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Enhanced Oral Bioavailability and Antioxidant Profile of Ellagic Acid by Phospholipids

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Ellagic acid (EA) has been reported as a potent antioxidant from natural resources with several nutritional benefits. The major disadvantage of this phytoconstituent is its rapid elimination from the body after administration. To overcome this limitation, a novel dietary formulation of EA with phospholipid was developed to investigate the effect of this complex on carbon tetrachloride induced liver damage in rats. The antioxidant activity of the complex (equivalent of EA = 25 and 50 mg/kg of body weight) and free EA (25 and 50 mg/kg of body weight) was evaluated by measuring various enzymes in oxidative stress condition. The complex significantly protected the liver by restoring the activity of superoxide dismutase, catalase and liver glutathione, and thiobarbituric acid reactive substances with respect to the carbon tetrachloride treated group ($P < 0.05$ and < 0.01). The complex provided better protection to rat liver than free EA at the same dose. The serum concentration of EA obtained from the complex (equivalent to 80 mg/kg of EA) was higher (C_{max} = 0.54 μg/mL) than that of pure EA (80 mg/kg) (C_{max} = 0.21 μg/mL), and the complex maintained effective concentration for a longer period of time in serum. The experimental outcome highlighted better hepatoprotective activity of the EA complex due to its potential antioxidant property compared with the free EA tested at the same dose level.

KEYWORDS: Ellagic acid; phospholipid complex; carbon tetrachloride; antioxidant; hepatoprotective activity; pharmacokinetic

INTRODUCTION

Many molecules developed as dietary supplements suffer from poor water solubility (*1*, *2*), which may substantially limit their bioavailability. Ellagic acid (EA) (**Figure 1**) [2,3,7,8-tetrahydroxychromeno[5,4,3-*cde*]chromene-5,10-dione], a dimeric derivative of gallic acid, is a polyphenolic antioxidant that occurs in its free form as a glycoside or is found as ellagitannins in fruits and nuts of several plants (*3*). Even though ellagic acid exhibits good antioxidant activity, it has solubility problems and has been classified under the Biopharmaceutical Classification System (BCS) as a class IV substance with low solubility (< 10 mg/mL in phosphate buffer, pH 7.4) and low permeability (0.13×10^{-6}) (*4*). Apart from poor solubility and permeability, EA has poor stability at a physiological pH of 7.4 (*5*, *6*). The metabolism and distribution of ellagic acid has been studied in animals, and the low bioavailability of EA has been proved (*7*, *8*). Experimental evidence using animal models reflects that orally administered EA is poorly absorbed and rapidly eliminated from the body, which limits its potential as a systemic antioxidant due to its inability to attain required tissue concentrations (*9*, *10*).

Several studies have reported the antioxidant, antimutagenic, anti-inflammatory, and cardioprotective activities of EA (*11*). The effect of EA, which is abundant in whiskey, has been proved to exhibit gastroprotective action against gastric lesions induced by NH_4OH or reperfusion in the ischemic stomach, probably due to its antioxidant activity (*12*). Ellagic acid also has been shown to be a potent anticarcinogenic agent (*13*). It can also inhibit the growth of microorganisms by sequestering metal ions that are critical for microbial growth and metabolism (*14*). One of the studies reported a better protection by ellagic acid than vitamin E against oxidative stress (*15*). Another study has demonstrated that oral administration of EA protects the system from alcohol toxicity by decreasing the liver marker enzymes, lipid peroxidative markers, NO, and PCC and increasing the antioxidant cascade in a dose-dependent manner (*16*).

Despite these pharmacological benefits, the low oral bioavailability of EA is attributed to its low aqueous solubility, metabolism in the GIT, irreversible binding to cellular DNA and proteins, and first-pass effect. Phospholipid is an important carrier system for the poorly soluble molecules. It provides the required sustained/controlled release in vivo due to rapid clearance from the body and plays a major role in delivery technology as it is better absorbed in vivo. Our recent studies with quercetin, curcumin (having poor oral absorption), and naringenin (having faster elimination) demonstrated that complexation with phospholipid enhances the bioactivity of these phytomolecules (*17*−*19*). The aim of this work was to develop an effective dietary supplement by EA−phospholipid complex to improve

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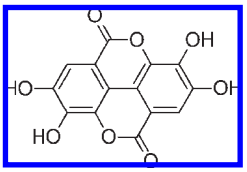

Figure 1. Structure of ellagic acid.

its bioavailability and evaluate the antioxidant activity of the complex in CCl_4-induced oxidative stress in rats in comparison to the parent molecule.

The therapeutic efficacy of EA had been evaluated in several in vivo model systems, often with conflicting results. The reason being that a sufficiently high concentration of EA is not present in plasma or target cells after oral administration due to its low solubility. Doyle and Griffiths (*7*) have isolated several metabolites of EA from blood, bile, urine, and feces; they have not detected EA in plasma and suggested that EA would be metabolized by intestinal micro-organisms under the condition of oral administration.

Several human studies have been done on the bioavailability and pharmacokinetics of EA. The pharmacokinetics of EA in human was studied by Seeram et al., after oral administration of pomegranate juice (*20*). The study demonstrated that consuming 180 mL of the juice concentrate was associated with maximum plasma concentrations of EA of 0.06 mmol/L after 1 h. The pharmacokinetic profile indicates that EA has poor absorption and rapid elimination after oral administration of pomegranate leaf extract, and part of it was absorbed from the stomach. Gary et al. studied the pharmacokinetics of EA in human volunteers fed freeze-dried black raspberries and found that, overall, $< 1\%$ of these compounds were absorbed and excreted in urine (*21*). These pharmacokinetic hurdles lead to poor oral bioavailability of EA and hindered its development as a potential nutritional supplement. Thus, the bioavailability studies performed on the prepared EA−phospholipid complex will certainly give insight into the effect of solubility in the pharmacokinetics and hence therapeutic efficacy of the same.

MATERIALS AND METHODS

Hydrogenated soy phosphatidylcholine (HSPC) was procured from Lipoid, Ludwigshafen, Germany; ellagic acid (EA) was purchased from Sigma Chemical, St. Louis, MO. Thiobarbituric acid, trichloroacetic acid, sodium carboxymethyl cellulose, *n*-hexane, and other chemicals were obtained from Loba Chemie, Mumbai, India, and SD Fine Chemicals, Kolkata, India. Dichloromethane was obtained from Qualigen Fine Chemicals, Mumbai, India. Glutathione, bovine serum albumin, tris base, nitroblue tetrazolium, and 5,5-dithiobis(2-nitrobenzoic acid) reagent were purchased from SRL Chemicals, Mumbai, India.

Preparation of EA−Phospholipid Complex. The complex was prepared with EA and HSPC at a molar ratio of 1:4 on the basis of our earlier reported method (*17*−*19*). Briefly, weighed amounts of EA 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 to 2−3 mL, and 10 mL of *n*-hexane was added to it with continuous stirring. The EA−phospholipid complex was precipitated, and the precipitate was filtered and dried under vacuum to remove traces of solvents. The resultant EA−phospholipid complex (yield = 89% w/w) was kept in an amber-colored glass bottle flushed with nitrogen and stored at room temperature.

Determination of EA Content in the Complex. The content of EA in the complex was determined spectrophotometrically with a Cecil CE 7200 spectrophotometer. A calibration curve was obtained for ellagic acid by dissolving increasing amounts of the EA, exactly weighed, in the desired volume of methanol. Approximately 5 mg of the complex was dissolved in 1 mL of methanol in a 10 mL volumetric flask, and the volume was adjusted to 10 mL. A portion of the sample was adequately diluted and analyzed at 306 nm to value the concentration of EA in the complex. Baseline was established for each measurement by placing a methanol solution of HSPC at the same concentration of the sample in the reference compartment. The experiments were carried out in triplicate.

High-Performance Thin Layer Chromatography (HPTLC) Study. The EA−phospholipid complex and pure EA were dissolved in methanol and spotted on the silica gel 60F254 precoated TLC plates, and a chromatogram was developed in chromatographic chambers using toluene/ethyl acetate/formic acid (5:5:1) as solvent system at a room temperature of 30 °C, at an angle of 70°. After development of the chromatogram, the plates were scanned with the help of a Camag TLC scanner 3, and the R_f values of the spots were recorded.

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 220 °C in a nitrogen atmosphere (60 mL/min). The peak transitions onset temperature of EA, phospholipid, EA−phospholipid complex, and physical mixture of EA and phospholipid were determined and compared with the help of a Mettler DSC 30 S (Mettler Toledo, U.K.).

Scanning Electron Microscopy (SEM) of the Complex. The complex was streaked onto a clean and dry microscope slide, and the sample was air-dried at room temperature. The morphology of the complex was observed using a scanning electron microscope (FEI quanta 200 - MK 2).

Animals. Male albino rats (Wistar strain) weighing 150−220 g were used for this study. Animals were housed in groups of seven or eight 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) (composition: 66% carbohydrate, 23% protein, 4.5% fat, fortified with amino acids, minerals, and vitamins) and water ad libitum. All rats were fasted overnight before the experiment. The experiment was performed within the ethical guidelines as provided by a committee for the purpose of control and supervision of experiments on animals (CPCSEA), and the institutional animal ethics committee approved the study.

Liver Function Test and Antioxidant Activity. Rats were divided into six groups of six animals each. Group I received only distilled water with Tween 80 (1% v/v) po for 7 days and served as vehicle control. Group II animals received a single dose of an equal mixture of carbon tetrachloride and olive oil (50%, v/v, 5 mL/kg ip) on the seventh day. Group III and IV animals were treated with EA suspension in distilled water with Tween 80 (1%, v/v) at a dose level of 25 or 50 mg/kg, respectively, per day po, for 7 days. On the seventh day, a single dose of an equal mixture of carbon tetrachloride and olive oil was administered (50%, v/v, 5 mL/kg ip). Group V and VI animals were treated with the EA−phospholipid complex at doses equivalent to 25 and 50 mg/kg of EA, respectively, per day po, for 7 days, and on the seventh day, a single dose of an equal mixture of carbon tetrachloride and olive oil (50%, v/v, 5 mL/kg ip) was administered.

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 histopathological and biochemical estimation. The liver was washed with ice-cold saline, and the homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged, and the supernatant was used for the assay of oxidative stress biomarkers, namely, reduced glutathione (GSH) (*22*), glutathione peroxidase (GPx) (*23*), glutathione-*S*-transferase (GST) (*24*), glutathione reductase (GRD) (*25*), superoxide dismutase (SOD) (*26*), and catalase (CAT) (*27*, *28*). Thiobarbituric acid reactive substances (TBARS) were also measured according to the method of Ohkawa (*29*). Protein concentration was determined (*30*) using purified bovine serum albumin as standard. However, serum was used to measure the activity of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminate (SGPT) (*31*), serum alkaline phosphatase (SALP) (*32*), and total bilirubin (*33*).

Histopathological Studies. Immediately after sacrificing the rats, the livers were dissected out and preserved in neutral buffered formalin. Livers were serially sectioned and microscopically examined after staining with hematoxylin and eosin with a magnification of 400×.

Estimation of Ellagic Acid in Rat Serum. Male albino Wistar rats were divided into two groups (n = 6/group/time point), one group for

administration of EA at a dose of 80 mg/kg and the other group for administration of the complex at a dose equivalent to 80 mg/kg of EA. 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 and then centrifuged at 3000 rpm for 10 min, and serum was separated and kept at −20 °C prior to analysis. Estimation of EA was done by HPLC technique (*10*) with slight modification. The HPLC system consisted of a 515 HPLC pump (Waters), a Rheodyne 7725i manual injector (Waters), a 996 photodiode array detector (Waters), and a Millennium 32 chromatogram workstation. A Hypersil C-18 column, 4.6 × 150 mm, 5 μm, was used. The mobile phase consisted of methanol/0.2% phosphoric acid water solution (25:55, v/v). It was filtered through a 0.22 μm Millipore filter and degassed prior to use. The flow rate was 0.8 mL/min. Detection was performed at a wavelength of 254 nm under a constant temperature (40 ± 1 °C).

Preparation of Stock Solution and Calibration Curves. Ten milligrams of standard EA was dissolved in a 10 mL volumetric flask in methanol, and then 0.1 mL of this solution was taken and serially diluted to a final concentration of 0.1 ppm with methanol. The stock solution at various concentrations (0.01−2 μg/mL) was spiked into blank serum to obtain the calibration curve for EA. Five hundred microliters of serum spiked with EA was taken. To it were added 150 μL of 1 M potassium dihydrogen phosphate solution and 15 μL of 50% phosphoric acid. Then each sample was vortex-mixed with 5 mL of acetonitrile for 1 min, centrifuged at 3000*g* for 15 min at 8−10 °C. The supernatant liquor was gently evaporated to dryness in a water bath at 37 °C. The residue was dissolved in 0.1 mL of methanol and was taken for HPLC analysis. Calibration curves were obtained after determining the peak area ratios of standard serum spiked with various concentrations of EA and plotting the peak area ratios against a corresponding concentration of EA.

Extraction of EA from Serum and Preparation of Sample. A 1.0 mL portion of rat plasma was adjusted to pH 2.5 with 0.3 mL of 1 M potassium dihydrogen phosphate solution and 30 μL of 50% phosphoric acid. Then each sample was vortex-mixed with 5 mL of acetonitrile for 1 min, centrifuged at 3000*g* for 15 min at 8−10 °C. The supernatant liquor was gently evaporated to dryness in a water bath at 37 °C. The residue was dissolved in 0.1 mL of methanol. Each proceeding sample was stored at 0−4 °C until further analysis.

Pharmacokinetic Parameters. The main pharmacokinetic parameters of the EA−phospholipid complex were obtained with the help of a computer-designed program, WINNONLIN - 4.1, and the parameters were compared to those of free EA. Maximum concentration (C_{max}) and time to reach maximum concentration (t_{max}) are the values obtained directly from the concentration−time curve. Area under the concentration−time curve ($AUC_{0\text{-}tn}$ and $AUC_{0\text{-}t\alpha}$), elimination half-life ($t_{1/2,el}$), elimination rate constant (K_{el}), clearance (cl), and volume of distribution (V_d) were determined. Relative bioavailability (F) was calculated as a ratio of the plasma AUC (0−infinity)(*34*, *35*).

Statistical Analysis. All data were expressed as mean ± SEM. The standard error was calculated by dividing the standard deviation by the square root of the number of observations. The Dunnett multiple-comparison test was carried out to compare populations using GraphPad Prism software (San Diego, CA). Statistical significance was defined as a P value of <0.05 or <0.01. P values of <0.05 were considered to be significant.

RESULTS

Content of EA in the Complex. The amount of EA present in the EA−phospholipid complex was 29 ± 1.7% w/w as estimated by UV−vis spectrophotometry. The results showed that the amount of EA intercalated in the phospholipid layer was 85.72 ± 2.6%.

HPTLC Study. The HPTLC chromatograms of pure EA and the EA−phospholipid complex showed that pure EA has a R_f value of 0.36, whereas the complex has a R_f value of 0.45.

DSC. 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.

(**Figure 2**) shows the DSC thermograms of pure EA (**A**), phospholipid (**B**), EA−phospholipid complex (**C**), and a physical mixture of EA and phospholipid (**D**). The thermogram of EA showed a sharp peak at 164.81 °C. The thermogram of phospholipids exhibited two different peaks; the first one (72.69 °C) was mild, which appeared because of the hot movement of the phospholipid polar headgroup. The second peak (149.17 °C) was very sharp and appeared due to phase transition from gel to liquid crystalline state. The nonpolar hydrocarbon tail of phospholipids may be melted during this phase, yielding a sharp peak. The thermogram of the complex showed two peaks, one at 79.94 °C and another at 147.83 °C, which are different from the peaks of the individual components of the complex. The physical mixture of the two showed a broad peak at 73.52 °C and a narrow peak at 147.27 °C. The former has an onset temperature similar to that of the complex, and the latter has an onset temperature similar to that of EA. It may be assumed that with the rise in temperature the phospholipids become melted and EA was dissolved in phospholipids, partly forming the complex. The thermogram of the complex exhibits a single peak, which differs from the peak of EA and phospholipids. It is evident that the original peaks of EA and phospholipids disappear from the thermogram of complex, and the phase transition temperature is lower than that of phospholipids, thus confirming the formation of the complex.

SEM. The scanning electron microscopic view, as shown in (**Figure 3**), indicated the presence of spherical structures in the complex. The vesicles consisted of hydrogenated soy phosphatidylcholine (HSPC), and EA was intercalated in the lipid layer. The particle size of the EA−phospholipid complex was found to be in the range of 1−3 μm.

Liver Function Test. Hepatic damage induced by CCl_4 caused a significant rise in marker enzymes SGPT, SGOT, and SALP and also in serum bilirubin. Pretreatment with EA at 25 mg/kg dose cannot significantly lower the enzyme activity, whereas pure EA at a dose of 50 mg and EA complex at a dose of 25 or 50 mg/kg gave a significant reduction ($P < 0.01$). The result of the liver function tests are given in **Table 1**.

Antioxidant Activity. The antioxidant activity of the EA−phospholipid complex is shown in (**Figure 4**). Glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) activities in liver homogenates were reduced in CCl_4-treated animals when compared to vehicle control rats. Pretreatment with pure EA at the 25 mg/kg dose level failed to produce significant increases in the GSH, GPX, SOD, and CAT activities when compared to CCl_4-treated animals but the EA−phospholipid complex at the dose equivalent of 25 or 50 mg/kg showed a significant increase in the activity of CAT ($P < 0.01$). Pure EA at a dose level of 50 mg/kg produced significant increases in GSH, GPX, SOD, and CAT activities ($P < 0.01$), whereas showed only moderately significant activity of GRD when compared to CCl_4 ($P < 0.005$). The TBARS in liver homogenates of CCl_4-treated rats significantly increased ($P < 0.01$) when compared to vehicle control rats. Pretreatment with both pure EA and EA complex at both dose levels showed significant change ($P < 0.01$). The EA−phospholipid complex at the dose equivalent of 25 or 50 mg/kg produced a significant effect ($P < 0.01$) comparable to the higher dose of the pure EA (50 mg/kg).

Histopathological Studies. Through electron microscopy, histological observation of liver tissue of the normal rats showed hepatic cells with well-preserved cytoplasm, nucleus, nucleolus, and central vein. In the CCl_4-treated group, histological observation showed fatty degeneration, damage of parenchymal cells, steatosis, and hydropic degeneration of liver tissue. Prominent damage of the central lobular region appeared in the liver.

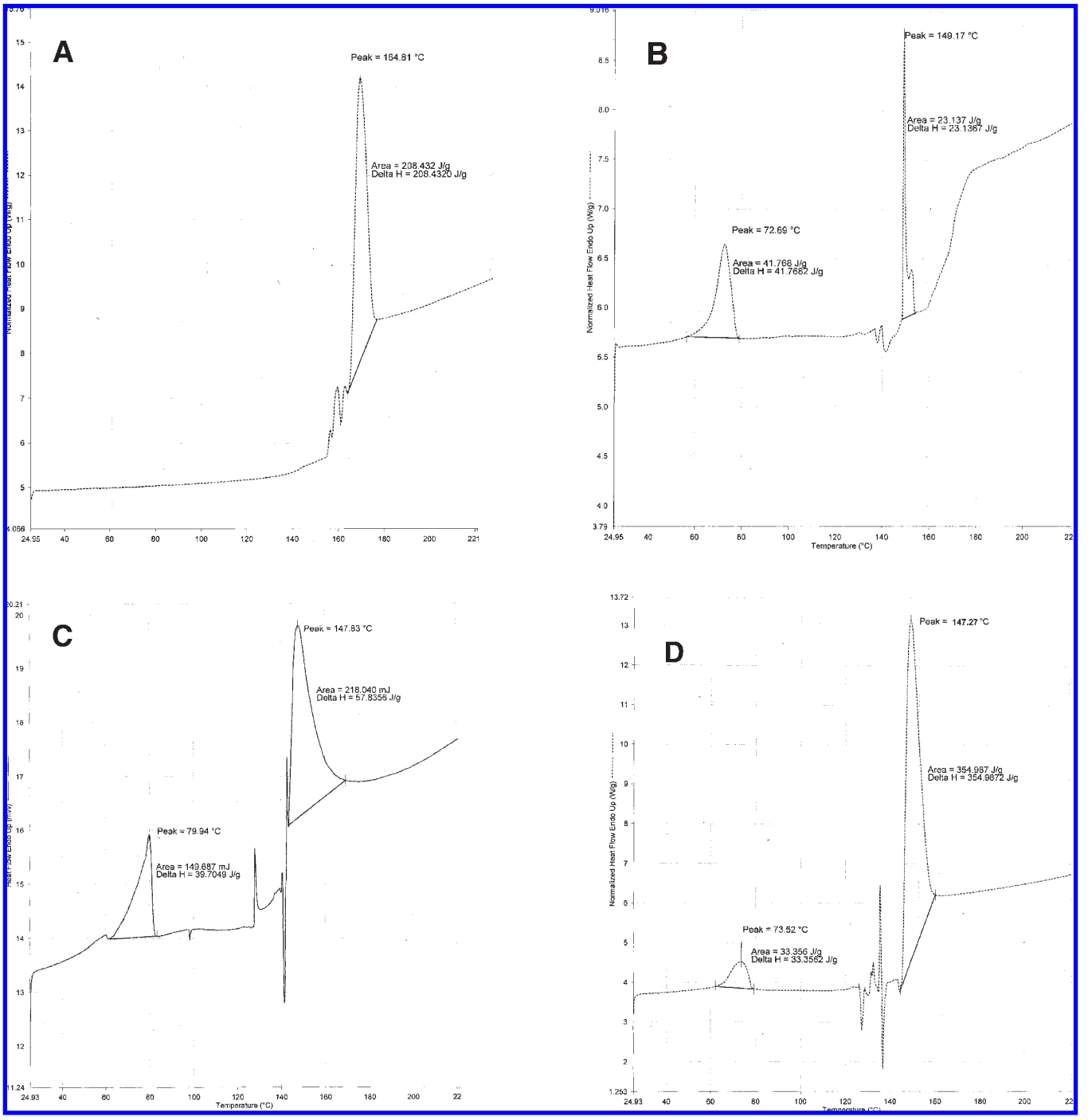

Figure 2. DSC thermograms of pure EA (**A**), phospholipid (**B**), EA−phospholipid complex (**C**), and physical mixture of EA and phospholipid (**D**).

Pretreatment with free EA at a lower dose showed little sign of amelioration, whereas at high dose, free EA reduced the degree of degeneration as compared to the lower dose. Pretreatment with the EA−phospholipid complex in both lower and higher doses retrieved the morphologic changes toward normal physiology in a dose-dependent manner.

Concentration of EA in Rat Serum. Peak serum concentration of 0.21 μg/mL was attained at 0.5 h when pure EA was administered. Also, in the case of complex, the peak concentration (0.54 μg/mL) appeared at 2 h, and concentration was maintained significantly for a longer period of time (**Figure 5**).

Pharmacokinetic Parameters. Table 2 shows the pharmacokinetic parameters of the EA−phospholipid complex and pure EA in rat. C_{max} was increased in the case of the complex. The elimination half-life of EA 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 area under the curve (AUC) 0 to infinity for the EA−phospholipid complex was 2.53 units, whereas that of pure EA was 0.89 unit. Thus, the EA−phospholipid complex has a relative bioavailability of 2.84 compared to that of pure EA. The results indicate that the relative bioavailability of the EA−phospholipid complex is 2.84-fold compared to the normal EA.

DISCUSSION

Development of valuable delivery systems for food sources is very much necessary because of the beneficial role of food in the management of various diseases (*36*, *37*). Continuing

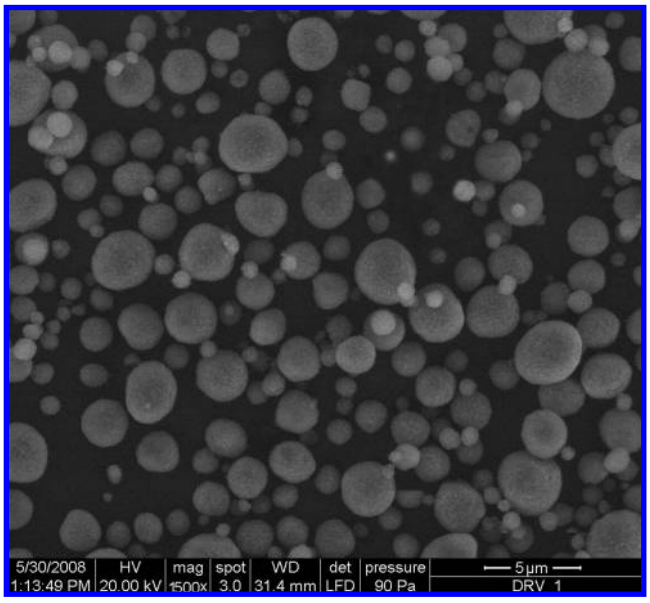

Figure 3. Scanning electron microscope image showing the spherical structures of the complex.

research to explore the nutritional benefits of the natural molecules as well as to develop proper delivery systems for enhancing the potential of those molecules is very much essential (*38*). The benefits of dietary molecules, especially polyphenols, have been recognized well only in the past decade, which is evident from various studies progressing in the field of polyphenols. These polyphenols are found abundantly in plants and have been shown to scavenge a wide range of reactive oxygen species (*39*−*41*). Ellagic acid is one of those polyphenols that have received the attention of researchers. It has a wide array of biological properties, such as radical scavenging, chemopreventive, antiviral, and antibacterial properties. Although EA exhibits good antioxidant activity, it is metabolized by intestinal microorganisms upon oral administration and rapidly eliminated from the body due to short plasma half-life, which makes it unavailable for sufficient nutritional benefit. To eliminate this, the present study dealt with the preparation and evaluation of a novel phospholipid complex of EA.

Carbon tetrachloride causes hepatocellular degeneration and centrilobular necrosis (*42*, *43*) and impairs different enzymatic systems (*44*). In CCl_4 hepatotoxicity, it is metabolized by cytochrome P-450 to produce the trichloromethyl radical, which

Table 1. Liver Function Test after CCl_4 Treatment[a]

treatment	SGPT (10^3 U/L)	SGOT (10^3 U/L)	SALP (U/L)	total bilirubin (mg/dL)	total protein (mg/mL)
group I (normal)	43.28 ± 0.78**	47.18 ± 2.37**	118.50 ± 2.51**	0.77 ± 0.06**	5.84 ± 0.27**
group II (CCl_4)	87.44 ± 1.89	126.40 ± 3.70	190.70 ± 3.26	1.62 ± 0.02	3.12 ± 0.33
group III	78.77 ± 3.56	106.10 ± 6.26	184.40 ± 4.08	1.31 ± 0.14	3.87 ± 0.16
group IV	69.77 ± 3.42*	91.25 ± 4.68*	166.90 ± 3.15*	1.18 ± 0.16*	4.18 ± 0.22
group V	68.92 ± 4.35*	93.25 ± 5.58*	161.90 ± 2.36*	0.92 ± 0.08*	4.03 ± 0.28
group VI	45.43 ± 1.06**	65.37 ± 5.23**	142.30 ± 3.35**	0.68 ± 0.06**	5.07 ± 0.26**

[a] Values are mean ± SEM of six animals. SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; SALP, serum alkaline phosphatase; CCl_4, carbon tetrachloride. Significant with respect to CCl_4-treated group: * $P < 0.05$; ** $P < 0.01$.

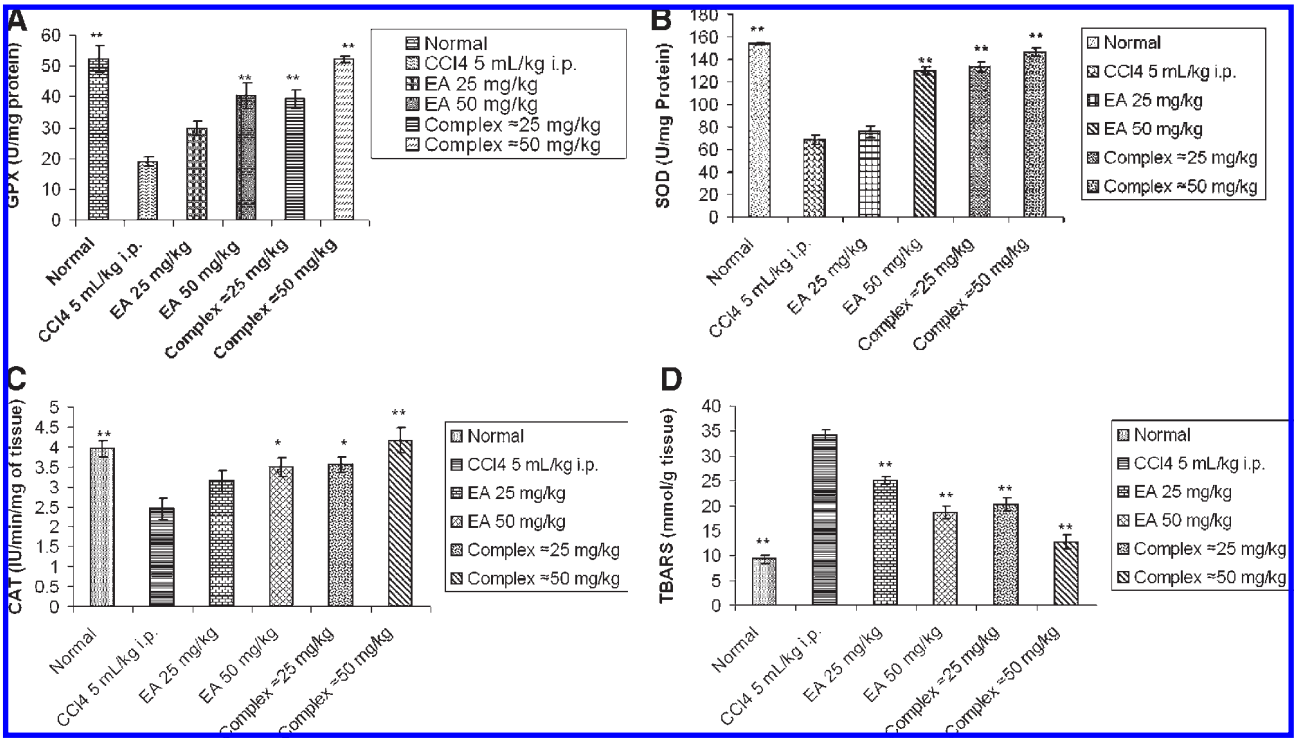

Figure 4. Effect of EA and its phospholipid complex on different oxidative stress biomarkers: (**A**) glutathione peroxide (GPX); (**B**) superoxide dismutase (SOD); (**C**) catalase (CAT); (**D**) thiobarbituric acid reactive substances (TBARS) activity in rat liver. * $P < 0.05$, and ** $P < 0.01$ (significant with respect to CCl_4 treated group). Values are mean ± SEM of six animals.

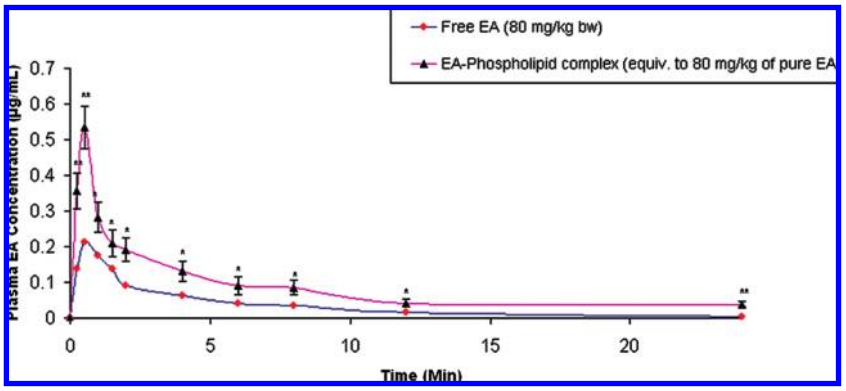

Figure 5. Effect of free EA and its phospholipid complex on serum concentration of EA in rats. * $P < 0.01$ and ** $P < 0.0001$ (significant with respect to pure EA treated group). Values are mean ± SEM of six animals.

Table 2. Main Pharmacokinetic Parameters of Free EA (80 mg/kg, po) and EA−Phospholipid Complex (Equivalent to 80 mg/kg of Free EA, po) Orally Administered in Rats ($n = 6$)

pharmacokinetic parameter	pure EA	EA−phospholipid complex
C_{max} (μg mL^{-1})	0.21	0.54
T_{max} (h)	0.5	0.5
area under concentration−time curve (AUC_{0-tn}) (μg mL^{-1} h)	0.72	2.05
area under concentration−time curve ($AUC_{0-t\infty}$) (mL^{-1} h)	0.89	2.53
elimination half-life ($t_{1/2,el}$) (h)	6.11	8.32
elimination rate constant (K_{el}) (h^{-1})	0.11	0.08
clearance (cl) (L h^{-1})	89640.03	31681.15
volume of distribution (V_d) (L)	789766.58	380301.38

initiates a cascade of free radical reactions resulting in an increase in lipid peroxidation and a reduction in some enzyme activities. Many compounds with antioxidant property have been investigated for protective activity against CCl_4-induced hepatotoxicity (*39*, *45*). EA has been found to have antioxidant activity to a significant extent (*15*). It has been shown to decrease the liver marker enzymes in rats exposed to alcohol-induced liver toxicity (*16*).

The bioavailability of lipophilic molecules when administered orally as solid 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 dissolution in the lumen of the gastrointestinal tract. Improved bioavailability can be achieved by the use of delivery systems that can enhance the rate or extent of solubilization of the molecules into aqueous intestinal fluids. Phospholipids play a major role in delivering poorly soluble compounds and several other benefits, which gives a rationale for selecting phospholipids for increasing the bioavailability of the molecules.

In the present experiment we prepared an EA−phospholipid complex by a simple and reproducible method. The physicochemical investigations showed that EA formed a complex with phospholipids. Liver function tests and subsequent antioxidant studies show that in general pretreatment with pure EA at low dose (25 mg/kg) failed to give significant change in the activity of the oxidative stress biomarkers present in the liver, whereas EA at high dose (50 mg/kg) produced a significant change in the activity, playing a protective role. The complex of EA prepared at both lower and higher doses (≈25 and 50 mg/kg) showed significant activity. The complex at the lower dose (≈25 mg/kg) showed results almost similar to those of the pure EA at double the dose. The results were statistically significant as compared to the CCl_4-treated group. Pharmacokinetics study showed that the release of EA from pure EA suspension was almost complete within 12 h, whereas the complex extended the release of EA up to 24 h. Serum concentration and pharmacokinetics study also substantiate the claim of sustained release of EA from the complex. Thus, from the present study it may be interpreted that the phospholipid complex of EA can produce better nutritional efficacy in rats for a longer period of time as compared to the molecule itself by virtue of the better absorption and bioavailability of the molecule, which is supported by the pharmacokinetic study.

ABBREVIATIONS USED

EA, ellagic acid; NH_4OH, ammonium hydroxide; GIT, gastrointestinal tract; DNA, deoxyribonucleic acid; CCl_4, carbon tetrachloride; HSPC, hydrogenated soy phosphatidylcholine; HPTLC, high-performance thin layer chromatography; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; GSH, reduced glutathione; GPx, glutathione peroxidase; GST, glutathione-*S*-transferase; GRD, glutathione reductase; SOD, superoxide dismutase; CAT, catalase; TBARS, thiobarbituric acid reactive substances; SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminate; SALP, serum alkaline phosphatase; HPLC, high-performance liquid chromatography; AUC, area under curve; SEM, standard error mean; UV−vis, ultraviolet−visible.

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