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Optimization and Evaluation of Piperine Loaded Herbosomes for their Antioxidant and Hepatoprotective Potential

GAYATRI JOSHI*, ABHISHEK TIWARI and PRASHANT UPADHYAY

Faculty of Pharmacy, IFTM University Moradabad-244102, India

*Corresponding author: Tel: +91 5944 280820; E-mail: gayatrijoshi044@gmail.com

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Piperine is classified as a class II drug in the biopharmaceutical classification system due to its low aqueous solubility. As a result, piperine herbosomes were created to improve the dissolution rate and *in vivo* liver protecting activity of piperine and physico-chemical characteristics were used to confirm herbosome formation. The piperine-herbosome formulation revealed spherical particle size of all formulations from P1-P10 and found142.4 ± 0.98 nm for best piperine-herbosome formulation (P2) and a PDI value of 0.237, indicating a homogeneous population of piperine loaded vesicles. *In vitro* drug release rate and percent entrapment efficiency were determined for all formulations P1-P25 and found to be 95.306 ± 0.21 and 97.306 ± 0.65 in 12 h, respectively for best piperine-herbosome formulation (P2). It exemplifies the complex's long-term releasing capability. This information suggests that it may have a longer retention time inside the body, extending the duration of effect. The antioxidant potential of pure piperine was determined using the DPPH scavenging method, with an IC_{50} value of 107.59 ± 0.11 g/mL compared to a formulation with an IC_{50} value of 93.926 ± 0.03 g/mL. Swiss albino mice of either sex were utilized for the evaluation of hepatoprotective activity. On the 8th day, the hepatotoxicity was caused by giving a single oral dosage of CCl_4 (0.5 mL) and the parameters were evaluated on the 9th day. This formulation has the best optimized based on drug content and drug entrapment. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin were among the biochemical markers measured. In comparison to normal control (161 ± 0.31 IU/L, 52.78 ± 0.28 IU/L, 121.12 ± 0.14 IU/L and 0.633 ± 1.44 IU/L) and P2 formulation (163.23 ± 0.49 IU/L, 66.9 ± 0.05 IU/L, 128.3 ± 1.15 IU/L and 0.645 ± 0.67 IU/L respectively).

Keywords: Piperine, Herbosomes, Antioxidant, Hepatotoxicity, Serum glutamic oxaloacetic transaminase, Alkaline phosphatase.

INTRODUCTION

Liver is the largest organ in the human body and it has a variety of functions that affect a variety of life processes. Liver illnesses (cirrhosis, viral hepatitis and hepatocellular cancer) are currently a serious public health concern with a global mortality rate of 2 million fatalities every year. In most of the under developed nations, medicines for liver infections are too expensive. For the treatment of liver diseases, most of the traditional dose forms are accessible. However, they have a wide range of adverse effects on different body tissues. There is a need for an alternate strategy to dealing with liver disorders and interest in herbal products is growing at the moment because they have little or no negative effects. This has sparked an increased interest for the use of traditional natural remedies that, as far as anyone knows, offer hepatoprotective properties [1]. The piperine alkaloid, which is responsible for the pungency of black pepper, is an important phytochemical. It is beneficial to a wide range of biological activities and also enhances liver function. The clinical potential of piperine is hindered by its low oral bioavailability, fast metabolism and poor water solubility. Piperine has an aqueous solubility of 40 mg/L at 18 ºC and is classified as a BCS Class II medication because to its low solubility in water and poor dissolution. To tackle the problem of poor solubility, the most recent breakthrough is the introduction of new noval lipid-based formulations. Piperine distribution has been improved using a variety of formulation strategies and methodologies, including liposomes, nanocrystal gel formulations and self-micro emulsifying drug delivery systems (SMEDDS) [2]. The goal of the study was to design,

define and test the hepatoprotective effect of a piperine-loaded herbosome (PLH), as well as to evaluate the optimized formulation in a rat model for both increased aqueous solubility and oral bioavailability of piperine. In addition, after oral administration of PLH to rats, a preliminary pharmacological investigation was performed to assess the *in vivo* antioxidant capacity.

EXPERIMENTAL

The phytoconstituent piperine was obtained from Vital plants Nursery in Delhi, India. 1,2-Dipalmitoyl-Sn-glycero-3-phosphocholine (DPPC) and cholesterol were procured Yarrow Chem, Mumbai.

Animals: Adult albino Wister rats, both male and female were used and obtained from I.V.R.I. University in Bareilly, India. The Animal Ethics Committee of Devsthali Vidyapeeth College of Pharmacy, with registration number DVCP/IAEC/2019/004, authorized the research protocols utilized in this study.

Preparation of standard solution for calibration curve of piperine: Piperine (10 mg) was accurately weighed and transferred into a 100 mL volumetric flask, then the drug was dissolved and diluted up to the mark with methanol to make a 100 g/mL stock solution, from which 1 mL was pipette out in a 10 mL volumetric flask and volume made up to the mark and the solution was scanned in a UV-visible spectrophotometer between wavelengths of 200-400. Fig. 1 shows the peak detected at 324 nm [3,4].

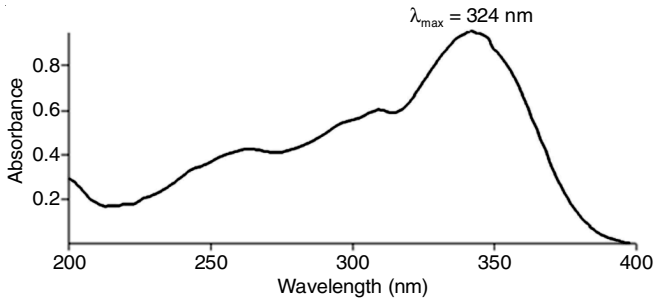

Fig. 1. UV absorption spectra of piperine

Standard calibration curve of piperine in methanol: Precisely weighed piperine (10 mg) was transferred to a 100 mL volumetric flask and the volume was made up with methanol (100 g/mL stock solution). From this, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL solution was pipetted out in a 10 mL volumetric flask and volume increased to the mark, yielding 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 g/mL solution with an R^2 value of 0.9.

Formulation of piperine-herbosomes: Different molar ratios of piperine loaded herbosome (PLH) as shown in Table-1 were used to make the PLH (1:1, 1:2, 1:3, 1:4 and 1:5). Piperine and phospholipid (DPPC) were accurately weighed and mixed in a 100 mL RBF with a 1:2 mixture of chloroform and methanol (20 mL). A water bath was employed to keep the reflux reaction temperature at 60 ºC for 30 min. The flask's contents were then condensed to form a dry residue. The dry material was hydrated using phosphate buffer. A bath sonicator was then used to sonicate the solution [5-12].

TABLE-1
COMPOSITION OF PIPERINE HERBOSOMES FORMULATIONS

Compositions	Quantity
Piperine	40 mg
DPPC	100 mg
Cholesterol	80 mg
Chloroform:methanol (1:2)	30 mL
Phosphate buffer	30 mL

Validation of the experimental design and selection of the best formulation: The formulation with the smallest particle size, highest percent entrapment efficiency and highest percent *in vitro* drug release for 12 h was chosen as the optimal formulation based on response characteristics. Polynomial equations were developed for each response using design expert software 13.0.0 (State-Ease, Inc., USA) to validate the design (Table-2). To evaluate the response, a statistical model with interactive and polynomial terms was used:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_1^2 X_2 + \beta_8 X_1^2 X_2^2$$

where X_1 and X_2 are the coded levels of the independent variables and X_1X_2 are the interaction and polynomial terms, respectively and 0 is the arithmetic mean of all quantities outcomes from nine runs, β_1 to β_8 are the coefficients computed from the observed experimental values of Y and 1 to 8 are the coefficients computed from the observed experimental values of Y.

Characterization of complexes

Drug content analysis: Added 10 mg piperine-loaded herbosome (PLH) in pH 6.8 phosphate buffer (50 mL) and shaked continuously for 2 h in mechanical stirrer and kept

TABLE-2
FORMULATION DESIGN FOR PIPERINE LOADED HERBOSOMES

Std.	Run	Phospholipid	Drug	Temperature	Size	Entrapment (%)
1	6	1	0	387.12	82.78667	84.18939
2	5	.5	0	303.20	79.89407	76.86345
3	9	-.5	.5	289.60	82.98409	80.75689
4	11	-1	1	355.32	88.69867	87.73877
5	8	-.5	-.5	237.50	80.234509	78.76543
6	4	-.5	0	235.60	79.34096	74.76452
7	13	0	-.5	278.40	80.34093	76.45290
8	25	0	1	459.42	83.32045	73.87495
9	14	.5	.5	245.30	81.89203	79.56432

overnight for complete solubility. After that the solution was filtered and makes the aliquotes; this study was carried out with the help of UV spectrophotometer at 343 nm by using phosphate buffer (pH 6.8) as blank [13-16].

Particle size: Photon correlation spectroscopy (PCS) was used to calculate the average diameter and PDI of prepared PLH at a fixed angle at 25 ºC using a zeta sizer. The sample was dissolved in distilled water 10 times before being tested for particle size. The readings were taken three times and reported [4,13-15].

Zeta potential: The zeta potential can be determined by determining the particle charge and the particle velocity in an electric field. The PLH was dissolved in distilled water ten times before being examined with the help of Malvern, Version-6.01 Zeta-Sizer [4,13-15].

Morphology: PLH morphology of optimized formulation was analyzed using transmission electron microscopy [11-13].

Differential scanning calorimetric: DSC studies performed for measured difference in energy and temperature. Piperine, phospholipid and PLH were performed on a Perkin Elmer. The instrument measures the difference in the heat flow between the test sample and the reference was measured in the 30-300 ºC with the heating rate 30 ºC/min. All studies were carried out in triplicate [4,13-15].

Drug entrapment efficiency: Drug entrapment efficiency of different formulation was calculed and the free concentration of piperine in the continues medium, the drug entrapment efficiency (EE) of piperine encapsulated inside and absorbed on to the herbosomes was also determined. Piperine-loaded herbosomes (PLH) were diluted in methanol and centrifuged. Supernatant was then filter and analyzed by UV-vis spectrophotometer at 343 nm [4,13-15].

$$\text{Entrapment efficiency (\%)} = \frac{\text{Entrapped amount of drug}}{\text{Total amount of drug added}} \times 100$$

***in vitro* release studies:** The solvent used for *in vitro* drug release was pH 7.4 and apparatus was used for this study was franz diffusion cell. The 5 mL herbosomes formulations were put into donor compartment covered with a dialysis membrane and was placed such that it just touches the diffusion medium present in receptor compartment. The drug sample was withdrawn at the interval of 30, 60, 120, 180, 240, 300, 360, 420, 480, 540 min for the period of 12 h and analyzed drug released of PLH by a UV spectrophotometer at 343 nm using simulated tear fluid as a blank [4,13-15].

***in vitro* antioxidant activities:** For evaluation of piperine antioxidant activity by the help of DPPH free radical scavenging assay. The antioxidant activity of the piperine against DPPH reagent was analyzed by using UV spectrophotometer at 517 nm. Pure drug and PLH different concentrations of 50, 100, 150, 200, 250 µg/mL, were prepared. A 2.5 mL DPPH was mixed with 0.5 mL of test sample at different concentration for determination of absorbance. The % inhibition was calculated using the equation given below [16,17]:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

***In vivo* study**

Acute toxicity study: Piperine acute toxicity study was performed according to OECD guidelines [18]. Briefly, total 12 rats were used for study (male and females) rats were divided into two groups of 12 (6 males and 6 females). The test group was orally given to the piperine in a single dose of 2,000 mg/kg body weight, while the control group received only saline water. Both groups were examined for toxic signs, convulsion and death for 14 days. The animals that died within this period were subjected to necropsies [18].

Sub-chronic toxicity study: A total of 30 rats divided into six groups consisting of five each, adapted to the environment for 7 days. Group I (Normal) was given distilled water, Group II (CCl_4) rats received distilled water daily and 5th day received CCl_4. Groups III (CCl_4 + Silymarin). Group IV (CCl_4 + Piperine) were given fraction suspension and Group V (CCl_4 + piperine herbosomes). All groups were received dose orally and the treatment was continued until the 13th day. On the 14th day, the blood serum of rats was taken and then measured the total bilirubin, ALP, SGOT and SGPT [19].

RESULTS AND DISCUSSION

Particle size: Fig. 2 displays the distinctive size distribution of piperine loaded herbosomes of all the formulations (F1-F10). The mean particle size (5L1) varied from 142.4 nm ((P_2) to 335.84 nm (P7) (Table-3). A quadratic equation was formulated to express the effect of independent variables on mean particle size, which is as follows:

$$\text{Size } (Y_1) = 262.02 - 99.31X_1 + 45.7096X_2 - 36.78X_1X_2 \quad (1)$$

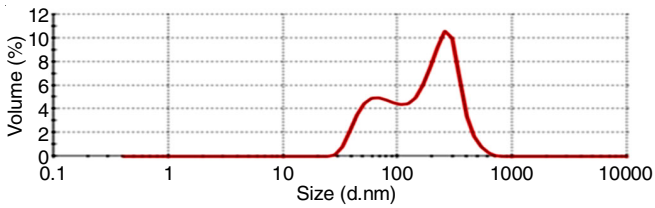

Fig. 2. Particle size of optimized formulations (P2)

TABLE-3
DRUG CONTENT AND DRUG ENTRAPMENT OF DIFFERENT FORMULATIONS OF PLH

Formulation	Particle size (Y_1)	Entrapment efficiency (%) (Y_2)	Percentage release (%) (Y_3)
P1	217.61 ± 0.31	68.75 ± 0.14	69.61 ± 0.28
P2	142.4 ± 0.98	97.18 ± 0.65	95.70 ± 0.21
P3	290.32 ± 0.36	87.73 ± 1.15	88.69 ± 0.50
P4	309.32 ± 0.78	59.07 ± 0.25	63.15 ± 0.05
P5	146.67 ± 0.32	82.36 ± 0.44	86.45 ± 0.36
P6	325.45 ± 0.49	55.78 ± 1.15	71.10 ± 0.05
P7	335.84 ± 1.41	52.10 ± 0.48	56.45 ± 0.47
P8	318.54 ± 0.25	84.18 ± 0.30	82.78 ± 0.58
P9	156.57 ± 0.51	91.80 ± 1.15	90.45 ± 0.23
P10	217.40 ± 1.06	55.98 ± 0.26	88.67 ± 0.15

As illustrated by the negative value preceding the variable in the quadratic equation, the variables X_1, X_2 had a negative impact on Y_1.

Entrapment efficiency (EE): In case of EE, the result ranged from 52.10% (P7) to 97.18% (P2), with an average of 73.59%, depending on the variable level selected (Table-3). The quadratic equation below demonstrated the influence of several independent variables on EE:

$$EE\ (Y_2) = 74.28 + 17.89X_1 - 10.22\ X_2 + 6.66X_1X_2 \quad (2)$$

It is evident from eqn. 2 that the variables X_1 and X_2 had positive impacts on the EE as shown by the positive value before the variable in the quadratic equation. Increased emulsifier-to-lipid ratios might result in an increase in EE; this could be due to the presence of a sufficient emulsifier that kept the piperine within the lipid particles and/or on their surface [20].

Percentage drug content: The percentage released ranged from 56.45% (P7) to 95.70% (P2), with an average of 79.30% (Table-3). The quadratic equation (eqn. 3) demonstrated the influence of several independent variables on the medication release:

$$\text{Drug content}\ (Y_3) = 69.93 + 17.52X_1 - 11.09X_2 + 6.82X_1X_2 \quad (3)$$

The beneficial influence of factors X_1 and X_2 on drug release may be observed in eqn. 3, as the positive value preceding these variables as indicated by the quadratic equation. The higher amount of drug release in the initial period could be related to the drug molecules present in the surface of the carriers. In addition, the higher emulsifier-to-lipid ratio could also have played a bigger role in enhancing the piperine release.

Increased particle size reduces percentage release and particle size is dependent on the polymer or drug ratio. Two way-ANOVA were applied to determine the significance and magnitude of interaction between independent and dependent variables (Table-3). The regression model was used to generate 3D surface to analyze interactions of the independent variables (Fig. 3) [21].

Zeta potential: The study zeta potential determines the storage stability of colloidal dispersion. The zeta potential values which were in the range of -32.3 mV (Table-4), which indicates optimized formulation showed stability and not cause aggregation (Fig. 4) [20,22,23].

Morphology: The piperine-loaded herbosome (PLH) optimized formulation P2 size and shape evaluated by the help of TEM and digital microscopy, which indicate the discrete spherical structures without aggregation (Fig. 5).

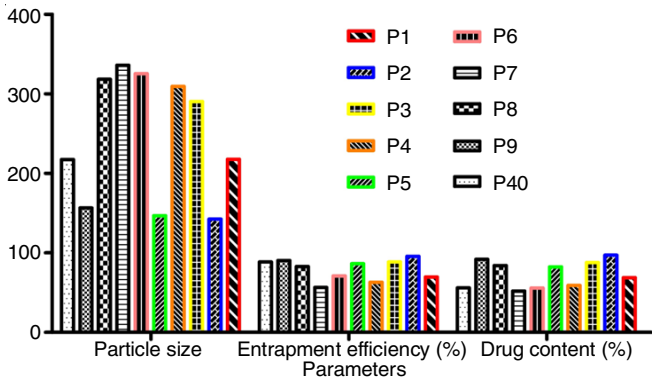

Fig. 3. Particle size, entrapment efficiency and drug content of priperiene loaded herbosomes (N = 6, data were expressed as Mean ± SEM, two way ANOVA, ***$p < 0.001$)

TABLE-4
PARTICLE SIZE ZETA PDI AND ZETA POTENTIAL OF OPTIMIZED FORMULATION (P2)

Formulation	Drug polymer ratio	Particle size	PDI	Zeta potential
P2	1:2	142.4	0.237	-32.3

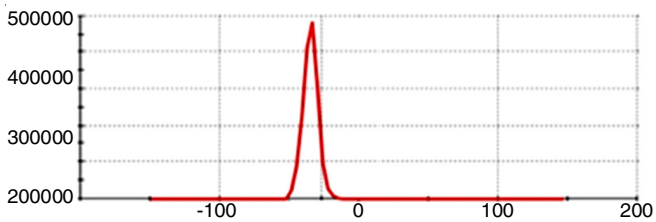

Fig. 5. Zeta potential of optimized formulations (P2)

Differential scanning calorimetry (DSC): According the DSC thermogram, a sharp endothermic peak of piperine and phospholipid melting point obtained at 131.39 and 231 ºC (Fig. 6). The melting point of phospholipid in blank NLC was decreased to 206 ºC. Because of the incorporation of piperine into the lipid matrix, the melting point of PLH was further reduced to 196 ºC as a result of disordered crystal structure arrangement [20,22,23].

***in vitro* release studies:** *In vitro* drug releases from the herbosome formulation were studied by the diffusion cell. The diffusion medium was 250 mL of phosphate buffer pH 6.8, stirred at 50 rpm at 37 ± 0.5 ºC. *in vitro* drug release of piperine

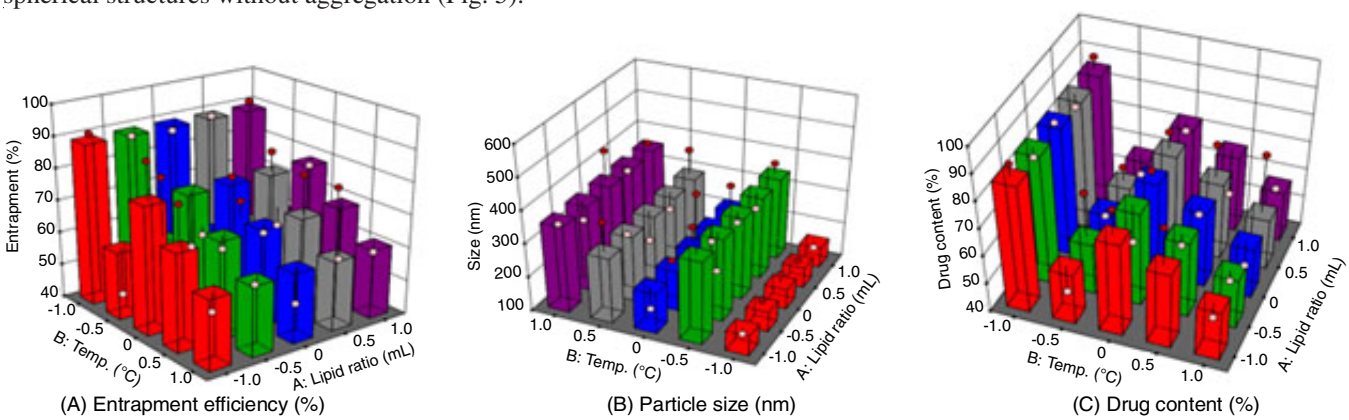

Fig. 4. Response surface plot showing effect of drug-lipid ratio (X_1) and temperature (X_2) on particle size, entrapment efficiency and release

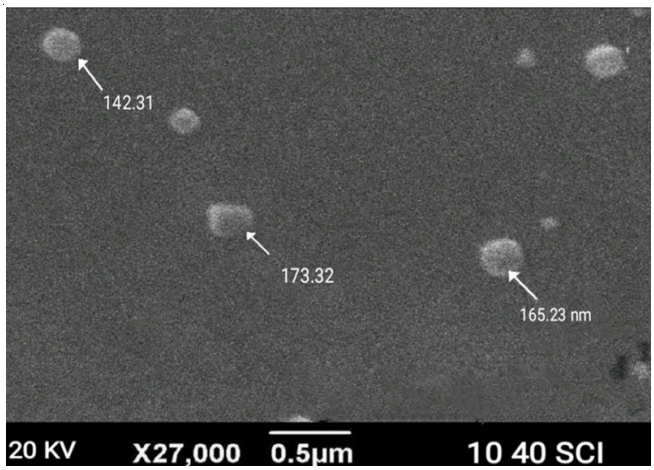

Fig. 6. TEM of optimized formulations P2

and optimized formulation are shown in Table-5. It is concluded that *in vitro* drug release of piperine pure are higher in comparison to the herbosomes formulations (Fig. 7) [20,22,23].

TABLE-5
COMPARISON OF *in vitro* RELEASE OF OPTIMIZED FORMULATION AND PURE DRUG

Time	CDR of pure drug (%)	CDR of optimized formulation (%)
0.5	0.159 ± 4.19	0.391 ± 8.39
1	3.047 ± 0.04	6.149 ± 8.17*
2	9.167 ± 6.49	11.388 ± 1.41
3	18.336 ± 3.10**	17.149 ± 2.73
4	29.313 ± 8.25	23.190 ± 3.21**
5	36.345 ± 0.87	27.405 ± 2.23
6	48.683 ± 8.17**	37.813 ± 8.56*
7	52.045 ± 5.74	41.747 ± 6.19
8	61.978 ± 4.16	50.907 ± 6.39
9	74.810 ± 7.87**	54.558 ± 4.16**
10	80.112 ± 3.31	58.178 ± 4.06
11	86.781 ± 5.64	62.906 ± 1.25**
12	95.306 ± 1.15	74.286 ± 1.45

Antioxidant activity

DPPH radical scavenging activity: Piperine and its herbosomes complex were tested for DPPH radical scavenging properties. The typical antioxidants, ascorbic acid and quercetin had IC_{50} values of 7.84 and 10.04 g/mL, respectively. Table-6 shows the scavenging activity of pure piperine and PLH concentrations, with IC_{50} values of 93.57 and 107.92 g/mL, respectively. PLH has a higher DPPH scavenging activity than pure piperine (Fig. 8). All the experimental procedures were carried out in triplicates and using Minitab software, the coefficient of determination (R^2-value) and significant differences between means ($p < 0.05$) were determined using one-way statistical analysis (ANOVA).

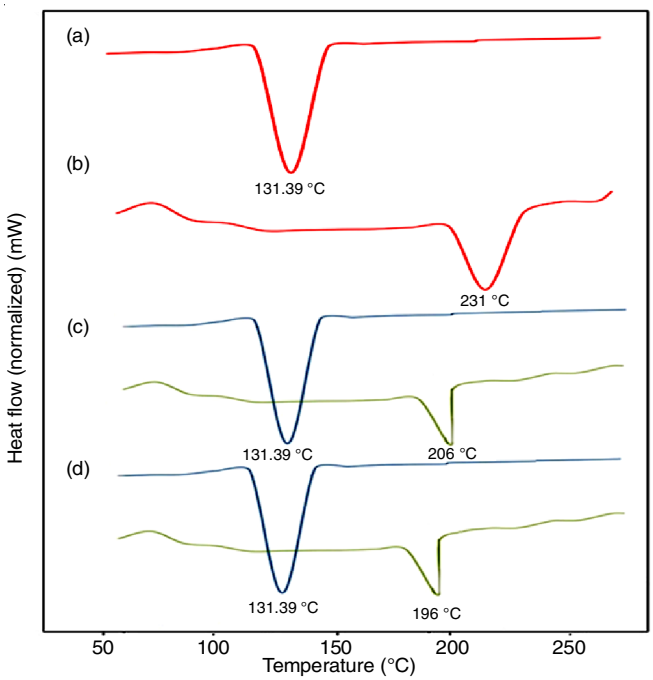

Fig. 7. DSC of (a) piperine, (b) phospholipid, (c) blank herbosomes, (Dd piperine-loaded herbosomes (P2)

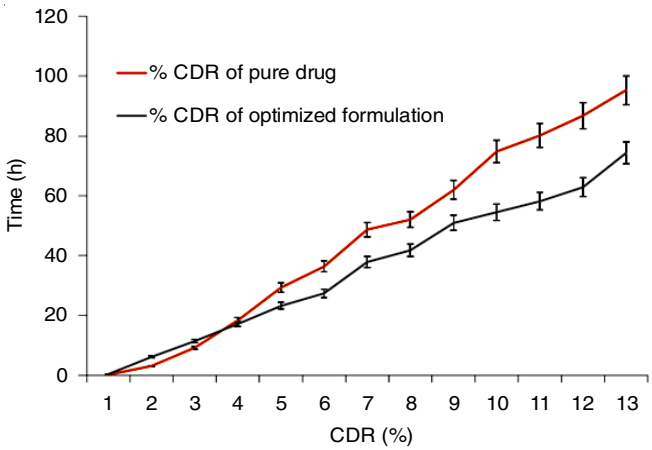

Fig. 8. Comparison of *in vitro* release of optimized formulation and pure drug (N = 6, data were expressed as Mean ± SEM, One way ANOVA followed by Dunnett's test, All groups were compared with control, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Acute toxicity study: After given a 2000 mg/kg dose orally to the rats no sign and symptoms of toxicity observed during 14 days.

Sub-chronic study: CCl_4-induced rats were used in the hepatoprotective activity test. Because it is a hepatotoxicant, it was designed to cause liver damage in rats. Piperine, which has hepatoprotective properties, can help protect the liver from injury and repair liver function that has been harmed by CCl_4. Organ histology or monitoring the amounts of total bilirubin,

TABLE-6
DPPH RADICAL SCAVENGING ACTIVITY

Sample name	IC_{50} value (µg/mL)	Regression Equations	R^2 – value	P value
Pure piperine	107.5719 ± 0.11	I = 16.62c+ 0.400	0.84	0.01
PLH	93.9267 ± 0.03	I =16.41c + 0.232	0.86	0.01

TABLE-7
SGOT, SGPT, ALP AND TOTAL BILIRUBIN LEVEL

Group of treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dl)
Normal control	161.00 ± 0.31	52.78 ± 0.28	121.12 ± 0.14	0.633 ± 1.44
CCl_4	265.53 ± 0.98	109.7 ± 0.21	220.30 ± 0.65	1.921 ± 1.23
CCl_4 + Silymarin	177.16 ± 0.78	89.4 ± 0.05	142.22 ± 0.25	0.972 ± 0.21
CCl_4 + Pure piperine	162.11 ± 0.36	65.03 ± 0.50	135.22 ± 1.15	0.68 ± 1.15
CCl_4 + P2	163.23 ± 0.49	66.90 ± 0.05	128.30 ± 1.15	0.645 ± 0.67

#Values were performed in triplicates and represented as mean ± SD

ALP, SGOT and SGPT enzymes in the blood can reveal liver disease. Table-7 shows the average SGOT and SGPT values of the group treatment. As in the negative control group, the data demonstrated an increase in SGOT and SGPT levels in rat blood following CCl_4 treatment. SGOT, bilirubin and ALP levels in normal rats were 161.00 ± 0.31 UI/L, 0.633 ± 1.44, 121.12 ± 0.14 and SGPT 52.78 ± 0.28, respectively. The serum protein level is reduced by pure piperine and PLH. Piperine-loaded herbosomes produce far greater effects than piperine alone, which is equivalent to silymarin. The presence of an elevated liver enzyme implies both acute and chronic liver injury. When liver cells are destroyed, the SGPT enzyme is secreted into the blood stream, where it can be evaluated using laboratory tests.

Conclusion

The present study has shown that phospholipid base molecular aggregates have a lot of potential for improving solubility, oral bioavailability, antioxidant activity and *in vivo* liver protection. Because phospholipids entrap both hydrophobic and hydrophilic moieties, we use them as a carrier to overcome this problem. In comparison to pure piperine, the P2 formulation has shown more prolonged liver protective activity on the basis of their SGOT, SGPT, ALP and total bilirubin. As a result of its prolonged release property, the formulation tackles the problem of poor solubility and improve hepatoprotectiove activity, while also lowering the frequency of administration. According to the findings, the P2 formulation has a sustained releasing feature, which means it has the potential to work for a long time inside the body. The results of the study have shown that P2 formulation can aid with poor solubility, bioavailability and hepatoprotective efficacy when compared to piperine at the same dose.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. B. Chopra, A.K. Dhingra, R.P. Kapoor and D.N. Prasad, *Open Chem. J.*, **3**, 75 (2016); https://doi.org/10.2174/1874842201603010075
2. L. Gorgani, M. Mohammadi, G.D. Najafpour and M. Nikzad, *Compr. Rev. Food Sci. Food Saf.*, **16**, 124 (2017); https://doi.org/10.1111/1541-4337.12246
3. C.R. Filburn, R. Kettenacker and D.W. Griffin, *J. Vet. Pharmacol. Ther.*, **30**, 132 (2007); https://doi.org/10.1111/j.1365-2885.2007.00834.x
4. K. Maiti, K. Mukherjee, A. Gantait, B.P. Saha and P.K. Mukherjee, *Int. J. Pharm.*, **330**, 155 (2007); https://doi.org/10.1016/j.ijpharm.2006.09.025
5. A. Singh, A.P. Singh and N. Verma, *Asian J. Chem.*, **23**, 5189 (2011).
6. S. Tripathy, D.K. Patel, L. Barob and S.K. Naira, *J. Drug Deliv. Ther.*, **3**, 147 (2013); https://doi.org/10.22270/jddt.v3i3.508
7. V. Srikanth, Ch. Laxmaiah, B.G. Shankar, P. Navee. B. Chiranjeeb and T.S. Gouda, *Int. J. Pharm. Rev. Dev*, **3**, 175 (2011).
8. P. Rathore and G. Swami, *Int. J. Pharm. Sci. Res.*, **3**, 737 (2012).
9. S. Moscarella, A. Giusti, F. Marra, C. Marena, M. Lampertico, P. Relli, P. Gentilini and G. Buzzelli, *Curr. Ther. Res. Clin. Exp.*, **53**, 98 (1993); https://doi.org/10.1016/S0011-393X(05)80160-2
10. M. Thomas, *Int. J. Pharm. Pharm. Sci.*, **2**, 10 (2010).
11. R.B. Chandrashekhar, R.Y. Narsimha and R.V. Malla, *Int. J. Pharm. Sci. Nano Technol.*, **1**, 87 (2018); https://doi.org/10.37285/10.37285/ijpsn.2008.1.1.8
12. W.W. Epstein, D.F. Netz and J.L. Seidel, *J. Chem. Educ.*, **70**, 598 (1993); https://doi.org/10.1021/ed070p598
13. A.N. Sahu and S. Sharma, *Pharmacognosy Res.*, **8**, 29 (2016); https://doi.org/10.4103/0974-8490.171102
14. J.J. Bhise, O.G. Bhusnure, S.R. Jagtap, S.B. Gholve and R.R. Wale, *J. Drug Deliv. Therap.*, **9**, 924 (2019); https://doi.org/10.22270/jddt.v9i3-s.2863
15. S. Bose and B. Michniak-Kohn, *Eur. J. Pharm. Sci.*, **48**, 442 (2013); https://doi.org/10.1016/j.ejps.2012.12.005
16. G. Nahak and R.K. Sahu, *J. Appl. Pharm. Sci.*, **1**, 157 (2011).
17. A. Szerlauth, S. Muráth, S. Viski and I. Szilagyi, *Heliyon*, **5**, e02763 (2015); https://doi.org/10.1016/j.heliyon.2019.e02763
18. S. Chunlaratthanaphorn, N. Lertprasertsuke, U. Srisawat, A. Thuppia, A. Ngamjariyawat, N. Suwanlikhid and K. Jaijoy, *Songklanakarin J. Sci. Technol.*, **29(Suppl. 1)**, 109 (2007).
19. E.P. Sabina, A.D.H. Souriyan, D. Jackline and M.K. Rasool, *Asian Pac. J. Trop.*, **3**, 971 (2010); https://doi.org/10.1016/S1995-7645(11)60011-4
20. S. Giacomelli, D. Gallo, P. Apollonio, C. Ferlini, M. Distefano, P. Morazzoni, A. Riva, E. Bombardelli, S. Mancuso and G. Scambia, *Life Sci.*, **70**, 1447 (2002); https://doi.org/10.1016/S0024-3205(01)01511-9
21. Q. Lv, A. Yu, Y. Xi, H. Li, Z. Song, J. Cui, F. Cao and G. Zhai, *Int. J. Pharm.*, **372**, 191 (2009); https://doi.org/10.1016/j.ijpharm.2009.01.014
22. A. Salunke and N. Upmanyu, *Adv. Pharm. Bull.*, **11**, 286 (2020); https://doi.org/10.34172/apb.2021.041
23. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, Ghaziabad, India, Ed.: 5 (2007).