

Curcumin–phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats

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Abstract

A novel formulation of curcumin in combination with the phospholipids was developed to overcome the limitation of absorption and to investigate the protective effect of curcumin–phospholipid complex on carbon tetrachloride induced acute liver damage in rats. The antioxidant activity of curcumin–phospholipid complex (equivalent of curcumin 100 and 200 mg/kg body weight) and free curcumin (100 and 200 mg/kg body weight) was evaluated by measuring various enzymes in oxidative stress condition. Curcumin–phospholipid complex significantly protected the liver by restoring the enzyme levels of liver glutathione system and that of superoxide dismutase, catalase and thiobarbituric acid reactive substances with respect to carbon tetrachloride treated group ($P < 0.05$ and < 0.01). The complex provided better protection to rat liver than free curcumin at same doses. Serum concentration of curcumin obtained from the complex (equivalent to 1.0 g/kg of curcumin) was higher (C_{\max} 1.2 $\mu\text{g/ml}$) than pure curcumin (1.0 g/kg) (C_{\max} 0.5 $\mu\text{g/ml}$) and the complex maintained effective concentration of curcumin for a longer period of time in rat serum. The result proved that curcumin–phospholipid complex has better hepatoprotective activity, owe to its superior antioxidant property, than free curcumin at the same dose level.

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1. Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione], a low molecular weight polyphenol derived from the rhizomes of turmeric (*Curcuma longa* Linn.), is a yellow pigment, widely used as a coloring agent and spice in many foods. Interests in this dietary polyphenol has grown in recent years due to its vast array of beneficial pharmacological effects including antioxidant, anti-inflammatory, anticarcinogenic (Ruby et al., 1995; Bush et al., 2001; Gescher et al., 2001; Sharma et al., 2001a,b; Ireson et al., 2002; Lukita-Atmadja et al., 2002; Perkins et al., 2002; Shao et al., 2002), hypocholesterolemic (Rao et al., 1970; Patil and Srinivasan, 1971), antibacterial (Ramaprasad and Sirsi, 1956), wound healing, antispasmodic, anticoagulant, antitumor (Ammon and Wahl,

1991) and hepatoprotective (Despande et al., 1998; Subramanian and Selvam, 1999; Park et al., 2000) activities. It is also a potent free radical scavenger, having superoxide anions, singlet oxygen, hydroxyl radicals scavenging and lipid peroxidation inhibitory activities (Tonnesen and Greenhill, 1992; Reddy and Lokesh, 1994; Subramanian et al., 1994; Ramirez Bosca et al., 1995).

Despite the promising biological effects of curcumin, its poor oral bioavailability in both rodents and humans, as reported by several workers (Wahlstrom and Blennow, 1978; Pan et al., 1999; Ireson et al., 2001), has restricted its use in the management of human ailments. Poor oral absorption (Ammon and Wahl, 1991) due to its extremely low aqueous solubility or extensive pre-systemic metabolism may be responsible for the unfavorable pharmacokinetics of this molecule. In rodents, curcumin undergoes avid metabolism by conjugation and reduction and its absorption after oral dosing is characterized by poor systemic bioavailability (Holder et al., 1978; Wahlstrom and Blennow, 1978; Ireson et al., 2001; Sharma et al., 2001a,b).

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Keeping in view, the promise of curcumin as a therapeutically active agent and its poor oral absorption, it is pertinent to develop a new formulation of curcumin, which can increase its oral absorption and enhance its therapeutic activity.

Several studies have indicated the beneficial role of phospholipids in enhancing the therapeutic efficacy of some molecules having poor oral absorption. Silybin is one such molecule having poor oral bioavailability. Efforts have been given to prepare phospholipids complex of silybin and to increase its oral bioavailability, therefore, therapeutic efficacy. It was observed that the silybin–phospholipids complex has significant upper hand over the pure molecule in protecting liver and exerting antioxidant activities (Carini et al., 1992; Conti et al., 1992; Morazzoni et al., 1993; Comoglio et al., 1995; Yanyu et al., 2006). Our recent study with quercetin–phospholipid complex showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride (Maiti et al., 2005).

Aim of the present study was to prepare phospholipids complex of curcumin, determining its physicochemical properties and to evaluate its antioxidant activity in carbon tetrachloride-intoxicated rats in comparison to pure curcumin to substantiate the claim that complexation between curcumin and phospholipid can enhance the therapeutic efficacy of the parent molecule. The concentration of curcumin in blood serum of rats, fed with both free curcumin and the complex was determined along with the main pharmacokinetic parameters.

2. Materials and methods

2.1. Materials

The phospholipid, hydrogenated soy phosphatidyl choline (HSPC) was purchased from Lipoid, Ludwigshafen, Germany. Curcumin was purchased from Simga (Sigma Chemical, St. Louis, MO, USA); ethylene diamine tetra acetic acid (EDTA), thiobarbituric acid, trichloroacetic acid, sodium carboxy methyl cellulose, sodium dodecylsulphate, *n*-hexane and other chemicals were obtained from Loba Chemie, Mumbai, India. Glutathione, glutathione reductase, bovine serum albumin, tris base, nitroblue tetrazolium, 5,5-dithiobis(2-nitrobenzoic acid), phenazine methosulphate, *n*-octanol were purchased from SRL chemicals, Mumbai, India.

2.2. Preparation of curcumin–phospholipid complex

The complex was prepared with curcumin and HSPC at a molar ratio of 1:1. Weighed amount of curcumin and HSPC were taken in a 100 ml round bottom flask and 20 ml of dichloromethane was added. The mixture was refluxed at a temperature not exceeding 60 °C for 2 h. The resultant clear solution was evaporated and 10 ml of *n*-hexane was added to it with continuous stirring. The curcumin–phospholipid complex was precipitated and the precipitate was filtered and dried under vacuum to remove traces of solvents. The resultant curcumin–phospholipid complex (yield 88%, w/w) was kept in

an amber colored glass bottle, flushed with nitrogen and stored at room temperature.

2.3. Determination of curcumin content in the complex

The content of curcumin in the complex was determined by HPLC method as described by Jayaprakasha et al. (2002) with slight modification. Approximately 5 mg of the complex was dissolved in 1 ml of methanol and the volume was adjusted to 10 ml with methanol. Twenty microliters aliquot of the solution was injected into a HPLC system (Hewlett-Packard, Palo Alto, CA), fitted with a Waters- μ -Bondapak C₁₈ column (300 mm \times 4.6 mm i.d.) (Waters Corp., Milford, MA). The mobile phase consisted of methanol, 2% acetic acid and acetonitrile at a ratio of 5:30:65. The elution was carried out with a flow rate of 1.0 ml/min at ambient temperature and a wavelength of 425 nm was used for detection.

2.4. Microscopic view of the complex

Leica (type DC 300F) Microsystems AG, CH-9435 Heerbrugg was used for microscopic characterization of the complex. The complex was suspended in distilled water and a drop was placed on a slide and covered with a cover slip. Microscopic view of the complex was observed at a magnification of 400 \times .

2.5. Differential scanning calorimetry (DSC) of the complex

The samples were sealed in the aluminum crimp cell and heated at the speed of 10 °C/min from 0 to 300 °C in nitrogen atmosphere (60 ml/min). The peak transition onset temperature of curcumin, phospholipid, curcumin–phospholipid complex and physical mixture of curcumin and phospholipid were determined and compared with the help of a Mettler DSC 30 S (Mettler Toledo, UK).

2.6. High performance thin layer chromatography (HPTLC) study

Curcumin–phospholipid complex and pure curcumin were dissolved in methanol and the methanolic solutions were spotted on the Silica Gel 60F₂₅₄ pre-coated TLC plates and chromatogram was developed in chromatographic chambers using dichloromethane:methanol 99:1 as solvent system at a room temperature of 30 °C, at an angle of 70°. After development of chromatogram, the plates were scanned with the help of Camag TLC scanner 3 and the *R_f* values of the spots were recorded.

2.7. Solubility studies

Determination of solubility characteristics of curcumin, curcumin–phospholipid complex and physical mixture of curcumin and phospholipid were obtained by adding excess of the samples to 5 ml of water or *n*-octanol in sealed glass container at room temperature. The liquids were shaken for 24 h and centrifuged at 5000 rpm for 10 min. The supernatant was filtered,

and 1 ml of filtrate mixed with 9 ml of methanol. Twenty microliters aliquot of the resulting solution injected in HPLC and concentration of curcumin was measured at 425 nm.

2.8. Antioxidant activity

2.8.1. Animals

Male albino rats (Wistar strain) weighing 180–200 g were used for this study. Animals were housed in groups of 7–8 in colony cages at an ambient temperature of 20–25 °C and 45–55% relative humidity with 12 h light/dark cycles. They had free access to pellet chow (Brook Bond, Lipton India) and water *ad libitum*. The experiment was performed with the ethical guidelines as provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.8.2. Dosing

The rats were divided into six groups of six animals each. Group I received only distilled water with Tween 20 (1%, v/v) p.o. for 7 days and served as normal. Group II animals received single dose of equal mixture of carbon tetrachloride and olive oil (50%, v/v, 5 ml/kg i.p.) on the seventh day. Groups III and IV animals were treated with curcumin suspension in distilled water with Tween 20 (1%, v/v) at a dose level of 100 and 200 mg/kg, respectively, per day p.o., for 7 days. On the seventh day, a single dose of equal mixture of carbon tetrachloride and olive oil was administered (50%, v/v, 5 ml/kg i.p.). Groups V and VI animals were treated with curcumin–phospholipid complex at doses equivalent to 100 and 200 mg/kg of curcumin, respectively, per day p.o., for 7 days and on the seventh day, a single dose of equal mixture of carbon tetrachloride and olive oil (50%, v/v, 5 ml/kg i.p.) was administered.

2.8.3. Enzyme estimation

All animals were sacrificed by cervical decapitation under light ether anesthesia on the eighth day. Immediately after sacrifice, the livers were dissected out for biochemical estimation. The liver was washed with ice-cold saline, and the homogenate prepared in 0.1 M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of marker enzymes namely reduced glutathione (GSH) (Anderson, 1985), glutathione peroxidase (GPx) (Mohandas et al., 1984), glutathione-S-transferase (GST) (Habig et al., 1974), glutathione reductase (GRD) (Dubler and Anderson, 1981), superoxide dismutase (SOD) (Kakkar et al., 1984) and catalase (CAT) (Rigo and Rotilio, 1977) as per reported methods. Thiobarbituric acid reactive substances (TBARS) were also measured according to the method of Ohkawa et al. (1979). Protein concentration was determined (Lowry et al., 1951) using purified bovine serum albumin as standard.

2.9. Estimation of curcumin in rat serum

Male albino Wistar rats (150–200 g) were divided into two groups ($n = 6$ /group/time point), one group for administration of curcumin at a dose of 1.0 g/kg and the other group for administration of the complex at a dose equivalent to 1.0 g/kg of curcumin.

Under ether anesthesia, jugular vein blood samples were collected from both groups of rats into centrifuge tubes at different time points. Blood was allowed to clot at room temperature for about 1 h, centrifuged at 3000 rpm for 10 min and serum was separated and kept at -20°C prior to analysis.

Estimation of curcumin was done by HPLC technique. Methanol, 2% acetic acid and acetonitrile at a ratio of 5:30:65 was used as mobile phase with a flow rate of 1.0 ml/min at ambient temperature and a wavelength of 425 nm was used for detection.

2.9.1. Extraction of curcumin from serum and preparation of sample

One milliliter of serum sample, after being equilibrated to room temperature was transferred into a 10 ml volumetric flask and 5 ml methanol was added to it. The content was shaken vigorously and heated at $75\text{--}80^{\circ}\text{C}$ for 25–30 min. The volume was made up to 10 ml with methanol and the turbid solution was transferred into a 15 ml centrifuge tube. The solution was centrifuged at 5000 rpm for 10 min and supernatant was separated. The supernatant was filtered and 20 μl was subjected to HPLC analysis.

2.9.2. Preparation of standard solution

Ten milligrams of standard curcumin was dissolved in a 10 ml volumetric flask in methanol. 0.1 ml of this solution was taken and serially diluted to a final concentration of 0.1 ppm with methanol.

2.9.3. Validation of extraction and quantification method

Curcumin from rat serum was separated completely by the extraction process and standard curves ranging from 0.05 to 2.0 $\mu\text{g/ml}$ were linear ($r = 0.9925$). Minimum detection level of curcumin was 25 ng/ml. The validation of the method for extraction and quantification of curcumin from rat serum was done by performing recovery rate experiments. Three concentration ranges of high, middle and low were selected and extraction as well as quantification of curcumin from those regions was carried out as per the procedures described earlier. The recover rates of curcumin from high, middle and low concentration ranges were 83.25, 84.29 and 82.70%, respectively. The inter-days relative standard deviation (R.S.D.) were 2.47, 3.24 and 2.12% respectively and intra-days R.S.D. were 3.56, 2.78 and 3.48%, respectively.

2.10. Pharmacokinetic parameters

The main pharmacokinetic parameters of curcumin–phospholipid complex were obtained with the help of a computer designed program “WINNONLIN-4.1” and the parameters were compared to that of free curcumin. Maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) are the values obtained directly from concentration–time curve. Area under the concentration–time curve ($\text{AUC}_{0\text{--}t_{\text{n}}}$ and $\text{AUC}_{0\text{--}t_{\infty}}$), elimination half life ($t_{1/2\text{el}}$), elimination rate constant (K_{el}), clearance (cl) and volume of distribution (V_{d}) were determined.

Relative bioavailability (F) was also calculated using the formula:

$$F = \frac{\text{total amount of drug absorbed from curcumin-phospholipid complex } (A_{\max \text{ complex}})}{\text{total amount of drug absorbed from curcumin}} \times 100 = \frac{(V_d K_{el} AUC_{0-t\alpha})_{\text{complex}}}{(V_d K_{el} AUC_{0-t\alpha})_{\text{curcumin}}} \times 100$$

as the volume of distribution and elimination rate constants of curcumin is different for the complex and pure curcumin treated rats.

2.11. Statistical analysis

The data were expressed as mean \pm standard error mean (S.E.M.). For antioxidant activity, the statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's test. For serum concentration study, animal data were analyzed by Student's " t " test. P -values <0.05 were considered as significant.

3. Results

3.1. Content of curcumin in complex

Content of curcumin in the complex, as estimated by HPLC, was 32.04% (w/w), and the complex has much higher solubility in water or n -octanol than curcumin or physical mixture of curcumin and phospholipid (Table 1).

3.2. Microscopic observations

The microscopic view, as shown in Fig. 1, indicated the presence of spherical structures of the complex. The vesicles consisted of hydrogenated soy phosphatidyl choline (HSPC), and curcumin was intercalated in the lipid layer.

3.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a fast and reliable method to screen drug-excipient compatibility and provides maximum information about the possible interactions. In DSC, an interaction is concluded by elimination of endothermic peak(s), appearance of new peak(s), change in peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy. Fig. 2 shows the DSC thermograms of pure curcumin (A), phospholipid (B), curcumin-phospholipid complex (C) and physical mixture of curcumin and phospholipid

(D). The thermogram of curcumin showed a single peak with an onset of 143.65 °C and maximum occurrence at 157.60 °C.

Thermogram of phospholipids exhibit two different peaks; the first one (72.84 °C) is mild, which appears because of the hot movement of phospholipids polar head group. The second peak (147.00 °C) is very sharp and it appears due to phase transition from gel to liquid crystalline state. The non-polar hydrocarbon tail of phospholipids may be melted during this phase, yielding a sharp peak. One broad peak, with an onset at 58.48 °C and maximum occurrence at 70.25 °C, appeared in the thermogram of the complex, which is different from the peaks of the individual components of the complex. Physical mixture of curcumin and phospholipids shows two peaks, one at 70.25 °C and other at 157.60 °C. The former has the same onset temperature (58.48 °C) of the complex and the later has same onset temperature (143.65 °C) of curcumin. It may assume that with the rise in temperature the phospholipids become melted and curcumin got dissolved in phospholipids, partly forming the complex. The thermogram of the complex exhibits a single peak which differs from the peak of curcumin and phospholipids. It is evident that the original peaks of curcumin and phospholipids disappear from the thermogram of complex and the phase transition temperature is lower than that of phospholipids. Began et al. (1999) suggested that curcumin can interact with phospholipids and the interaction is hydrophobic in nature. There is also some contribution of hydrogen bonding apart from hydrophobic interaction in the curcumin-phospholipids interaction. The -OH groups of the phenol rings of curcumin are involved in hydrogen bonding whereas the aromatic rings could be involved in hydrophobic interaction. The interaction of curcumin with the polar part of phospholipids molecules make the long hydrocarbon tail of phospholipids to turn freely and 'envelop' the polar head of phospholipids containing the curcumin molecule. As a result there is a decrease in the sequence of the phospholipids hydrocarbon chains and the second sharp peak of phospholipids disappears and lowers the phase transition temperature.

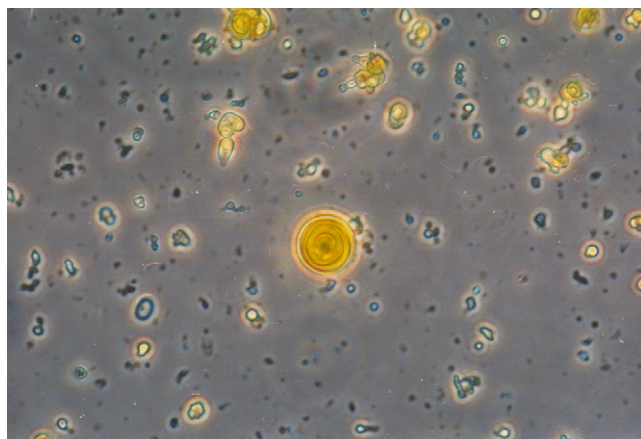


Fig. 1. Microscopic views of curcumin-phospholipid complex with a magnification of 400 \times . Curcumin was intercalated in the phospholipid layer.

Table 1
Solubility of curcumin, curcumin-phospholipid complex and physical mixture of curcumin and phospholipid in water and n -octanol at 25 °C

| Samples | Solubility in water ($\mu\text{g/ml}$) | Solubility in n -octanol (mg/ml) |
|-----------------------------------------------|------------------------------------------|------------------------------------|
| Curcumin | 8.33 \pm 0.23 | 4.08 \pm 0.28 |
| Curcumin-phospholipid complex | 26.67 \pm 1.34 | 45.99 \pm 2.32 |
| Physical mixture of curcumin and phospholipid | 12.40 \pm 0.52 | 8.87 \pm 0.31 |

Values are mean \pm S.E.M. ($n=3$).

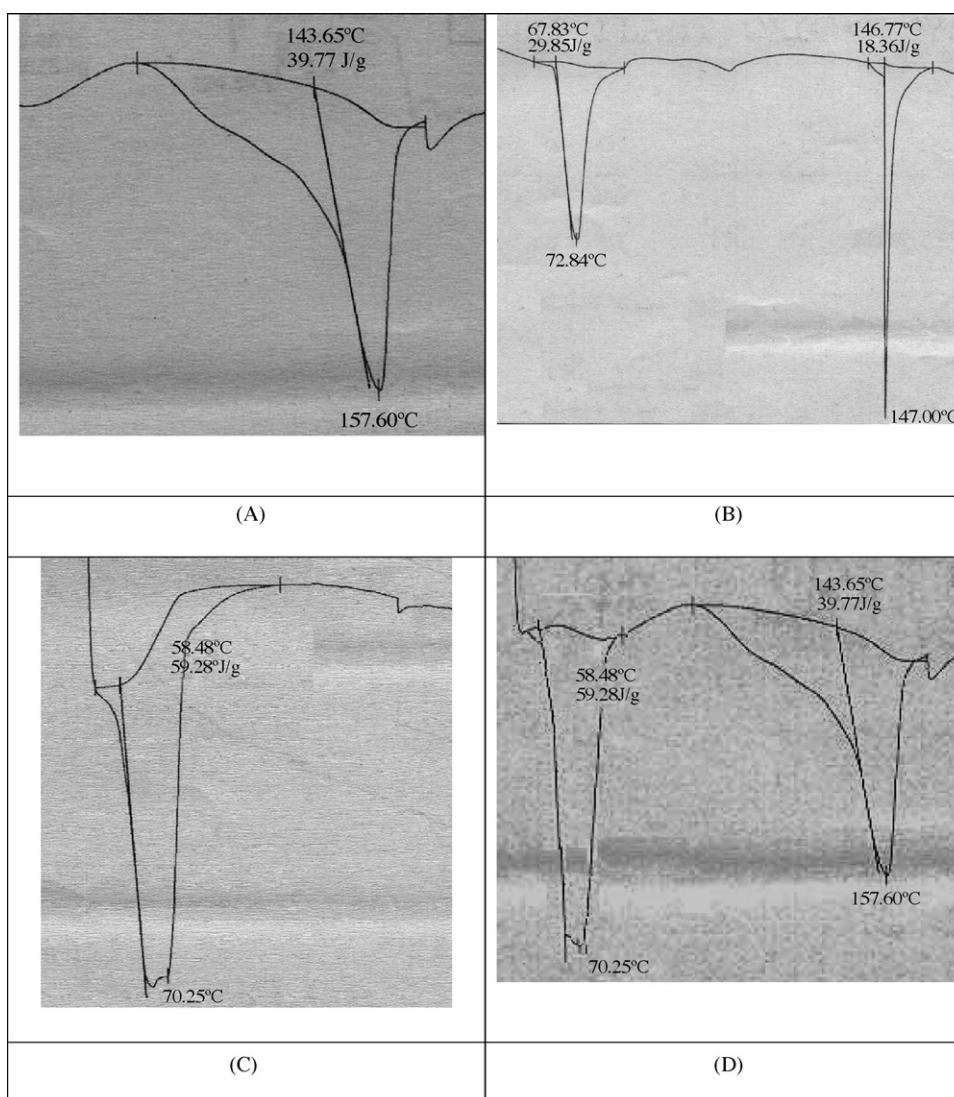


Fig. 2. DSC thermograms exhibiting the peak transition onset temperature of pure curcumin (A), phospholipid (B), curcumin–phospholipid complex (C) and physical mixture of curcumin and phospholipid (D).

3.4. High performance thin layer chromatography (HPTLC) study

Figs. 3 and 4 showed the HPTLC chromatograms of pure curcumin and the curcumin–phospholipid complex, respectively. It was observed that pure curcumin has a R_f value of 0.46, whereas the complex was having the R_f value of 0.56.

3.5. Antioxidant activity

The results of the antioxidant activities of curcumin and the complex at different dose levels were shown in Table 2 and Figs. 5–7.

3.5.1. Liver glutathione system

Animals intoxicated with CCl_4 showed impaired levels of reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GRD). Activities of all these enzymes in CCl_4 treated

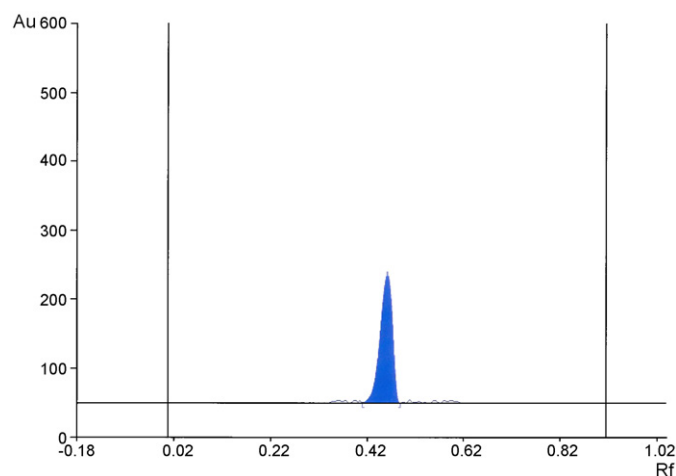


Fig. 3. HPTLC chromatogram of pure curcumin with a R_f value of 0.46.

Table 2
Effect of curcumin–phospholipid complex on the enzymes of liver glutathione system of CCl₄ intoxicated rats

| Parameters | Group I | Group II | Group III | Group IV | Group V | Group VI |
|-------------------------------------------------------------------|-----------------|---------------|-----------------|-----------------|-----------------|-----------------|
| GSH (nmol/mg protein) | 44.75 ± 3.84** | 21.33 ± 1.54 | 30.32 ± 2.14* | 38.83 ± 1.70** | 37.38 ± 2.61** | 43.73 ± 1.88** |
| GPx (nmol of NADPH oxidized/min/mg protein) | 308.90 ± 3.81** | 172.70 ± 4.90 | 225.90 ± 3.28** | 282.50 ± 7.17** | 279.70 ± 7.05** | 303.90 ± 4.67** |
| GST (nmol of CDNB conjugate formed/min/mg protein) | 294.50 ± 7.47** | 160.70 ± 4.91 | 187.50 ± 8.19 | 200.10 ± 6.94** | 194.00 ± 7.16* | 252.50 ± 8.82** |
| GRD [nmol of oxidized glutathione (GSSG) utilized/min/mg protein] | 20.52 ± 0.34** | 9.68 ± 0.40 | 12.21 ± 0.67 | 15.90 ± 0.86** | 15.24 ± 0.28** | 20.04 ± 1.49** |

Values are mean ± S.E.M. ($n=6$). * $P<0.05$, ** $P<0.01$ (significant with respect to CCl₄-treated group).

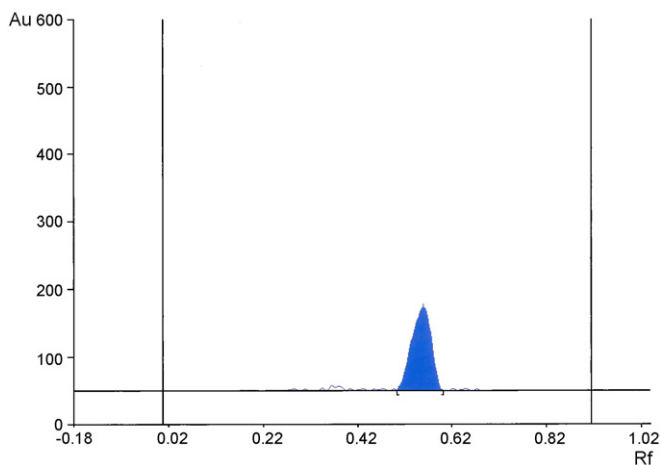


Fig. 4. HPTLC chromatogram of curcumin–phospholipid complex with a R_f value of 0.56.

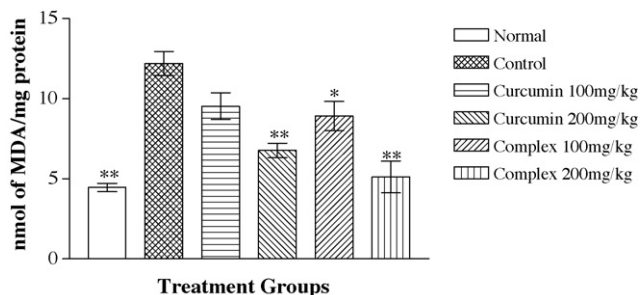


Fig. 5. Effect of curcumin–phospholipid complex on TBARS. Values are mean ± S.E.M. ($n=6$). * $P<0.05$, ** $P<0.01$ [significant with respect to control (CCl₄-treated group)].

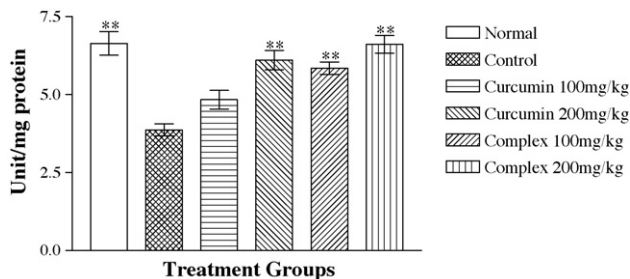


Fig. 6. Effect of curcumin–phospholipid complex on SOD. Values are mean ± S.E.M. ($n=6$). ** $P<0.01$ [significant with respect to control (CCl₄-treated group)].

rats were reduced significantly ($P<0.01$) as compared to normal animals. Pretreatment of animals with curcumin as well as curcumin–phospholipid complex increased the reduced levels of these enzymes. The complex at both the dose levels produced better results than the respective doses of free curcumin (Table 2).

3.5.2. Thiobarbituric acid reactive substance (TBARS)

TBARS level of liver homogenates in CCl₄-challenged rats significantly increased ($P<0.05$) when compared to normal rats (4.45 nmol of MDA/mg of protein). Treatment with free curcumin (200 mg/kg) as well as curcumin–phospholipid complexes (100 and 200 mg/kg) showed significant ($P<0.05$) decrease in TBARS levels in liver homogenate when compared to CCl₄-treated animals (Fig. 5).

3.5.3. Superoxide dismutase (SOD)

SOD level was significantly reduced in CCl₄-treated animals. Treatment with free curcumin at 100 mg/kg did not produce any significant result but the complex at the same dose significantly increased the SOD levels ($P<0.01$) in liver homogenate when compared to CCl₄-treated animals. At 200 mg/kg dose level, free curcumin as well as curcumin–phospholipid complex showed significant increase in SOD levels ($P<0.01$) (Fig. 6).

3.5.4. Catalase (CAT)

Significant reduction of CAT level occurred in CCl₄-treated animals as compared to normal ($P<0.01$). In pre-treated groups of free and complexed curcumin (100 and 200 mg/kg), the level of CAT increased significantly ($P<0.01$) (Fig. 7).

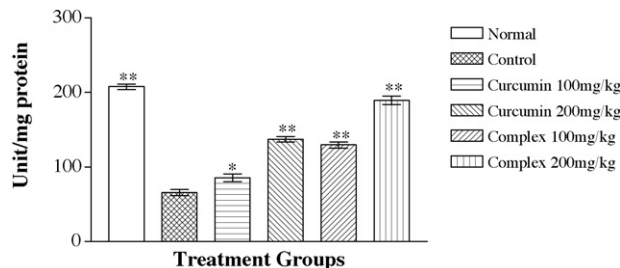


Fig. 7. Effect of curcumin–phospholipid complex on catalase. Values are mean ± S.E.M. ($n=6$). * $P<0.05$, ** $P<0.01$ [significant with respect to control (CCl₄-treated group)].

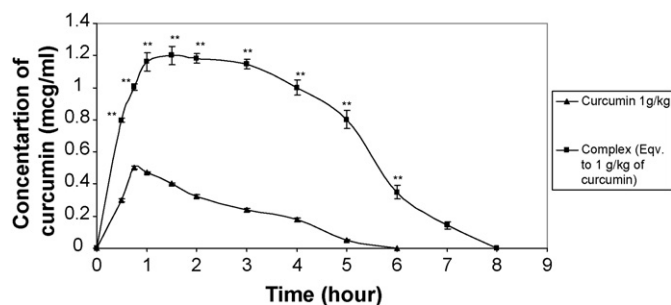


Fig. 8. Effect of complexation on serum concentration of curcumin in rats. Values are mean \pm S.E.M. ($n=6$ /group/time point). ** $P<0.001$ (significant with respect to pure curcumin-treated group).

Table 3

Main pharmacokinetic parameters of free curcumin (1 g/kg, p.o.) and curcumin–phospholipid complex (equivalent to 1 g/kg of free curcumin, p.o.) in rats ($n=6$)

| Pharmacokinetic parameters | Curcumin | Curcumin–phospholipid complex |
|------------------------------------------------------------------------------------------|----------|-------------------------------|
| C_{max} ($\mu\text{g ml}^{-1}$) | 0.50 | 1.20 |
| T_{max} (h) | 0.75 | 1.50 |
| Area under concentration–time curve (AUC_{0-t_n}) ($\mu\text{g ml}^{-1} \text{h}$) | 1.32 | 5.90 |
| Area under concentration–time curve (AUC_{0-t_x}) ($\text{ml}^{-1} \text{h}$) | 1.68 | 8.73 |
| Elimination half life ($t_{1/2el}$) (h) | 1.45 | 1.96 |
| Elimination rate constant (K_{el}) (h^{-1}) | 0.48 | 0.35 |
| Clearance (cl) (l h^{-1}) | 92.26 | 22.33 |
| Volume of distribution (V_d) (l) | 192.21 | 63.82 |

3.6. Concentration of curcumin in rat serum

Fig. 8 shows the result of serum concentration study of curcumin and curcumin–phospholipid complex in rats. Peak serum concentration of $0.5 \mu\text{g/ml}$ attained rapidly within 1 h when pure curcumin was administered. But in case of complex, the peak concentration ($1.2 \mu\text{g/ml}$) appeared at 1.5 h and the concentration was much higher than pure curcumin. The higher concentration was also maintained for a longer period of time before being dropped down.

3.7. Pharmacokinetic parameters

Table 3 shows the main pharmacokinetic parameters of curcumin–phospholipid complex and curcumin in rat. C_{max} was increased from 0.50 to $1.2 \mu\text{g/ml}$ in case of the complex, so did the T_{max} . The elimination half life of curcumin was increased when it was in the complex form with phospholipids and eventually the clearance of the molecule in complex form was also lowered. The complex persisted for a longer period of time in body with a higher relative bioavailability of 125.80%.

4. Discussion

Development of valuable drug delivery system from natural resources is very much necessary because of the beneficial role

of herbal drug in the management of varied diseases (Mukherjee, 2001, 2002a). Continuing research is very much essential to explore the therapeutic efficacy of the natural molecules as well as to develop proper delivery system for enhancing the therapeutic potential of those molecules (Mukherjee, 2003).

Carbon tetrachloride (CCl_4) causes hepatocellular degeneration, centrilobular necrosis (Kim et al., 1990; Valles et al., 1994) and impairs different enzymatic systems (Glende et al., 1976). In CCl_4 hepatotoxicity, it is metabolized by cytochrome P-450 to produce the trichloromethyl radical, which initiates a cascade of free radical reactions resulting in an increase in lipid peroxidation and a reduction in some enzyme activities (Recknagel et al., 1989). Many compounds with antioxidant property have been investigated for protective activity against CCl_4 induced hepatotoxicity (Mukherjee, 2002b; Rai et al., 2006). Curcumin is known to enhance the bile acid production and secretion (Kerry, 1991). It also increases the excretion of cholesterol in bile with a concomitant reduction in bile cholesterol saturation and elevated faecal fat excretion (Rao et al., 1970; Kerry, 1991). It may be possible that enhanced biliary excretion from the system may accelerate the clearance of toxic metabolites of CCl_4 . Curcumin is also a powerful free radical scavenger, which can exert its radical scavenging activities, thus, in turn can protect the liver from the adverse effects of CCl_4 (Shih and Lin, 1993; Ruby et al., 1995).

The bioavailability of lipophilic drugs when administered orally as solid dosage forms is notoriously low. There are usually several factors responsible for this, but a particularly widespread problem is poor absorption due to slow and/or incomplete drug dissolution in the lumen of the gastro-intestinal tract. In this case, improved bioavailability can be achieved by the use of delivery systems, which can enhance the rate and/or the extent of drug solubilizing into aqueous intestinal fluids. Phospholipids play a major role in drug delivery technology. There are numerous advantages of phospholipids in addition to solubilizing property while considering them for a carrier system.

In the present experiment we prepared curcumin–phospholipid complex by a simple and reproducible method. The physicochemical investigations showed that curcumin formed a complex with phospholipids. The complex has enhanced aqueous or *n*-octanol solubility. The antioxidant activity of the complex was significantly higher than pure curcumin in all dose levels tested. Free curcumin at the dose of 200 mg/kg prevented the adverse conditions in rats created by CCl_4 intoxication. The complex also restored the normal condition of rat liver enzymes. Lower dose of pure curcumin (100 mg/kg) failed to produce significant result in most of the occasions but as complex it gave almost similar effects to that of pure curcumin in double dose (200 mg/kg). The complex at a dose equivalent to 200 mg/kg of curcumin gave better results than the free molecule (200 mg/kg) and restored the normal enzyme levels. This enhanced therapeutic efficacy of curcumin in terms of hepatoprotection, obtained from curcumin–phospholipid complex may be due to better absorption and bioavailability of the molecule, which was supported by the pharmacokinetic study in rats.

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