Functionalized nanocolloidal constructs for targeted delivery of antioxidants

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INTRODUCTION AND OBJECTIVES

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. 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.

The physiologic and biologic features of the liver and hepatocytes give galactosylated carriers the opportunity to deliver drugs to hepatocytes via asialoglycoprotein receptor-mediated endocytosis. Delivery of drugs using liposomes bound to asialoglycoprotein receptors in a specific manner would provide significant therapeutic benefits in hepatic disease with a homogenous intrahepatic membrane distribution of its intercalated components. Silibinin has been reported to possess many pharmacological activities (Morazzoni *et al* 1995) such as anti-inflammatory, anti-tumor and anti-fibrotic effects, and to positively influence some risk factors of atherosclerosis. However, during *in vivo* studies these effects are restrained by its very low bioavailability (Morazzoni *et al* 1995 and Basaga *et al* 1997). 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.

MATERIALS AND METHODS

Silibinin(SY), Egg phosphatidylcholine (PC), cholesterol (CH), phosphatidylethanolamine (PE), p-aminophenyl-β-D-galactopyranoside and *Ricinus communis* lectin were purchased from Sigma Chemicals Co. (USA). 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, India. All other reagents were of analytical grade and were used as procured.

Liposomes (LP-SY) were prepared using the method reported by El-Samaligy *et al* (2006) with slight modification. Galactosylation of liposomes (GL-LP-SY) was done according to the method reported by Mandal and Das (2005). TEM was used to examine the ultrastructure of liposomes. The size of the liposomes was measured by an automated photocorrelation spectroscopy with Zetasizer Nano ZS 90. Percent entrapment efficiency was determined after separation of unentrapped drug (Fry et al, 1978). The presence of galactose residues on the surface of liposomes was detected qualitatively by agglutination of the vesicles with *Ricinus communis* lectin. The drug release from liposomes was studied by dialysis cell membrane method (El-Samaligy *et al.,* 2006). Biodistribution, fluorescence microscopy and intrahepatic distribution studies were carried out. Various formulations were evaluated regarding silibinin's hepatoprotective activity against CCl_4-induced oxidative stress in albino rats.

RESULTS AND DISCUSSION

The prepared LP-SY formed of PC:PE:Chol at 7:1:2 molar ratio showed promising drug encapsulation efficiency of 60.25±2.23%. The vesicle size distribution of both of the formulations was found to be below 200 nm. TEM of the formulation suggested that the vesicles were spherical in shape and unilamellar in nature (see Figure 1).

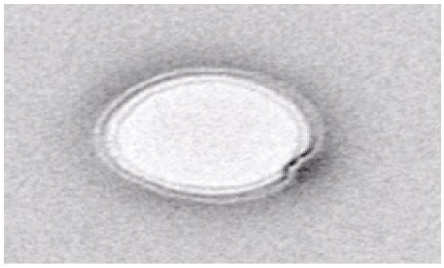

Figure 1 TEM of Galactosylated Liposome (50,000X)

Aggregation of galactosylated liposomes by *Ricinus communis* revealed the presence of galatose residues on the surface of liposomes. Liposomes either uncoated or galactosylated showed sustain release potential. *In vivo* studies revealed that liposomal formulations exhibited extensive localization in liver and spleen. Separation of the liver cells showed that galactosylated liposomes were preferentially taken up by the hepatocytes (79% of the total hepatic uptake in 1 h) (see Figure 2).

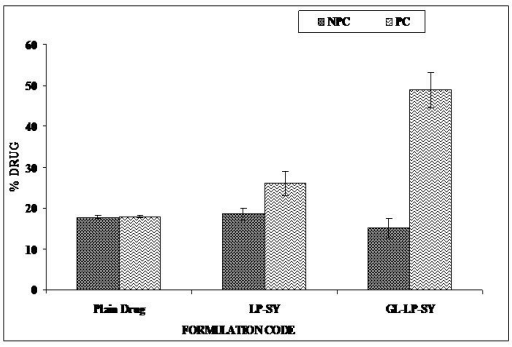

Figure 2 Hepatic cellular localization of Silibinin from different formulations following intravenous administration in rats. Drug content was determined 60 min post-injection in parenchymal and non-parenchymal cells. Each value represents mean ± SD (n = 3).

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 (SGOT) and serum glutamic pyruvate transaminase (SGPT). The introduced galactosylated silibinin produced a significant decrease in both transaminase levels when challenged with CCl_4 intraperitonially.

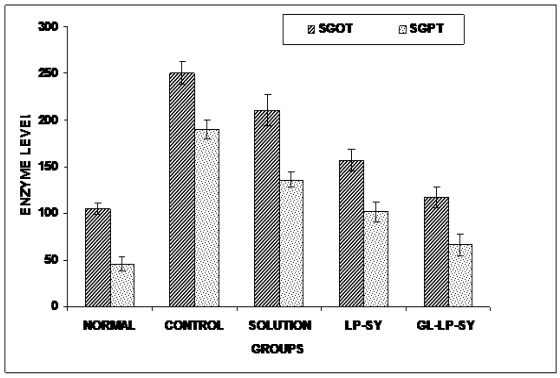

Figure 3 Serum enzyme levels of various groups. Each value represents means ± SD (n = 3).

CONCLUSION

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

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

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ACKNOWLEDGEMENTS

Author Devyani Dube acknowledges University Grant Commission (UGC), New Delhi, India for providing financial assistance.