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Research Article

Exploring the Effect of Hesperetin–HSPC Complex—A Novel Drug Delivery System on the *In Vitro* Release, Therapeutic Efficacy and Pharmacokinetics

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Abstract. Hesperetin is known to exhibit a variety of pharmacological activities in mammalian cell systems. Although it shows appreciable bioavailability when administered orally, its faster elimination from body creates the need of frequent administration to maintain effective plasma concentration. To overcome this limitation, a phospholipid complex of hesperetin was prepared and evaluated for antioxidant activity and pharmacokinetic profile. The hesperetin content of the complex was determined by a spectrophotometer and the surface characteristics of the complex were studied by means of microscope. The antioxidant activity was evaluated in carbon-tetrachloride-intoxicated rats at a dose level of 100 mg/kg body weight, p.o. The complex was studied for *in vitro* drug release characteristics and effect of complexation on serum concentration of hesperetin in rats was also studied along with main pharmacokinetic parameters. The results showed that the complex has a sustained release property and enhanced antioxidant activity (P<0.05 and <0.01) as compared to free hesperetin at the same dose level. Pharmacokinetic study depicted that the complex has higher relative bioavailability and acted for a longer period of time. The study therefore suggests that phospholipid complex of hesperetin produced better antioxidant activity than free drug at the same dose level and the effect persisted for a longer period of time, which may be helpful in solving the problems of faster elimination of the molecule.

KEY WORDS: antioxidant; bioavailability; elimination; hesperetin–phospholipid complex; *in vitro* release; pharmacokinetic; sustained release.

INTRODUCTION

Hesperetin, which is derived from the hydrolysis of hesperidin (hesperetin 7-rhammnoglucoside) (1), is an important constituent belonging to the class of flavonoids called flavanones. It shows a vast array of biological activities contributing towards protection of human health and is abundant in citrus fruits (2). It has been found to possess antioxidant and free-radical scavenging (3–6), blood-lipid lowering (7,8), and anti-carcinogenic activities (9). Hesperetin has been shown to inhibit chemically induced mammary (10), urinary bladder (11), and colon (9,12) carcinogenesis in laboratory animals. In addition, it is used for the treatment of hemorrhoids (13) and in the prevention of post-operative thromboembolism (14). Other possible effects of hesperetin are on lipid metabolism. It has been reported to regulate apolipoprotein B secretion by HepG2 cells, possibly through inhibition of cholesterol ester synthesis (7), and to inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase and acyl coenzyme A:cholesterol *O*-acyltransferase in rats (15,16). An increase of high-density lipoprotein levels in hypercholesterolemic human subjects after consumption of orange juice was also reported (17). In spite of this wide range of therapeutic efficacy, shorter half-life, and rapid clearance of hesperetin from the body restricts its use as a potent phyto molecule. Erlund *et al*. (2001) reported that the elimination half-life of hesperetin is 2.2 h in humans (18). Therefore, to maintain steady plasma concentration of hesperetin inside the body so as to exert its therapeutic activity, frequent administration of the drug is required and it necessitates the need for development of a dosage form, which can maintain the concentration of hesperetin in blood for a longer period of time.

Hydrogenated soy phosphatidyl choline (HSPC) is an important phospholipid carrier system for the drug molecules and facilitates them with required sustained/controlled release *in vivo* and play a major role in drug delivery technology. Our recent studies with quercetin, curcumin (having poor oral absorption), and naringenin (having rapid elimination) demonstrated that complexation with phospholipid enhances the bioactivity of these phytomolecules (19–21). So the aim of this work was to develop a hesperetin–HSPC complex, a novel formulation of hesperetin, to evaluate the antioxidant activity of the complex in carbon-tetrachloride-intoxicated rats in comparison to parent molecule and to study the main pharmacokinetic parameters of complex and hesperetin in rats.

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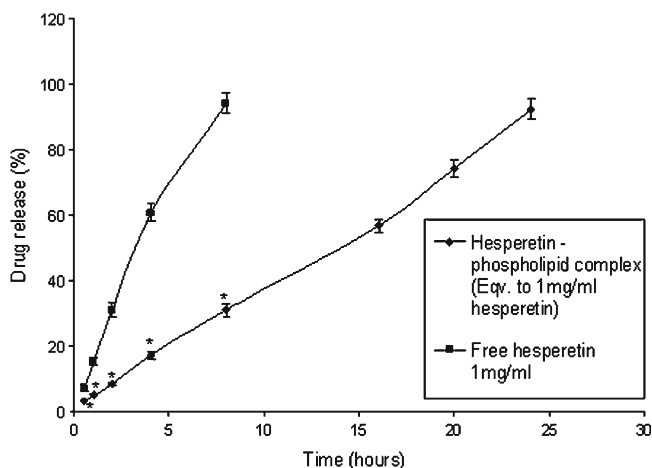

Fig. 1. *In vitro* release study of hesperetin from pure hesperetin suspension and hesperetin–phospholipid complex at different time points. Values are mean ± SEM (n=3). *P<0.001 (significant with respect to free hesperetin)

EXPERIMENTAL

Materials and Methods

HSPC was procured from Lipoid, Ludwigshafen, Germany; hesperetin (purity 95%) was purchased from Sigma Chemical, St. Louis, MO, USA. Thiobarbituric acid, trichloroacetic acid, sodium carboxy methyl cellulose, n-hexane, and other chemicals were obtained from Loba Chemie, Mumbai, India and S.D. Fine Chemicals, Kolkata, India. Dichloromethane were obtained from Qualigen Fine Chemicals, Mumbai, India. Glutathione, bovine serum albumin, tris base, nitroblue tetrazolium, 5,5′-dithiobis(2-nitrobenzoic acid) reagent were purchased from SRL chemicals, Mumbai, India.

Preparation of Hesperetin–HSPC Complex

The complex was prepared with hesperetin and HSPC at a molar ratio of 1:1 as per reported method (19–21). Briefly, weighed amount of hesperetin [mol. wt., 302.27] and HSPC [mol. wt., 782] 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. Resultant clear solution was evaporated to 2–3 ml and 10 ml of n-hexane was added to it with continuous stirring. Hesperetin–HSPC complex was precipitated and the precipitate was filtered and dried under vacuum to remove traces of solvents. The resultant hesperetin–HSPC complex (yield 92% w/w) was kept in an amber colored glass bottle, flushed with nitrogen, and stored at room temperature.

Determination of Hesperetin Content in the Complex

Content of hesperetin in the complex was determined spectrophotometrically with a Cecil CE 7200 Spectrophotometer. A calibration curve was obtained for hesperetin by dissolving increasing amounts of the drug, 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 289 nm to value the concentration of hesperetin in the complex. Baseline was established for each measurement placing methanol solution of HSPC at the same concentration of the sample in the reference compartment. The experiments were carried out in triplicate.

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.

In vitro Release Study of Hesperetin from Pure Hesperetin Suspension and Complex

In vitro drug release from the complex was determined using dialysis sacks (retains proteins with molecular weight >12,000), purchased from Sigma, 250 -7U, USA. The sacks were washed as per the instruction given by the manufacturer. After proper pretreatment, one end of the sack was tied and 1 ml of pure hesperetin suspension/hesperetin–phospholipid complex was placed inside the sack. The other end of the sack was tied and then suspended vertically into a beaker containing 200 ml of phosphate buffer saline (PBS) pH 7.4. The experiment was conducted under sink condition and Tween 20 was added to the dissolution medium. The content of the beakers were stirred at 50 rpm using a magnetic stirrer at 37±1°C. For a period of 24 h, the sacks were opened at different time points, 0.5 ml of sample was withdrawn and amount of drug release was determined spectrophotometrically at 289 nm in triplicate.

Liver Function Test and Antioxidant Activity

Animals

Male albino rats (Wistar strain) weighing 150–220 g, were used for this study. Animals were housed in groups of seven to eight in colony cages at an ambient temperature of 20–25°C and 45–55% relative humidity with 12 h light/dark cycle. 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 the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and the institutional animal ethics committee has approved the study.

Dosing

Rats were divided into five groups of 15 animals each. Group I received only distilled water with Tween 20 (1% v/v) p.o. for 7 days and served as vehicle control. 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 (22). Group III animals were treated with hesperetin suspension in

distilled water with Tween 20 (1% *v*/*v*) at a dose level of 100 mg/kg, per day p.o., for 7 days. Group IV animals received 100 mg/kg of hesperetin on the first, third, fifth, and seventh days. Group V animals were treated with hesperetin–phospholipid complex equivalent to 100 mg/kg of hesperetin on the alternate day (first, third, fifth, and seventh) like group IV animals up to seventh day. On the seventh day, a single dose of equal mixture of carbon tetrachloride and olive oil was given (50% *v*/*v*, 5 ml/kg i.p.) to group III, IV, and V animals 1 h after hesperetin (free or complexed) treatment. The dose of hesperetin (100 mg/kg) was selected based upon the LD50 value (data not mentioned).

Enzyme Estimation

Animals were fasted and 24 h after intoxication, they were anesthetized with ether to collect their blood from retro orbital plexus on the eighth day. Serum was separated by centrifugation and then liver function tests were performed by measuring serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) (23). Serum alkaline phosphatase (SALP) (24) and total bilirubin (25) were also measured. Five animals from each group, chosen randomly, were sacrificed by cervical decapitation under light ether anesthesia after blood collection. Liver was dissected out immediately after sacrifice for biochemical estimation, washed in ice-cold saline and homogenated in 0.1 M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged and supernatant was used for the assay of glutathione peroxidase (GPX) (26), superoxide dismutase (SOD) (27) and catalase (CAT) (28). Thiobarbituric acid reactive substances (TBARS) and protein concentrations (purified bovine serum albumin as standard) were also measured as per the reported methods (29,30). The same experimental protocol was repeated on the ninth and tenth days. For liver function test, 15 animals were used on day 8, which was further reduced to ten and five animals on days 9 and 10, respectively, as five animals on each of the eighth and ninth day were sacrificed for enzyme assay. For antioxidant activities on the eighth, ninth, and tenth day, five animals were used for all the groups. There was no drug treatment on the eighth, ninth, and tenth day.

Estimation of Hesperetin in Rat Serum

Male albino Wistar rats were divided into two groups (n=6/group/time point), one group for administration of hesperetin at a dose of 100 mg/kg and the other group for administration of the complex at a dose equivalent to 100 mg/kg of hesperetin. 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 3,000 rpm for 10 min and serum was separated and kept at −20°C prior to analysis.

Estimation of hesperetin was done by HPLC technique as described by Yang *et al.* (31) with slight modification. The HPLC system (Shimadzu, Japan) was accommodated with an UV spectrophotometric detector (Shimadzu, Japan) and RP C-18 column (Lichrospher 100, 5 μm, 250×4 mm). The mobile phase comprised of acetonitrile and 1% acetic acid at a ratio of 35:65. The flow rate was 1.0 ml/min and a wavelength of 289 nm was used for detection.

Preparation of Stock Solution and Calibration Curves

Ten milligrams of standard hesperetin 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. The stock solution at various concentrations (0.1–10 μg/ml) was spiked into blank serum to obtain the calibration curve for hesperetin. Five hundred microliters of serum spiked with hesperetin was taken. To this, 350 μl of 0.12 mol of sodium acetate buffer (pH 5.0)/l, 150 μl of 0.03 mol of ascorbic acid/l and 150 μl of 0.1 (N) hydrochloric acid were added. The serum mixture was partitioned with 1 ml of ethyl acetate containing 5, 7-dimethoxy coumarin (2.5 μg/ml, internal standard). Ethyl acetate layer was evaporated to dryness, reconstituted with 50 μl of acetonitrile and 20 μl was taken for HPLC analysis. Calibration curves were obtained after determining the peak area ratios (hesperetin to internal standard) of standard serum spiked with various concentrations of hesperetin and plotting the peak area ratios against corresponding concentration of hesperetin.

Extraction of Hesperetin from Serum and Preparation of Sample

The serum samples were hydrolyzed enzymatically to cleave the glucuronic acid and sulfate conjugates of hesperetin. The result exhibits total hesperetin present in serum. One milliliter of serum sample after being equilibrated to room temperature was added with 700 μl of a crude preparation from *Helix pomatia* (type HP-2, containing β-glucuronidase and sulfatase, Sigma Chemical Co., USA) in sodium acetate buffer (pH 5.0) and 300 μl of 0.03 mol of ascorbic acid/l and incubated at 37°C for 4 h. After incubation, the serum sample was mixed with 300 μl of 0.1 (N) hydrochloric acid and partitioned with 2 ml of ethyl acetate containing 5, 7-dimethoxy coumarin (2.5 μg/ml). Ethyl acetate layer was evaporated to dryness, reconstituted with acetonitrile and 20 μl was taken for HPLC analysis.

Validation of Extraction and Quantification Method

Hesperetin from rat serum was separated completely by the extraction process and standard curves ranging from 0.10 to 10.0 μg/ml were linear (r=0.9941). Minimum detection level of hesperetin was 50 ng/ml. The validation of the method for extraction and quantification of hesperetin 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 hesperetin from those ranges was carried out as described earlier. The recovery rates of hesperetin from high, middle, and low concentration ranges were 85.38, 86.33, and 84.67%, respectively. The inter-days relative standard deviations (R. S. D.) were 3.47, 2.24, and 3.92%, respectively, and intra-days R.S. D. were 4.06, 2.98, and 3.12%, respectively.

Pharmacokinetic Parameters

The main pharmacokinetic parameters of hesperetin–phospholipid complex were obtained with the help of a computer-designed program "WINNONLIN-4.1" and the parameters were compared to that of free hesperetin. 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 ($AUC_{0\text{-tn}}$ and $AUC_{0-t\alpha}$), elimination half-life ($t_{1/2}$el), elimination rate constant (K_{el}), clearance (cl) and volume of distribution (V_d) were determined. Relative bioavailability is a ratio of total amount of drug absorbed from hesperetin–phospholipid complex to total amount of drug absorbed from hesperetin. Amount of drug absorbed (A_{max}) from a dosage form is a function of V_d, K_{el}, and $AUC_{0-t\alpha}$. Therefore, relative bioavailability (F) was calculated using the formula:

$$F = \frac{\text{Total amount of drug absorbed from hesperetin} - \text{phospholipid complex}(A_{max}\text{complex})}{\text{Total amount of drug absorbed from hesperetin}(A_{max}\text{hesperetin})} \times 100$$

$$= \frac{(V_d \times K_{el} \times AUC_{0-t\alpha})\text{complex}}{(V_d \times K_{el} \times AUC_{0-t\alpha})\text{hesperetin}} \times 100$$

(as the volume of distribution and elimination rate constants of hesperetin is different for the complex- and pure-hesperetin-treated rats).

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). For liver function test and antioxidant activities, the statistical analysis was carried out using one-way analysis of variance followed by Dunnett's test. For *in vitro* drug release and serum concentration study, the statistical analysis was carried out using Student's *t* test. *P*-values less than 0.05 were considered as significant.

RESULTS

Content of Hesperetin in Complex

Amount of hesperetin, present in the hesperetin–phospholipid complex was 25±1.3% *w/w* as estimated by UV–VIS spectrophotometer. The results showed that the amount of hesperetin intercalated in the phospholipid layer of the multilayer preparation was 89.92±5.7%.

Microscopic Observations

The microscopic view indicated the presence of spherical structures of the complex with a mean size of 5–6 µm. The vesicles consisted of HSPC, and hesperetin was intercalated in the lipid layer.

In Vitro Release Study

From the *in vitro* release study, it was evident that the release of hesperetin from complex was sustained and persisted for a longer period of time as compared to free hesperetin (Fig. 1). Release of hesperetin (solubility 0.3 mg/ml) from pure hesperetin suspension was almost completed within 8 h whereas the complex extended the release of hesperetin up to 24 h.

Liver Function Test

Hepatic damage induced by CCl_4 caused significant rise in marker enzymes SGPT, SGOT, SALP, and also in serum bilirubin. Pretreatment with pure hesperetin at 100 mg/kg dose level in alternate days cannot lower the elevated enzyme levels significantly whereas animals treated with same dose of pure hesperetin for seven consecutive days produced ameliorative effect on day 8 (P <0.05). On days 9 and 10, it failed to exhibit significant protection of liver as evident from the liver function test. On the contrary, pretreatment with hesperetin–phospholipid complex on alternate days significantly protected the rat liver against the toxic effect of CCl_4 on days 8, 9 (P<0.01), and day 10 (P<0.05). The result of liver function tests is described in Table I.

Antioxidant Activity

Antioxidant activity of the hesperetin–phospholipid complex is shown in Figs. 2, 3, 4, and 5. Glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) levels in liver homogenates was significantly (P< 0.01) reduced in CCl_4-treated animals when compared to vehicle control rats. Pretreatment with pure hesperetin in alternate days failed to produce significant increase in the GPX, SOD, and CAT level when compared to CCl_4-treated animals but hesperetin–phospholipid complexes at the same treatment schedule showed significant increase in these enzyme levels (P<0.01) in liver homogenate as compared to CCl_4-treated animals on the eighth and ninth days. It also produced significant increase in enzymatic levels on the tenth day (P<0.05). Even hesperetin pretreatment for seven consecutive days hardly can increase the reduced level of these enzymes. The level of thiobarbituric-acid-reactive substance (TBARS) in liver homogenates of CCl_4-challenged rats significantly increased (P<0.05) when compared to vehicle control rats. Pretreatment with hesperetin–phospholipid complex (equivalent to 100 mg/kg of hesperetin) showed significant (P<0.05) decrease in TBARS levels in liver homogenate when compared to CCl_4-treated animals whereas free hesperetin upon daily or alternate day administration failed to bring down the increased level of TBARS.

Table I. Liver Function Test After CCl_4 Treatment

Treatment	SGPT (U/l)			SGOT (U/l)			SALP (U/l)			Total Bilirubin (mg/dl)		
	Day 8[a]	Day 9[b]	Day 10[c]	Day 8[a]	Day 9[b]	Day 10[c]	Day 8[a]	Day 9[b]	Day 10[c]	Day 8[a]	Day 9[b]	Day 10[c]
Vehicle control (Group I)	38.73±1.48**	38.78±2.43**	39.67±1.30**	37.47±1.15**	39.40±1.40**	39.80±1.77**	130.50±2.20**	132.70±2.14**	132.30±4.25**	0.65±0.01**	0.66±0.01**	0.67±0.03**
CCl_4 treated (Group II)	83.90±2.80	85.22±2.14	86.00±2.96	116.00±4.47	118.90±5.75	117.80±7.20	192.10±4.14	193.20±4.31	193.00±7.50	1.08±0.00	1.09±0.03	1.11±0.05
CCl_4 + Hesperetin for 7 days (100 mg/kg) (Group III)	74.69±2.25*	75.99±2.91	78.03±2.79	90.07±1.40**	99.30±4.91*	102.20±7.71	179.60±2.72*	183.40±4.92	184.80±6.11	0.92±0.04**	0.95±0.06*	0.97±0.09
CCl_4 + Hesperetin for alternate days (100 mg/kg) (Group IV)	77.60±2.19	79.46±3.36	81.30±3.09	102.9±4.30*	105.60±5.96	106.60±6.62	182.40±3.08	185.60±4.24	186.20±3.26	0.95±0.04*	0.96±0.04	0.99±0.07
CCl_4 + Hesperetin – phospholipid complex for alternate days (Eqv. to 100 mg/kg of hesperetin) (Group V)	49.67±1.43**	51.96±2.05**	56.47±1.64**	55.69±1.51**	63.02±2.44**	71.00±5.18**	156.10±1.91**	166.50±2.69**	170.60±4.21*	0.74±0.01**	0.77±0.02**	0.81±0.02*

SGOT serum glutamate oxaloacetate transaminase, *SGPT* serum glutamate pyruvate transaminase, *SALP* serum alkaline phosphatase, *CCl_4* carbon tetrachloride

[a] Values are mean ± SEM of 15 animals

[b] Values are mean ± SEM of ten animals

[c] Values are Mean ± SEM of five animals

*$P<0.05$, **$P<0.01$ (significant with respect to CCl_4-treated group)

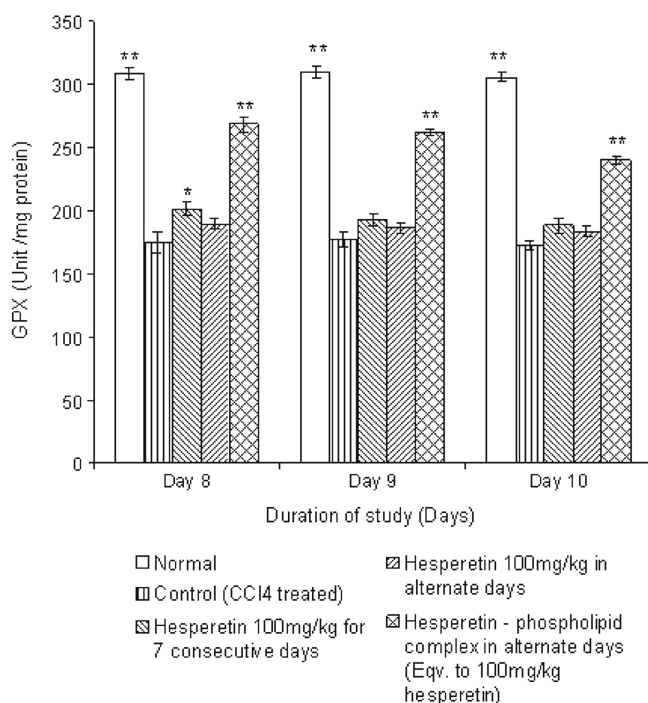

Fig. 2. Effect of hesperetin–phospholipid complex on glutathione peroxide (GPX) level in rat liver. [**P*<0.05, ***P*<0.01 (significant with respect to CCl_4-treated group). Values are mean ± SEM of five animals]

Concentration of Hesperetin in Rat Serum

Figure 6 shows the result of serum concentration study of hesperetin and hesperetin–phospholipid complex in rats. Peak serum concentration of 6.12 μg/ml attained at 4 h when pure hesperetin was administered. But in case of complex, the

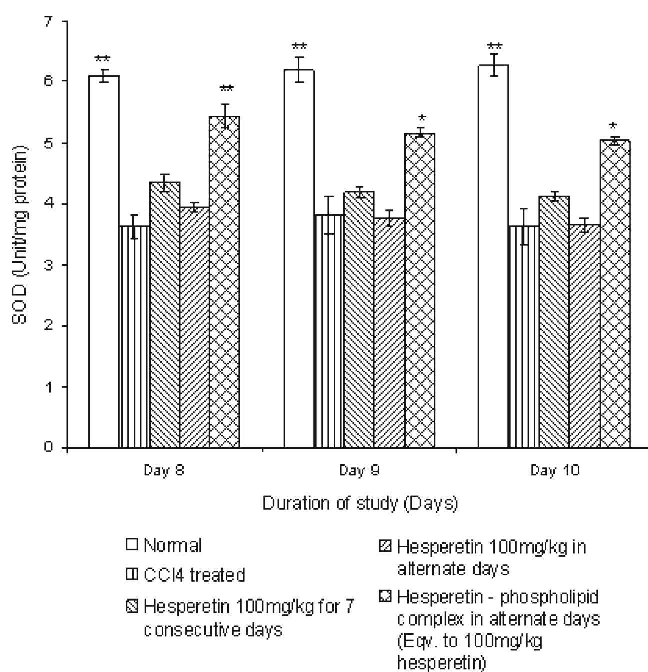

Fig. 3. Effect of hesperetin–phospholipid complex on SOD level in rat liver. [**P*<0.05, ***P*<0.01 (significant with respect to CCl_4-treated group). Values are mean ± SEM of five animals]

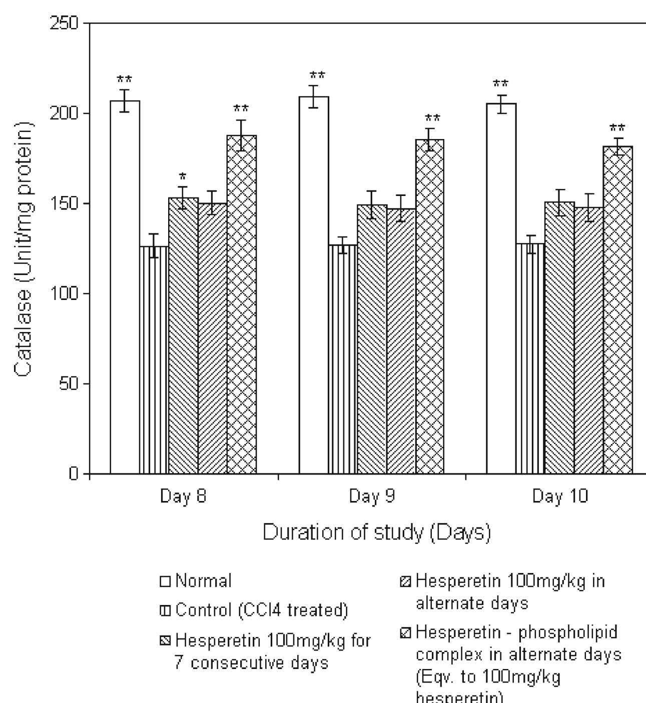

Fig. 4. Effect of hesperetin–phospholipid complex on catalase level in rat liver. [**P*<0.05, ***P*<0.01 (significant with respect to CCl_4-treated group). Values are Mean ± SEM of 5 animals]

peak concentration (9.20 μg/ml) appeared at 6 h and concentration was also maintained for a longer period of time.

Pharmacokinetic Parameters

Table II shows the main pharmacokinetic parameters of the hesperetin–phospholipid complex and hesperetin in rat. $C_{\max}$ was increased in case of the complex, so did the $T_{\max}$.

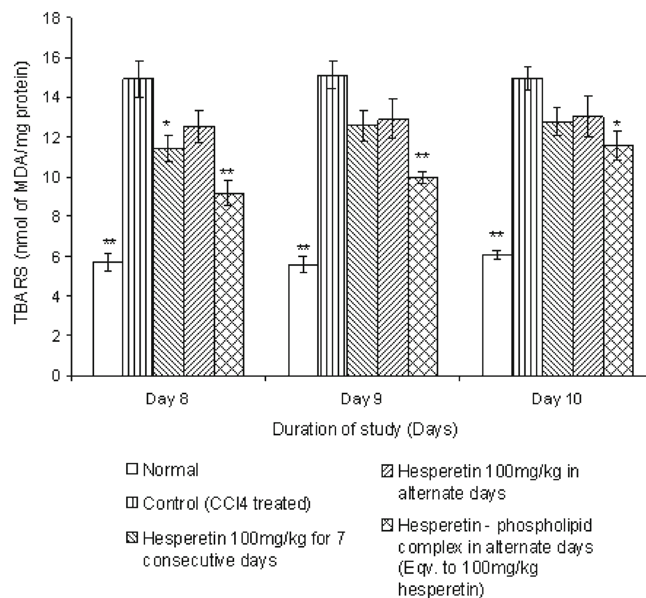

Fig. 5. Effect of hesperetin–phospholipid complex on TBARS level in rat liver. [**P*<0.05, ***P*<0.01 (significant with respect to CCl_4-treated group). Values are mean ± SEM of five animals]

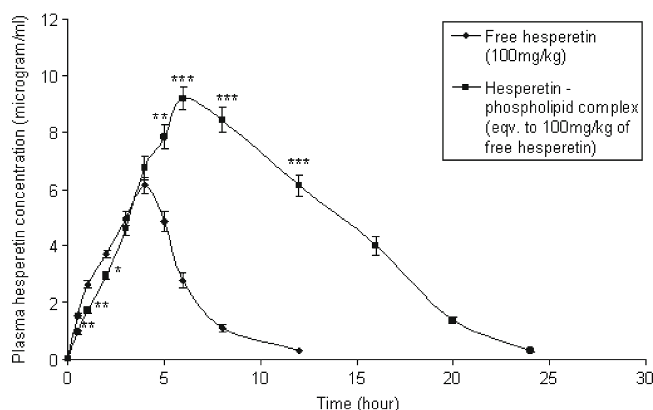

Fig. 6. Effect of complexation on serum concentration of hesperetin in rats. Values are mean ± SEM (n=6/group/time point). *$P<0.05$; **$P<0.001$, and ***$P<0.0001$ (significant with respect to free hesperetin treated group)

The elimination half-life of hesperetin 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 133.31%.

DISCUSSION

Molecules like hesperetin having enormous therapeutic potentials as a drug can play a major role in the management of varied diseases. Various active constituents of plant origin are gaining considerable interest day by day due to their potential antioxidant and free-radical scavenging activity (32). Therefore, it is very much essential to explore them as potent therapeutic agents through some novel drug delivery systems, which could maximize the healing properties of these molecules (33,34).

The present study dealt with the preparation and evaluation of a novel phospholipid complex of hesperetin, which increases its therapeutic efficacy. To understand the sustained activity of the complex, the study was designed to evaluate the protective activity on alternate day's therapy. From the liver function tests and the antioxidant activity studies, it is observed that pretreatment with pure hesperetin (100 mg/kg) on alternate days neither reduced the elevated levels of SGOT, SGPT, SALP, total bilirubin, and TBARS nor increased the depleted levels of GPX, SOD, and CAT. The results were statistically insignificant as compared to the CCl_4-treated group. Similar results were obtained from the animals receiving 100 mg/kg of pure hesperetin daily for 7 consecutive days prior to CCl_4 administration. For both the liver function tests and the antioxidant activity studies, the complex produced statistically significant ($p<0.01$ and <0.05) results (Table I and Figs. 2, 3, 4, and 5) as compared to the CCl_4-treated group. The results of the liver function tests and the antioxidant activity studies were supported by the hesperetin *in vitro* drug release study. The sustained action of the complex over 24 h along with the increase in the half-life of the drug may be a reason for the protective effect of the complex over 3 days.

The results of these physiological parameters showed less variation in spite of variation of their body weight. This may be due to several reasons, to spell the least; dose of the drug to be administered was calculated based on the body weight of the individual rats. Further, animals living in temperate climates are continually adapting to seasonal demands of reproduction and survival. It is well documented that experimental animals show seasonal changes in both reproductive and non-reproductive physiological and behavioral characteristics like reduction of metabolic rate and appetite during the winter with respective increases during spring and summer (35). Similarly, there are wide variations in the apparent relative potency for individual chemicals; given the uncertainties in the experimental and epidemiological data; it was not possible to rule out body weight of rats. But, in some cases, the variations depend on the body surface area. Due to small sample sizes and wide variations for individual chemicals, however, these comparisons are of limited predictive value. For reactive chemicals that are spontaneously inactivated by metabolism, body weight scaling may provide a more accurate scaling. But for some drugs with sustained effects, the body weight variations do not affect. Another factor affecting is the effective dose of the drug inside the body. Irrespective of the body weight, the effective dose may not give enough variations in the parameters (35).

In the present experiment, we prepared a hesperetin–phospholipid complex by a simple and reproducible method and the results obtained from that study showed that the complex produced a sustained release effect. Release of hesperetin from pure hesperetin suspension was almost completed within 8 h whereas the complex extended the release of hesperetin up to 24 h. The result produced by the complex may be a combined effect of sustained release property of the complex as well as protective effect of HSPC on hesperetin. Complexation plays a major role in sustaining the release of hesperetin from complex, which is evident from the experimental result. Serum concentration and pharmacokinetic study also substantiate the claim of sustained release of hesperetin from the complex.

Table II. Main Pharmacokinetic Parameters of Free Hesperetin (100 mg/kg, p.o.) and Hesperetin–Phospholipid Complex (eqv. to 100 mg/kg of Free Hesperetin, p.o.) in Rats (n=6)

Pharmacokinetic parameters	Hesperetin	Hesperetin–phospholipid complex
C_{max} [μg ml^{-1}]	6.12±0.30	9.20±0.41
T_{max} [h]	4.0	6.0
Area under concentration–time curve ($AUC_{0\text{-tn}}$) [μg ml^{-1} h]	30.47±2.34	109.72±6.72
Area under concentration–time curve ($AUC_{0-t\alpha}$) [ml^{-1} h]	31.24±2.54	151.90±8.43
Elimination half-life ($t_{1/2}$el) [h]	1.78±0.09	3.86±0.13
Elimination rate constant (K_{el}) [h^{-1}]	0.380±0.002	0.140±0.001
Clearance (cl) [lh^{-1}]	0.490±0.003	0.130±0.001
Volume of distribution (V_d) [l]	1.310±0.08	0.970±0.005

CONCLUSION

The present study showed that phospholipid complex of hesperetin can produce better therapeutic efficacy in rats for a longer period of time as compared to the molecule itself by virtue of its sustained release property and by decreasing the rate of elimination of the molecule from the body. The study shows the potential of the hesperetin–phospholipid complex as a drug delivery system. The complex can enhance the therapeutic efficacy of valuable citrus flavonoid hesperetin by reducing its elimination from the body and can act in the body for a prolonged period of time. The study also indicates that there is ample scope of using hesperetin as a phospholipid complex in the management of different ailments.

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