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Original research article

Silymarin liposomes improves oral bioavailability of silybin besides targeting hepatocytes, and immune cells

Nitesh Kumar [a], Amita Rai [b], Neetinkumar D. Reddy [a], P. Vasanth Raj [a,1], Prateek Jain [a], Praful Deshpande [a], Geetha Mathew [a], N. Gopalan Kutty [a], Nayanabhirama Udupa [a], C. Mallikarjuna Rao [a,*]

[a] Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka 576104, India
[b] Manipal Acunova, Manipal, Karnataka 576104, India

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ABSTRACT

Background: Silymarin, a hepatoprotective agent, has poor oral bioavailability. However, the current dosage form of the drug does not target the liver and inflammatory cells selectively. The aim of the present study was to develop lecithin-based carrier system of silymarin by incorporating phytosomal–liposomal approach to increase its oral bioavailability and to make it target-specific to the liver for enhanced hepatoprotection.
Methods: The formulation was prepared by film hydration method. Release of drug was assessed at pH 1.2 and 7.4. Formulation was assessed for *in vitro* hepatoprotection on Chang liver cells, lipopolysaccharide-induced reactive oxygen species (ROS) production by RAW 267.4 (murine macrophages), *in vivo* efficacy against paracetamol-induced hepatotoxicity and pharmacokinetic study by oral route in Wistar rat.
Results: The formulation showed maximum entrapment (55%) for a lecithin–cholesterol ratio of 6:1. Comparative release profile of formulation was better than silymarin at pH 1.2 and pH 7.4. *In vitro* studies showed a better hepatoprotection efficacy for formulation (one and half times) and better prevention of ROS production (ten times) compared to silymarin. In *in vivo* model, paracetamol showed significant hepatotoxicity in Wistar rats assessed through LFT, antioxidant markers and inflammatory markers. The formulation was found more efficacious than silymarin suspension in protecting the liver against paracetamol toxicity and the associated inflammatory conditions. The liposomal formulation yielded a three and half fold higher bioavailability of silymarin as compared with silymarin suspension.
Conclusions: Incorporating the phytosomal form of silymarin in liposomal carrier system increased the oral bioavailability and showed better hepatoprotection and better anti-inflammatory effects compared with silymarin suspension.

Introduction

Silymarin, a hepatoprotective agent, obtained from single herb *Silybum marianum*, is widely used in the treatment of liver diseases. A mixture of flavolignan isomers, namely silybin, isosilybin, silydianin, silychristin is collectively expressed as silymarin [1]. Among these isomers, the most active component is silybin, which accounts for 60–70% of the total content of silymarin and is considered as the marker of silymarin [2].

Many experimental studies have proved the hepatoprotective activity of silymarin [3]. One of the major limitations of silymarin is poor oral-bioavailability. The oral absorption of silymarin is only about 23–47% [4], leading oral bioavailability to 0.73% [5]. Therefore, a higher dose of silymarin is required to improve therapeutic efficacy. The reasons suggested for its poor bioavailability includes the following: poor enteral absorption [6], instability in gastric environment [4] and poor solubility [4]. Thus, enhancement of bioavailability of silymarin is a challenging task. Although substantial advancement has been made in improving

* Corresponding author.
E-mail addresses: mallik.rao@manipal.edu, mallikin123@gmail.com (C.M. Rao).
[1] Present address: Faculty of Pharmacy, AIMST University, Malaysia.

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the bioavailability of silymarin through various dosage forms, little information is available on the measures adopted by researchers to make silymarin target specific to promote hepatocytes' regeneration and to prevent inflammation in liver [7].

The process of repair in the liver is largely by the regeneration of hepatocytes. However, inflammation in liver is one of the major problems associated with hepatocyte toxicity. If inflammation is not controlled sufficiently, the cellular phase of inflammation through macrophages (Kupffer cells) and fibroblast (stellate cells) promote fibrosis to replace dead cells [8]. Therefore, a formulation of silymarin that would target the liver in general and inflammation in particular would be beneficial over a formulation of silymarin that would just enhance the bioavailability of silymarin. In this context, the present study is aimed at developing a formulation of silymarin with the help of liposomal and phytosomal combination. This is based on the fact that phytosomal silymarin is more stable in the gastric environment [9] to enhance the bioavailability of silymarin while the liposomal silymarin is having the highest ability to get captured by macrophages, Kupffer cells and infiltrated WBC *viz.*, neutrophil, monocytes, *etc.* through phagocytosis process and modulate their actions [10]. This phenomenon makes silymarin in formulation to target inflammation.

Materials and methods

Materials

Triton-X 100, trypsin and dithiothreitol (DTT) were purchased from Himedia lab Pvt. Ltd. (Mumbai, India). Lecithin (Soya L-α-Phosphatidylcholine or SPC), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Dulbecco's modified eagle's medium (DMEM), minimum essential medium (MEM), fetal bovine serum (FBS) and silymarin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in the study were of analytical grade.

Cell lines

Chang liver cells and RAW 264.7 were purchased from National Center for Cell Sciences (Pune, India).

Animals

Wistar rats of 4–6 weeks age, weighing 180–200 g were selected for study. The animals were acclimatized to for one week in controlled temperature and humidity conditions with 12:12 h light and dark cycle. The rats were fed standard food pellets and water *ad libitum*. The study was conducted after obtaining clearance from the Institutional Animal Ethics Committee of KMC.

Table 1
Optimization of lipid and drug ratio.

Formulation	SPC:cholesterol: drug (mg)	Molar ratio (SPC:C)	% Entrapment efficiency
L1	294:00:10	10:00	36.11
L2	255:15:10	09:01	32.84
L3	240:15:10	08:01	18.69
L4	180:15:10	06:01	47.22
L5	240:30:10	04:01	32.22
L6	180:30:10	03:01	35.53
L7	180:77:10	1.5:1	11.06

SPC:cholesterol:drug ratio without freeze-drying at sonication of frequency – 80 Hz, time – 2 min and pulse – 4 s.

Preparation of liposomes

Preformulation studies

Silymarin was evaluated for its physicochemical interaction with lecithin (SPC) and cholesterol (C) at a ratio of 1:1:1 using differential scanning calorimetry (DSC).

Development of silymarin-liposomes

Liposomes were prepared by lipid film hydration method [11]. Silymarin (S) 10 mg, different quantities of SPC and cholesterol were taken into a round bottom flask and dissolved in methanol-chloroform mixture (1:9) (Tables 1 and 2). Later the solvent was evaporated under vacuum at 40 °C in a rotary evaporator to develop thin film. The solvent traces from film was removed by drying overnight in a vacuum desiccator. The film was hydrated with phosphate buffer saline (PBS, pH 7.4), containing varied amount of cryoprotectant (mannitol and sucrose) (Table 2) at 100 RPM and at 50 °C for 1 h to prepare a liposomal suspension. The liposome vesicle size was reduced under high-pressure homogenization at 20,000 psi for 5 cycles. The liposomes were kept overnight in deep freezer at −80 °C. The frozen liposomes were lyophilized at reduced pressure and stored at 4 °C in airtight containers for further experiments.

Physicochemical characteristics of liposomes

Particle size and zeta potential

Mean particle size, polydispersity index (PDI) and zeta potential of liposomes were determined by Malvern NanoZS (Malvern Instruments Ltd., Worcestershire, UK) after suitable re-dispersion in water.

Drug entrapment efficiency in liposomes [12]

Liposomal suspension (1 ml) was centrifuged at 1000 rpm for 10 min to separate unentrapped particles. Supernatant was collected and again centrifuged at 64,000 × *g* at 4–8 °C for

Table 2
Q6 Optimization of various liposomal parameters for formulation L4.

Parameters		Preformulation of liposome (SPC:C 6:1)						Optimized
Freeze drying		NO	Yes	Yes	Yes	Yes	Yes	Yes
Cryoprotectant[a]		Nil	Nil	Mannitol 10%	Sucrose 10%	Mannitol 15%	Sucrose 15%	Sucrose 5%
Yield		98%	98%	97%	95%	68.75%	75.63%	98.5%
Particle size (nm)		100	701	1005	400	1205	405	329
Zeta potential		>−70 mV	>−70 mV	>−70 mV	>−70 mV	>−70 mV	>−70 mV	−70.5 mV
HPH[b] (Cycle, bars)		NA	10, 15,000	10, 15,000	10, 15,000	15, 20,000	10, 20,000	10, 20,000
% Entrapment	Sil A&B*	47.22	53.39	41.28	53.27	52.40	47.29	58.94
	Sil A[c]	NA	40.89	39.16	50.50	63.55	39.63	59.24
	Sil B[d]	NA	65.90	43.41	56.03	41.25	54.95	58.64

[a] Amount in w/v.
[b] High pressure homogenization.
[c] Silybin A.
[d] Silybin B.

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