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Original Article

PREPARATION AND OPTIMIZATION OF KUPFFER CELL TARGETED CATECHIN LOADED SPHERICAL PARTICLES

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Abstract

Liver targeting of the drugs, specifically to the Kuppfer cells can be achieved after i.v. administration of drug loaded spherical particles. This mode of administration of drugs enhances its overall delivery to the liver via passive targeting. The purpose of this study was: 1) To optimize pharmacokinetics and kupffer cell (KC) uptake of catechin after i.v. administration of catechin-polycaprolactone nanoparticles and microparticles 2) To optimize particle size for improvement in targeting to the KC with catechin polycaprolactone spherical particles (CSP). A w/o/w solvent evaporation technique was used to prepare CSP and particles were characterized for in vitro parameters, pharmacokinetics (PK) and KC uptake. To optimize the particle size, a series of nano- and micro-particles encapsulating the same drug amounts were investigated for hepatoprotective activity. Ten different CSP of sizes ranging from 200 nm to 25 µm were successfully prepared. PK parameters suggested that nanoparticles offered better PK and enhanced KC uptake compared to microparticles. Nanoparticles resulted in better hepatoprotection in CCl_4 induced liver fibrosis model compared to microparticles (Hepatoprotection rank: 365 nm > 1.1 µm > 3.2 µm = 6.1 µm < 10.4 µm) suggesting that liver uptake, particularly KC uptake is more with nanoparticles. Particles of sizes greater than 6 µm lead to reduction in liver uptake and increase in lung uptake. The study concludes that nanoparticules are better and optimized for catechin delivery to KC compared to microparticles.

Key words: *Catechin, Fibrosis, Spherical particles, Polycaprolactone, Kupffer cell, Targeting.*

Kupffer Hücrelerine Hedeflenen Kateşin Yüklü küresel Partiküllerin Optimizasyonu ve Hazırlanması

İlaçların karaciğere özellikle Kupffer hücrelerine hedeflenmesi ilaç yüklü küresel partiküllerin iv uygulanması ile başarılabilir. Bu uygulama modu, ilaçların pasif hedeflendirme ile karaciğere toplam taşınmasını arttırmaktadır. Bu çalışmanın amacı, 1) kateşinin, kateşin-polikaprolakton nanopartiküllerinin ve mikropartiküllerinin iv uygulanmasından sonar farmakokinetiğini ve Kupffer hücreleri (KC) tarafından alımını optimize etmek, 2) Kateşin polikaprolakton küresel partikülleri (CSP) ile KC'ne hedeflemenin iyileştirilmesi için partikül büyüklüğünü optimize etmektir. CSP'nin hazırlanması için S/Y/S çözücü buharlaştırma tekniği kullanılmış ve partiküllerin in vitro parametreler, farmakokinetik ve KC alımı için karakterize edilmiştir. Partikül büyüklüğünü optimize etmek için, aynı miktarda ilaç içeren nano ve mikropartikül serileri hepatoprotektif aktivite için incelenmiştir. 200 nm – 25 µm arasında partikül büyüklüğüne sahip CSP'ler başarılı bir şekilde hazırlanmıştır. PK parametreleri mikrokürelere göre nanopartiküllerin daha iyi PK ve daha yüksek KC alımına neden olduğunu göstermiştir. Nanaopartiküller CCl4 ile indüklenen karaciğer fibroz modelinde daha iyi hepatoprotektif etki göstermişlerdir (hepatoprotektif etki sırası: 365 nm > 1.1 µm > 3.2 µm = 6.1 µm < 10.4 µm) ki bu durum karaciğer alımının özellikle de KC alımının nanopartiküller ile daha yüksek olduğuna işaret etmektedir. Partikül büyüklüğünün 6 µm'den büyük olması karaciğer alımını azaltıp akciğer alımını artırmıştır. Çalışma, KC'ye kateşin taşınması için nanopartiküllerin mikropartiküllere göre daha iyi olduğunu göstermiştir.

Anahtar kelimeler: *Kateşin, Fibrosis, Küresel partiküller, Polikaprolakton, Kupffer hücreleri, Hedefleme.*

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INTRODUCTION

Fibrosis in the liver is a consequence of chronic insult to the liver (1). If not controlled fibrosis proceeds towards irreversible cirrhosis. There is no proper treatment for this disease (2). Thus there is a need to find effective treatment for fibrosis. Catechin is a molecule which previously demonstrated promise in fibrosis. Catechin has been shown to reduce liver fibrosis via its antioxidant mechanism (3). Its strong antioxidant mechanism and inhibition of lipid peroxidation are the lead reasons for its effects. Also it demonstrated anti-inflammatory activity which is also helpful in the amelioration of liver fibrosis (4). Several liver cells, including hepatic stellate cells, hepatocytes and kupffer cells are known to be involved in this pathology (1). It has been proposed by popular scientists that fibrosis occurs when oxidative stress activates stellate cells thereby promoting the production of TGF-β1 which in turn causes production of extracellular matrix thereby leading to inflammation and also further oxidative stress (5). Consequently, inflammation and oxidative stress at several liver cells including KC further results in the progress towards the formation of fibrosis (6). Thus, catechin which is both an antioxidant and also anti-inflammatory can be targeted to KC to demonstrate its usefulness in fibrosis. The main objective of this study was to investigate the delivery of catechin to KC using polymeric nanoparticles.

Drug targeting to specific cells can increase the intracellular levels of the drugs and can improve the therapy tremendously (7). Drug targeting is essential to achieve significant levels of the drugs in the target cells or tissues. Targeted drug delivery systems are referred to carriers that concentrate at the target cell either through local delivery or via whole body circulation. Drug targeting to the liver is of interest in several studies (8,9). Our group is identifying potent ways of treating various liver disorders including fibrosis especially focusing on the drug delivery systems and liver targeting (10-12). In this study, the aim was to deliver catechin a known therapeutic agent useful in liver fibrosis with drug targeting strategies. Catechin can be targeted to KC and its delivery into these cells can be enhanced with catechin encapsulated in the particulate systems. To find means of optimization of effective particle size of CSP that can be used in targeting KC, the first aim of this study was to formulate a catechin encapsulating polycaprolactone spherical particles of different sizes. The aim is to prepare CSP both in the nano- and micro- size. The second aim was to demonstrate more benefits of catechin nanoparticles over catechin microparticles in enhancing the PK properties of this drug in a rat model. Drug delivery systems like liposomes and nanoparticles are known to enhance PK properties thereby improving the therapeutic index of the drug (13). The third aim was to optimize the particle size for passive targeting of catechin nano- and micro-particles to KC. Particle size is an important determinant of the efficacy in the therapy against phagocytic cells such as KC (14). Here, the objective was to investigate the KC uptake properties of CSP of sizes above 150 nm. Particles above 150 nm are taken up by KC in the liver (15). Some particles of this size which are present in the circulation are also taken up by sinusoidal endothelial cells (SEC) (16). Particles of less than 150 nm may have access to several other cells in the liver (17). The fourth aim was to optimize particle size to determine the size that can show a better reversal of fibrosis. A CCl_4 induced liver fibrosis model was used in the study. The role of KC in this model is well elucidated. KC are involved in several types of chemical induced liver damage, including damage related to CCl_4 (18). KC are important mediators of fibrosis development in this animal model.

EXPERIMENTAL

Materials

Catechin was procured from Yucca Enterprises, Mumbai, India, Poly-ε-caprolactone (Mwt. 20,000) was procured from Sigma Aldrich, Germany, Poly vinyl alcohol (cold water soluble) was procured from Qualikems Fine Chemicals Pvt Ltd, New Delhi, dichloromethane was

procured from SD fine chemicals limited, Mumbai. All other reagents were of analytical grade. A probe sonicator (Homogenizer 150 VT) used to prepare nanoparticles was procured from M/S Biologics, Inc USA and was used. A Zeta sizer 3000 HAS from Malvern Instruments, Malvern, UK, was used to measure the particle size. A HPLC from Cyber Labs, USA was used to analyze the plasma samples. A UV- Visible spectrophotometer (Elico, SL 164 Double beam, Hyderabad) was used to analyze drug loading and release study samples. Magnetic stirrer from Remi industries, Mumbai was used to prepare the microparticles. An Ultracentrifuge from Remi industries, Mumbai was used to recover the particles after preparation.

Methods

Preparation of catechin nanoparticles and microparticles

Double emulsion (W/O/W) solvent evaporation method was employed in the preparation of catechin nanoparticles and microparticles. To prepare nanoparticles, a probe sonicator was used to prepare the emulsion while a magnetic stirrer was used to prepare the emulsion in the case of the preparation of the microparticles. The oil phase consisted of weighed amount of catechin that was dissolved in specific volume of dichloromethane. Polycaprolactone was also dissolved in the same organic solvent. The compositions taken and the specification for both nanoparticles and microparticles is mentioned in Table 1. The aqueous phase consisted of PVA in water. A 5ml PVA solution was added drop wise manner while under probe sonication or stirring to obtain a w/o emulsion. The above primary emulsion was added to PVA solution in drop wise manner and allowed for ultrasonication or stirring, and this resulted in the formation of a w/o/w emulsion. This emulsion was placed on magnetic stirrer to ensure complete evaporation of dichloromethane, leaving nanoparticle and microparticle suspension. To recover nanoparticle suspension ultracentrifugation was used. The nanoparticle suspension was centrifuged at 12,500 rpm for 20 mins. The supernatant was collected and the pellet was washed with PBS, resuspended and was again allowed for centrifugation. After this centrifugation the supernatant was collected and this was added to previously collected supernatant. The pellet was collected and allowed for complete dryness. The powdered particles were collected, weighed and used for further evaluation. Microspheres were collected by filtration using a whatman filter paper. Catechin amount in the total volume of collected supernatant was determined. The amount encapsulated is the amount initially taken subtracted from the amount in the supernatant volume. Encapsulation percentage was determined using the following formula:

Encapsulation = (Amount of the drug initially taken – Amount of the drug in the supernatant)/Total weight of the microspheres

In vitro characterization of nanoparticles and microparticles

The nanoparticle and microparticle formulations were evaluated for particle size, drug-excipient interaction, organic solvent content and in vitro drug release. Nanoparticles were also evaluated for particle charge. Percentage yield was calculated using:

$$Percentage\ yield = \frac{Practical\ weight}{Theoritical\ weight}\ x\ 100$$

Nanoparticles and microparticles were evaluated for their particle size, polydispersity index 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. The morphology of the particles and their surface characteristics were determined using a scanning electron microscope. Residual organic content was determined using a gas chromatography using a previously published method (19). Drug – polymer interaction was

investigated using FTIR. FTIR of drug, polymer, placebo nanoparticles and drug loaded particles were taken using a Thermo Nicolet Nexus 670 Spectrophotometer with KBr pellets. For the release studies, a dialysis membrane was used. A volume of 5 ml particle suspension was placed in donor compartment. Receiver compartment consisted of 50 ml phosphate buffered saline. The RPM of the receiver compartment was maintained by using magnetic stirrer and bead (50rpm). Samples (5ml) were removed from the receptor compartment and replaced with fresh medium immediately. These were analyzed using spectrophotometrically at 280nm wave length using UV spectrophotometer (Elico SL 164 Double beam) and the values were taken.

In-vivo Studies

Male Wister rats (150–180g) were purchased from Mahaveer enterprises, Hyderabad. Animals were maintained in an air-conditioned room at 22 ± 2 ^{0} C and relative humidity of 45–55% under a 12h light: 12 h dark cycle. The animals had free access to standard food pellets and water was available *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee of Vaagdevi College of Pharmacy, Warangal, India (Registration No: 1047/ac/07/CPCSEA) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India. Catechin nanoparticles and microparticles were suspended in normal saline solution. Catechin solution for *iv* administration was prepared by dissolving the drug in 5% dimethyl sulfoxide (DMSO) solution.

For the evaluation of PK of the catechin formulations in rat, a standard graph of catechin in plasma was developed by following a previously published HPLC method (20). Plasma drug levels were assayed using the same technique. 5% DMSO solution and blank particles injected into the rats did not yield any peak at catechin peak. PK of the formulation was performed on male wistar rats. Rats were divided into three groups each group contains six rats.

Group 1 receives catechin solution (10 mg) intravenously.
Group 2 receives catechin nanoparticlar (365 nm) suspensions (equivalent to 10 mg of catechin) intravenously
Group 3 receives catechin microparticular (2.2 μm) suspension (equivalent to 10 mg of catechin) intravenously.

Catechin levels in the plasma were determined at 30 min, 1, 3, 6, 12, 18, 24 hours. For nanoparticular and microparticular formulations along with above time intervals samples were also collected after, 3, 6 and 9 days. Drug levels in various tissues like liver, kidney, brain were determined by isolating tissues from the rats. The tissues were chopped into small pieces and minced with dichloromethane. The resulted solution was evaporated to dryness and reconstituted with mobile phase and concentrations in the tissues were analyzed by performing HPLC with mobile phase methanol:water (20:80). Standard curve in the individual tissues was prepared following same method used for plasma. To determine the drug levels in the liver cells of interest, KC and SEC, three remaining rats were used. Cells were isolated at the end of the study from the liver as described in detail elsewhere (21). The drug from these cells was extracted using dichloromethane. This was evaporated to dryness and reconstituted with mobile phase and HPLC was performed.

Carbon tetrachloride induced liver damaged model was used in the optimization of the particle size. Male wistar rats (150-180g) were divided into 10 groups containing three rats each.

Group 1 received normal saline (1 ml/rat) daily for 9 days.
Group2 received carbon tetrachloride formulation (0.7 ml/kg).

Group 3 received catechin solution (1.1 mg/kg) intravenously, daily for 9 days.
Group 4 received catechin nanoparticular suspension (365 nm) (equivalent to 10 mg/kg of catechin) intravenously at day one.
Group 5 received catechin microparticle suspension (1.1μm) (equivalent to 10 mg/kg of catechin) intravenously at day one.
Group 6 received catechin microparticle suspension (3.2μm) (equivalent to 10 mg/kg of catechin) intravenously at day one.
Group 7 received catechin microparticle suspension (6.1μm) (equivalent to 10 mg/kg of catechin) intravenously at day one.
Group 8 received catechin microparticle suspension (10.4μm) (equivalent to 10 mg/kg of catechin) intravenously at day one.
Group 9 received placebo polycaprolactone nanoparticles containing highest polymer amounts used in the particles.
Group 10 received DMSO control (5% DMSO solution in water).

All groups received CCl_4 at 3, 6 and 9th day of the study except normal control. Carbon tetrachloride composition administered was a of mixture of CCl_4 and olive oil (25:75). Animals were anaesthetized on the last day of study and blood (3 ml) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (Ultracentrifuger, Remi Laboratories, Mumbai) at 3000 rpm for 15 min and SGPT and SGOT were estimated on the same day spectrophotometrically using Coral Clinical Systems kit by modified IFCC method. Histological slides were prepared as described previously (22). The slides were observed for various features under a microscope. The drug levels in various tissues including brain, lung and liver at the end of the study were determined using the HPLC method described previously.

Statistical analysis

The data were presented as mean ± standard deviation (STDEV) and were analysed by 2-tailed Students t-test using MiniTAB software (Minitab Inc. State College, PA, USA). $P < 0.05$ was used in determining statistical significant difference.

RESULTS

Preparation and Characterization of Catechin Nanoparticles and Microparticles

Catechin nanoparticles and microparticles were successfully prepared by double emulsion solvent evaporation method using polycaprolactone. Drug polymer interactions in nanoparticles and microparticles were studied by FTIR; from the spectras we observed that there is no interaction between the drug and polymer (data not shown). Three nanoparticular formulations (CNP1, CNP2, CNP3) were prepared by taking different drug to polymer ratios (Table 1). Seven different catechin microparticular formulations (CMP1 through CMP7) were prepared by taking 50 mg of drug and 200 mg of the polymer and then varying PVA and solvent ratios (Table 1).

Table 1. Compositions and methodology of preparation of nanoparticles and microparticles

Formulation	Drug (mg)	Polymer (mg)	PVA (%W/V)	W1/O/W2 (ml)	Size of the particles
CNP1	150	150	3	5:5:30	205 nm
CNP2	150	200	3	5:5:30	365 nm
CNP3	150	400	3	5:5:30	500 nm
CMP1	50	200	3	5:5:30	1.1 μm
CMP2	50	200	0.5	5:5:30	2.2 μm
CMP3	50	200	3	5:10:30	3.2 μm
CMP4	50	200	0.5	5:10:30	6.1 μm
CMP5	50	200	0.5	5:15:40	10.4 μm
CMP6	50	200	0.5	5:15:30	17.3 μm
CMP7	50	200	0.5	5:25:30	25 μm

The results for particle size, zeta potential and encapsulation efficiency are shown in Table 2. As the polymer amounts used in the preparation of nanoparticles increased, the size of the nanoparticles.

Table 2. Particle size, zeta potential and encapsulation efficiency of katechin nano and microparticles

Formulation Code	Particle Size (mean ± SD) nm for CNPs, μm for CMPs	Polydispersity Index (PDI)	Zeta Potential (mean ±SD)	% Encapsulation Efficiency (mean ± SD)
CNP1	204 ± 1.63	0.32	-2.56 ± 0.54	78.41 ± 2.15
CNP2	364 ± 3.25	0.10	-2.91 ± 0.32	74.25 ± 1.24
CNP3	500 ± 1.63	0.52	-2.85 ±0.25	83.75 ± 3.45
CMP1	1.1 ± 0.12	0.21	-1.61 ± 0.56	76.25 ± 2.37
CMP2	2.2 ± 0.15	0.32	-2.44 ± 0.32	78.58 ± 1.23
CMP3	3.2 ± 0.19	0.23	-2.33 ± 0.54	76.25 ± 1.56
CMP4	6.1 ±0.23	0.34	-2.54 ± 0.65	74.35 ± 1.72
CMP5	10.4 ±0.45	0.13	-2.67 ± 0.23	76.25 ± 1.21
CMP6	17.3 ±0.52	0.26	-2.83 ± 0.32	75.34 ± 1.29
CMP7	25 ± 0.75	0.12	-2.32 ±0.17	75.26 ± 2.34

For the all three nanoformulations the drug release was above 40% during first 3 days and it was complete by the end of the 9th day. The release study was performed for 9 days because in vivo we aimed to test the formulations in a 9-day CCl_4 fibrosis model. For all the microsphere formulation drug release was sustained for upto 9 days. Data from the release studies indicated that drug release was mainly diffusion controlled and followed Higuchi pattern. Residual organic solvent content in all the formulations was not detectable. SEM pictures taken for both nanoparticles and microparticles indicated that the particles were spherical and had a smooth surface (data not shown). Thus, using the methodology employed in our study, different nanoparticle and microparticle formulations encapsulating catechin and that can sustain the release of the drug were developed.

In vivo Drug Release, KC uptake and the Hepatoprotective Activity of Catechin Nanoparticles and Microspheres

Drug levels in the plasma, tissues and KC were determined using HPLC. The retention time of the drug was 12.5 min. Plasma profile of the drug after administration of nanoparticular, microsphere, and *i.v.* solutions demonstrated that the drug release into the plasma was sustained for 9 days with both nanoparticular and microparticular formulation after intravenous administration while with solution administration the drug levels in the plasma disappeared within 24 hours (Fig 1; IV data not shown in the figure). Peak serum concentration of 2.2µg/ml was observed within 30 min when catechin solution was given intravenously. Peak serum concentration of 6.6µg/ml was observed within 1 hour when catechin nanoparticles (CNP1) suspension was given intravenously. Peak serum concentration of 4.4µg/ml was observed within 1 hour when catechin microspheres (CMP3) suspension was given intravenously.

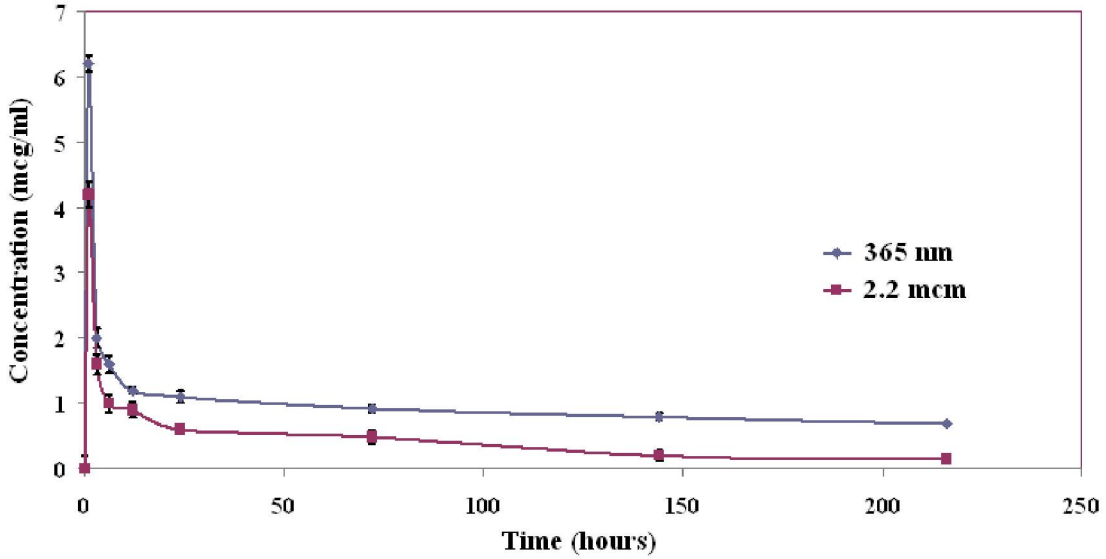

Figure 1. In vivo release of catechin after administration of catechin spherical particles

All the pharmacokinetic parameters with microsphere and nanosphere group was statistically significant when compared with the control group at $p<0.05$ excepting for vd of the nanoparticles. Table 3 shows the pharmacokinetic parameters obtained after administration of drug and formulations.

Table 3. Pharmacokinetic parameters obtained after administration of catechin formulations

Parameter	Catechin iv bolus	Catechin microspheres iv	Catechin nanoparticles iv
Cmax(µg/ml)	2.2±0.024	4.4±0.041*	6.6±0.15*
Ke(per hour)	0.14±0.004	0.0101±0.005*	0.004±0.0008*
$T_{1/2}$(hours)	6.52±0.01	68.43±1.06*	155.4134±1.57*
Vd(Litres)	10.85±2.32	15.561±0.35*	12.23±0.45
Clearance(L/h)	0.4498±0.05	0.157±0.02*	0.0386±0.004*
AUC_0^∞ (µg/ml/h)	12.713±2.658	95.164±5.68*	387.649±10.54*

Values indicate mean ± standard deviation mean (STDEV). n=3. * $p < 0.05$ compared with catechin iv bolus

Fig 2 shows the drug levels in the KC and SEC at the end of the study. In KC, the uptake was higher for both the particles tested. But in SEC only nanoparticles were taken and not the microparticles.

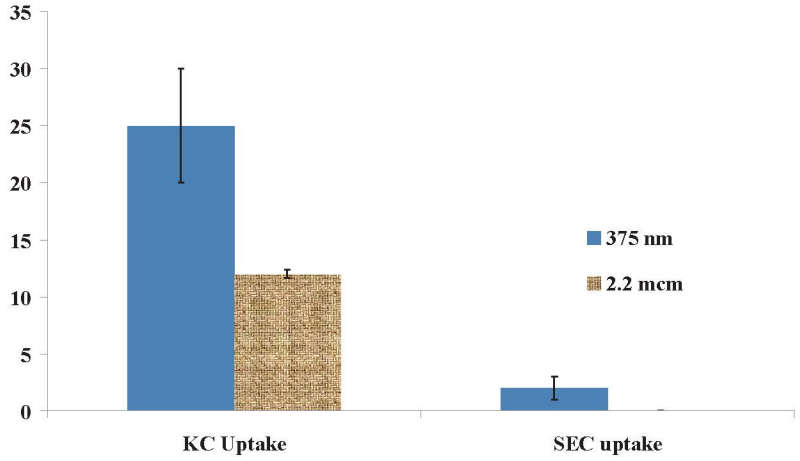

Figure 2. Kuppfer cells and sinusoidal endothelial cell uptake of catechin at the end of pharmacokinetic study

Data obtained from hepatoprotective study is shown in Fig 3. Nanoparticular 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 ($p < 0.05$). Nanoparticles resulted in better hepatoprotection in CCl_4 induced liver fibrosis model compared to microparticles (Hepatoprotection rank, as indicated by SGOT and SGPT: 365 nm > 1.1 µm > 3.2 µm = 6.1 µm < 10.4 µm). The reversal in the levels of SGOT and SGPT was statistically significant ($p < 0.05$) for the particulate groups excepting for 10.4 µm group compared to CCl_4 group.

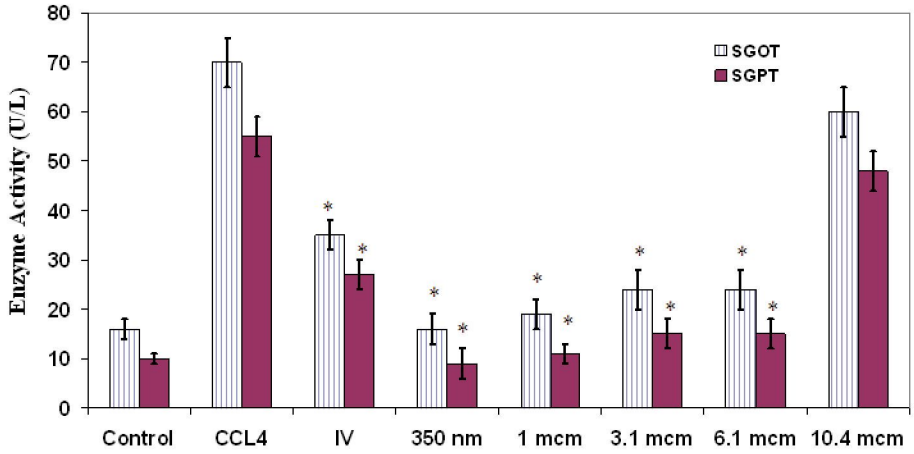

Figure 3. Effect of Catechin and its forms on Liver enzyme levels in rats with CCl_4 induced hepatotoxicity. Values indicate mean ± standard deviation mean (STDEV). n=3. * $p < 0.05$ compared with catechin iv bolus.

Nanoparticles completely reversed histological changes observed in the CCl_4 toxicity model. Microparticles partially reversed histological changes observed in the CCl_4 toxicity model. Livers of animals in the CCl_4 group on gross examination demonstrated scattered yellow and white areas attributed to fatty and necrotic changes. Light microscopic examination HE stain slides of vehicle control animals demonstrated normal architecture of the liver with hepatic cords and plates radiating out from the central vein. Few normal scattered kupffer cells were also demonstrated. CCl_4 alone exhibited centrilobular necrosis, microvesicular and macrovesicular fatty changes, scattered lymphomononuclear infiltrate in hepatic parenchyma. Treatment with nanoparticles and microparticles exhibited reversal of these changes induced by CCl_4 with few foci of necrosis of hepatocytes and fatty changes and signs of regenerative activity. However reversal was significant with nanoparticles compared to microparticles. The drug levels in various tissues like liver, lung, brain at the end of the study were estimated and are shown in Fig 4. From tissue distribution studies it was observed that drug was more concentrated in the liver compared to lung and brain.

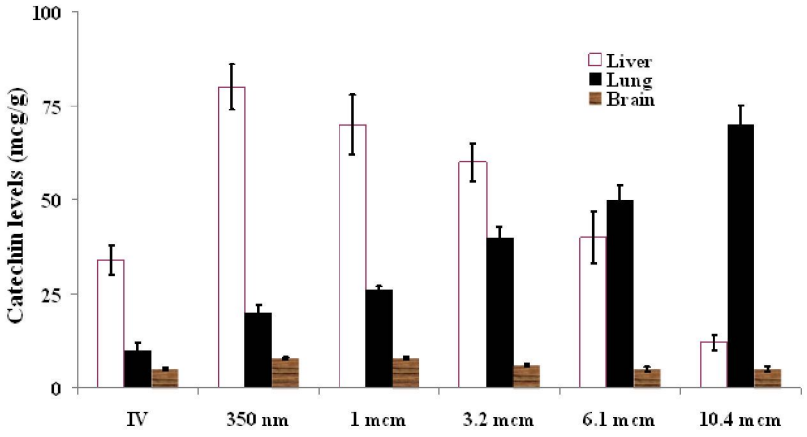

Figure 4. Catechin Levels in various Tissues at the End of the Study upon IV administration of Various size of CS particles.

DISCUSSION

Intravenous nanoparticles or microparticles are one alternative to enhance systemic drug delivery of drugs. Such a delivery can result in sustained systemic release of the drug and achieve drug targeting (23). Saturation solubility of the drug in aqueous medium can be enhanced with these delivery systems (24, 25). Thus, after intravenous administration, Cmax of the drug can be enhanced. This mode of administration can also be used for drugs which undergo extensive first pass metabolism since the drugs are directly administered into the systemic circulation. A nanoparticular or microparticular formulation containing catechin when injected by i.v. route can lead to enhanced therapy for liver protection by promoting sustained and targeted release. Previous studies have indicated that both microparticles and nanoparticles can be injected intravenously to achieve targeted therapy to the liver (8,9). Microparticles of sizes greater than 6 μm are targeted to achieve pulmonary delivery of the drugs while the sizes below 6 μm are invariably taken up by the KC cells in the liver (26). Drugs when encapsulated in particles of sizes less than 6 μm and injected intravenously are taken up by KC in the liver and then slowly releases the drug into the systemic circulation after forming a deport in KC. This type of targeting approach is called passive targeting. Stealth technology that prevents this engulfment of particles into KC and thereby promoting more sustained systemic delivery is currently more popular (27). Although stealth technology is currently popular, the passive

targeting of drugs to KC where these cells are involved in the pathology can be used to enhance the therapy. Thus, in this study KC uptake of catechin encapsulated in particles which is useful in liver fibrosis was investigated. KC is known to be involved in liver fibrosis (28). Previously, dexamethasone targeting to KC for enhanced therapy in liver fibrosis has been investigated (29). In their study, dexamethasone coupled to mannosylated albumin ($Dexa_5$-Man_{10}-HSA) was designed to selectively deliver this anti-inflammatory drug to the KC. The synthesis of this conjugate is difficult. On the other hand, the use of spherical particles for the same purpose can be utilized with added advantages. By now, the preparation and manufacture methods for nanoparticles and microparticles is well known to the pharmaceutical industry and their toxicity issues are also resolved to a better extent (30). Thus, particulate approach can be utilized for targeting to KC with added advantages. Although it is known that particulates can be taken up by KC, the optimization of the size for the same purpose has not been investigated systematically. For the first time, we are investigating this issue by taking catechin as the model drug.

Appropriate size of the particles encapsulating catechin for better delivery can be optimized based on the in vivo KC uptake and hepatoprotective activity. Also the knowledge of PK of drugs encapsulated in spherical particles will help in the best design of suitable formulation for further use. Generally, with delivery systems, the drug absorption is delayed, the drug biodistribution is restricted and altered and drug metabolism, clearance and volume of distribution are decreased (31,32). These changes can be drastically different with intravenously injected nanoparticles compared to microparticles. A biodegradable and well-known polymer polycaprolactone was used in the preparation of CSP. Solvent evaporation technique was used to prepare CSP of different sizes. To prepare spherical particles of biodegradable polymers, several methods can be used (32). Depending on the type of the polymer and the drug, a suitable method can be opted and this can affect the characteristics of the microspheres. A w/o/w emulsion solvent evaporation technique can be used to prepare hydrophobic catechin polycaprolactone spherical particles. The intention is that the inner water phase may increase the porosity of the particle and thus then can facilitate smooth drug release from the formed spherical particles once taken up by KC. The particles with different sizes were obtained with the use of varying proportions of organic solvents, PVA concentrations and different drug:polymer ratios. Particles of sizes varying between 350 nm and 25 μm were successfully prepared. Particle size and entrapment efficiency of the catechin nanoparticles were increased with increase in polymer content for up to 400 mg. This may be due to availability of more polymer to coat the drug. All the microspheres sustained the release of the drug from the formulation for more than 9 days suggesting the suitability of their use for in vivo studies. The in vivo study was aimed for 9 days. From the release data we observed that increase in the polymer content delays the drug release due to increase in particle size and reduced surface area available for drug release. FTIR studies indicated no interaction between the drug and the polymer at the end of the fabrication. Scanning electron microscopy indicated spherical nature of the particles and the smoothness of the surface.

PK and KC/SEC uptake were investigated in healthy rats with selected nanoparticle (350 nm) and microparticle (2.1 μm) formulations. Upon their injection, several PK parameters were altered. Cmax was several fold higher with nanoparticles and microparticles compared to solution form administration. The Vd was decreased with nanoparticles when compared to microparticles and i.v. solution administration, the clearance was decreased for both microparticles and nanoparticles when compared to the solution form. Nanoparticles had significantly lower clearance compared to microparticles. AUC was significantly increased with both microparticles and nanoparticles. Nanoparticles demonstrated 30 times enhancement in AUC. Cellular uptake of catechin indicated that both microparticle and nanoparticles are taken up into KC while only nanoparticles are taken up by SEC. Further, KC cell uptake of nanoparticles is greater than that of microparticles. The results of the PK study suggest that nanoparticles are taken up into KC with more advantages compared to microparticles. Thus, it can be said that the nano range particles are optimized for the best effect at KC. However, we

continued to investigate the improvement in the activity with different particle sizes. The reversal of fibrosis and the biochemical markers in CCl_4 also determines the role of particle size in targeting the KC with catechin loaded particles. To generate fibrosis in rats, a CCl_4 administered model was selected. Within 9 days, SGOT and SGPT were increased. Further histopathological changes were also seen in this model at the end of 9 days. The particles of sizes ranging from nano to micro have been injected into CCl_4 model to determine the hepatoprotective activity. Hepatoprotection rank was 350 nm > 1 μm > 3.1 μm = 6.2 μm < 10.4 μm. This ranking further suggests that nanoparticles are better than microparticles for achieving the hepatoprotective activity. It is further confirmed that as the particle size is increased the hepatoprotective activity decreased for the same amount of the drug. Particles of sizes ~ 3 μm and 6 μm demonstrated similar hepatoprotective activity suggesting that the efficacy of these two sizes for liver protection is the same. However, it is always important that the carrier:drug ratio is low (7) and this can happen when the particle size is smaller for particles demonstrating similar hepatoprotective activity. Since the drug administered is the same in the particles of different sizes, it can be inferred that smaller size particles demonstrated low carrier:drug ratio. From tissue distribution studies it was observed that drug was more concentrated in the liver compared to lung and brain. This is true upto a particle size of 6 μm. Beyond this size the liver uptake decreased while the lung uptake increased. The drug accumulation order in the different tissues is as follows: liver, lung and brain. Thus, nanosize particles also resulted in enhanced liver uptake compared to microparticles.

CONCLUSIONS

Catechin is a drug that can be used in the treatment of fibrosis. When encapsulated in spherical particles and administered intravenously, the potency of the drug to treat fibrosis increases several fold. This increase is mainly attributed to its enhanced targeting to the KC, one of the cell known to be involved in the pathology. Within the spherical particles, it was demonstrated that nanoparticles are better than microparticles in enhancing KC uptake. Also, particles offered better PK profile and parameters to the drug compared to in vivo solution administration. Within particles, nanoparticles offered better PK profile compared to microparticles for catechin.

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