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Original Article

FORMULATION OF CURCUMINOID LOADED SOLID LIPID NANOPARTICLES IN ORDER TO IMPROVE ORAL BIOAVAILABILITY

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ABSTRACT

Objective: Solid lipid nanoparticles (SLNs) of Curcuminoids were formulated and characterized in order to improve poor oral bioavailability of Curcumin. *In vivo* pharmacokinetics study in rats was conducted to demonstrate improved oral bioavailability.

Methods: High pressure homogenization followed by ultrasonication method was adopted to formulate solid lipid nanoparticles of Curcumin. Compritol 888 ATO and Precirol ATO 5 were explored as solid lipids with LIPOID S 75 being used as surfactant. Freeze dried solid lipid nanoparticles were compared with marketed formulation of Curcumin (Adcumin®) in rat plasma using High Pressure Liquid Chromatography (HPLC) method using ultraviolet (UV) detector.

Results: Particle size measurements performed on Solid lipid nanoparticles of Curcumin revealed the mean particle size of 200-300 nm for optimized formulations and entrapment efficiency of close to 80%. Sucrose and Dextrose were suitable cryoprotectants to prepare freeze dried solid lipid nanoparticles. Curcumin loaded solid lipid nanoparticles exhibited sustained release pattern during *in vitro* release kinetics.

Conclusion: *In vivo* pharmacokinetics study in Swiss albino rats revealed that encapsulation of Curcumin into solid lipid nanoparticles increased oral bioavailability of Curcumin to 12 folds when compared with marketed formulation of Raw Curcumin (Adcumin®).

Keywords: Curcumin, Solid Lipid Nanoparticles, Anti-neoplastic, Oral bioavailability, High pressure homogenization, Ultra sonication, Lyophillization.

INTRODUCTION

Curcumin is one of the well-known natural chemopreventive compounds, obtained from rhizomes of Curcuma longa (turmeric) that has shown high efficacy and potency against many diseases. It consists of 3 structurally-related curcuminoids; Curcumin (Curcumin I, «80%), demethoxycurcumin (curcumin II, «15%) and bisdemethoxycurcumin (Curcumin III, «5%). It has shown tremendous potential as a potent antioxidant, anti-inflammatory, antiproliferative, antimetastatic, anti-angiogenic, anti-diabetic, hepatoprotective, antiatherosclerotic, anti-thrombotic and anti-arthritic. It also enhances wound healing, inhibits human immunodeficiency viral replication and blunts the progression of various other diseases of pulmonary and cardiac origin [1].

However, in the scientific world it is well known for its potential to inhibit carcinogenesis induced by environmental carcinogens and other chemical agents [2]. It induces apoptosis in oncogenic cells by inhibiting various intracellular second messengers like NF-kB, AP-1, c-Jun, Jak-STAT pathway and various others [3]. However, major mechanisms of action of Curcumin are induction of endogenous antioxidants, enhanced expression of CYP1A1 enzymes and inhibition of IkB kinase.

Although, Curcumin exhibited a ken of potent activities, it is a lipophilic compound with very low aqueous solubility [4]. Due to low solubility, it shows solubility-limited bioavailability which makes it a Biopharmaceutical Classification System (BCS) class II drug[4]. Various studies with Curcumin in rodents and humans have shown very low bioavailability with limited efficacy.

Such low bioavailability limits its usefulness and hence its introduction into the clinical setting. Furthermore, the high doses required to achieve therapeutic concentrations in blood are often undesirable by the patients. In various clinical studies, daily doses from 8-12 g given to human volunteers were found to give very low plasma concentrations (<1 ug/ml) insufficient to exert any clinically useful pharmacological activity [5].

To combat this problem and to achieve therapeutic concentrations, various novel drug-delivery systems like microemulsions, liposomal vesicles, nanoparticles, phospholipid complexes were prepared [6-8]. But their frequent dosing and complex manufacturing requirements limited their introduction into the clinical setting and an effective drug-delivery approach of such a high potential compound is still not available.

Among modern drug delivery carriers solid lipid nanoparticles seemed to be a promising colloidal carrier system. Solid lipid nanoparticles made from biodegradable solid lipids exist in the submicron size range and can be prepared by several methods. The advantages of SLN are as follows: possibility of controlled drug release and drug targeting, protection of incorporated compound against chemical degradation, no biotoxicity of the carrier, avoidance of organic solvent and no problems with respect to large scale production.

In this study, oral bioavailability of Curcumin was improved by encapsulating Curcumin in to Solid lipid nanoparticles. The primary goal was to optimize the formulation with respect to drug to lipid ratio and achieving desired particle size for the freeze dried product. The pattern of drug release was also investigated and compared with raw Curcumin.

Present study focuses on formulation of solid lipid nanoparticles using Compritol 888 ATO and Precirol ATO 5 as solid lipids and LIPOID S 75 as surfactant. LIPOID S 75 is a patented natural phospholipid designed by LIPOID Germany and contains Lecithin with approximately 70% of Phosphatidylcholine. This is the first study that uses LIPOID S 75 as an ingredient in solid lipid nanoparticles of Curcumin.

MATERIALS AND METHODS

Materials

Curcumin was a gift sample from Natural Remedies, Bangalore. LIPOID S 75 was a kind gift sample from Lipoid Germany. Compretol A 888, Precirol,Glycerylmonostearate, Polysorbate 80, Poloxamer

188, Labrafac, Mygliol, Captexwere gift samples from Gattefosse, India. Dialysis polyvinylidenedifluoride (PVDF) membrane of a 1,000,000 molecular weight cut off (MWCO) was obtained from Spectra/Por (Becthai, Bangkok, Thailand). Sucrose, Trehalose, Mannitol and Glucose were purchased from Carbosynth. HPLC grade methanol and acetonitrile were procured from Merck. All other chemicals used were of analytical grade. Ultrapure water (HPLC grade) was used for all the experiments.

Preparation of curcumin loaded solid lipid nanoparticles

Nanoparticles were prepared by high pressure homogenization followed by ultrasonicationtechnique. The lipid was melted at a temperature slightly higher than 40 °C. The drug was dissolved in the melted lipid with continuous stirring. This constituted the lipid phase. The aqueous phase was prepared by dissolving surfactant into purified water. Lipid phase was slowly added to an aqueous phase with continuous stirring. The mixture was homogenized at 1000rpm for 30 min a Remi's high pressure homogenizer at to yield a uniform suspension. The homogenized suspension was subjected to ultrasonication for 3 min to yield particles in the nanometer size range. The lipid nanoparticle dispersions were washed twice with deionized water. The suspension was further freeze dried to yield Curcuminoid loaded solid lipidnanoparticles. Finally, 2% (w/v) sucrose was added to the dispersion before shock-frozen in liquid nitrogen and lyophilization at 0.30 mmHg and −30 °C for 24 h using Microprocessor control freeze dryer. The lipids used for preparation of nanoparticles were Compritol 888 ATO and Precirol ATO 5. The surfactants used for the process were Lipoid S 75, Poloxamer 188, Tween 80 and Tween 20. All the samples/batches were prepared in triplicates. Lipid and surfactants were used in varying concentrations and optimum concentrations were finalized by preliminary experiments.

Physical characterization of curcumin loaded solid lipid nanoparticles

Particle size and zeta potential measurements

Particle size and polydispersity index were determined using a Malvern Zetasizer Nano ZS (Malvern Instrument, UK) based on quasi-elastic light scattering. Briefly, 1 mg/ml of nanoparticulate Curcumin solution was prepared in double distilled water and sonicated for 30 s in an ice bath. Size measurements were performed in triplicates following the dilution (100 ml diluted to 1 ml) of the NPs suspension in MilliQ water at 25 ºC. Zeta potential was measured in the same instrument at 25 ºC using the above protocol. All measurements were performed in triplicates.

Fourier transform infrared (FTIR) spectral study

FTIR spectra were taken in to observation (Shimadzu) to investigate the possible chemical interactions between the curcumin and the polymer matrix. Void Nanoparticles, native Curcumin, nano particulateCurcumin were crushed with Potassium Bromide (KBr) to get the pellets by applying a pressure of 300 kg/cm^2. FTIR spectra of the above sample were obtained by averaging 32 interferograms with resolution of 2 cm^{-1} in the range of 1000-4000 cm^{-1}.

Entrapment efficiency

The percentage of drug incorporated during nanoparticle preparation was determined by centrifuging the drug loaded nanoparticles at 15,000 rpm for 15 min and separating the supernatant. The pellet obtained was washed twice with water and dissolved in acetonitrile followed by estimation of the drug in triplicate by a validated UV spectrophotometric method.

***In vitro* quantification of curcumin**

Waters Alliance system with UV detector and analytical column Waters Symmetry [10] ° RP-18(150 x4.6 mm, 5µm) from Waters was used for curcumin quantification. Curcumin was eluted isocratically at a flow rate of 1 ml/min using mobile phase concentration of Acetonitrile: Citrate buffer pH 3.0(60:40). Injection volume was 20 µland retention time of curcumin was 4.8 min. Limit of detection and limit of quantification for curcumin was 10 and 20 ng/ml respectively. Curcumin was determined in the concentration range of 0.2–10µg/ml. Peaks were measured at a wavelength of 480 nm. The analytical method was validated according to the guidelines of the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Parameters validated included precision (repeatability (intra-day) and intermediate precision (inter day)) and accuracy. Both the intra- and inter-day relative standard deviations (RSD) of QC standards were less than 3% over the selected range. A good accuracy of the method was verified with recovery values of 98–101%.`

***In vitro* release kinetics**

The release of drug from nanoparticulateCurcumin (Cu-SLN) was carried out by dissolving 100 mg of nanoparticles in 15 ml Phosphate buffer solution (PBS)(0.01 M, pH 7.4) and the solution was divided in 30 eppendorf (500 \ ml each) tubes, as experiment was performed in triplicates. The tubes were kept in a shaker at 37 °C at 150 rpm (Wadegati Lab equip, India). Free curcumin is completely insoluble in water; therefore, at predetermined intervals of time, the solution was centrifuged at 3000 rpm for 10 min (Remi, Germany) to separate the released (pelleted) curcumin from the nanoparticulate curcumin. The released curcumin was re dissolved in 1 ml of methanol and 20 ml of this solution was injected in the HPLC to determine the amount of curcumin released with respect to different time intervals.

Freeze drying

In this study, freeze drying was employed as a means to impart stability or improve shelf life of the developed formulations. Freeze drying using an automated system (AdVantage, VirTis, USA) was adapted that was previously optimized for curcumin nanoparticles. To study the protective effect of various types and concentrations of cryoprotectants (e. g. carbohydrates), freeze-thaw cycles were carried out as a pre-test.

Stability studies

Cu-SLNs were stored in vials at 5±3 °C for 1 year, and the samples were withdrawn at 0, 6 and 12 months as per ICH guidelines. The average size, total drug content and the entrapment efficiency were determined at each time point. Accelerated stability studies were carried out at 25 °C/60% RH for 3 months.

***In vivo* quantification of curcumin**

An already reported method was used for *in vivo* quantification of Curcumin. Shimadzu HPLC system with analytical column Li Chrospher® 100 RP-18 (250 mm×4.6 mm, 5_m) from Merck (Darmstadt, Germany), linked with a Nucleosil, C18 guard column from Macherey-Nagel (Germany) was used for *in vivo* curcumin quantification. Curcumin was eluted by a gradient flow at a rate of 1 ml/min using mobile phase of acetonitrile, 2.8% acetic acid and methanol in concentrations described in table 1 and curcumin was eluted at 7.8 min. 17-Estradiol acetate was used as internal standard. Curcumin was estimated at 425 nm and 17-estradiol acetate at 280 nm. Limit of detection and limit of quantification for curcumin was 5 and 10 ng/ml respectively.

The analytical method was validated according to the guidelines of the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Parameters validated included precision (repeatability (intra-day) and intermediate precision (inter-day)) and accuracy. Both the intra-and inter-day RSD of QC standards were less than 5% over the selected range. A good accuracy of the method was verified with recovery values of 96–101%.

Study design for *in vivo* pharmacokinetic studies

For *in vivo* pharmacokinetic studies, male Wistar rats weighing 250–300 g were used. The protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of KadiSarvaVishvavidyalaya, Gujarat, India. Theanimals were divided into two groups (n12). Group 1 (VH)was administered 50 mg/kg body weight (bw) of C-SLNs; and Group 2 was administered 50 mg/kg free curcumin (Adcumin®) (C-S; solution of curcumin in 25% Tween 80) per orally using anoral dosing cannula. The blood

samples (0.5 mL) were withdrawn from retro orbital plexus under mild ether anesthesia and collected into heparinized microcentrifuge tubes (containing 20 mL of1000 IU heparin/ml of blood) at different time intervals. After each sampling, 1 ml of dextrose–normal saline was administered to prevent changes in the central compartment volume and electrolytes. Plasma was separated by centrifuging the blood samples at 4000 rpm for 10 min at 41 °C. After centrifugation, the plasma obtained was stored at 20 °C until analysis

RESULTS AND DISCUSSION

Preparation of curcumin loaded solid lipid nanoparticles

The choice of particular solid lipid, drug to lipid ratio and concentration of surfactants are the driving factors for preparation of stable Curcuminoid loaded solid lipid nanoparticles. Screening of suitable lipid phase and drug to lipid ratio are two most important parameters. The effect of surfactant concentration and drug to lipid ratio was studied on characteristics of Curcuminoid loaded SLNs. Table 2 describes the details of experimental designs and the respective outcome.

Various formulations of Curcumin loaded SLNs were designed varying drug to lipid ratio and concentration of surfactants. As described in Material and Methods, Compretol A888 and Precirol ATO 5 were used as solid lipid while LIPOID S 75was used as surfactant (Stabilizer). Particle size distribution, Drug loading and Entrapment efficiency were the parameters used to conclude suitable drug to lipid ratio and optimum surfactant concentration.

Table 1: Gradient for analytical method for *in vivo* quantification of Curcumin

Time	Acetonitrile %	Acetic acid %	Methanol %
0	35	55	10
4	45	25	30
6	40	30	30
8	35	55	10

Table 2: Design of experiments for formulation of solid lipid nanoparticles of Curcumin and their outcomes

Batch number coding	Curcumin	Compritol 888 ATO (%)	Precirol ATO 5 (%)	Lipoid S 75	Propylene glycol (%)	Water (%)	Particle size distribution d(0.9)	Entrapment efficiency
Cu SLNs A	0.1	-	20	5	1	73.9	0.322±0.089	38.5±1.2
Cu SLNs B	0.5	-	20	5	1	73.5	0.289±0.014	32.7±4.9
Cu SLNs C	1	-	20	5	1	73	0.222±0.032	70±5.3
Cu SLNs D	0.1	20	-	5	1	73.9	0.242±0.039	56±11.2
Cu SLNs E	0.5	20	-	5	1	73.5	0.259±0.049	41.7±0.5
Cu SLNs F	1	20	-	5	1	73	0.289±0.085	80.1±0.9
Cu SLNs G	0.1	-	20	8	1	70.9	0.334±0.011	70.6±4.1
Cu SLNs H	0.5	-	20	8	1	70.5	0.482±0.020	65.6±2.3
Cu SLNs I	1	-	20	8	1	70	0.358±0.059	68.6±6.8
Cu SLNs J	0.1	20	-	8	1	70.9	0.526±0.043	61.1±1.1
Cu SLNs K	0.5	20	-	8	1	70.5	0.449±0.114	65.2±1.2
Cu SLNs L	1	20	-	8	1	70	0.305±0.144	41±3.2

It was found that at drug to lipid ratio of 1:20, both the lipids viz. Compritol 888 ATO and Precirol ATO 5 led to solid lipid nanoparticles with highest entrapment efficiency and particle size within 200-300 nm.

Table 3: Freeze drying studies with different cryoprotectants and particle size measurement

Cu-SLN			
S. No.	**Cryoprotectant**	**d(0.9) before freeze drying**	**d(0.9) after freeze drying**
1	Sucrose	0.242±0.039	0.237±0.045
2	Dextrose	0.242±0.039	0.256±0.067
3	Mannitol	0.242±0.039	0.382±0.021
4	No Sugar	0.242±0.039	1.012±0.101

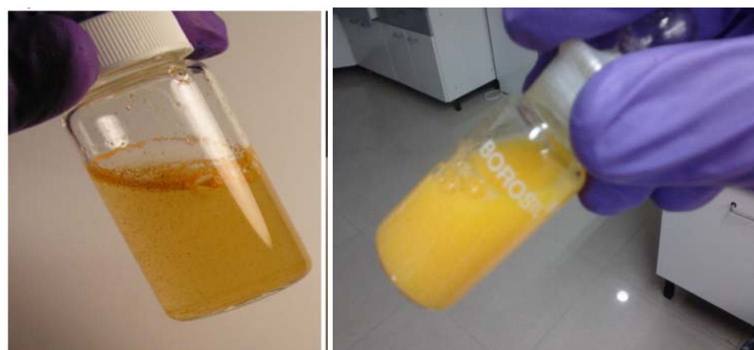

Fig. 1: Raw Curcumin and Curcumin loaded solid lipid nanoparticles in dispersion form

Zeta potential and FTIR measurement for optimized formulations

Zeta potential measured for Formulation C and F indicated high charge on particles that helps the formulation nanoparticles repel each other in order to have long term stability. Further to confirm encapsulation of Curcumin into nanoparticles, FTIR measurement was conducted for native Curcumin, nanoparticulate Curcumin and void nanoparticles. The broadening of peak at 3390 cm^{-1}indicates enhancement of hydrogen bonding. Marker peaks for Curcumin were not found in void Curcumin that indicates an encapsulation of Curcumin into nanoparticles.

Freeze drying

Evident from the literature, SLN dispersions shows an increase in particle size in a short period of time during the storage due to Ostwald ripening and hydrolysis reactions. Particle size of solid lipid nanoparticles is reported to cross the nanometric range within a week. Lyophilization offers chemical and physical stability by preventing Ostwald ripening and hydrolysis reactions [9].

Cryoprotectants have been used to decrease SLN aggregation due to the stress during the process of freeze-drying. Various cryoprotectants were screened at the concentrations of 5 % w/v. Mannitol resulted into a collapsed cake which was non dispersible. Sucrose and dextrose worked well with Cu-SLNs. The particle size also remained similar before and after freeze drying.

Solubility and stability studies for curcumin

To confirm solubility of nano curcumin in aqueous media, we found that nano Curcumin resulted into a well dispersed aqueous liquid. IN contrast, native Curcumin is poorly wettable and poorly soluble in aqueous media. One of the major challenges in drug delivery of Curcumin is poor stability at physiological pH as well.

In order to demonstrate stability of nanoformulations at physiological pH, we incubated our formulations and native Curcumin in PBS (pH: 7.4) and estimated concentration with time using HPLC method. It was observed that native Curcumin underwent rapid degradation and only 3% of Curcumin remained intact after 4 hours of incubation. However, nanoparticulate Curcumin was stable at the same conditions.

***In vitro* release kinetics for Nanocurcumin**

In vitro release kinetics studies for Curcumin loaded SLNs exhibited a sustained release pattern. Sustained release was observed over a period of 7 days. Initial burst release can be attributed to dissociation of surface absorbed Curcumin into lipid matrix while sustained released over a period of 7 days can be attributed to release of Curcumin from nanoparticles. Native Curcumin was visibly insoluble in PBS and after 7 days, only 6% drug releases were observed.

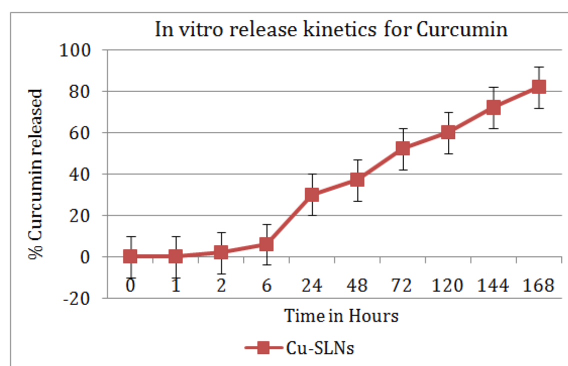

Fig. 2: *In vitro* release kinetics of Curcumin loaded SLNs

***In vivo* pharmacokinetics studies**

Curcuminoid loaded SLNs were designed in order to improve extremely poor bioavailability associated with Curcumin, a drug with tremendous therapeutic potential. Plasma levels of Curcumin after administration as SLNs were compared with those attained after oral administration of raw Curcumin (Adcumin®). Negligible plasma levels were attained after administration of raw Curcumin. The plasma levels were so low that they were below detection limit of the method for the first few time points. Encapsulation of Curcumin into SLNs led to 12 fold increase in bioavailability. The relative pharmacokinetic parameters Cmax, Tmax and Area under curve (AUC) are listed in table 4.

Table 4: *In vivo* pharmacokinetics for Curcumin loaded solid lipid nanoparticles

Formulation	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-\infty}$ (ng-/ml-h)	Relative BA
Adcumin®	35.5±10.6	2	40.8±30.5	NA
Cu-SLNs	624.5±65.4	6	488±81.5	1196%

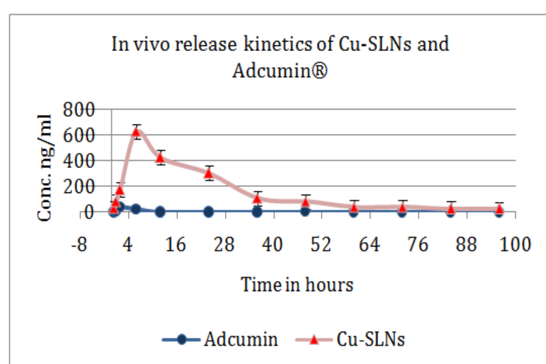

Fig. 3: *In vivo* release kinetics of Curcumin loaded SLNs and Adcumin®

DISCUSSION

Curcumin derived from the common food spice turmeric has been used for centuries as a remedy for many disorders as mentioned in the foregoing sections. Scientific research over the past decade has shown the compound to possess the preventive and therapeutic value against the wide variety of diseases. Despite its promising pharmacological activity, Curcumin's low oral bioavailability has remained a major hurdle. Biologists, who have studied Curcumin extensively, are puzzled by its bioavailability problems. History of use for over 100 years, on the other hand has prevented pharmaceutical scientists, who specialize in addressing this issue, from this pursuit. After the research for Curcumin hit a roadblock due to the poor biopharmaceutical properties, most importantly the low solubility and poor intestinal permeability, it has become a molecule of interest to the drug delivery scientists. Considering the potential of nanoparticles as oral drug delivery system, the present investigation involved development and characterization of polymeric Curcumin nanoparticles with a view to improve its oral bioavailability.

Curcumin loaded SLNs were prepared by hot homogenization followed by ultrasonication method. The process yielded SLNs with 200-300 nm with higher entrapment efficiency. Both the solid lipids selected for the study Compritol 888 ATO and Precirol ATO 5 yielded SLNs with the desired particle size with high entrapment efficiency. For the purpose of further *in vivo* studies and characterization, SLNs

formulated with Compritol 888 ATO were selected. The SLNs were also subjected to *in vitro* release studies. The selection of dissolution medium for such poorly soluble drugs has always been a challenge. For the present study, Phosphate buffer pH 7.4 was selected as the dissolution medium. The results of the *in vitro* release studies not only exhibited improved solubility but also sustained release pattern for Curcumin from the nanoparticles. The release of drug from nanoparticles was affected by several parameters which include physiochemical properties of drug and polymer, size of particles, type of surfactant/stabilizer used for particle preparation, amount of drug loading and nature of release medium and the study may not provide true insight considering the difficulties involved in establishing IVIV.

Apart from solubility and permeability, stability of Curcumin also presents a major challenge in the formulation of dosage forms. Curcumin is highly sensitive to light and oxidation. It is well known that a lyoprotectant is necessary to decrease nanoparticle aggregation during the lyophilization process. It was demonstrated that encapsulation of Curcumin into SLNs also improved stability of Curcumin. Compared to native Curcumin, which got degraded by 97% in only 3 h, freeze dried Curcumin loaded SLNs were stable for 6 months at 2-8ºC. Thus freeze drying of Cu-SLNs with sucrose or dextrose as cryoprotectants, improved long term stability of Curcumin. These findings are in agreement with the results of other researchers suggesting that the transformation of SLNs into a dry powder can prevent the aggregation of nanoparticles and improve the stability of light and oxygen sensitive substances [9].

In vivo pharmacokinetics studies in Rat plasma revealed that encapsulation of Curcumin into SLNs increased relative bioavailability of Curcumin by 12 folds. Marketed capsule formulation of Curcumin (Adcumin) was selected for comparison. It was found that native Curcumin showed negligible plasma levels when administered through oral route. While nanoparticulate Curcumin led to sustained release of Curcumin into plasma making it bioavailable. Raw Curcumin through oral route thus may only be used for ailments that require local action while nanoparticulate Curcumin may also be useful for malignancies that require systemic action.

Past research to improve Curcumin bioavailability has shown that bioavailability of Curcumin can be increased by combining it with Piperine, a well-known inhibitor of hepatic and intestinal glucuronidation [10]. Shaikh *et al.* [11] prepared polymeric nanoparticles of Curcumin and demonstrated that nanoparticulate encapsulation of Curcumin improved bioavailability by 9 folds compared to Curcumin administered with Piperine as absorption enhancer. Solid lipid nanoparticles present advantages over polymeric nanoparticles in the way that solid lipid nanoparticles are more biologically accepted as they are made from biodegradable solid lipids and they offer higher potential for sustained release as depicted in the present study.

CONCLUSION

Curcumin loaded solid lipid nanoparticles were successfully formulated using high pressure homogenization followed by ultra-sonication technique. The mean particle size could be achieved in the range of 200-300 nm with entrapment efficiency of more than 70%. The *in vivo* pharmacokinetics studies in rats have demonstrated that encapsulation into nano carriers can significantly improve oral bioavailability and stability of Curcumin. The major hurdle of bringing this molecule, with tremendous therapeutic potential to clinic is possible by means of nanotechnology and further trials in human volunteers need to be conducted to establish safety and efficacy.

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CONFLICT OF INTERESTS

Declared None

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