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ORIGINAL ARTICLE

Preparation and evaluation of galactosylated vesicular carrier for hepatic targeting of silibinin

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Abstract

Purpose: Silibinin, the main flavonolignan of Silymarin, is used in the treatment of liver diseases of varying origins. Aiming at improving its poor bioavailability of oral products, galactosylated liposomes were introduced in this work for silibinin delivery and targeting to the lectin receptors present on the hepatocytes. *Methods*: Small unilamellar liposomal vesicles were prepared and *p*-aminophenyl-β-D-galactopyranoside was covalently coupled. The drug release from liposomes was studied by dialysis method. Plasma, tissue distribution and intrahepatic distribution of free, plain liposomal and galactosylated liposomal encapsulated silibinin were determined following a bolus intravenous injection in albino rats. Various formulations were evaluated regarding silibinin's hepatoprotective activity against CCl_4-induced oxidative stress in albino rats. The degree of protection was measured using biochemical parameters like serum glutamic oxalacetate transaminase and serum glutamic pyruvate transaminase. *Results*: Aggregation of galactosylated liposomes by *Ricinus communis* revealed the presence of galactose residues on the surface of liposomes. After 24 hours, cumulative drug release percent from galactosylated liposomes was found to be moderate (30.9 ± 1.73%). The results of tissue distribution study indicated extensive localization of liposomal formulations in liver cells (galactosylated liposomes, 61.27 ± 3.84% in 1 hour). Separation of the liver cells showed that galactosylated liposomes were preferentially taken up by the hepatocytes (79% of the total hepatic uptake in 1 hour). The introduced galactosylated silibinin produced a significant decrease in both transaminase levels when challenged with CCl_4 intraperitonially. *Conclusion*: A positive outcome of these studies gave an insight that galactosylated liposomes are more effective and suitable for targeted delivery of silibinin to hepatocytes.

Key words: *Galactosylated liposomes; hepatocytes; silibinin; targeting*

Introduction

For drug delivery, the physiochemical characterization of the drug carriers conjugate is particularly important in determining whether or not the complex is suitable for administration in vivo. In fact, liposomes are accepted as potent drug carriers not only for its biocompatible nature but also because these phospholipid vesicles do not elicit negative biological responses that generally occur when a foreign material is introduced in the system. Moreover, these lipid vesicles are nontoxic, nonimmunogenic, noncarcinogenic, nonthrombogenic, and biodegradable. The mammalian liver contains primarily parenchymal cells (PCs) (hepatocytes) and reticuloendothelial cells (Kupffer cells, endothelial cells, and stellate cells). The membrane composition of liposomes is crucial for its targeting and function. It was observed that galactosylated liposome-entrapped material was largely taken up by hepatocytes. Mannose-grafted liposome-entrapped material was enriched in the sinusoids, that is, Kupffer cell, endothelial cells, and stellate cells of liver. The high endocytic activity of the sinusoidal lining cells makes them most competent to internalize colloidal particles, particularly liposomes. So, natural targeting of liposomes can take place by these cells and it is reasonable to assume that galactosylated liposome administration increases intracellular accumulation of vesicular content in hepatocytes[1]. Furthermore, because of the presence of galactosyl receptor on the surface of hepatocytes, galactosylated

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liposomes are effective in the site-specific drug delivery to hepatic tissues with a homogenous intrahepatic membrane distribution of its intercalated components[2]. Delivery of drugs using liposomes bound to asialoglycoprotein receptors (ASGPRs) in a specific manner would provide significant therapeutic benefits in hepatic disease. ASGPR-mediated targeting of pharmaceuticals to hepatocytes is a promising approach to achieve cell (hepatocyte)-specific delivery after systemic administration because (i) the ASGPRs are specifically expressed in hepatocytes, (ii) molecules entering the systemic circulation easily get access to the cells through the discontinuous endothelium of the liver, and (iii) the liver has a rich blood flow[3].

To this end, extensive studies on chemical modification of liposomes with asialoglycoproteins[4–6] or low-molecular weight glycolipid have been carried out to achieve effective targeting to hepatocytes[7–17].

Han et al.[18] evaluated the potential of galactosylated bovine serum albumin as hepatocyte-directed and more effective liver targeting carrier of drug such as methotrexate for liver diseases. Gal-C4-Chol is extensively used for ASGPR-mediated targeting of genes and lipophilic drugs[10–12,15,19]. Gal-C4-Chol when combined with neutral lipids [distearoylphosphatidylcholine (DSPC) and Chol] was taken up by the liver PCs. Wang et al.[17] synthesized and incorporated a novel galactosylated lipid with a mono-galactoside moiety (CHS-ED-LA) into liposomes that enhanced the liver targetability of liposomal doxorubicin (DOX). Moreover, the results of intrahepatic distribution and competitive inhibition studies provided evidence of the recognition of galactosylated residues of CHS-ED-LA by ASGPR on the surface of PCs. Galactosylated liposomes have been used for targeting dimercaptosuccinic acid (DMSA)[20] and Qurecetin[21] to hepatocytes. Regarding therapeutic purpose, it is necessary to develop a strategy that could provide an even distribution of antioxidants for complete protection against hepatotoxicity. For better protection against oxidative attack the antioxidant should reach the liver with homogenous intrahepatic distribution. This study aims at developing nontoxic antioxidant formulation that can selectively be targeted to liver PCs.

Silibinin is the main flavonolignan of silymarin, a standardized extract from *Silybum marianum* L. (Asteraceae), used for their hepatoprotective effects in human medicine[22]. Membrane-stabilizing and antioxidant properties of silibinin are well documented in vitro and accepted by most investigators as the major protective mechanisms[23,24]. Silibinin has been reported to possess many pharmacological activities[24], such as anti-inflammatory, antitumor, and antifibrotic effects, and to positively influence some risk factors of atherosclerosis[25,26]. However, during in vivo studies these effects are restrained by its very low bioavailability[24,27,28].

The objective of this study is to target silibinin to the site of action through its incorporation in galactosylated liposomal formulation for parenteral administration. Galactosylation of liposomes is done to achieve site-specific delivery. Liposomes are mainly composed of phospholipids and hence can themselves serve as hepatoprotectant, and when silibinin is entrapped in liposomes a synergistic action can be produced. So a liposomal drug delivery system may be ideal in the case of silibinin. Silibinin produces hepatoprotective activity against tetrachloride-induced oxidative stress in albino rats in a dose-dependent manner; such bioassay can provide useful data in evaluating the efficiency of the introduced galactosylated liposomal formula in comparison to its solution.

Materials and methods

Chemicals

Silibinin (SY), egg phosphatidylcholine, cholesterol, phosphatidylethanolamine (PE), *p*-aminophenyl-β-D-galactopyranoside, Triton X-100, *Ricinus communis* lectin, and Sephadex G-50 were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Glutaraldehyde (Loba-Chemie, Mumbai, India), HEPES, and Hanks' balanced salt solution were purchased from Himedia Labs Ltd. (Mumbai, India). Aspartate aminotransferase diagnostic kit and alanine aminotransferase diagnostic kit were purchased from Bayer Diagnostics (Baroda, India). All other reagents were of analytical grade and were used as procured.

Methods

Preparation of liposomes

Liposomes (LP-SY) were prepared using the method reported by El-Samaligy et al.[29] with slight modifications. Briefly, the liposomal components were weighed and dissolved in 10 mL chloroform. The organic solvent was evaporated using a rotary evaporator (Steroglass, Perugia, Italy) to produce a thin film. The film was redissolved in 10 mL ether. Silibinin solution in 10 mL acetone together with phosphate-buffered saline (PBS, pH 7.4) was added. The organic solvents were evaporated using rotary evaporator. The liposomal suspension was kept overnight in the refrigerator. The resulting liposome suspension after sonication was extruded through 0.4- (five times) and 0.22-μm (10 times) polycarbonate membrane filters (Millipore, Bedford, MA, USA) using an extruder device to get liposomes of desired size range.

Galactosylation of liposomes

Galactosylated liposomes (GL-LP-SY) were prepared by covalent coupling of *p*-aminophenyl-β-D-galactopyranoside to PE liposomes[30] with some modification. LP-SY (1 mL) suspension in PBS (pH 7.4) was mixed with 20 mg *p*-aminophenyl-β-D-galactopyranoside (contained in 2 mL PBS, pH 7.4). Glutaraldehyde was added slowly to the liposomal suspension up to 10 mM final concentration and the mixture was incubated for 5 minutes at 25°C. The uncoupled glycoside and glutaraldehyde were removed by dialysis against the same buffer.

Shape and size determination

Transmission electron microscope (TEM) (Philips Morgagnii, Eindhoven, The Netherlands) was used to examine the ultrastructure of liposomes. To prepare samples, copper grids were coated with a solution of collodion and then a drop of liposomal dispersion was deposited and left for 15 minutes in contact. Finally, the grids were picked up, blotted with filter paper, left for drying for 3 minutes, and then observed under the TEM. The size of the liposomes was measured by an automated photocorrelation spectroscopy with Zetasizer Nano ZS 90 (Malvern Instruments, Worcestershire, UK).

Determination of silibinin entrapment efficiency in the prepared liposomes

Entrapment efficiency was determined after separation of unentrapped drug by Sephadex G-50 minicolumn using centrifugation technique[31,32]. The amount of drug entrapped in the vesicles was then determined by disrupting the vesicles using 0.2% Triton X-100, it was then filtered and the drug content was determined spectrophotometrically (GBC Cintra 10 UV/Vis spectrophotometer; GBC Scientific Equipments, Victoria, Australia) at 326 nm[33].

Percent ligand attachment

The presence of galactose residues on the surface of liposomes was detected qualitatively by agglutination of the vesicles with *R. communis* lectin. The coupling of the glycoside on liposomes was monitored quantitatively by determination of the free amino groups present on the liposomes before and after coupling of the ligand. This was determined by titrating amino groups of liposomal PE with trinitrobenzene sulfonic acid in the presence of 0.2% Triton X-100[34].

In vitro drug release studies

The drug release from liposomes was studied by dialysis cell membrane method[29]. Two milliliters of each LP-SY and GL-LP-SY was taken in dialysis tube (cellulose dialysis membrane, 2.4 nm porosity; Himedia Labs Ltd., Mumbai, India) and placed in a receiving compartment containing 100 mL PBS (pH 7.4), which was continuously stirred using magnetic stirrer at 37 ± 1°C. After appropriate time interval (1 hour), 1 mL of sample was withdrawn and analyzed for drug content. Equal volume of fresh media was added to replace the withdrawn sample. Silibinin release was measured at 326 nm using UV spectrophotometer (GBC Cintra 10 UV/Vis spectrophotometer). Every experiment was performed in triplicate.

In vivo study

Chromatography

C18 reverse phase analytical column (3 mm, 4.6 × 250 mm) (ESA, Bedford, MA, USA) was employed in all the high-performance liquid chromatography (HPLC) analyses. The linear gradient system was employed at room temperature (Table 1). The solvent flow rate throughout the run was 0.6 mL/min and the column effluent was monitored by UV absorbance at 270 nm[35].

Plasma and tissue distribution study

The studies were performed on albino rats of either sex weighing between 115 and 130 g. The animals were housed in clean cages and maintained in controlled temperature (23 ± 2°C) and light cycle (12 hours light and 12 hours dark). They were fed with standard diet and water. Rats were divided into four groups with 18 rats in each group. They were fasted overnight before administration of dose. To the first group, plain drug solution (50 mg/kg) was administered. LP-SY and GL-LP-SY containing the drug in equivalent dose were administered intravenously to each rat of the second and third group, respectively. Fourth group served as control. After 30 minutes, 1, 2, 4, 6, and 24 hours three animals from each group were picked. Blood samples were collected from the retro orbital plexus of the rat's eye and serum was separated by centrifuging at 290 × *g* for 3 minutes. Diethyl ether (5.5 mL) was added to 1 mL of plasma and shaken for 10 minutes. After centrifugation at 290 × *g*, the organic phase was collected and evaporated

Table 1. Protocol for linear gradient system employed for HPLC.

Time (minutes)	Solution system
0–5	75% A and 25% B
5–15	75% A and 25% B to 50% of both A and B
15–20	50% of A and B to 30% A and 70% B
20–25	Isocratic 30% A and 70% B
25	End of run

A, 7.5% methanol in 100 mM acetate buffer containing 50 mM triethylamine (TEA) and 1 mM 1-octanesulfonic acid (OSA) (pH 4.8). B, 80% methanol in 100 mM acetate buffer containing 50 mM TEA and 1 mM 1-OSA (pH 4.8).

under a stream of nitrogen. The residue was redissolved in methanol, vortexed for 3 minutes, centrifuged, and supernatant was used for HPLC analysis. After collection of blood samples the animals were killed and different organs (liver, spleen, lungs, and kidney) were excised, isolated, washed with distilled water, and were blot dried. For tissue distribution studies, each tissue sample (lung, liver, kidney, and spleen) obtained from different time intervals was assessed for silibinin. Briefly tissues (50–150 mg) were suspended in 50 mM Tris–HCl (pH 7.4); the volume being three times the volume of the tissues taken and homogenized thoroughly at room temperature using a homogenizer (York, Mumbai, India). To the homogenate was added equal volume of butanol:methanol (95:5 v/v) and mixed with the homogenizer for 5 seconds. The samples were centrifuged at 160 × *g* at 4°C for 5 minutes and the organic layer was collected. The butanol:methanol extraction was repeated twice more and the three butanol extracts were combined and stored at −80°C until HPLC analysis.

Intrahepatic distribution

At predetermined time intervals after plain drug, LP-SY and GL-LP-SY administration, the liver was perfused with Ca^{2+}, Mg^{2+}-free perfusion buffer, 10 mM *N*-2-hydroxyethyl piperazine-*N*-2-ethanesulfonic acid (HEPES), 137 mM NaCl, 5 mM KCL, 0.5 mM NaH_2PO_4, and 0.4 mM Na_2HPO_4, (pH 7.2) for 10 minutes. Then, the liver was perfused with perfusion buffer supplemented with 5 mM $CaCl_2$ and 0.05% (w/v) collagenase (type I) (pH 7.5) for 10 minutes. As soon as the perfusion started, the vena cava and aorta were cut and the perfusion rate was maintained at 3–4 mL/min. Following the discontinuation of perfusion, the liver was excised and its capsular membranes were removed. The cells were dispersed in ice-cold Hanks–Hepes buffer containing 0.1% bovine serum albumin by gentle stirring. The dispersed cells were filtered through cotton mesh sieves, followed by centrifugation at 50 × *g* for 1 minute. The pellets containing liver PCs were washed twice with Hanks–Hepes buffer by centrifuging at 50 × *g* for 1 minute. The supernatant so obtained had nonparenchymal cells (NPC). PCs and NPCs were separately resuspended in ice-cold Hanks–Hepes buffer. Cell numbers and viability were determined by the Trypan Blue exclusion method[36]. PCs and NPCs were processed in a manner similar to that of tissue as described in previous paragraph and drug content was determined by HPLC analysis.

Hepatoprotective activity testing (enzyme assay and histopathological examination)

The method described by Saraf and Dixit[37] was employed with some modification (Table 2). Animals were divided into five groups, each group having three rats. On fifth day, blood samples were collected from the retro orbital plexus of the rat's eye and serum was separated by centrifuging at 290 × *g* for 3 minutes. Collected serum was biochemically tested for transaminase levels of both types: serum glutamic oxalacetate transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT). The animals were then killed. The liver was immediately removed and fixed in 10% formalin and sectioned serially. The sections were microscopically examined using Nikon ECLIPSE E200 (Nikon Instech Co., Kawasaki, Japan) after staining with hematoxylin and eosin to analyze any pathological changes.

Statistical Analysis

Statistical analysis was performed on the data obtained in the in vitro and in vivo studies by one-way analysis of variance with Tukey–Kramer multiple comparisons post-test using GraphPad InStatTM software (GraphPad Software Inc., San Diego, CA, USA). Throughout, the level of significance was chosen as less than 0.05 (i.e., $p < 0.05$).

Results and Discussion

Preparation of liposomes

The prepared LP-SY formed of PC:PE:Chol at 7:1:2 molar ratio showed promising drug encapsulation

Table 2. Protocol for hepatoprotectivity activity testing.

Group	Day 1	Day 2	Day 3	Day 4
I (control)	DW	DW	DW	DW
II (toxin)	DW	CCl_4 + DW	CCl_4 + DW	DW
III	SY-Sol^n	CCl_4 + SY-Sol^n	CCl_4 + SY-Sol^n	SY-Sol^n
IV	LP-SY	CCl_4 + LP-SY	CCl_4 + LP-SY	LP-SY
V	GL-LP-SY	CCl_4 + GL-LP-SY	CCl_4 + GL-LP-SY	GL-LP-SY

CCl_4, 50% CCl_4 in liquid paraffin, 2 mL/kg body weight.

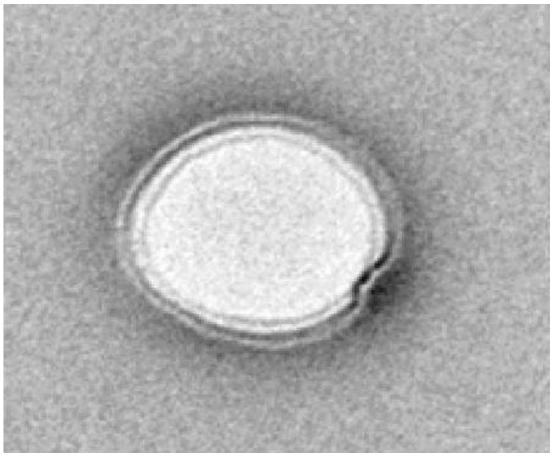

Figure 1. TEM of galactosylated liposome (×50,000) (bar 50 nm).

efficiency of 60.25 ± 2.23%. The interaction between the drug and the lipid membrane in terms of complexation represents one of the possible encapsulation mechanisms of the drug into the liposomal vesicles[38]. Complex formation between the drug and the phospholipids was investigated, and it was found that a complex is formed between phospholipids and flavonoids[39]. Similar complexes were also investigated by Moribe et al.[38] and known to have higher bioavailability[40]. The vesicle size distribution of both of the formulations was found to be below 200 nm (LP-SY 156.75 ± 15.38 nm and GL-LP-SY 173.29 ± 23.86 nm). There was a slight increase in size of the liposomal formulation after the attachment of the ligand. TEM of the formulation suggested that the vesicles were spherical in shape and unilamellar in nature (Figure 1).

Galactosylation of liposomes

The results of the agglutination of liposomes induced by *Ricinus communis* lectin suggested that surface ligand anchoring and the process used did not affect the ligand affinity and avidity toward their recognition motifs. The titration of liposomal PE groups with trinitrobenzene sulfonic acid revealed that about 58.54 ± 4.28% of the total amino groups were modified through covalent coupling of *p*-aminophenyl-β-D-galactopyranoside (data not shown).

Drug release studies

Drug release studies were carried out for both LP-SY and GL-LP-SY. Cumulative drug release percent was significantly higher with LP-SY as compared to that of GL-LP-SY. After 24 hours significantly low (30.9 ± 1.73%) cumulative drug release percent of GL-LP-SY indicated prolonged release carrier potential (Figure 2).

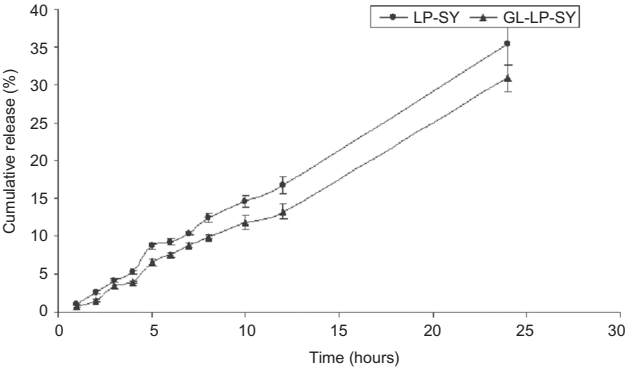

Figure 2. In vitro drug release of silibinin from uncoated and galactosylated liposomal formulation at 37°C in PBS, pH 7.4 (n = 3).

Hepatic drug uptake studies

The silibinin levels in the blood for free drug, LP-SY, and GL-LP-SY after 1 hour were found to be 58.13 ± 1.93, 28.90 ± 2.39, and 17.67 ± 1.53, respectively (Figure 3).

Nanocarriers show natural affinity toward liver and are passively targeted, and further inclusion of specific ligand (galactoside) significantly enhances the rate and extent of uptake by the PCs. So, liposomal formulations as compared to silibinin solution were rapidly cleared from the circulation. Estimation of drug accumulated in various organs (Figure 4) revealed that liposomal administration of drug significantly altered the pattern of biodistribution. Although free drug was also accumulated in liver, lung, spleen, and kidney to a significant extent, the rate, extent, and duration of accumulation were significantly high upon liposomal incorporation of drug. The accumulation of drug in all organs except liver, which showed moderate accumulation, was negligible after 24 hours.

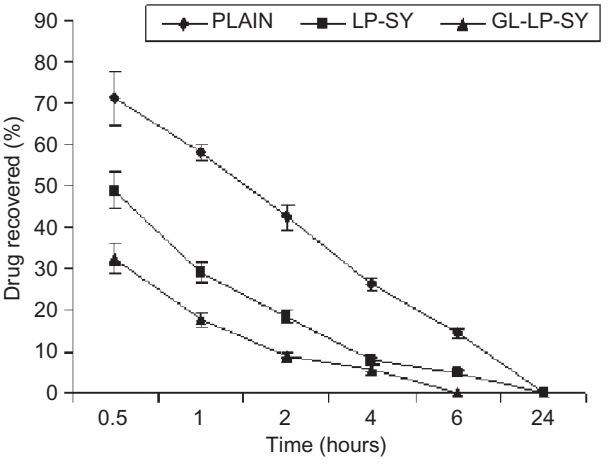

Figure 3. Plasma concentration profile of silibinin after tail vein intravenous administration of free silibinin, LP-SY, and GL-LP-SY. Each value represents means ± SD ($n = 3$).

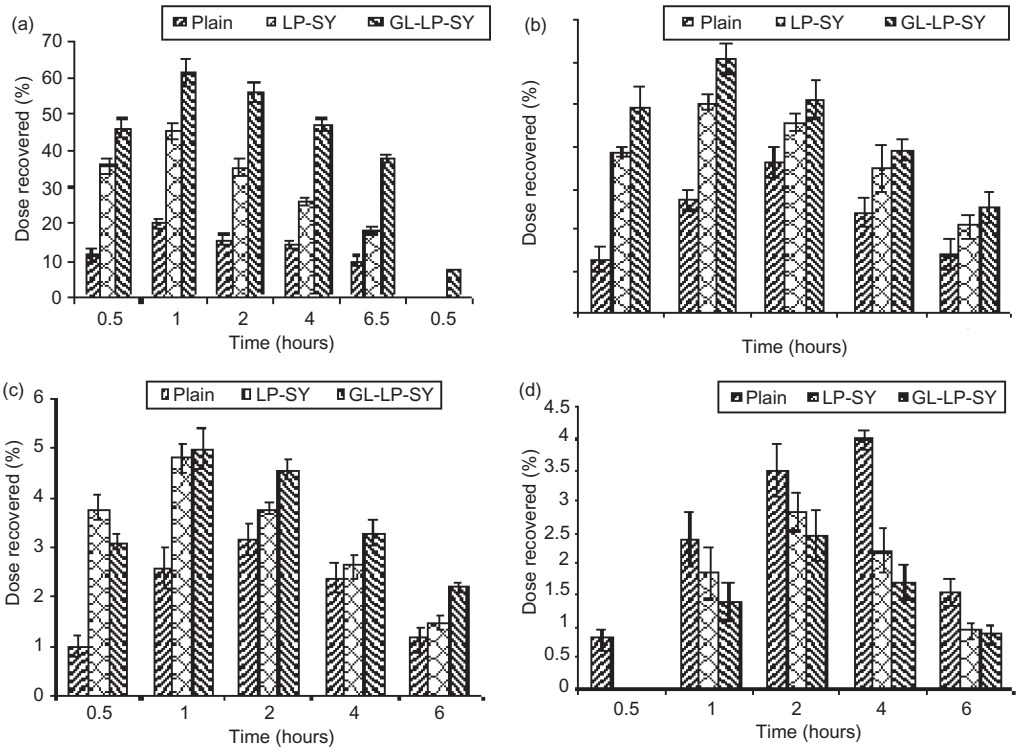

Figure 4. Tissue distribution of silibinin-loaded liposomal formulations in various tissues as a function of time after a single intravenous dose (50 mg/kg) to rats. (a) Liver, (b) spleen, (c) lung, and (d) kidney. Each value represents means ± SD ($n = 3$).

The amount of liposomes accumulated in liver was significantly higher than that of the free drug. Intrahepatic distribution showed that uptake of GL-LP-SY by PC was significantly higher than that by NPC (Figure 5). Fenestrations in the endothelial cell lining in the sinusoids are of the order of about 200 nm as mean diameter. The particles smaller than this size can readily pass through the sinusoidal endothelial cells and be taken up by the hepatocytes. Therefore, small unilamellar liposomes may easily access to hepatocytes directly but large multilamellar vesicles may not. This anatomical and physiological characteristic of the endothelial cells might account for size-dependent distribution of liposomes to the hepatocytes. Further the galactose-specific lectin (ASGPr) on the surface of hepatocytes could account for greater uptake of the galactosylated liposomes[41].

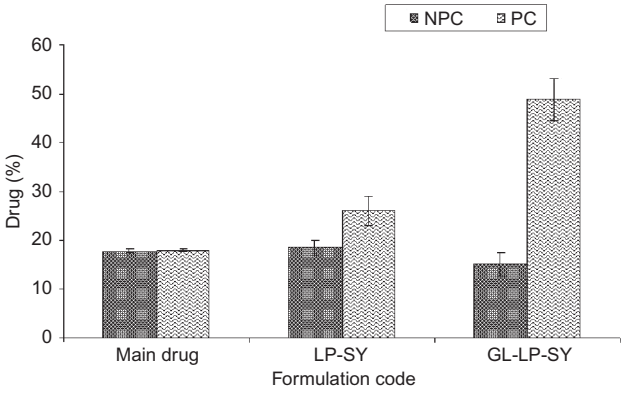

Figure 5. Hepatic cellular localization of silibinin from different formulations following intravenous administration in rats. Drug content was determined 60 minutes post-injection in parenchymal and nonparenchymal cells. Each value represents mean ± SD ($n = 3$).

Enzyme assay and histopathological examination

Estimation of the serum enzymes is a useful quantitative marker of the extent and type of hepatocellular damage. Alanine aminotransferase is a cytosolic enzyme primarily present in the liver. The levels of this enzyme in serum increase because of leakage of this cellular enzyme into plasma if there is any hepatic injury. SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle, and kidney. Increased serum level of SGOT is also associated with liver damage. Acute CCl_4 administration resulted in a significant increase in serum SGOT to 250.19 ± 12.08 U/L compared with normal value that was 104.94 ± 6.16 U/L (Figure 6). Administration of silibinin solution and LP-SY produced a significant decrease in SGOT levels to reach 210.48 ± 16.25 U/L and 157.02 ± 11.75 U/L, respectively. The SGOT level in the case of GL-LP-SY-treated group was found to be 117.04 ± 10.66 U/L, which

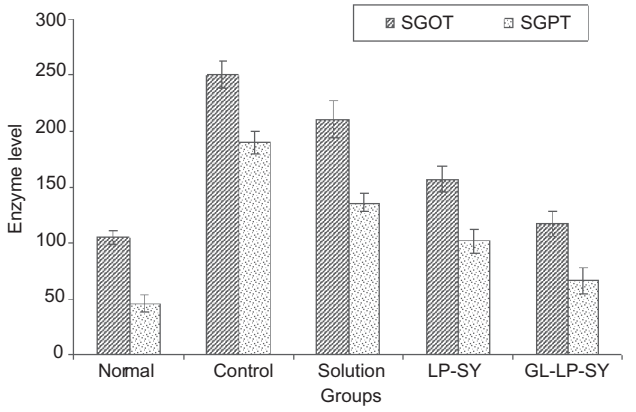

Figure 6. Serum enzyme levels of various groups. Each value represents means ± SD ($n = 3$).

is almost same as that of the normal group. At the same time, acute CCl_4 administration resulted in a significant increase in serum SGPT to 189.51 ± 10.22 U/L compared to normal value, which was analyzed as 45.65 ± 6.79 U/L. Silibinin solution produced nonsignificant change in serum SGPT to reach 135.91 ± 8.12 U/L. Significant decrease in SGPT levels upon administration of LP-SY and GL-LP-SY to reach 101.66 ± 10.81 U/L and 65.71 ± 11.39 U/L, respectively, was produced.

CCl_4 is known to cause hepatic damage with a marked elevation in blood SGPT and SGOT. GL-LP-SY delivery controlled the increase of these enzyme activities that were produced by the treatment of CCl_4. This strongly suggested the possibilities of silibinin targeting specifically to hepatic PCs and nonspecifically to other hepatic phagocytic cells.

Histopathological studies (Figure 7) showed CCl_4-induced massive necrosis, hepatic lesions, ballooning, and vacuolar degeneration of hepatocytes. Administration of silibinin solution also revealed the severe damage observed upon CCl_4 administration in which focal necrosis, ballooning, vacuolar degeneration as well as dilatation and congestion in portal vessels were still present. By microscopic examination, the massive area of necrosis and hepatic lesions induced by CCl_4 were remarkably reduced by administration of LP-SY. GL-LP-SY showed better responses, than LP-SY and silibinin solution, in the hepatocytes structure, necrotic areas, and ballooning damage by comparing Figure 7c–e. GL-LP-SY minimized the vacuolar degeneration and liver tissue restored its normal structure. These results were in good agreement with the results of the serum SGPT and SGOT levels in which IV administration of GL-LP-SY produced better protection against CCl_4-induced damage in comparison with silibinin solution.

Galactosylated liposome-encapsulated silibinin (GL-LP-SY) as compared to its free form (solution) showed increased protection, which could be explained by the observation that liposome-loaded compounds interact with cells at a much faster rate than that of free components[42]. Moreover, by utilizing the galactosyl receptor of hepatic PCs, galactosylated liposomes could be targeted to those nonphagocytic cell[19].

Conclusion

Silibinin is a very potent hepatoprotectant drug and its poor bioavailability is attributed to its poor eternal absorption, degradation by gastric fluid, or its poor water solubility. As a result, it needs to be incorporated in a dosage form that makes it more effective by delivering it to the site of action. In this study, as compared to

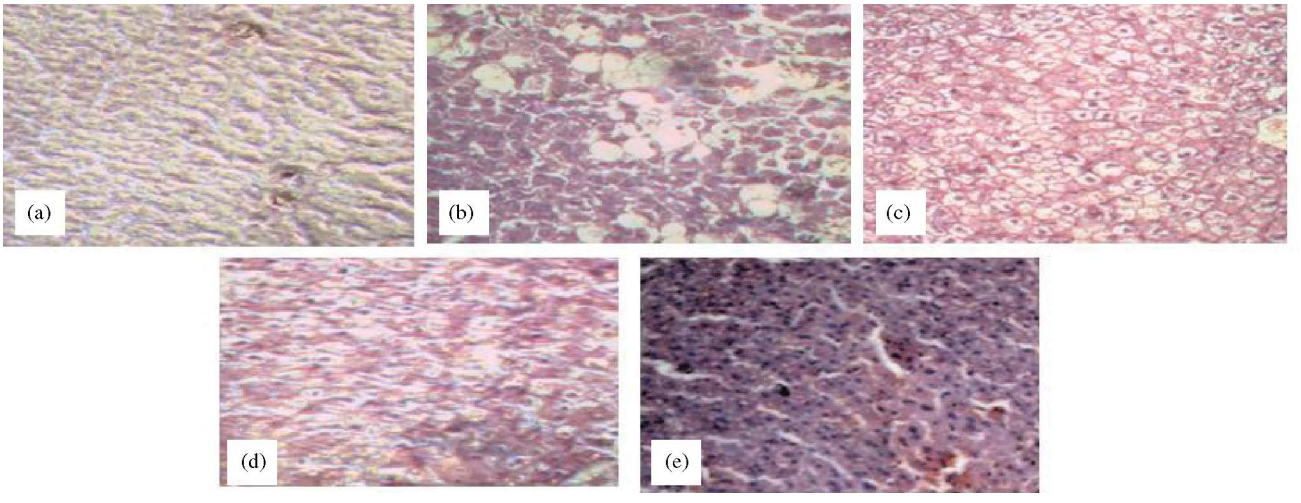

Figure 7. Photomicrographs (at ×100) of liver section showing structural integrity of hepatic architecture. (a) Control rat, (b) toxic control rat, (c) silibinin solution, (d) LP-SY, and (e) GL-LP-SY.

the previous work done, we made an attempt to deliver silibinin to its target site through bypassing the oral route.

Liposomes either uncoated or galactosylated have sustain release potential. Slow release in case of GL-LP-SY may be attributed to the presence of protective coat of galactose on liposomal surface, which interferes with the drug release. This release potential may be beneficial in case the drug has to be delivered to the specific cells.

The extent of drug uptake from liposomal formulations is an important determinant of the dosage of the formulation. The dose of liposomal formulation can be decreased, as there is greater uptake of vesicular carrier by cells. The release of liposome-encapsulated drug into tissues is due to liposome cell interaction followed by endocytosis[43]. A large number of ligands are known to bind with one of the components of liposomes containing a variety of drugs, allowing the therapy to be tailored to suit the needs of individual patients[44–46].

The results of our investigation indicate that the damage in rat liver caused by CCl_4 intoxication was prevented by the treatment with GL-LP-SY. This observation is in correlation with the biochemical and histopathological examinations of CCl_4-intoxicated rat liver in which most of the hepatocytes that suffered degeneration were protected completely by the treatment with silibinin in galactose-grafted liposomes.

We hypothesize that silibinin in galactosylated liposomes may be more protective than free silibinin or liposomal silibinin because of the enhanced intracellular accumulation of silibinin by selective tissue-targeted delivery. This approach of delivering a nontoxic herb origin antioxidant silibinin selectively to the liver offers a variety of clinical applications in human hepatic diseases.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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