital part of the cellular antioxidant defence mechanism (Broide et al., 2000). Silymarin displays antioxidant properties by increasing SOD in erythrocytes and lymphocytes (Manibusan et al., 2007). SOD activity in the CCl₄ treated group was observed to be less than in the control group. Both the phytosomes and milk thistle extract treated groups had elevated SOD levels, but the phytosomes were more efficient than milk thistle extract ($p < 0.05$). CAT is an antioxidant enzyme present in most aerobes that serves as a defence system against H₂O₂, which can cause intracellular damage (Singh et al., 2009). CAT activity of liver homogenate in the CCl₄ treated group was measured to be

lower than the normal group. Liver CAT activities in the phytosomes and milk thistle extract groups were higher than the CCl₄ toxicant group, but there was no significant difference between them ($p > 0.05$).

Silymarin is reported to be a renal protectant against nephrotoxicity associated with the use of acetaminophen, cisplatin, vincristine and cyclosporine, as well as in radiotherapy (Esera et al., 2012). There was a decrease of TP in the CCl₄ treated group, but there was no significant change after treatment with phytosomes or milk thistle extract. There was no significant difference between the four groups in albumin and globulin levels ($p > 0.05$).

The significant improvement in liver function in the milk thistle extract and phytosomes treated groups compared to CCl₄ toxicant group could be attributed to the hepatoprotective and antioxidant effect of silymarin (Lia et al., 2012). Silymarin is effective in the treatment of various types of liver diseases including hepatitis, cirrhosis, fatty infiltration of the liver (chemical and alcohol induced fatty liver), and inflammation of the bile duct (Wellington and Jarvis, 2001). The superior effect of

phytosomes in decreasing the SGPT level and increasing SOD activity compared to uncomplexed silymarin could be due to increased lipophilicity and therefore, the permeability of phytosomes. Phytosomes are better able to transition from a hydrophilic environment into the lipid-friendly environment of the outer cell membrane, and from there into the cell, finally reaching the blood (Lu et al., 2009). In aqueous media, phytosomes present in a cell-like structure, such as the valuable components of the herbal extract, are protected from destruction by digestive secretions and gut bacteria (Marczylo et al., 2007). Moreover, phosphatidylcholine is not only a carrier for the bioactive flavonoids of the phytosomes, but it is also a bioactive nutrient with documented clinical efficacy for liver disease, including alcoholic hepatic steatosis, drug-induced liver damage, and hepatitis (Yan et al., 2004). Phosphatidylcholine is also a surface active agent that increases the aqueous solubility of the milk thistle extract active components. The insignificant difference in the SGOT, SALP CAT, GR and GPX levels between the milk thistle extract and silymarin phytosomes treated groups could be due to other constituents of milk thistle extract such as apigenin, silybonol, myristic, oleic, palmitic and stearic acids, and betaine hydrochloride, which may have a hepatoprotective effect (Jacobs et al., 2002). Additionally, phosphatidylcholine is the main phospholipid in plant and animal tissues and is a component of milk thistle extract, which improved its antioxidant and hepatoprotective effects.

Conclusions

Silymarin phytosomes were more efficient hepatoprotectant than milk thistle extract. Because milk thistle extract is not pure silymarin, other components of the extract could play a role in its antioxidant and hepatoprotective effects.

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Declaration of interest

The authors report no declarations of interest.

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