IJPSR (2019), Volume 10, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 August 2018; received in revised form, 28 January 2019; accepted, 09 March 2019; published 01 April 2019

PARENTERAL ADMINISTERED SUSTAINED RELEASE PIPERINE MICROPARTICLES INTENDED FOR TREATMENT OF LIVER FIBROSIS

B. Chandra Shekhar Reddy [*1], Krishnaveni Bommineni [2] and Jithan Aukunuru [3]

Department of Pharmaceutics [1], Department of Pharmacognosy [2], Vaagdevi Pharmacy College, Warangal - 506005, Telangana, India.
Department of Pharmaceutics [3], Omega College of Pharmacy, Hyderabad - 501301, Telangana, India.

Keywords:

Piperine, Microparticles, Liver fibrosis, Pharmacokinetics, Hepatoprotective activity

Correspondence to Author:
Dr. B. Chandra Shekhar Reddy

Associate Professor,
Department of Pharmaceutics,
Vaagdevi Pharmacy College,
Warangal - 506005, Telangana,
India.

E-mail: reachbcsr@gmail.com

ABSTRACT: The objective of this work was to formulate and characterize microparticles containing piperine, and evaluate their activity against carbon tetrachloride (CCl_4)-induced liver toxicity. Piperine microparticles were formulated by o/w emulsion solvent evaporation technique using poly-ε-caprolactone as a polymer. Four different microparticle formulations (PM1, PM2, PM3, and PM4) were prepared by varying the drug/polymer ratio. The particles were characterized for particle size, drug content, surface morphology, and *in-vitro* drug release. The pharmacokinetics and pharmacodynamics of the piperine formulations in male Wistar rats were evaluated following intraperitoneal administration, using piperine solution as reference. The hepatoprotective activity of the formulation was determined in a CCl_4-treated rat model and also compared with piperine solution. Piperine microparticles were successfully prepared using o/w emulsion solvent evaporation technique. The microparticles sustained the release of the drug both *in-vitro* and *in-vivo* for up to 10 days and offered better pharmacokinetic properties than the free drug itself. Microparticle formulation tested *in-vivo* demonstrated better pharmacokinetics and pharmacodynamics compared to the reference. Drug levels in the liver were significantly higher with the microparticular formulation. The piperine microparticles produced a significant decrease in both transaminase levels when challenged with CCl_4 intraperitoneally. Positive results of these studies gave an insight that microparticles are more effective and suitable for targeted and sustained drug delivery to the liver.

INTRODUCTION: Statistics indicate that liver fibrosis/cirrhosis is one of the leading causes of death in several parts of the world. For the past 20 years, liver cirrhosis has been extensively studied. In the initial stages, this disease is characterized by liver fibrosis.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(4).1935-43

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(4).1935-43

Liver fibrosis is caused because of a variety of reasons. When fibrosis becomes cirrhosis, life-threatening complications occur which include variceal bleeding, ascites formation, and hepatorenal syndrome, among others.

These complications are an economic burden to the society, and epidemiological studies indicate that this burden would increase tremendously shortly [1]. There is no clinically useful drug to treat this disorder. Clinically trial and preclinical data showed that several hepatoprotective drugs could cure or reverse the progression of this disease. Clinical interventions to stop the progression of this

disease or to heal it are in the investigational stages. Several hepatoprotective drugs are included in this category. Hepatoprotective, anti-inflammatory and antioxidants drugs along with several other direct antifibrotic drugs can reduce/reverse liver fibrosis. The drugs that demonstrated promise against liver fibrosis are not sufficiently compelling and cause too many adverse effects. Therefore, drug targeting and sustained release dosage forms are options to maximize efficacy and minimize adverse drug reactions. The objective of this study is to develop targeted and sustained release piperine microspheres and further evaluate the efficacy of these delivery systems when compared to conventional modes of drug delivery in a rat liver fibrosis model.

Piperine is a natural alkaloid which previously demonstrated hepatoprotective activity[2]. It is an active alkaloid isolated from *Piper longum* and *Piper nigrum*. Its validated use in liver fibrosis has been demonstrated with the demonstration of its hepatoprotective activity. In a tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity model, piperine demonstrated a reduction in both *in-vitro* and *in-vivo* lipid peroxidation, enzymatic leakage of GPT and AP and prevented the depletion of GSH and total thiols in the intoxicated mice. Inhibition of the reactive oxygen species (ROS) production and replenishment of GSH by piperine along with its antifibrotic properties may in part be responsible for its hepatoprotective activity [3].

Since, there is strong evidence regarding its use in hepatoprotection; we aimed to develop a sustained release dosage form for piperine delivery to the liver. Biodegradable polyester biomaterial polycaprolactone (PCL) has been very widely used because of their biodegradation characteristics, and they have been approved by the Food and Drug Administration [4]. PCL degrades slowly when compared to PLGA and is more suitable for long-term drug delivery. PCL is also less expensive compared to PLGA copolymers. Hence, in this work, low molecular weight PCL has been chosen for the preparation of piperine-loaded microsphere. Microspheres prepared from low molecular weight PCL can reduce the degradation time of microspheres and its associated debris after the complete release of the drug.

The present study aims to formulate sustained release system such as biodegradable microspheres that can improve patient compliance, reduce side effects and increase tissue levels of the drugs can be conveniently used to treat fibrosis with added advantages. The microsphere preparation, piperine solubility, and microsphere characterization were investigated by size measurement, entrapment efficiency, *in-vitro* release, FTIR, SEM, stability testing and for sterility. Also, pharmacokinetic, cellular uptake and pharmacodynamic studies were also evaluated.

MATERIALS AND METHODS:

Materials: Piperine and Poly-ε-caprolactone (mol wt, 14,000) were procured from Sigma-Aldrich, Germany. Polyvinyl alcohol (PVA, cold-water-soluble) was procured from Qualikems Fine Chemicals Pvt Ltd, New Delhi. Dichloromethane and HPLC grade Methanol were procured from Finar Chemicals, Ahmedabad, India. All other reagents were of analytical grade.

Methods:

Preparation of Piperine microparticles: Emulsion (O/W) solvent evaporation method was employed in the preparation of piperine microspheres using polycaprolactone (PCL) as the polymer [5]. Four different microparticle formulations PMP_1, PMP_2, PMP_3 and PMP_4 containing drug: the polymer in the ratio of 1:1, 1:2, 1:3 and 1:4 respectively, were prepared. For the preparation, piperine (100 mg) and PCL (100, 200, 300 and 400 mg) was dissolved in 20 ml of dichloromethane (DCM) by vortexing. The mixture (organic phase) was added drop-wise to 50 ml of 0.5% PVA solution while stirring by use of magnetic stirrer to obtain an O/W emulsion [6-7]. Stirring was continued until the total evaporation of the solvent (DCM). The microspheres were then recovered by filtration, washed three times with double distilled water and dried at room temperature for 12 h. Four formulations (PMP1-PMP4) were prepared and varying concentration of PCL. Blank microspheres without piperine were prepared in a similar way as that of the microparticle formulation.

***In-vitro* Characterization of Microspheres:** The microsphere formulations were evaluated for percent entrapment, particle size, surface

morphology, drug-excipient interaction, and *in-vitro* drug release. Percentage yield was also calculated.

Percentage yield = Practical weight × 100/Theoretical weight

Determination of Particle Size: The mean particle size of the piperine microspheres were determined by optical microscopy. The eyepiece micrometer was calibrated using a stage micrometer, and the calibration is undertaken to find out the measure of each division using the stage micrometer. After calibration, the eyepiece micrometer is used for determining the size of microspheres. The microspheres were mounted on a slide and observed under the microscope. At least 200 microspheres were measured for each preparation and the mean diameter was calculated. The shape of the microspheres was visualized, and the photographs were taken with the aid of a binocular microscope (QUASMO, ANISO, PZRM 700 India 9001-2000).

Determination of Encapsulation Efficiency: An accurately weighed 20 mg microspheres were dissolved in 1ml chloroform and 9 ml methanol. The absorbance associated with the dissolved piperine was determined. A 20 mg of blank microspheres were also dissolved in the same solvent and absorbance measured at the same wavelength. The absorbance associated with the piperine was a subtraction of the absorbance of the piperine microspheres and the blank microspheres. Drug content was calculated from the standard curve. Percentage Encapsulation Efficiency (EE) was calculated by using the formula:

Encapsulation Efficiency = Actual drug content × 100 / Theoretical drug content

Particle Shape and Surface Morphology: Morphological characterization of the microspheres was done by using Scanning electron microscope (JEOL JSM -5200). The samples were coated to 200A° thickness with gold-palladium using before microscopy. Microcapsules before dissolution study were only subjected to SEM study.

Fourier Transform Infrared Spectral (FTIR) Analysis: FTIR spectra were taken on to investigate the possible chemical interactions between the drug and the polymer in the microsphere formulation. An FTIR analysis was carried out by using Bruker alpha spectro-photometer. Samples were crushed with KBr to get the pellets. The spectra of piperine, PCL, empty microspheres and piperine-loaded microspheres (optimized) were recorded.

***In-vitro* Release Study:** The *in-vitro* release study was performed in a diffusion cell developed in house. An inverted cylindrical test tube cut to a height of 8 cm was used as a donor cell. The receiver compartment consisted of 100 ml of phosphate buffer (pH 7.4). A dialysis membrane soaked in warm water for 30 min was placed at the lower end of the cylindrical portion. Microspheres containing 50 mg of drug was suspended into 5 ml of pH 7.4 buffer and placed in the donor compartment. The donor compartment was inserted into the receiver compartment such that the height was sufficient for the drug to be released into the receiver. The system was stirred using a magnetic stirrer and bead. Samples (5 ml) were removed from the receiver compartment and replaced with the same volume of fresh medium immediately. The samples were analyzed by UV spectrophotometer at 343 nm.

Stability Testing: Stability studies of Piperine microparticles was done as per the ICH guidelines at 4 °C ± 2 °C, 25 ± 0.5 °C (60% RH) and 40 ± 0.5 °C (75% RH). Freshly prepared microparticles were sealed in vials and kept in stability chambers (Thermolab Scientific equipment, India) for 25 °C and 40 °C and in refrigerator for 4 ± 2 °C for 4 weeks. Microparticles were withdrawn after one month and tested for any changes in the physical appearance, particle size, and drug content.

***In-vivo* studies:** Male Wister rats (weighing 150 – 180 g each) were purchased from Mahaveer Enterprises, Hyderabad, India, and were maintained in an air-conditioned room at 22 ± 2 ºC and relative humidity of 45 – 55 % in a 12/12 h light/dark cycle. The animals had free access to standard food pellets and water was available *ad libitum*. All the animal experiments were conducted according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India [8] and the study protocol was approved by Institutional Animal Ethical Committee of Vaagdevi College of Pharmacy, Warangal, India (ref. no. 1047/ac/07/ CPCSEA).

International guidelines issued by the International Council for Laboratory Animal Science were also followed [9]. These conditions were maintained throughout the experiment. The study was performed in two groups of six rats each.

Group 1 received Piperine solution containing 30 mg/kg intraperitoneally; Group 2 received PMP1 piperine microspheres equivalent to 30 mg/kg of drug suspended in normal saline and injected intraperitoneally. Blood samples were collected at different time intervals throughout 24 h. For the microparticle formulations, samples were also collected at days 3, 6 and 9 after administration. Drug levels in the plasma samples were evaluated by HPLC. Drug levels in various tissues like liver, kidney, lung, and brain were determined by isolating tissues from the rats. HPLC standard curve for the drug in plasma was also generated [10]. The collected blood samples were centrifuged at a speed of 3000 rpm for 10 min and plasma was separated into other micro centrifuge tube by using micro pipette and stored in the deep freeze. The drug was extracted from the plasma by adding 500 µl of ethyl acetate, and vortexed on a cyclomixer for 20 min. The organic phase was separated and collected into another micro centrifuge tube and allowed to dryness. These dried samples were reconstituted in 200 µl of Methanol: distilled water (75:25 v/v) and analyzed at 343 nm wavelength using HPLC. The following pharmacokinetic parameters were determined using Kinetica pharmacokinetic data analysis software: elimination rate constant (K_E), the volume of distribution (Vd), elimination half-life (t1/2), clearance (CL), mean residence time (MRT) and area under the curve (AUC).

Tissue Distribution Studies: Drug level in tissues like liver, kidney, lung, and brain were determined by isolating tissues from the rats. The tissues were chopped into small pieces and minced with ethyl acetate. 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 and water (75:25 v/v).

Evaluation of Hepatoprotective Activity: Carbon tetrachloride (CCl_4)-induced liver damage model was used in the evaluation of the hepatoprotective activity. For this purpose, another set of male Wistar rats were divided into four groups each containing 6 rats. Group 1 received normal saline (1 ml/rat) daily for 9 days and served as normal control. Group 2 received CCl_4 (dissolved in 3 times its volume of olive) at a dose of 0.7 ml/kg intraperitoneally on days 1, 3 and 6 served as toxic control. Group 3 received the piperine solution in a dose of 10 mg/kg intraperitoneally daily for 9 days. Group 4 received piperine microspheres suspension equivalent to 90 mg/kg of drug intraperitoneally on day 1. All the groups received CCl_4 at days 1, 3 and 6 of the study except normal control.

The animals were anesthetized on the last day of the study and blood was collected from the rat's retro-orbital plexus of the eye (1 ml). Plasma was separated from the blood samples by centrifugation at 3000 rpm for 15 min. Hepatoprotective activity was quantified by the serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT) levels present in the plasma. After draining the blood, liver samples were excised, washed with normal saline and processed separately, for histological observations. The liver was immediately removed and fixed in formalin, serially sectioned and microscopically examined after staining with hematoxylin and eosin to analyze pathological changes. The body weights of the rats were also monitored.

Statistical Analysis: The data were expressed as mean ± standard deviation (SD) and statistical analysis was carried out by one-way ANOVA followed by Student's Newman-Keuls test. The level of significance used was $P<0.05$. The statistical software used was Graph Pad Prism, USA, versions 4 and 5.

RESULTS AND DISCUSSION: Piperine microspheres were efficiently prepared to utilize polycaprolactone by O/W emulsion solvent evaporation process. The mean particle sizes of piperine microspheres with different d/p ratio were shown in **Table 1**. Increase in the concentration of polymer increased in mean particle size. It could be suggested that a higher concentration of polymer may lead to increased frequency of collisions, resulting in fusion of semi particles and finally producing bigger particles thereby increasing the size of microspheres [11]. Encapsulation efficiency of

PCL microparticles containing piperine is presented in **Table 1**. Results show that enhance the concentration of polymer resulted in improvements in encapsulation efficiency. This could be due to the enhancement of viscosity because of the higher concentration of polymer in emulsion droplets, which in turn prevents drug diffusion to the external aqueous phase and eventually raises drug entrapment efficiency. As the d/p ratio decreases, the quantity of polymer present was inadequate to cover the drugs completely. The results were by the previous reports[12] which states that when the d/p ratio is increased it ends in an increase in viscosity of primary emulsion and thereby lowers the partition of drugs into external phase and consequently raises drug entrapment efficiency.

TABLE 1: PARTICLE SIZE AND ENTRAPMENT EFFICIENCY OF PIPERINE MICROPARTICLES

Formulation code	Particle size (μm)	% Drug entrapment
PMP1	5.5 ± 1.25	62.5 ± 3.0
PMP2	6.2 ± 1.8	65 ± 4.5
PMP3	8.2 ± 2.25	68 ± 5.5
PMP4	10.5 ± 2.2	72 ± 4.5

The morphological characterization of microspheres would help to correlate the particle size and surface characteristics with other determined properties. The microspheres obtained were free-flowing. The microspheres were being circular without any pores as demonstrated using scanning electron micrographs **Fig. 1**. The smooth surface of the microspheres reveals the complete removal of the organic solvents during the fabrication process. The presence of loosely bound drug on the surface of the microspheres is evident in the photomicrographs.

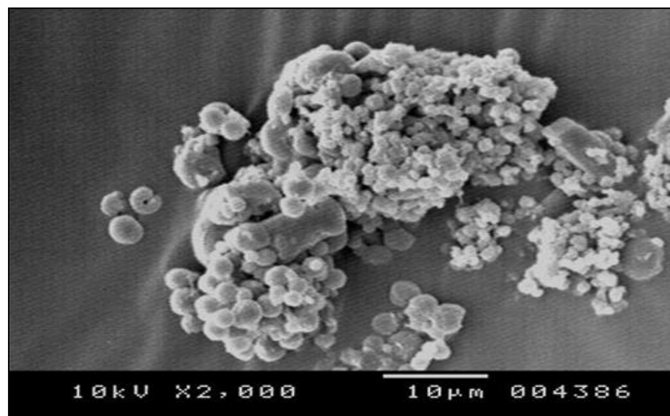


FIG. 1: SCANNING ELECTRON MICROSCOPIC (SEM) IMAGE OF PIPERINE MICROPARTICLES

Drug-Polymer Interactions: The FTIR spectra of Piperine, Polycaprolactone, Placebo microparticles, and drug-loaded microparticles were shown in **Fig. 2**. The FTIR spectra of piperine showed characteristic peaks at 2939 cm^{-1} due to aliphatic C-H stretching, C-O-C stretching at 1250 cm^{-1}, C-N stretching at 1192 cm^{-1} and C-N stretching at 1632 cm^{-1}. FTIR spectra of polycaprolactone showed characteristic peaks at 2942 cm^{-1} due to aliphatic C-H stretching, ester stretching at 1720 cm^{-1}, C=C aromatic stretching at 1364 cm^{-1}, C-O-C stretching at 1238 cm^{-1}, and C-N stretching at 1161 cm^{-1}. IR spectra of placebo microparticles showed the same characteristic peaks which are seen in IR spectra of polycaprolactone. No additional peaks were seen in IR spectra of piperine microparticles. The FTIR spectra concluded that all of the piperine, polycaprolactone, placebo microparticles and drug-loaded microparticles exhibited the characteristic bands which confirm no interaction.

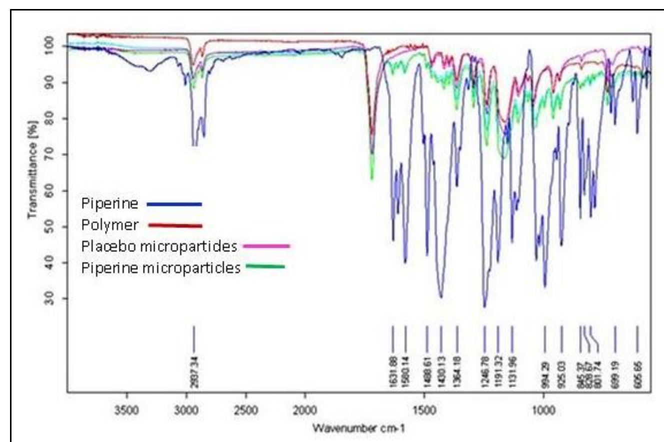


FIG. 2: FTIR SPECTRA OF PIPERINE, POLY-CAPROLACTONE, PLACEBO MICROPARTICLES, AND PIPERINE MICROPARTICLES

In-vitro drug release rates were shown in **Fig. 3.** Release data shows that raise in the polymer content detain the drug release because of enhanced particle size and decreased surface area. Samples were withdrawn at an interval of 24 h, and absorbances were measured by UV spectrophotometer at 343 nm. The release rates of all the microspheres demonstrated a pattern of increasing release. At the end of 24 hrs an average of 25% of the drug was released from all batches of microspheres. A biphasic drug release style has been observed, *i.e.,* burst release accompanied by delayed release. The initial burst effect could be described with the release of some drug loosely bound on the surface of the microspheres [13].

An additional cause for the burst release may be due to unstable nature of the inner water emulsion droplets throughout the solvent evaporation that ends in their coalescence and probably have brought the drug to discover at the surface of the microparticles [14]. The release of drug from microspheres with a lower concentration of polymer was much more rapid than those with higher polymer concentration. Drug release from PMP1 was greater than PMP2, PMP3, and PMP4. This could be due to a rise in polymer concentration, which in turn resulted in the progress of very dense, least porous polymer matrix leading to reduced release rate. This increase in release rate could be correlated with the smaller particle size and hence increased the surface area of the microsphere.

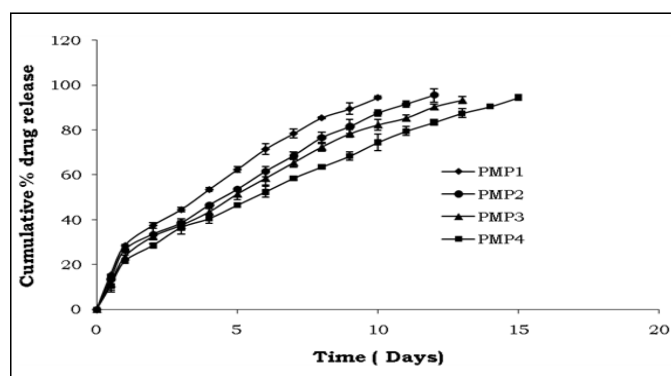


FIG. 3: CUMULATIVE % RELEASED *vs*. TIME PLOTS OF PIPERINE MICROPARTICLES

To determine the process of drug release the experimental data had been fitted into various kinetic equations. The R^2 values range between 0.9585–0.9690 (Zero order), 0.9566–0.9681 (First-order), 0.9920–0.9901 (Higuchi), 0.9906–0.9899 (Peppas). Release rates of drug from all these formulations could be best stated by the Higuchi equation, as the graphs show high linearity. From the Higuchi model, it is evident that the drug is released by a diffusion process. For confirmation, the data was fitted into the Korsmeyer–Peppas formula. The slope (n) values for the Peppas model ranged from 0.581-0.610. All the formulations follow nonfickian diffusion. The slow and continuous release of the drug might be due to diffusion of drug through polymer as well as on account of erosion involving hydrophobic polymer [15]. Furthermore, characterization of the crystal form of the drug in the polymer might reveal any adequate basis for the slow release [16].

Stability Studies: Stability studies have been performed for Piperine MP1 formulation to check any changes in physical appearance, particle size, and drug content. It was observed that there was no color change of microparticles. No considerable changes were shown in the particle size of the formulation after storage for 1 month. The percentage residual drug content has been estimated and was found in the range of 98.5-97.5, 98.5-96.5 and 98-95 respectively at 4 °C ± 2 °C, 25 ± 0.5 °C and 40 ± 0.5 °C. Not any substantial variations in percent residual drug content were seen in the micro particular formulation showing all formulations were stable.

***In-vivo* Studies:** HPLC method has been utilized to calculate the drug quantities in plasma and tissues. Extraction efficiency was 92%. The retention time was 6.6-7.0 min, and the minimum detection level was 10 ng/ml. The mean plasma concentration-time curves of piperine after intraperitoneal administration of piperine solution and piperine microspheres to rats were shown in **Fig. 4**. From plasma profile, it was seen that the microspheres extended the drug release for 9 days. This might be due to a reduction in the elimination and metabolism. The pharmacokinetic parameters of piperine solution and piperine microspheres calculated according to one compartmental method. The major PK parameters are shown in **Table 2**.

The MRT of microsphere formulation was 8.4 times higher than that of the solution, which suggested that encapsulation of the piperine into microsphere could produce a remarkably sustained release of the drug *in-vivo*. The K_E and CL values of the microspheres significantly decreased compared with those of the solution, suggesting that the microsphere formulation was more slowly removed from plasma compared with the solution. The $t_{1/2}$ and AUC (0- T) values of microsphere formulation were significantly higher than the drug solution. AUC (0- T) of microspheres was 6.6 times greater than the solution. The apparent volume of distribution at steady state (*V*dss) of piperine microspheres was slightly lower than that of piperine solution. These results indicated that Piperine microspheres could significantly retard the clearance of piperine in blood to give high plasma levels compared to piperine solution.

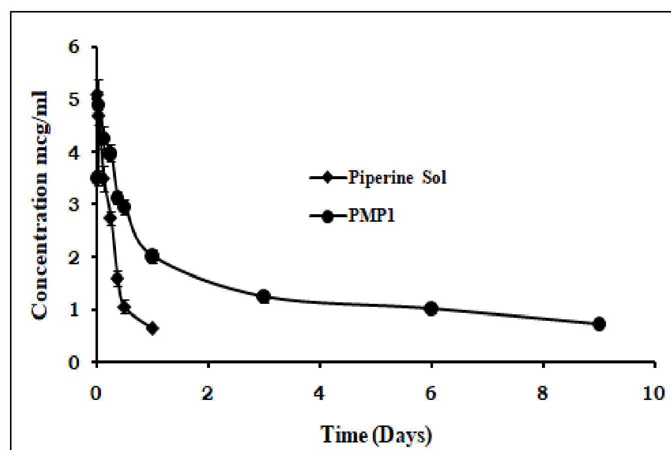


FIG. 4: PLASMA CONCENTRATION-TIME PROFILES OF PIPERINE FORMULATIONS

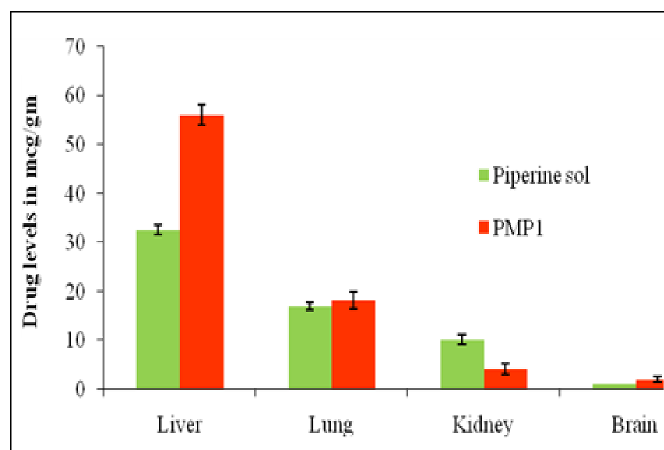


FIG. 5: PIPERINE LEVELS IN VARIOUS TISSUES

TABLE 2: PHARMACOKINETIC PARAMETERS FOR PIPERINE MICROPARTICLES

Parameter	Piperine Sol	PMP1
C_{max} (µg/ml)	5.1 ± 0.2	4.9 ± 0.2
Ke (h^{-1})	0.039 ± 0.004	0.0061 ± 0.001
$t_{1/2}$(h)	17.34 ± 0.25	112.63 ± 1.23
MRT (h)	18.94 ± 0.3	160.79 ± 4.5
Vd(L)	12.69 ± 0.25	12.16 ± 0.25
Clearance(L/h)	0.507 ± 0.02	0.074 ± 0.01
$AUC_{0-\infty}$(µg.h/ml)	59.12 ± 2.29	400.5 ± 3.5

Distribution data of piperine in plasma and other tissues including liver, lung, kidney, and brain has been measured after administrating piperine solution and piperine microspheres to rats **Fig. 5**. From experimental studies, it was witnessed that drug is more in the liver in comparison to lungs, kidney, and brain. Drug amounts inside liver were greater due to improvements in the degree of the drug obtained in the liver cells when given in the form of particulates.

The Piperine formulations were screened for hepatoprotective activity in rats. To test the hepatoprotective activity, the formulation was administered to CCl_4 induced model. Carbon tetrachloride, a known hepatotoxin is a commonly used model for hepatoprotective drug screening, and the severity of the liver damage is measured by the levels of elevated cytoplasmic enzymes (SGOT and SGPT) in circulation [17]. Three rats died in the CCl_4 group, and no rats died in the other three groups during the whole experimental period. The body weight was decreased in CCl_4 treated groups in comparison with that in the normal group ($P<0.01$). There were no considerable differences among CCl_4 intoxicated groups and piperine treatment groups. However, piperine treated groups had a slight increase in body weight **Table 3**. Evaluation of the serum enzymes can be a beneficial quantitative marker of the degree and type of hepatocellular damage.

TABLE 3: EFFECT OF PIPERINE FORMULATIONS ON SGOT AND SGPT LEVELS

Groups	Initial body weight (g)	Bodyweight after 9 days (g)	SGPT (U/L)	SGOT (U/L)
Control	165 ± 10	180 ± 15	11.2 ± 3.5	18.5 ± 1.25
CCl_4	160 ± 15	148 ± 10	47.5 ± 3.5	75.6 ± 2.4
CCl_4 + Piperine Sol	165 ± 5	172 ± 5	25.5 ± 2.5	34.5 ± 3
CCl_4 + PMP1	170 ± 6	180 ± 15	18.5 ± 1.5	25.2 ± 2.6

Increased serum level of SGPT and SGOT is associated with liver damage. CCl_4 administration generated a significant rise in serum SGOT to 75.6 ± 2.4 U/L in comparison with the normal value that was 18.5 ± 1.25 U/L **Fig. 6**. Administration of piperine solution and piperine microspheres produced a substantial lowering in SGOT levels to reach 34.5 ± 3 U/L and 25.2 ± 2.6 U/L, respectively. Concurrently, CCl_4 administration triggered in a tremendous rise in serum SGPT to 47.5 ± 3.5 U/L in comparison with normal value, which was assessed as 11.2 ± 3.5 U/L. Piperine solution and microspheres produced a considerable change in serum SGPT to reach 25.5 ± 2.5 U/L. and 18.5 ± 1.5 U/L, respectively, were produced. Treatment with 100 mg dose of piperine microparticles intraperitoneally reversed the elevation of the liver enzymes found in CCl_4 treated rats.

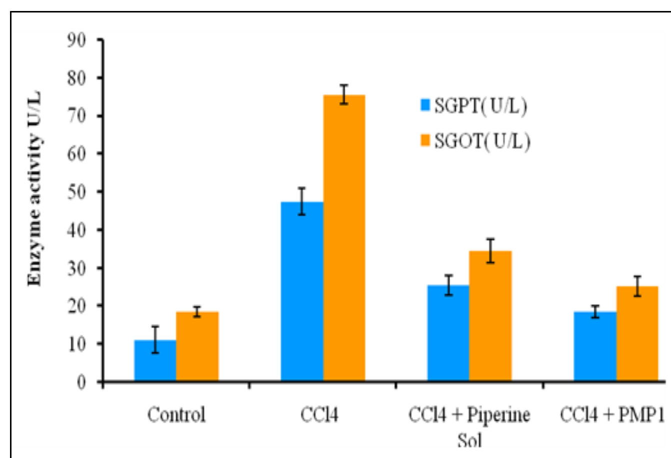
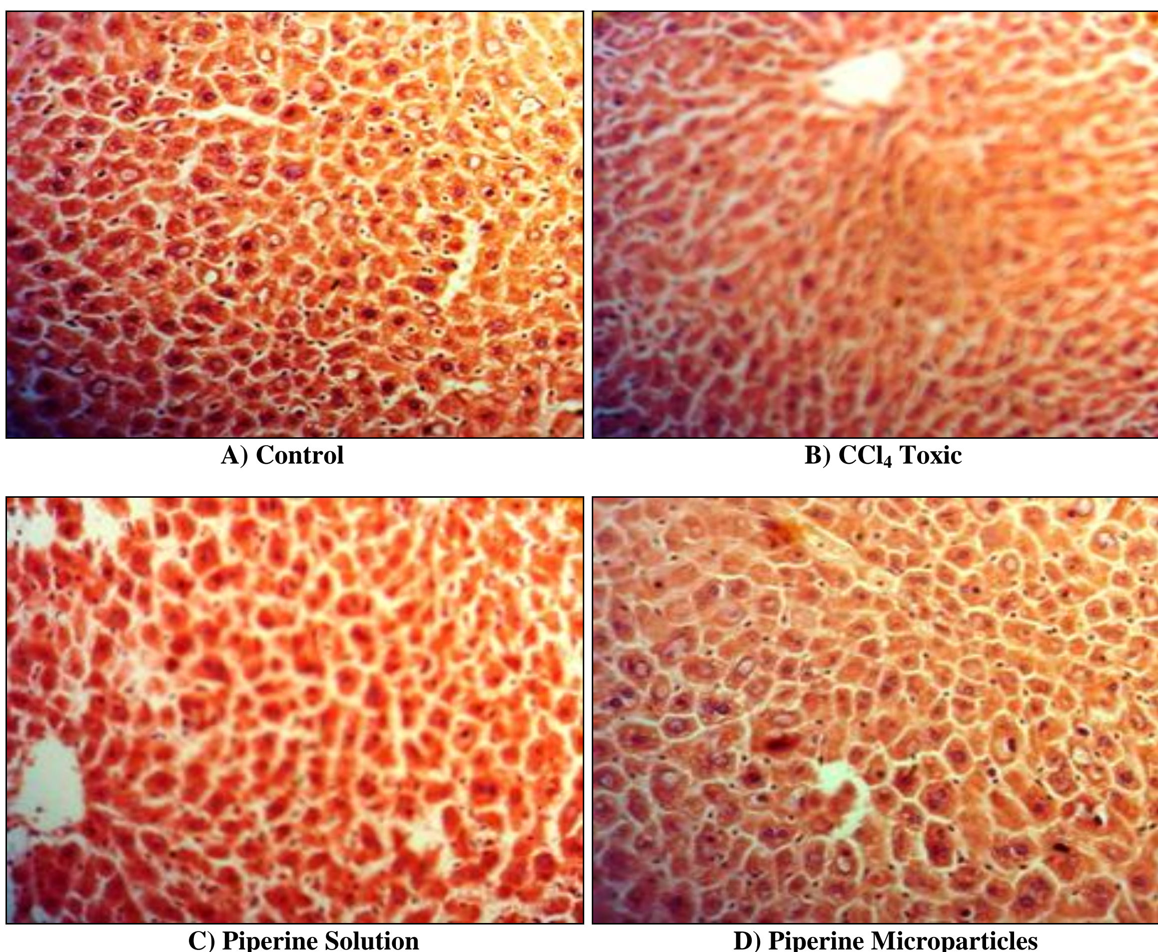


FIG. 6: EFFECT OF PIPERINE AND ITS FORMS ON SGOT AND SGPT LEVELS

Histopathological pictures were shown in **Fig. 7**. The regular hepatic structure is having unique hepatic cells, well-presented cytoplasm sinusoidal spaces and the central vein is shown by control animals **Fig. 7A**. Disarrangement regarding normal cells along with extreme centrilobular necrosis had been noticed in CCl_4 intoxication liver **Fig. 7B**. Administration of piperine solution did show a moderate change in regeneration with mild inflammation and some spotty necrosis **Fig. C**. Administration of piperine microparticles showed a greater number of regenerating liver cells; no inflammatory cells and fibrosis; liver tissue restored its normal structure **Fig. 7D**.



A) Control **B) CCl_4 Toxic**

C) Piperine Solution **D) Piperine Microparticles**

FIG. 7: HISTOPATHOLOGY OF LIVER

CONCLUSION: Piperine microparticles can be suitably prepared by emulsion solvent evaporation technique using polycaprolactone as a biodegradable polymer. The particles showed good encapsulation efficiency and sustained drug release both *in-vitro* and *in-vivo*. Piperine microparticles offer an effective approach for drug targeting of the liver.

ACKNOWLEDGEMENT: The authors are grateful to the management of Vaagdevi College of Pharmacy for the facilities, acknowledges for permitting to do Ph.D. work at this institute under the supervision of Prof. (Dr). Aukunuru Jithan, Ph.D.

CONFLICT OF INTEREST: The authors declare that the contents of this article have no conflict of interest.

REFERENCES:

1. Udompap P, Mannalithara A, Heo NY, Kim D and Kim WR: Increasing prevalence of cirrhosis among U.S. adults aware or unaware of their chronic hepatitis C virus infection. Journal of Hepatology 2016; 64: 1027-1032.
2. Gorgani L, Mohammadi M, Najafpour GD and Nikzad M: Piperine-The Bioactive Compound of Black Pepper: From Isolation to Medicinal Formulations. Comprehensive Reviews in Food Science and Food Safety 2017; 16: 124-40.
3. Meghwal M and Goswami T: Piper nigrum and piperine: an update. Phytotherapy Research 2013; 27: 1121-1130.
4. Woodruff MA and Hutmacher DW: The return of a forgotten polymer- polycaprolactone in the 21st century. Progress in Polymer Science 2010; 35(10): 1217-1256.
5. Riahi A: Oil-in-Water emulsion-solvent evaporation fabrication of polycaprolactone containing piroxicam for drug release. Medbiotech Journal 2018; 2(1): 117-123.
6. Behera AK, Barik BB and Joshi S: Poly-εCaprolactone based microspheres and nanospheres: a review. FS Journal of Pharmaceutical Research 2012; 1(2): 38-45.
7. Madan JR, Kadam V, Bandavane S and Dua K: Formulation and evaluation of microspheres containing ropinirole hydrochloride using biodegradable polymers. Asian Journal of Pharmaceutics 2013; 7(4): 184-188.
8. CPCSEA guidelines for laboratory animal facility. Indian Journal of Pharmacology 2003; 35(4): 257-274.
9. Demers G, Griffin G, De Vroey G, Haywood JR, Zurlo J and Bedard M: Harmonization of animal care and use guidance. Science 2006; 312: 700-701.
10. Veerareddy P and Vobalaboina V: Pharmacokinetics and tissue distribution of piperine lipid nanospheres. Pharmazie 2008; 63(5): 352-355.
11. Dhanarajua MD, Sathyamoorthya N, Sundara VD and Suresh CB: Preparation of poly (epsilon-caprolactone) microspheres containing etoposide by a solvent evaporation method. Asian Journal of Pharmaceutical Sciences 2010; 5(3): 114-122.
12. Sharma N, Madan P and Lin S: Effect of process and formulation variables on the preparation of parenteral paclitaxel-loaded biodegradable polymeric nanoparticles: A co-surfactant study. Asian Journal of Pharmaceutical Sciences 2016; 11(3): 404-416.
13. Kakade SM and Hassan DM: Effects of Formulation Parameters on the Characteristics of Biodegradable Microspheres of Goserelin Acetate. Asian Journal of Pharmaceutics 2018; 12(2): 691-697.
14. Farhangi M, Dadashzadeh S and Bolourchian N: Biodegradable gelatin microspheres as controlled release intraarticular delivery system: the effect of formulation variables. Indian Journal of Pharmaceutical Sciences 2017; 79(1): 105-112.
15. Raval A, Parikh J and Engineer C: Mechanism of controlled release kinetics from Medical devices. Brazilian Journal of Chemical Engineering 2010; 27(2): 211-225.
16. Censi R and Martino PD: Polymorph Impact on the Bioavailability and Stability of Poorly Soluble Drugs. Molecules 2015; 20(10): 18759-18776.
17. Lima TB, Suja A, Jisa OS, Sathyanarayanan S and Remya KS: Hepatoprotective activity of LIV-first against carbon tetrachloride-induced hepatotoxicity in albino rats. International Journal of Green Pharmacy 2010; 4(2): 71-74.

How to cite this article:
Reddy BCS, Bommineni K and Aukunuru J: Parenteral administered sustained release piperine microparticles intended for treatment of liver fibrosis. Int J Pharm Sci & Res 2019; 10(4): 1935-43. doi: 10.13040/IJPSR.0975-8232.10(4).1935-43.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Play store)