Liver targeted Piperine Nanoparticles: Preparation and Characterization

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ABSTRACT:

The objective of this study was to fabricate biodegradable nanoparticle formulation of piperine and evaluate their activity against CCl_4 liver toxicity. Piperine nanoparticles were fabricated by o/w emulsion solvent evaporation technique using polycaprolactone as the polymer. Four different nanoparticle formulations (PNP1, PNP2, PNP3 and PNP4) were prepared by varying the drug / polymer ratio. The nanoparticles were characterized for drug content, particle size, charge, in-vitro drug release and the drug-polymer interaction. The in-vivo properties of the formulations in male wistar rats were evaluated from pharmacokinetics and pharmacodynamics of piperine following *iv* administration of nanoparticles. Piperine solution was administered *iv* as a reference. Hepatoprotectivity of the formulation was determined in CCl_4 treated rat model. Piperine nanoparticles were successfully prepared using o/w emulsion solvent evaporation technique. The nanoparticles sustained the release of the drug both in-vitro and in-vivo for up to 10 days offered better pharmacokinetic properties than the free drug itself. Drug levels in the liver were significantly higher with the nanoparticular formulation. *IV* nanoparticulate administration reversed serum liver enzyme levels 98% compared to only 50% compared to drug solution. The developed piperine nanoparticles showed superior pharmacokinetic and hepatoprotective activity of piperine solution.

KEYWORDS: Piperine, Polycaprolactone, Nanoparticles, Solvent evaporation, Hepatoprotection.

INTRODUCTION:

Liver fibrosis and its end-stage disease cirrhosis are a major cause of mortality and morbidity throughout the world[1]. Fibrosis is a response to chronic liver injury or infection such as infectious diseases (e.g. viral hepatitis), metabolic derangements (non-alcoholic steatohepatitis), exposure to toxins (e.g. alcohol liver diseases), or autoimmune diseases (e.g. primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis) [2]. Hepatic fibrosis is normally preceded by way of continual liver damage and consequences from the progressive accumulation and reduced degradation or reworking of the extracellular matrix, which disrupts the normal architecture of the liver[3]. If left untreated, hepatic fibrosis may additionally progress into liver cirrhosis, hepatocellular carcinoma and loss of life. Liver transplantation is the only treatment available for patients with advanced stages of liver fibrosis. Therefore, new strategies for anti-fibrotic therapy are required. Unfortunately, there is no effective antifibrotic treatment approved for human use, and the point at which fibrosis becomes irreversible is not yet recognizable[4]. Currently, a large variety of drugs are being investigated for antifibrotic effects. These compounds can be classified according to their therapeutic effects, including reduction of inflammation, antioxidant properties or promotion of ECM degradation. The ideal antifibrotic therapy would be one that is liver-specific, well tolerated when administered for prolonged periods of time, effective in attenuating excessive collagen deposition without affecting normal ECM synthesis, effectively delivered and nontoxic to other organs[5].

Piperine is the principle pungent substance in pepper. It is a major constituent of piper nigrum and piper longum. Piperine showed chemopreventive, immunomodulatory, anticarcinogenic, stimulatory, antioxidant, hepatoprotective, antiinflammatory antibacterial, anticonvulsant, anti-epileptic action, antimicrobial, and antiulcer activities[6, 7]. Piperine demonstrated hepatoprotective activity for the first time and was reported by indu bala kour and aruna kapil from regional research laboratory, jammu, india[8]. Since then several direct and indirect evidence has been routinely reported regarding its hepatoprotectivity. It has anti-inflammatory, antioxidant and antifibrotic properties. Since there is a stromg evidence regarding its use in hepatoprotection. Our research group has already demonstrated the hepatoprotective activity of piperine with polycaprolactone-piperine biodegradable microspheres. However, our aim is to develop better means for further development of formulations containing piperine. Use of modern nanotechnology for nanoparticle formulations of piperine and its encapsulation in polymer matrixes are the most recent advancements to improve targeting of drugs to the cells involved in a disease can increase therapeutic effectiveness several-fold[9]. In this regard we have formulated piperine nanoparticles using a biodegradable polymer, polycaprolactone by emulsion solvent evaporation method. PCL is a biodegradable and biocompatible polymer with a very slow degradation rate, making it suitable for long-term delivery[10]. Other advantages of PCL include hydrophobicity, in vitro stability and low cost. Therefore, many investigations have focused on the application of PCL microspheres to drugs in recent years. In the present study, we aimed to develop a biodegradable nanoparticulate formulation which not only can sustain drug release but also lead to enhanced intracellular levels of the drug in liver cells, such as kupffer and sinusoidal endothelial cells following intravenous administration.

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. A

probe sonicator (Homogenizer 150 VT), used to prepare the nanoparticles, was procured from M/S Biologics, Inc USA. A zeta sizer (3000 HAS (Malvern Instruments, Malvern, UK) was used to measure the particle size. (HPLC (Cyberlab, USA) was used to analyze plasma samples while UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) was used to analyze drug loading and drug release samples. A magnetic stirrer (Remi Industries, Mumbai, India) was used to facilitate evaporation of dichloromethane while an ultracentrifuge (Remi, Mumbai) was employed to recover the nanoparticles after preparation. Male Wistar rats weighing 150 – 180 g were purchased from Mahaveer Enterprises, Hyderabad., India.

Methods:

Preparation of Piperine Nanoparticles by emulsion solvent evaporation method

Emulsion (O/W) solvent evaporation method was employed in the preparation of Piperine nanoparticles using poly-ε -caprolactone as the polymer[11]. Four different nanoparticle formulations PNP_1, PNP_2, PNP_3 and PNP_4 containing drug:polymer in the ratio of 1:1, 1:2,1:3 and 1:4, respectively, were prepared. For the preparation of nanoparticles, Piperine (100 mg) and Polycaprolactone (100, 200, 300 and 400 mg) was dissolved in 15 ml of dichloromethane by vortexing. The above organic phase was added drop wise to 50ml of 0.5 % PVA solution under ultra sonicator (150V/T probe) using a ultrahomogenizer (Biologics inc., USA) by keeping at 40w for 12min. This emulsion was placed on magnetic stirrer to ensure complete evaporation of dichloromethane, leaving nanoparticle suspension. 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.

Characterization of Piperine nanoparticles

The nanoparticle formulations were evaluated for particle size and charge, drug encapsulation, drug-excipient interaction and *in vitro* drug release[12].

Particle size, polydispersity index and zeta potential determination of Piperine nanoparticles

The nanoparticles 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 obtain suitable kilo-counts per second (kcps). Analysis was performed at 25 °C with an angle of detection of 90°. Each determination was made in triplicate.

Estimation of encapsulation efficiency of piperine nanoparticles

Encapsulation efficiency (EE) was calculated by estimating the amount of unentrapped drug. This was found by measuring the absorbance of the drug in supernatant, which was obtained after centrifugation of nanoparticle suspension and then applying equation 1.

$$EE\ (\%) = \frac{\text{Wt of drug added} - \text{Wt of free drug}}{\text{Wt of drug added}} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

The value obtained from equation 1 was compared with entrapped drug. For this determination, an accurately weighed amount of nanoparticles were taken in a test tube and dissolved in dichloromethane and the solvent allowed evaporating completely. An aliquot of methanol (10 ml) was added to the test tube which dissolved only the drug. The absorbance of this solution was measured and the amount of drug encapsulated is calculated.

Fourier transforms infrared spectroscopy analysis of Piperine nanoparticles

FTIR spectra were taken on to investigate the possible chemical interactions between the drug and the polymer in the nanoparticle formulation. An FTIR analysis was carried out by using Bruker alpha Spectrophotometer. Samples were crushed with KBr to get the pellets. The spectra of piperine, PCL, placebo nanoparticles and piperine-loaded nanoparticles (optimised) were recorded.

***In-vitro* release of prepared piperine nanoparticles**

The *in vitro* release study was performed in a diffusion cell set-up across a dialysis membrane[13]. An inverted cylindrical test tube cut to a height of 8 cm was used as a donor compartment. The receiver compartment consisted of 100 ml of phosphate buffer (pH 7.4, 37 °C) in a beaker placed over a water bath. A dialysis membrane which was pre-soaked in warm water for 30 min was placed at the lower end of the cylindrical setup and the membrane separated the donor compartment from the receiver compartment. Nanoparticles containing 20 mg of drug was suspended into 5 ml of pH 7.4 buffer and placed in the donor compartment. 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 spectrophotometrically at 343 nm wave length using UV spectrophotometer (Shimadzu UV-1800). Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from the piperine loaded particles.

Stability studies

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

Pharmacokinetics and tissue distributions of piperine nanoparticles

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 ^{0}C 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 the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India and the study protocol was approved by Institutional Animal Ethical Committee of Vaagdevi College of Pharmacy, Warangal, India[14] (ref no. 1047/ac/07/CPCSEA). International guidelines issued by the International Council for Laboratory Animal Science were also followed[15]. These conditions were maintained throughout the duration of the experiment.

The study was performed in two groups of three rats each.

Group 1 received 1ml of Piperine solution (30 mg/kg) intravenously; Group 2 received 1ml of Piperine nanoparticle formuation equivalent to 30 mg/kg intravenously.

Blood samples were collected at different time intervals over a period of 24 h. For the nanoparticle formulations samples were also collected days 3,6 and 9 after administration. Drug levels in the plasma samples were evaluated by HPLC[16]. 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. 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 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 200µl 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), volume of distribution (Vd), elimination half-life (t1/2), clearance (CL), mean residence time (MRT) and area under curve (AUC).

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 of piperine nanoparticles in CCl_4 induced model.

Carbon tetrachloride (CCl_4)-induced liver damage model was used in the evaluation of hepatoprotective activity[17]. For this purpose another set of male Wistar rats were divided into four groups each containing 6 rats. Group1 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 1st and 4^{th} day and served as toxic control. Group 3 received the drug solution in a dose of 10 mg/kg intravenously daily for 9 days. Group 4 received piperine nanoparticle (PNP1) suspension equivalent to 90 mg/kg of drug intravenously on day 1. All the groups received CCl_4 at days 1, 3and 6 of the study except normal control.

The animals were anaesthetized on the last day of the study and blood was collected from the rat's retro orbital plexus of eye (1ml). 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 analyse pathological changes[18]. 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 nanoparticles were successfully prepared by o/w emulsion solvent evaporation method using polycaprolactone as biodegradable polymer and polyvinyl alcohol as an emulsifier. Results regarding particle size, charge, PDI and entrapment efficiency of piperine nanoparticles are presented in (Table 1). The mean particle size ranged from 235-570 nm. Increase in the polymer concentration used in the preparation ended in increase in the size of the nanoparticles. The polydispersity indices (PDI) of all the nanoparticles were found to be below 0.4, suggesting all the nanoparticles were being homogenous in distribution. To estimate the physical stability of the systems, the zeta potential was assessed. As shown in (Table 1), all the zeta potentials of nanoparticles were in the range of -35 to -45mv, indicating long term stability of aqueous dispersions. The encapsulation efficiencies of all the nanoparticles were found to be increased as the polymer concentration is increased. Increase in particle size of the nanoparticles may be related to a rise in viscosity using elevating polymer levels that ended in bigger emulsion droplets and finally in increased particle size. The percent entrapment of the nanoparticles improved as polymer concentration is enhanced. The encapsulation efficiency of the nanoparticles of all the formulations was above 70%.

This could be due to the enhancement of viscosity because of the greater concentration of polymer inside emulsion droplets, which often will limit this migration involving drug to the outer aqueous phase[19]. Particle size and % entrapment of the formulations were being elevated with rising the polymer concentration might be because of excessive quantity of polymer available for covering the drug. Upon raising the polymer quantity, number of layers has been increased; that ended in improvement with size and entrapment effectiveness.

Table 1: Particle size, Zetapotential, Polydispersity index and % drug entrapment efficiency of Piperine nanoparticles

Formulation Code	Particle size (nm)	PDI	Charge	%Entrapment
PNP1	240 ± 1.72	0.23±0.02	-38±0.05	74±2.5
PNP2	320 ± 1.69	0.18±0.03	-40±1.6	77±2.1
PNP3	395± 1.63	0.28±0.02	-41±0.9	79±1.5
PNP4	565± 1.23	0.33±0.01	-42±0.7	82±1.25

*(Mean ± SD)

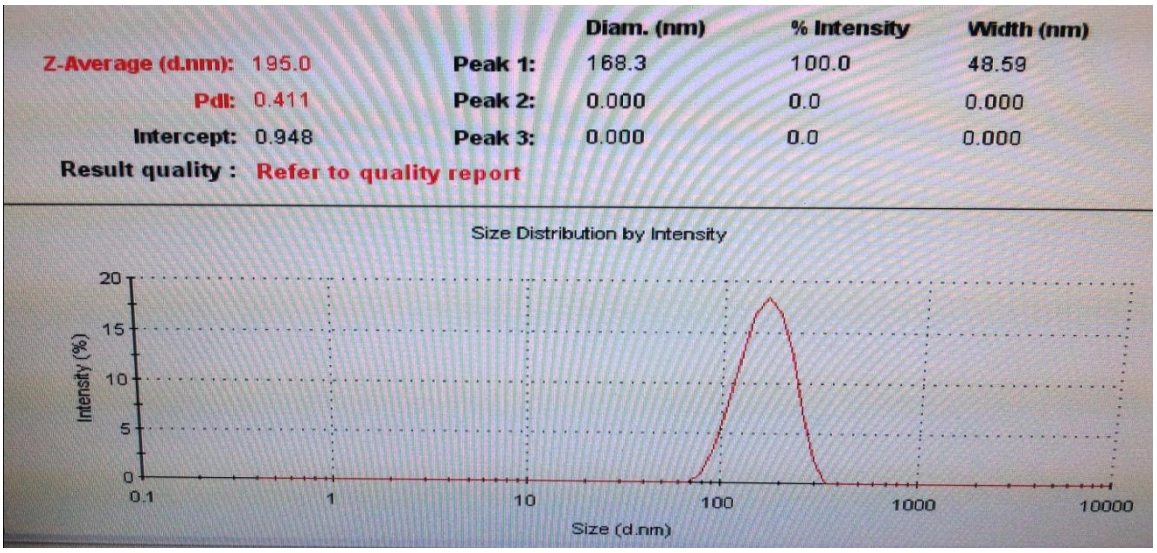

Fig. 1: Particle size of Piperine nanoparticles (PNP1)

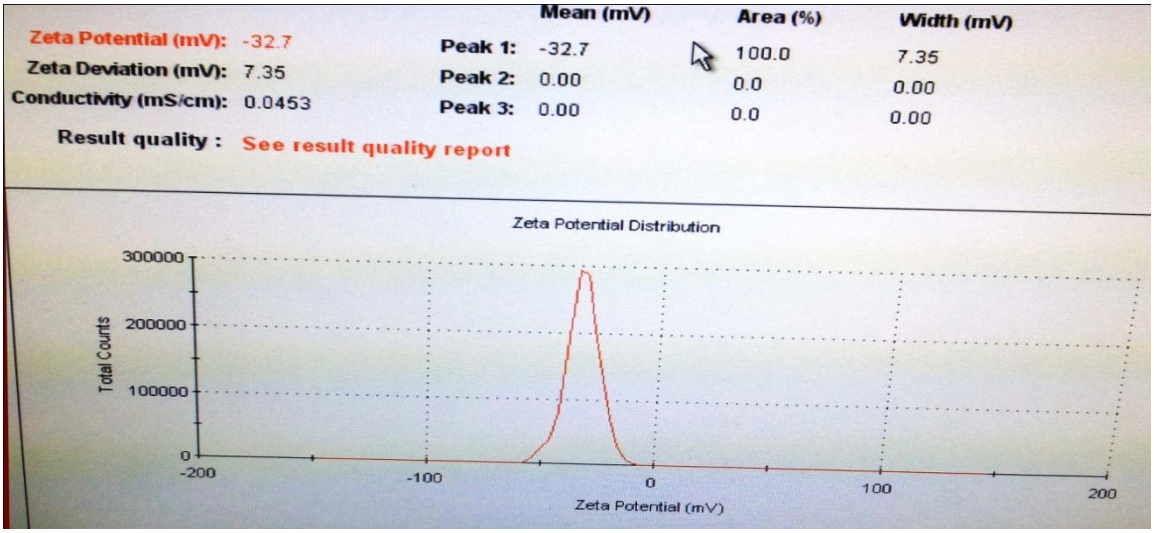

Fig. 2: Zetapotential of Piperine nanoparticles (PNP1)

Drug- polymer interactions

The FTIR spectra of Piperine, Polycaprolatone, Placebo nanoparticles and piperine loaded nanoparticles were found shown in Fig 3. 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 nanoparticles showed same characteristic peaks which are seen in IR spectra of polycaprolactone. No additional peaks were seen in IR spectra of piperine nanoparticles. The FTIR spectra concluded that all of the piperine, polycaprolactone, placebo nanoparticles and drug loaded nanoparticles exhibited the characteristic bands which confirm no interaction.

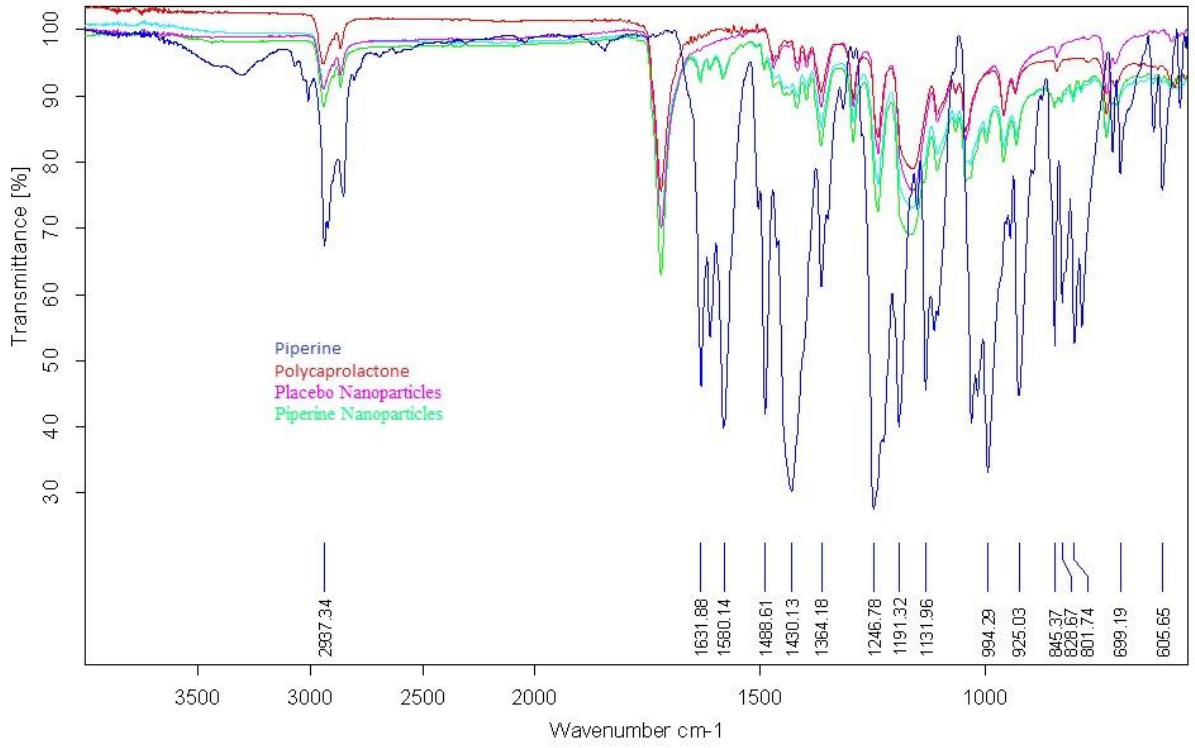

Fig. 3: Ftir spectra of piperine, polycaprolactone, placebo nanooparticles and piperine nanoparticles

In vitro drug release rates are shown in the (Fig.4). Release data shows that raise in the polymer content detains the drug release because of enhanced particle size and decreased surface area. Samples were withdrawn at an interval of 24 hours and absorbances were measured by UV spectrophotometer at 343 nm. The release data shows that all the preparations prolonged the drug release for more than 12 days. A biphasic drug release style has been observed, i.e., burst release accompanied by delayed release. In the initial 24 hours, the drug that has been not entrapped is released. Later the entrapped drug released moderately, so a gradual increase was seen in the release. The initial burst effect could possibly be described with the release of some drugs loosely bound on the surface of the nanoparticles[20]. An additional cause for the burst release may be due to unstable nature of the inner water emulsion droplets throughout the solvent evaporation that ended in their coalescence and probably have brought the drug to discover at the surface of the nanoparticles. The release of drug from nanoparticles with lower concentration of polymer was much more rapid than those with higher polymer concentration. Drug release from PNP1 was greater than PNP2, PNP3 and PNP4. This might be due to raise in polymer concentration, which in turn resulted in 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 surface area of nanopartices. Log percent cumulative drug released, plotted as a function of log time yielded curves, the slope of is the diffusional release exponent (n). The values of diffusional n were 0.68, 0.58, 0.63 and 0.634 for PNP1, PNP2, PNP3 and PNP4 respectively, which indicate that drug release from all the formulations followed a non fickian pattern.

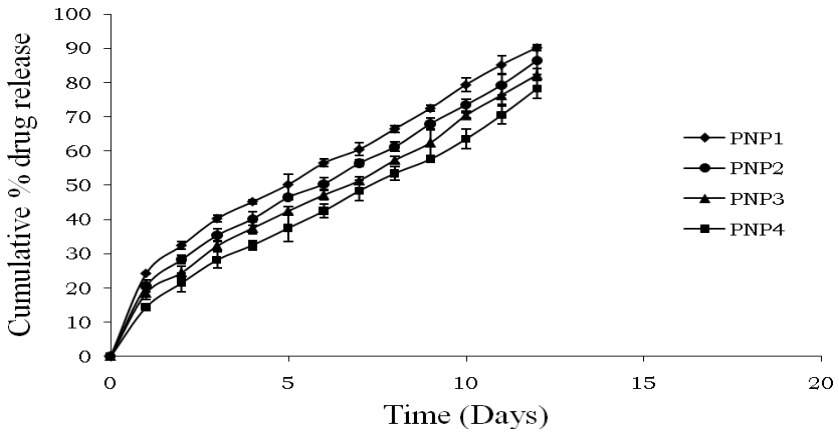

Fig.4 : Cumulative % released vs time plots of Piperine nanoparticles.

Stability studies:

Stability studies have been performed for piperine formulation in order to check any changes in physical appearance, particle size and drug content. It was observed that there was no color change of nanoparticles. No considerable changes were shown in the particle size of the formulation after storage for 1 month. The percentage residual drug content of nanoparticles were found in the range of 99 - 97.5, 98.5 – 96 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 nanoparticular formulation showing all formulations were stable.

Pharmacokinetic 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 10ng/ml. Chromatogram of Piperine in plasma was shown in Fig. Drug concentration in plasma following intravenous administration of piperine nanoparticles and the drug solution are presented in (Fig.5). The major PK parameters are given in (Table 2). The MRT of nanoparticle formulation was 17.5 times higher than that of the solution, which suggested that encapsulation of the piperine into nanoparticle could produce a remarkably prolonged release of the drug *in vivo*.The $t_{1/2}$ and AUC (0-∞) values of nanoparticles have been significantly greater than the drug solution. Maximum serum concentration of 5.2µg/ml has been seen within 30 min when piperine solution was given intravenously. Maximum serum concentration of 5.4 µg/ml has been seen within 1 hour when piperine nanoparticles (PNP1) suspension was given intravenously. AUC (0-∞) of nanoparticular formulation was 28 times higher than that of the drug solution. Vd value of nanoparticle formulation was less than piperine solution. The K_E and CL values of the nanoparticles significantly decreased compared with those of the solution, suggesting that the nanoparticles formulation was more slowly removed from plasma compared with the solution. All these results implied that nanoparticles did increase that piperine concentration in plasma, retarded its clearance and established sustained release property *invivo*. The final results of the research obviously show that a nanoparticle formulation comprising piperine is more preferable with pharmacokinetic properties and sustained plasma drug levels.

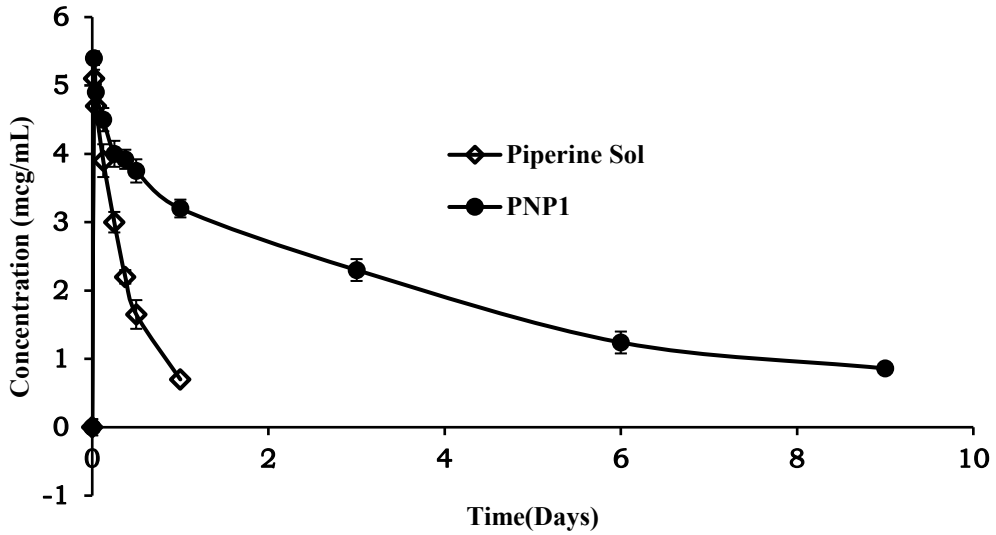

Fig.5: Plasma concentration-time profiles of Piperine formulation

Table 2: Pharmacokinetic parameters for Piperine nanoparticles

Parameter	Piperine Sol	Piperine NP1
C_{max} (µg/ml)	5.1±0.1	5.4±0.2
Ke (h^{-1})	0.071±0.005	0.0081±0.005
$t_{1/2}$(h)	9.70±0.60	84.92±1.5
MRT (h)	12.94±0.25	121.71±2.53
Vd(L)	6.94±0.3	7.35±0.5
Clearance(L/h)	0.496±0.02	0.06±0.01
$AUC_{0-\infty}$(µg.h/ml)	60.4±3.5	499.52±4.2

*(mean ± SD, n= 3)

The drug amounts in several tissues have been determined and the drug deposition order in various tissues is as follows: liver > lung > kidney > brain. Drug amounts in the liver had been greater due to small size of particles which were very easily taken up by the kupffer cells along with RES of liver.

Table 3: Effect of Piperine formulations on enzyme levels in rats with carbon tetrachloride (CCl_4) –induced hepatotoxicity (mean ± SD, n = 6)

Groups	Initial body weight (g)	Bodyweight after 9 days (g)	SGOT(U/mL)	SGPT(U/mL)
Control	165±10	180±15	14.6±1.37	9.3±0.83
CCl_4	160±15	148±10	59.2±3.6	35±2.3
CCl_4 + Piperine Sol	165±5	171±5	34.7±3.01	20.4±0.6
CCl_4 + PNP1	170±6	180±15	15.9±1.2	11.9±1.2

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[21]. One rat died in CCl_4 group, and no rats died in 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 the body weight (Table 3). Evaluation of the serum enzymes can be a beneficial quantitative marker of the degree and type of hepatocellular damage. Increased serum level of SGPT and SGOT is associated with liver damage. CCl_4 administration generated a significant raise in serum SGOT to 59.2 ± 3.6 U/L in comparison with normal value that was 14.6 ± 1.37 U/L. Administration of piperine solution and piperine nanoparticles produced a substantial lowering in SGOT levels to reach 34.7 ± 3.01 U/L and 15.9 ± 1.2 U/L, respectively. The SGOT level in the case of piperine nanoparticles which is almost same as that of the normal group. Concurrently, CCl_4 administration triggered in a tremendous raise in serum SGPT to 35 ± 2.3 U/L in comparison to normal value, which was assessed as 9.3 ± 0.83 U/L. Piperine solution and nanoparticles produced significant change in serum SGPT to reach 20.4 ± 0.6 U/L. and 11.9 ± 1.2 U/L, respectively, was produced. Upon administration of piperine nanoparticles was able to reduce all the elevated enzyme levels. Piperine nanoparticles might be mainly taken up by RES and transferred directly into nonparenchymal hepatic cells in liver. Non parenchymal cells play a crucial role in continuous infection with liberating cytokine. The curability of liver toxicity had been a lot more for nanoparticles compared to the solution because of smaller size particles were instantaneously taken on through RES of liver thus the drug was aggregated in the liver and the drug release was more focused at the cellular level of liver. This outcome in the hepatoprotection. The reversal of biochemical end points in a CCl_4 hepatotoxic model is more preferable with piperine nanoparticles in comparison to intravenously administered piperine solution.

Histopathological studies showed normal control animals confirmed normal hepatic structure along with specific liver parenchymal cells (Fig.6a). Whereas CCl_4 treatment caused the formation of necrotic area in the liver with considerably wide range of inflammatory cell infiltration encircling the centrilobular veins of the liver (Fig.6b). Major improvements in $CC1_4$-induced liver damage in rats (such as fibrosis and inflammation) have been witnessed following treatment with piperine. Administration of piperine solution did show moderate change in regeneration with mild inflammation and some spotty necrosis (Fig.6c). Administration of piperine nanoparticles showed a greater number of regenerating liver cells; no inflammatory cells and fibrosis; liver tissue restored its normal structure (Fig.6d). This kind of shows that these nanoparticles could be prospective utilization in the management of fibrosis with piperine. The outcomes could be extrapolated to additional drugs recommending the significant advantage of passive targeting of drugs to the liver.

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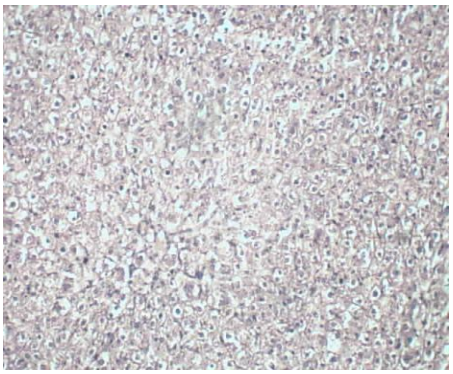
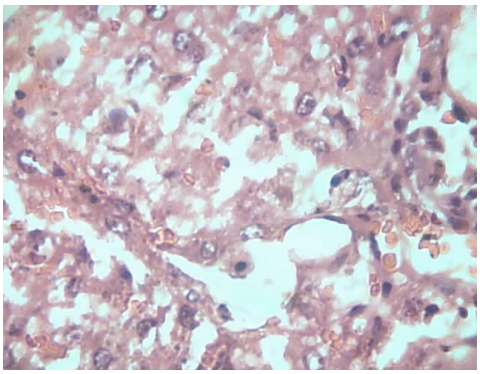
CONFLICT OF INTEREST

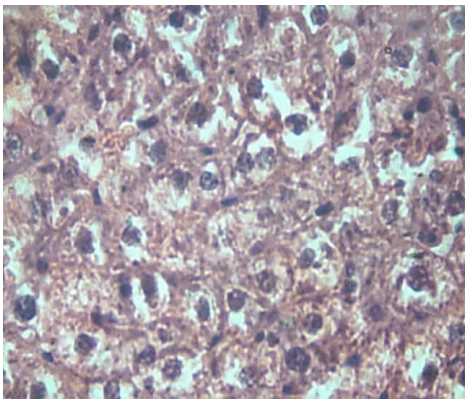
The authors declare that the contents of this article have no conflict of interest.

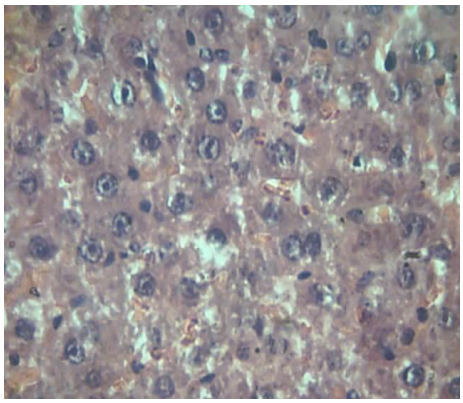




a) Control

b) CCL_4





c) Piperine Sol

d) Piperine NP1

Fig.6: Histopathology of Rat livers

REFERENCES

1. Lim YS, Kim WR. The global impact of hepatic fibrosis and end-stage liver disease. Clinics in liver disease. 2008 Nov 1; 12(4):733-46.
2. Koyama Y, Xu J, Liu X, Brenner DA. New developments on the treatment of liver fibrosis. Digestive diseases. 2016; 34(5):589-96.
3. Merlin NJ, V Parthasarathy, R Manavalan. Role of Flavanoids in Free Radical Induced Hepatic Fibrosis. Research J. Pharm. and Tech. 2(1): Jan.-Mar. 2009; Page 52-57.
4. Altamirano-Barrera A, Barranco-Fragoso B, Méndez-Sánchez N. Management strategies for liver fibrosis. Annals of hepatology. 2017; 16(1):48-56.
5. Bonepally CR, Gandey SJ, Bommineni K, Gottumukkala KM, Aukunuru J. Preparation, characterisation and in vivo evaluation of silybin nanoparticles for the treatment of liver fibrosis. Tropical Journal of Pharmaceutical Research. 2013; 12(1):1-6.
6. Samir Shah, Kintu Patel, Mohsin Pathan. Evaluation of the Effect of Piperine on Bioavailability and Pharmacokinetics of Macrolides in Rats. Asian J. Res. Pharm. Sci. 2018; 8(2):61-67.

7. Samir Shah, Kintu Patel, Mohsin Pathan. Evaluation of the Effect of Piperine on Bioavailability and Pharmacokinetics of Macrolides in Rats. Asian J. Res. Pharm. Sci. 2018; 8(2):61-67.
8. Bonepally CR, Aukunuru JV, Yellu NR, Vanga MR. Fabrication and investigations on hepatoprotective activity of sustained release biodegradable piperine microspheres. Int J Pharm Sci Nanotech. 2008;1:87-96.
9. Rinku Jaiswal, Shripal Singh, Hemant Pande. Magnetic Nanoparticles Activated Carbon: Preparation, Characterization and Application: A Review article. Asian J. Research Chem.2015; 8(12): 757-768.
10. Ch. Prabhakar, K. Bala Krishna. A Review on Polymeric Nanoparticles. Research J. Pharm. and Tech. 2011; 4(4): 496-498.
11. Chandrasekhar R B*, Krishnaveni B, Krishna Mohan G, Jithan AV. Preparation, Characterization and *In Vivo* Evaluation of Dexamethasone Nanoparticles for the treatment of Liver Fibrosis. *Research J. Pharm. and Tech. 2020; 13(12):6098-6104*.
12. 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.
13. Jameel Ahmed Mulla, Sarasija Suresh, Imtiyaz Ahmed Khazi. Formulation, Characterization and in vitro Evaluation of Methotrexate Solid Lipid Nanoparticles. Research J. Pharm. and Tech. 2009; 2 (4):685-689.
14. CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol. 2003; 35: 257-274.
15. Demers G, Griffin G, De Vroey G, Haywood JR, Zurlo J, Bedard M. Harmonization of Animal Care and Use Guidance. Science. 2006; 312: 700-701.
16. Sathesh Kumar. S, Felix Joe. V. Pharmacokinetics of Tacrine Loaded MPEG-PCL Polymeric Nanoparticles. Research J. Pharm. and Tech. 2017; 10(1): 135-140.
17. Rakesh N Pillai, Beny Baby, AJM Christina, Abin Abraham. Anti fibrotic effect of ethanolic extract of Cyclea peltata (H.F and T) roots, on carbon tetrachloride induced liver fibrosis. Research J. Pharm. and Tech. 2009; 2(1):201-205.
18. Konatham S, Nyathani H, Bonepally CR, Yeannameneni P, Aukunuru J. Liposomal delivery of curcumin to liver. Turk J Pharm Sci. 2010; 7: 89-98.
19. Ashok Reddy B, Satish S, Sankara G, Adithya Sarma, Sai Kishore V. Influence of Solvents on Entrapment Efficiency and Drug Release rate of Propranolol Hydrochloride from Ethyl Cellulose Microcapsules. Asian J. Research Chem. 2011; 4(1):143-146.
20. Archana A, Manikanta Kumar A, Manjunath Shetty KS. Formulation Development and Evaluation of Sumatryptan Pulsatile Drug Delivery using Pulsincap Technology. Research J. Pharm. and Tech. 2013; 6(12):1375-1379.
21. Prashant Tiwari, Kuldeep Kumar, Rajnikant Panik, Alok Pandey, Ashish Pandey, Pratap Kumar Sahu. Hepatoprotective Potentials of Butea monosperma Stem Bark Extract against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats. Research J. Pharmacology and Pharmacodynamics. 2011; 3(5): 281-284.