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Original article

LIPOSOMAL DELIVERY OF CURCUMIN TO LIVER

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Abstract

Curcumin is a major constituent of turmeric, a plant product used extensively in Ayurveda. Curcumin has poor bioavailability because of extensive systemic metabolism and poor oral absorption. In order to improve the bioavailability of Curcumin we developed small unilamellar vesicules (SUVs) encapsulating the active by thin film hydration followed by probe sonication method. SUV liposomal formulations selected can also lead to highest concentration in the liver cells because of phagocytosis and endocytosis of this formulation in these cells. Four formulations (CL1, CL2, CL3, CL4) were prepared by taking different drug to lipid ratio. The prepared formulations were evaluated for particles size, surface potential, entrapment efficiency, in vitro release, drug excipient compatibility. Particle size of all formulations was in the range of 110-240 nm and the entrapment efficiencies were in the range of 44-70 %. From the four formulations CL3 was selected as best formulation by considering the size, entrapment efficiency and release profiles. Pharmacokinetic parameters of the CL3 formulations were evaluated in rat. CL3 formulation was evaluated for hepatoprotective activity in CCl_4 induced liver toxicity model and drug levels in different tissues were determined. Formulation CL3 showed better in vivo performance compared to curcumin iv and oral solutions. Tissue levels especially in liver were more with formulation CL3 was more compared to curcumin iv and oral solutions.

Key words: *Curcumin, Small unilamellar vesicles, Hepatoprotective, Liver targeting*

Kurkumin'in Lipozom İçinde Karaciğere Taşınması

Kurkumin, Ayurveda'da yaygın olarak kullanılan bitkisel ürün "turmerik" in en önemli bileşenidir. Kurkumin'in sistemik metabolizmasının yüksek ve oral absorbsiyonunun zayıf olması nedeniyle düşük bir biyoyararlanıma sahiptir. Kurkumin'in biyoyararlınımını arttırmak amacıyla, etkin maddenin ince film hidrasyonu ve ardından prob sonikasyonu yöntemiyle enkapsüle edildiği küçük unilamelar veziküller (SUVs) geliştirilmiştir. Seçilen SUV liposom formülasyonları karaciğer hücrelerindeki fagositoz ve endositoz nedeniyle, en yüksek kurkumin konsantrasyonunu bu hücrelere taşıyabilmektedirler. Farklı ilaç lipit oranları alınarak 4 formulasyon (CL1, CL2, CL3, CL4) hazırlanmıştır. Hazırlanan formülasyonlar partikül büyüklüğü, yüzey potansiyeli, hapsedilme etkinliği, in vitro salım, ilaç eksipiyan geçimsizliği yönünden değerlendirilmiştir. Partikül büyüklüğü tüm formülasyonlarda 110 – 240 nm arasında, hapsedilme etkinliği ise % 44 – 70 arasında bulunmuştur. Partikül büyüklüğü, hapsedilme etkinliği ve salım profilleri dikkate alınarak dört formülasyon arasında CL3 en uygun formülasyon olarak seçilmiştir. CL3 formülasyonunun farmakokinetik parametreleri ratlarda incelenmiştir. CL3 formülasyonunun hepatoprotektif etkinliği CCl_4 ile indüklenmiş karaciğer toksisite modelinde incelenmiş ve farklı dokulardaki ilaç düzeyleri tayin edilmiştir. Kurkumin'in IV ve oral çözeltileri ile karşılaştırıldığında CL3 formülasyonu daha iyi bir in vivo performans göstermiştir. Kurkumin'in özellikle karaciğer olmak üzere doku düzeyleri, IV ve oral çözeltilerine kıyasla daha yüksek bulunmuştur.

Anahtar kelimeler: *Kurkumin, Küçük unilamelar vesiküller, Karaciğer koruyucu, Karaciğere hedeflenen*

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INTRODUCTION

Curcumin, an ayurvedic natural product has several therapeutic applications [1]. Despite curcumin's multiple medicinal benefits, low oral absorption of curcumin and its high systemic metabolism is suggested to be a big challenge in its application as a clinical agent. Lower serum and tissue levels of curcumin are observed irrespective of the route of administration due to extensive intestinal and hepatic metabolism and rapid elimination thus restraining curcumin's bioavailability [2, 3, 4]. Several formulation strategies previously addressed the issue of improving its bioavailability [5, 6, 7]. In this study, we addressed this issue by taking the help of liposomal delivery system which not only is useful in enhancing its systemic bioavailability by protecting the drug from the plasma enzymes but also could lead to higher intracellular concentration in the liver. This dual strategy is better helpful in several liver disorders. The diseases that this formulation could target include liver fibrosis and cirrhosis. In both these disorders, it is the abnormal oxidative stress and the inflammation which are the culprits [8, 9, 10]. Curcumin is both an antioxidant as well as antiinflammatory. Non-parenchymal cells such as Kupffer cells, fat storing stellate cells, sinusoidal endothelial cells and leukocytes (i.e., neutrophils and monocytes) have a significant role in the mechanism [11]. These cells are the target for the liposomal curcumin formulation. Interestingly, Kupffer cells in the liver are exposed to systemic circulation and are phagocytic in nature [12]. Sinusoidal endothelial cells also exposed to systemic circulation can actively take up particles by endocytosis [13]. In this stop different liposomal formulations encapsulating curcumin and that can be injected via the intravenous route were developed. Formulations were evaluated for the pharmacokinetic and tissue distribution studies. The hepatoprotectivity of the formulation was evaluated in CCl_4 induced liver toxicity model in rats.

EXPERIMENTAL

Materials

Curcumin was obtained from Yucca Enterprises, Mumbai, India. Soya lecithin was procured from Hi Media Laboratories Pvt Ltd, Mumbai, India. Cholesterol was procured from Molychem, Mumbai. Chloroform and Methanol were procured from Finar chemicals, Ahmedabad, India. Male Wistar rats (150-200gm each) purchased from Mahaveer Enterprises (Hyderabad, India) were used in this preclinical investigation. SGOT and SGPT kits were purchased from Coral Clinical Systems, Goa, India.

Preparation of curcumin liposomes

Multilamellar vesicles (MLVs) containing curcumin were prepared according to the thin film hydration method of Bangham et al. [14]. In this method the curcumin (25mg), soya lecithin (90mg, 100 mg, 110mg, 120mg) and cholesterol (20mg) were dissolved in chloroform (10ml) and the solution was dried in a rotary evaporator (Laborota 4000, Heidolph, Germany) under reduced pressure at 60^0C and 90 rpm to form a thin film of lipid. Then the dried lipid was hydrated with 10ml of normal saline solution and vortexed for one hour at 72^0 C (above the gel – liquid transition temperature of the soya lecithin) and 90 rpm to get dispersion of multilamellar liposomal vesicles. The liposomal suspension was then centrifuged at 5000 rpm for 30 min to separate the free drug and then the pellet of MLVs was resuspended in normal saline. The resulting suspension was subjected for sonication for 15min (3min each cycle, 5cycles, 150V/T probe) using a ultra-homogenizer (Biologics inc., USA) to get small unilamellar vesicles (SUVs).

Characterization of liposomes

Determination of the particle size and the surface potential

The prepared liposomes were evaluated for their particle size, polydispersity index (PDI) of size distribution and surface charge potential, by photon correlation spectroscopy (PCS) using Zetasizer 3000 HAS (Malvern Instruments, Malvern, UK). The formulations were diluted 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was performed at 25°C with an angle of detection of 90°.Each sample was measured in triplicate.

Encapsulation efficiency

Separation of unentrapped drug from the prepared liposomes was carried out by mini column centrifugation method. Liposomal suspension (0.2ml) was placed in Sephadex G-50 column (pre-saturated with empty liposomes) and centrifuged at 2000rpm for 3min. Elutes containing drug loaded liposomes were collected and observed under optical microscope to ensure the absence of unentrapped drug particles. Appropriate amount of elute was digested with chloroform and the clear solution thus obtained was analyzed using UV Visible spectrophotometer for the drug content estimation at a λmax of 425nm. Liposomes prepared without drug were treated in similar manner and served as blank for the above study. Studies were conducted in triplicate.

$$\% \text{ Entrapment efficiency} = \frac{\text{Entraped drug (mg)}}{\text{Total Drug Added (mg)}} \times 100$$

In vitro drug release studies

In vitro release studies were performed using dialysis membrane method. An aliquot of SUV formulation was placed inside a dialysis membrane (Molecular Weight Cut Off: 25,000) immersed in aqueous buffer of volume 100 ml (PBS). At predetermined time points the dialysate was sampled and the amount of curcumin was determined using a UV-Vis spectrophotometer as mentioned in the previous section. Drug release was monitored until no more drug was released for 30 min.

Drug- polymer interactions

Drug – polymer interaction was investigated using FTIR. FTIR of drug, lipid, cholesterol, placebo liposomes and drug loaded liposomes were taken using a Thermo Nicolet Nexus 670 Spectrophotometer with KBr pellets.

Animal studies

In vivo drug release and hepatoprotective activity of the formulation

In vivo drug release was investigated in male wistar rats. All the animal experiments were conducted according to the rules and guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India. The study was approved by Institutional Animal Ethical Committee of Vaagdevi College of Pharmacy, Warangal, registered under CPCSEA, India (Registration No: 1047/ac/07/CPCSEA). The rats were acclimatised with 12 hour dark and 12 hour light cycle at a temperature of 20° C at a humidity of 60% and were fed on standard diet for 10 days prior to the commencement of the experiment. The conditions continued during the next 10 days of experimentation. Rats were divided into three groups each group contains three rats. Group 1 received 1 ml of curcumin solution (15mg) intravenously. Group 2 received 1ml of curcumin liposomes (equivalent to 15mg of

curcumin) by intravenously. Group 3 received 1ml of curcumin oral suspension (15mg). After administration of the formulations blood samples were collected at 0.5, 1, 2, 3, 6, 12, 24 hours and 3, 6 and 9 days and plasma was separated. The drug was extracted from the plasma by adding 500 µl of ethyl acetate. Curcumin in these samples were estimated using a HPLC (Cyberlab, USA) at 230 nm. In case of liver, kidney, colon and brain, the tissue was crushed and then the drug was extracted with acetonitrile. The mobile phase consisted of acetonitrile/methanol/water/acetic acid at a ratio of 40:23:36:1, v/v/v/v. The column used was C_{18} ODS column and the size of silica used in this column is 5µm and the dimensions of the column are 4.6 × 250mm. A HPLC standard curve for the drug in the plasma and the liver was generated.

Hepatoprotective activity

Carbon tetrachloride induced liver damaged model was used in the evaluation of hepatoprotectivity of prepared formulations. Male wistar rats (150-180g) were divided into 5 groups containing three rats each. Group 1 received normal saline (1 ml/rat) daily for 9 days. Group2 received carbon tetrachloride (0.7 ml/kg), administered intraperitoneally on the 3rd, 6th and 9th day consisting of mixture of CCl_4 and olive oil (25:75). Group 3 received 1ml of curcumin oral suspension (100 mg/kg) daily for 9 days. Group 4 received 1ml of curcumin solution (10mg/kg) daily for 9 days. Group 5 received 1ml of curcumin liposomes (100mg/kg) by intravenously at day one. All groups received CCl_4 at 3, 6 and 9th day of the study except normal control. Hepatoprotective activity was quantified by the activity of SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase) levels and histological studies following a previously published report [15].

Statistical analysis

Results are expressed as means±S.E.M. of three rats per treatment group. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett test. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Curcumin liposomes were successfully prepared by thin film hydration-sonication method. Four formulations were prepared by taking different drug to lipid ratios. Compositions of liposomes were described in Table 1.

Table1. Compositions of different curcumin liposomes

Formulation code	CL1	CL2	CL3	CL4
Curcumin (mg)	25	25	25	25
Soya Lecithin(mg)	90	100	110	120
Cholesterol (mg)	20	20	20	20

The results for particle size, zeta potential and encapsulation efficiency were shown in Table 2.

Table 2. Mean particle size, zeta potential, PDI and % encapsulation efficiency of curcumin liposomes.

Formulation code	Mean Particle size (nm)[a]	Zeta potential [a]	PDI [b]	%Encapsulation efficiency [a]
CL1	120.12±12.56	-24.23±13.65	0.587	46.89±2.34
CL2	165.85±8.45	-12.26±8.65	0.398	55.89±2.12
CL3	198.26±3.56	-10.2±14.2	0.156	66.51±1.28
CL4	235.56±4.69	-13.69±10.36	0.345	68.35±1.95

a: values indicates mean ± standard deviation, b: PDI: Polydispersity index.

Drug excipient interactions were studied by FTIR; from the spectra we observed that there is no interaction between the drug and excipients. The in vitro release studies from curcumin liposomes were performed and release profiles were fitted into various release kinetic models (Table 3).

Table 3. Release kinetic models of curcumin liposomes

Formulation code	Zero order	First order	Higuchi model	Korsemeyer peppas model	
	R^2	R^2	R^2	R^2	n
CL1	0.8086	0.8497	0.9454	0.9237	0.653
CL2	0.8783	0.9232	0.9750	0.9456	0.702
CL3	0.9514	0.9873	0.9919	0.9614	0.729
CL4	0.9735	0.9938	0.9892	0.9624	0.730

From release profiles it was observed that increase in lipid concentration results in decrease of drug release rate (Fig.1). From the all four formulations the drug release was up to 25% from the first 2 days. The percent cumulative drug release was observed 67.4, 70.5, 74.78 and 76.9 and *in vitro* drug release sustained up to 9, 10, 12 and 14 days for CL1, CL2, CL3 and CL4 respectively.

Drug levels in the plasma and the tissues were determined using HPLC. The retention time of the drug was 9.6 min. Plasma profile of the drug after liposomal, oral and *i.v.* solution form administration was plotted (Fig 2). From the figure it clearly indicates that liposomal formulation sustains the drug release up to 9 days reflecting in an increased area under curve. Peak serum concentration of 2.2µg/ml was observed within 30 min when curcumin solution was given intravenously. Peak serum concentration of 1.12µg/ml was observed within 6 hours when curcumin solution was given orally. Peak serum concentration of 2.4µg/ml was observed within 1 hour when curcumin liposomes (CL3) suspension was given intravenously. Table 4 shows the pharmacokinetic parameters obtained after administration of drug and formulation.

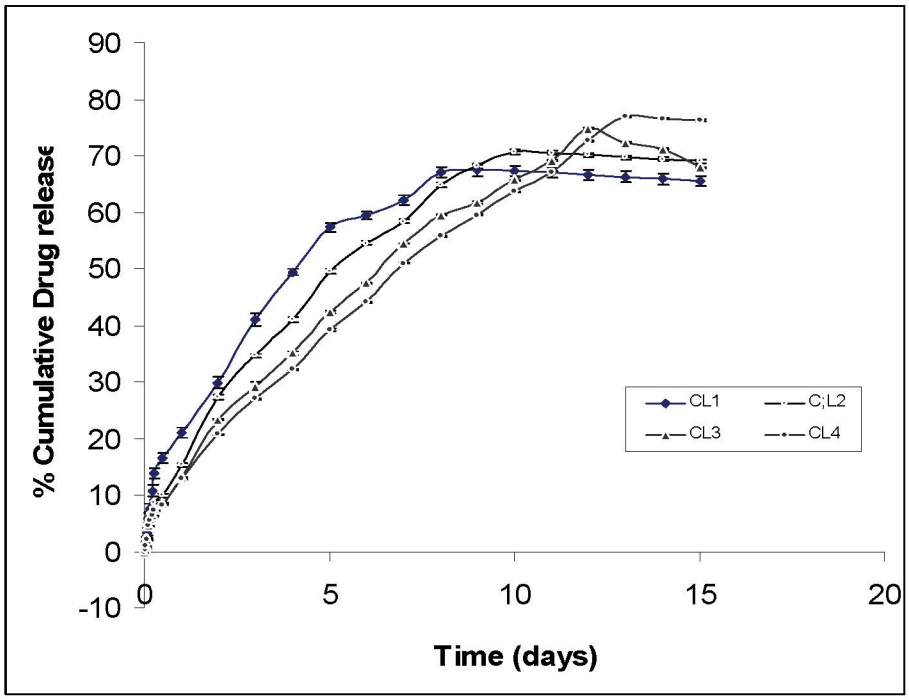

Figure 1: *In vitro* release profiles of curcumin liposomes (Mean ± S.D. n=3)

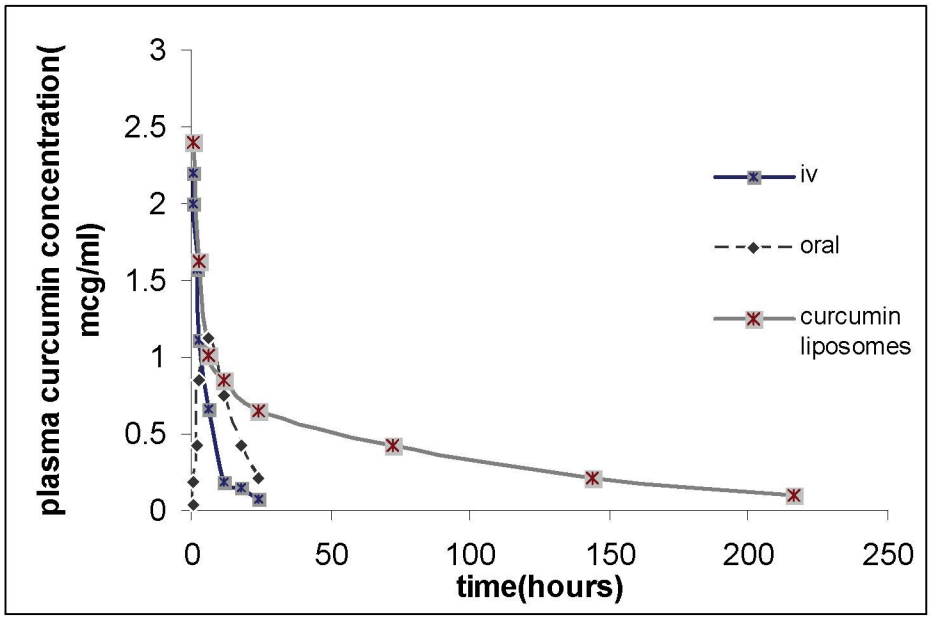

Figure 2: *In vivo* release profiles of curcumin formulations

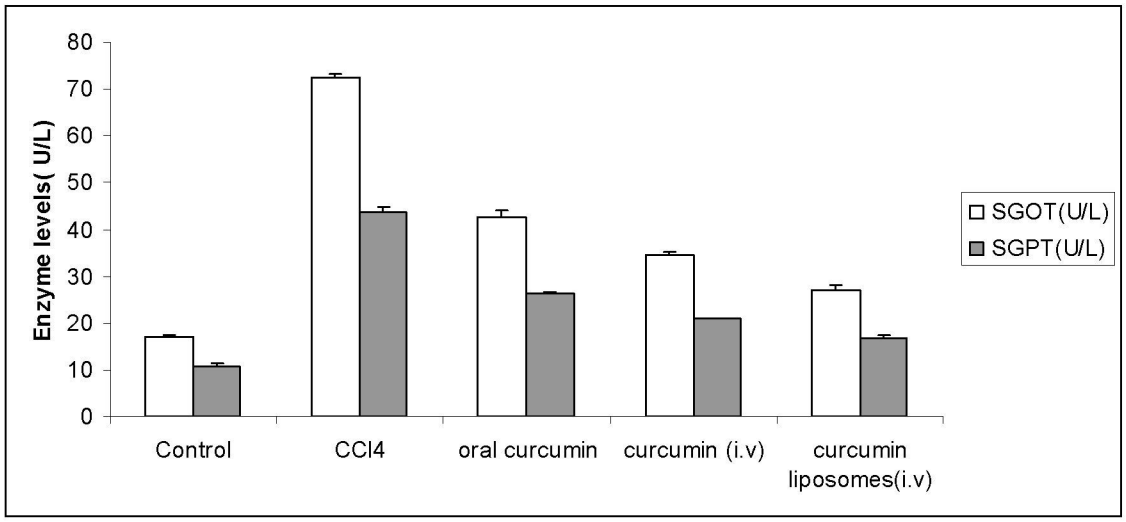

Figure 3: Effect of formulations on serum levels of enzymes in rats with CCl_4 induced liver toxicity

Table 4. Pharmacokinetic parameters obtained after administration of liposomal formulation and curcumin solutions

Parameter	Curcumin oral (15mg)	Curcumin iv bolus(15 mg)	Curcumin liposomes iv
Cmax(µg/ml)	1.12±0.056	2.2±0.024	2.4±0.041
Ke(per hour)	0.106±0.008	0.14±0.004	0.0101±0.005
$T_{1/2}$(hours)	6.53±0.45	4.69±0.3	68.43±1.06
Vd(Litres)	8.39±0.95	7.99±0.54	15.561±0.35
Clearance(L/h)	0.8±0.034	1.1798±0.06	0.157±0.02
AUC_0^{∞}(µg/ml/h)	16.835±2.548	12.713±2.658	95.164±5.687

Data obtained from hepatoprotective study was showed in Table 5.

Table 5. Effect of curcumin formulations on enzyme levels in rats with CCl4 induced hepatotoxicity (Mean ± SEM; n=3).

Groups	SGOT(U/L)	SGPT(U/L)
Normal control(saline)	17.06±0.3	11.38±0.52
CCl_4 treatement	72.46±0.66[b]	42.72±0.86[b]
CCl_4 + Curcumin oral(100mg/kg)	42.62±1.453[a]	26.54±0.55[a]
CCl_4 + Curcumin i.v (10mg/kg)	34.49±0.5772[a]	21.29±0.16[a]
CCl_4 + Curcumin liposomes (100mg/kg)	26.9± 1.321[a]	16.48± 0.66[a]

Note: [a] $p < 0.05$ vs CCl_4 treated, [b] $p < 0.05$ vs normal control

Liposomal formulation significantly reduces the elevated enzymes levels. From the table it was observed that all formulations were significant when compared to CCl_4 treated group ($p < 0.05$). From the formulations, curcumin liposomes were showed high hepatoprotective activity (Fig 3). The drug levels in various tissues like liver, brain, kidney, and colon were estimated and shown in table 6. Histopathology of liver has been shown in Fig 4.

Particle size and entrapment efficiency of the curcumin liposomes were increased with increasing the lipid content up to 100 mg. This is may be due to high amount of lipid is available to encapsulate the drug, upon increasing the lipid content number of layers coated the drug was increased, this resulted in an increase in the particle size and entrapment efficiency. Further increase in the lipid content to 120 mg there is no much increase in the entrapment due to the availability of the drug to be incorporated is low.

The in vitro drug release was fitted into various release models. From the release data we observed that increase in the lipid content delays the drug release due to increased particle size and reduced surface area available for drug release. From all the formulations, CL3 was selected as optimized formulation because less PDI, high entrapment efficiency and the prolonged release up to 12 days. All formulations release mechanism was non-fickian pattern ($n>0.5$; diffusional and dissolution mediated) [16]. From the pharmacokinetic data it was shown that the drug levels in plasma was lower in case of curcumin when given orally compared to that of curcumin and CL3 given intravenously. This may be due to lower bioavailability of curcumin when given orally. From the liposomal formulation the drug release was sustained up to 9 days due to decrease in the elimination of drug.

Table 6. Curcumin levels in various tissues

Tissue	Curcumin oral solution[a]	Curcumin i.v solution[a]	Curcumin liposomes[a]
Liver	15.25 ±0.57747	26.34 ± 1.139	35.49 ± 0.66
Colon	8.47 ± 0.586	9.343 ± 0.49	10.55 ± 0.39
Kidney	0.9 ± 0.032	1.11 ± 0.08	2.073± 0.06
Brain	Not detectable	Not detectable	0.0706 ± 0.007

a: values indicates Mean ± S.E.M (Standard Error Mean); n=3

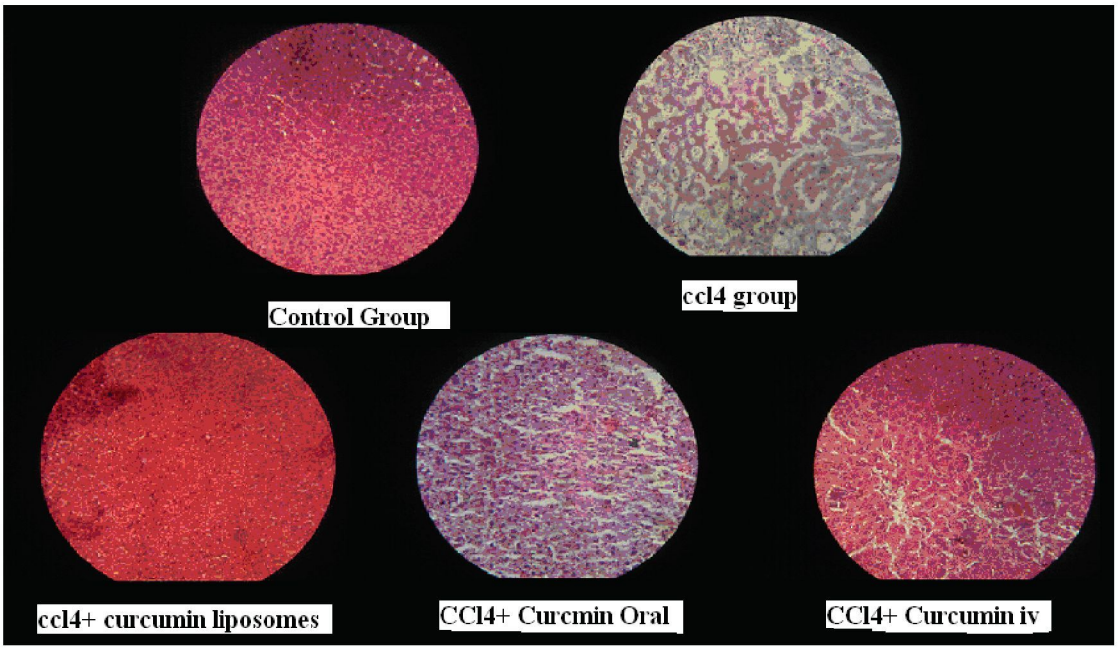

Figure 4. Histopathology of liver after administration of CCl_4 alone and in combination with Curcumin oral suspension., *IV bolus,* and Curcumin liposomes.

From the histopathological studies we observed that SGOT and SGPT levels were more in case of CCl_4 treated animals because of tissue injury caused by CCl_4 which releases the enzymes in to the blood stream. A fatty layer was observed in the histopathology of liver. Upon administration of curcumin formulations the enzyme levels was decreased due to the fibrosis caused by the CCl_4 was reversibly cured by the curcumin. From tissue distribution studies it was observed that drug was more concentrated in the liver compared to colon, kidney, and brain. The drug accumulation order in the different tissues as follows: liver > colon > kidney > brain. Drug levels in the liver were higher because of the increase in the level of the drug taken into the liver cells when administered in the form of particulates.

CONCLUSION

The small unilamellar vesicular formulations of curcumin were successfully prepared thin film hydration followed by probe sonication method. The drug release from the formulations was sustainable up to 15 days. The *in vitro* performance of the formulation was more compared to the respective oral and *i.v* formulations. From the hepatoprotectivity study it was concluded that the formulation was more effective. From the distribution studies it was concluded that liver targeting was achieved from the formulations.

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REFERENCES

1. **Chattopadhyay, I., Biswas K., Bandyopadhyay, U., Banerjee, R.K.,** "Turmeric and curcumin: Biological actions and medicinal applications" *Current Sci.*, 87 (1), 44-53, **2004.**
2. **Anand, P., Kunnumakkara, A.B., Newman, R.A., Aggarwal, B.B.,** "Bioavailability of curcumin: problems and promises" *Mol. Pharm.*, 4, 807–818, **2007.**
3. **Pan, M.-H., Huang, T. M., Lin, J. K.,** "Biotransformation of Curcumin through reduction and glucuronidation in mice". *Drug Metab. Dispos,*. 27, 486–494, **1999.**
4. **Sharma, R.A., Steward,W.P., Gescher, A.J.,** "Pharmacokinetics and pharmacodynamics of curcumin". *Adv. Exp. Med. Biol.*, 595, 453–470, **2007.**
5. **Bisht, S., Feldmann, G., Soni, S., Ravi, R., Karikar, C., Maitra, A.,** "Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): a novel strategy for human cancer therapy" *J. Nanobiotechnol.*, 5, 3, **2007.**
6. **Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P., Mukherjee, P.K.,** "Curcuminphospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats" *Int. J. Pharm.*, 330, 155–163, **2007.**
7. **Tiyaboonchai, W., Tungpradit, W., Plianbangchang, P.,** "Formulation and characterization of curcuminoids loaded solid lipid nanoparticles" *Int. J. Pharm.*, 337, 299–306, **2007.**
8. **Kumar, M., Sarin, S.K.,** "Is Cirrhosis of the Liver Reversible" *Indian J. Pediatrics,* 74, 393- 399, **2007**.
9. **Jaeschke, H., Gores, G.J., Cederbaum, A.I., Hinson, J.A., Pessayre, D., Lemasters, J.J.,** "Mechanisms of hepatotoxicity". *Toxicol. Sci.*, 65 (2), 166–76, **2002.**
10. **Patel, T., Roberts, L.R., Jones, B.A., Gores, G.J.,** "Dysregulation of apoptosis as a mechanism of liver disease: an overview" *Semin. Liver Dis.*, 18 (2), 105–14, **1998.**
11. **De Bleser, P.J., Niki, T., Rogiers, V., Geerts, A.,** "Transforming growth factor-beta gene expression in normal and fibrotic rat liver" *J. Hepatol.*, 26, 886-893, **1997.**
12. **Nagayama, S., Ogawara, K., Fukuoka, Y., Higaki, K., Kimura, T.,** "Time-dependent changes in opsonin amount associated on nanoparticles alter their hepatic uptake charactereistics" *Int. J. Pharm.*, 342: 215-221, **2007.**
13. **Ogawara, K., Yoshida, M., Higaki, K., Kimura, T., Shiraishi, K., Nishikawa, M., Takakura., Y, Hashida, M.,** "Hepatic uptake of polystyrene microspheres in rats: effect of particle size on intrahepatic distribution" *J. Control Release*, 59, 15-22, **1999.**
14. **Bangham, A.D., Standish, M. M., Watkins, J.C.,** "Diffusion of univalent ions across the lamellae of swollen phospholipids" *J. Mol. Biol.*, 13,238-52, ***1965.***
15. **Bonepally, C.R., Aukunuru, JV., Yellu, N.R., Vanga, M.R.** "Fabrication and Investigation on Hepatoprotective Activity of Sustained Release Biodegradable Piperine Microspheres" *Int. J. Pharm. Sci. and Nanotech.*, 1, 87-96, **2008.**

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