

Enhancing bioavailability and hepatoprotective activity of andrographolide from *Andrographis paniculata*, a well-known medicinal food, through its herbosome

Kuntal Maiti, Kakali Mukherjee, Venkatesh Murugan, Bishnu Pada Saha and Pulok K Mukherjee*

Abstract

BACKGROUND: *Andrographis paniculata* is a health food used extensively in Southeast Asia, India and China and contains the pharmacologically important phytochemical andrographolide. Although andrographolide has antihepatotoxic activity, its bioavailability from *A. paniculata* is restricted by its rapid clearance and high plasma protein binding. The aim of this study was to formulate a herbosome of andrographolide with a naturally occurring phospholipid in order to enhance the bioavailability and hepatoprotective activity of andrographolide in rats.

RESULTS: Andrographolide herbosome equivalent to 25 and 50 mg kg⁻¹ andrographolide significantly protected the liver of rats, restoring hepatic enzyme activities with respect to carbon tetrachloride-treated animals ($P < 0.05$ and $P < 0.01$ respectively). The rat plasma concentration of andrographolide obtained from the complex equivalent to 25 mg kg⁻¹ andrographolide ($C_{\max} = 9.64 \mu\text{g mL}^{-1}$) was higher than that obtained from 25 mg kg⁻¹ andrographolide ($C_{\max} = 6.79 \mu\text{g mL}^{-1}$), and the complex maintained its effective plasma concentration for a longer period of time.

CONCLUSION: The results proved that the andrographolide complex produced by this method has better bioavailability and hence improved hepatoprotective activity compared with andrographolide at the same dose. Andrographolide complexation is therefore helpful in solving the problem of rapid clearance and low elimination half-life associated with andrographolide from *A. paniculata*.

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Keywords: andrographolide–DSPC complex; herbosome; hepatoprotective; bioavailability; pharmacokinetic parameters

INTRODUCTION

Liver diseases of various origins remain a serious health problem and a major cause of mortality. In the absence of reliable hepatoprotective drugs in modern medicine, herbs and plants play an important role in the management of several liver disorders.^{1,2} Extensive literature related to the hepatoprotective activity of molecules from herbal sources have shown that there is a vast array of molecules with potent hepatoprotective efficacy. However, some of these molecules have poor solubility and hence limited bioavailability.³ Improvement of the oral bioavailability of such drugs by suitable techniques may reduce the variability in systemic drug levels and also enable dose reduction, leading to reduced side effects and lower cost.⁴

Andrographis paniculata Nees (Acanthaceae) is used as a health food in South East Asia, India and China. Andrographolide is a hepatoprotective herbal molecule obtained from *A. paniculata*. Its traditional uses include treatment of fever, inflammation, common cold, upper respiratory tract infection, tonsillitis, pharyngitis, laryngitis, pneumonia, tuberculosis, pyelonephritis, hepatic impairment, etc.^{5–7} According to the literature, andrographolide possesses potent

antihyperglycaemic,⁸ antipyretic,^{9,10} anti-inflammatory,^{11,12} anticancer,¹³ antileishmanial,¹⁴ antifertility,¹⁵ anti-HIV,¹⁶ cardiovascular,¹⁷ immunomodulatory¹⁸ and choleric¹⁹ activities. It also inhibits platelet-activating factor-induced platelet aggregation,²⁰ shows preventive activity against common cold^{6,7} and is useful in the management of respiratory tract infection.²¹ However, the most important use of andrographolide seems to be in the management of various liver disorders. Research on andrographolide has shown its effective use for several ailments, mainly in the treatment of cancer. A recent study revealed that andrographolide reverses hexachlorocyclohexane-induced experimental liver carcinogenesis in mice, suggesting it as a potential therapeutic/preventive agent for liver cancer.²² Further investigation into the mechanism of its anticancer properties has revealed

* Correspondence to: Pulok K Mukherjee, School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India. E-mail: naturalproductm@gmail.com

School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India

that it has multiple pharmacological activities. It has been shown to exert potent anticancer activity against human colorectal carcinoma Lovo cells by inhibiting cell cycle progression and also reducing MMP-7-mediated cellular events.²³ Andrographolide was also found to possess significant antinociceptive and antiedematogenic activities via nonopioid mechanisms.²⁴ Further, 14- α -lipoyl andrographolide derived from andrographolide was found to have significant activity against influenza A viruses, including the H5N1 avian influenza virus.²⁵

In Ayurveda, over 25 different polyherbal formulations containing *A. paniculata* are mentioned as popular remedies for the treatment of various liver disorders, and a significant number of investigations have substantiated the pharmacological potential of andrographolide as a hepatoprotective agent. Andrographolide showed significant dose-dependent protective activity against paracetamol-induced toxicity in an *ex vivo* preparation of isolated rat hepatocytes²⁶ and exhibited a protective effect against hepatotoxicity induced in mice by carbon tetrachloride (CCl₄) or *tert*-butyl hydroperoxide intoxication.^{27,28} It has also been shown to produce an ameliorative effect on galactosamine-induced acute hepatitis in rats²⁹ and to prevent beta hexachlorocyclohexane (BHC)-induced increase in γ -glutamyl transpeptidase and glutathione-S-transferase enzyme activities and lipid peroxidation.¹ The hepatoprotective effect provided by andrographolide was attributed to the antioxidant ability of the compound, which scavenges the free radicals generated during hepatotoxin challenge.²⁷ Apart from antioxidant activity, inhibition of cytochrome P-450, stimulation of hepatic regeneration²⁸ and inhibition of microsomal enzymes or lipid peroxidation²⁹ could explain the hepatoprotective effect of andrographolide. Although andrographolide has such enormous pharmacological potential, its early elimination from the body creates the need for an alternative delivery system that can maintain the concentration of andrographolide in blood for a longer period of time.

Likewise, several studies have indicated the beneficial role of soy lecithins containing phospholipids in maintaining the concentration of the drug in plasma for a longer time and also in enhancing the therapeutic efficacy of some molecules having poor oral absorption. Several phospholipids such as hydrogenated soy phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine have been employed for this purpose. Silybin is one such molecule having poor oral bioavailability. It was observed that silybin-phospholipid complexes had a significant advantage over the free molecule in protecting the liver and exerting antioxidant activity.³⁰ Previous studies in our laboratory with quercetin, curcumin, ellagic acid and naringenin as phospholipid complexes (herbosomes) showed that the complexes exerted better therapeutic efficacy than the free molecules in rat liver injury induced by CCl₄.^{31–35}

In view of the promise shown by andrographolide as a therapeutically active agent, in the present study we prepared a phospholipid complex of andrographolide (herbosome), determined its physicochemical properties and evaluated its hepatoprotective activity in CCl₄-intoxicated rats in comparison with free andrographolide to confirm whether phospholipid complexation can enhance the therapeutic efficacy of the parent molecule. The concentration of andrographolide in blood plasma of rats fed both free andrographolide and the andrographolide herbosome was determined along with the main pharmacokinetic parameters.

MATERIALS AND METHODS

Materials

The phospholipid 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) was purchased from Lipoid (Ludwigshafen, Germany). Andrographolide was obtained from Natural Remedies Pvt. Ltd (Bangalore, India) as a gift. Glutathione, glutathione reductase, bovine serum albumin, Tris base, nitroblue tetrazolium, 5,5-dithiobis(2-nitrobenzoic acid), phenazine methosulfate and *n*-octanol were purchased from SRL Chemicals (Mumbai, India). Ethylene diamine tetraacetic acid (EDTA), thiobarbituric acid, trichloroacetic acid, sodium carboxymethyl cellulose, sodium dodecylsulfate, *n*-hexane and all other chemicals were obtained from Loba Chemie (Mumbai, India).

Preparation of andrographolide-phospholipid complex

The complex was prepared by the reaction of 1 mol of andrographolide with 1 mol of DSPC according to the method developed in and patented by our laboratory.³⁵ The prepared complex was filtered and dried under vacuum to remove traces of hexane. The andrographolide complex (herbosome) thus obtained (yield 92.50% w/w) was kept in an airtight container until evaluation.

Microscopic view of complex

A DC 300F optical microscope (Leica Microsystems AG, Heerbrugg, Switzerland) was used for microscopic characterisation of the andrographolide complex. The complex was suspended in distilled water, then a drop of the suspension was placed on a slide, covered with a coverslip and viewed at a magnification of 400 \times .

Differential scanning calorimetry (DSC) of complex

DSC thermograms were recorded with a Mettler STAR[®] SW 8.10 DSC instrument (Mettler Toledo, Leicester, UK) by heating samples sealed in aluminium crimp cells from 35 to 250 °C at 10 °C min⁻¹. The peak transition onset temperatures of andrographolide, phospholipid, andrographolide complex and a physical mixture of andrographolide and phospholipid were determined and compared.

High-performance thin layer chromatography (HPTLC) study

Andrographolide and andrographolide complex were dissolved in methanol and spotted on silica gel 60 F₂₅₄-precoated TLC plates using a Linomat III applicator (Camag, Muttenz, Switzerland). Chromatograms were developed in a chromatographic chamber using chloroform/methanol (7:1 v/v) as solvent. After

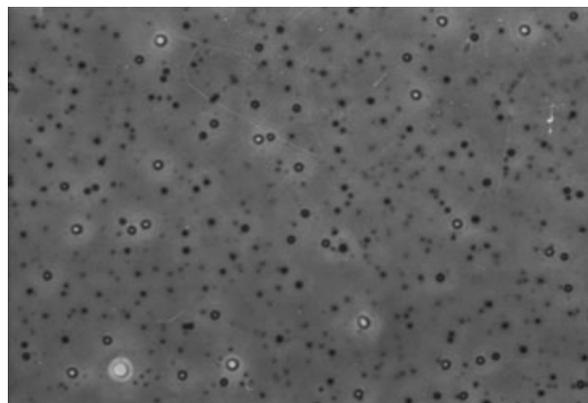


Figure 1. Photomicrograph of andrographolide complex.

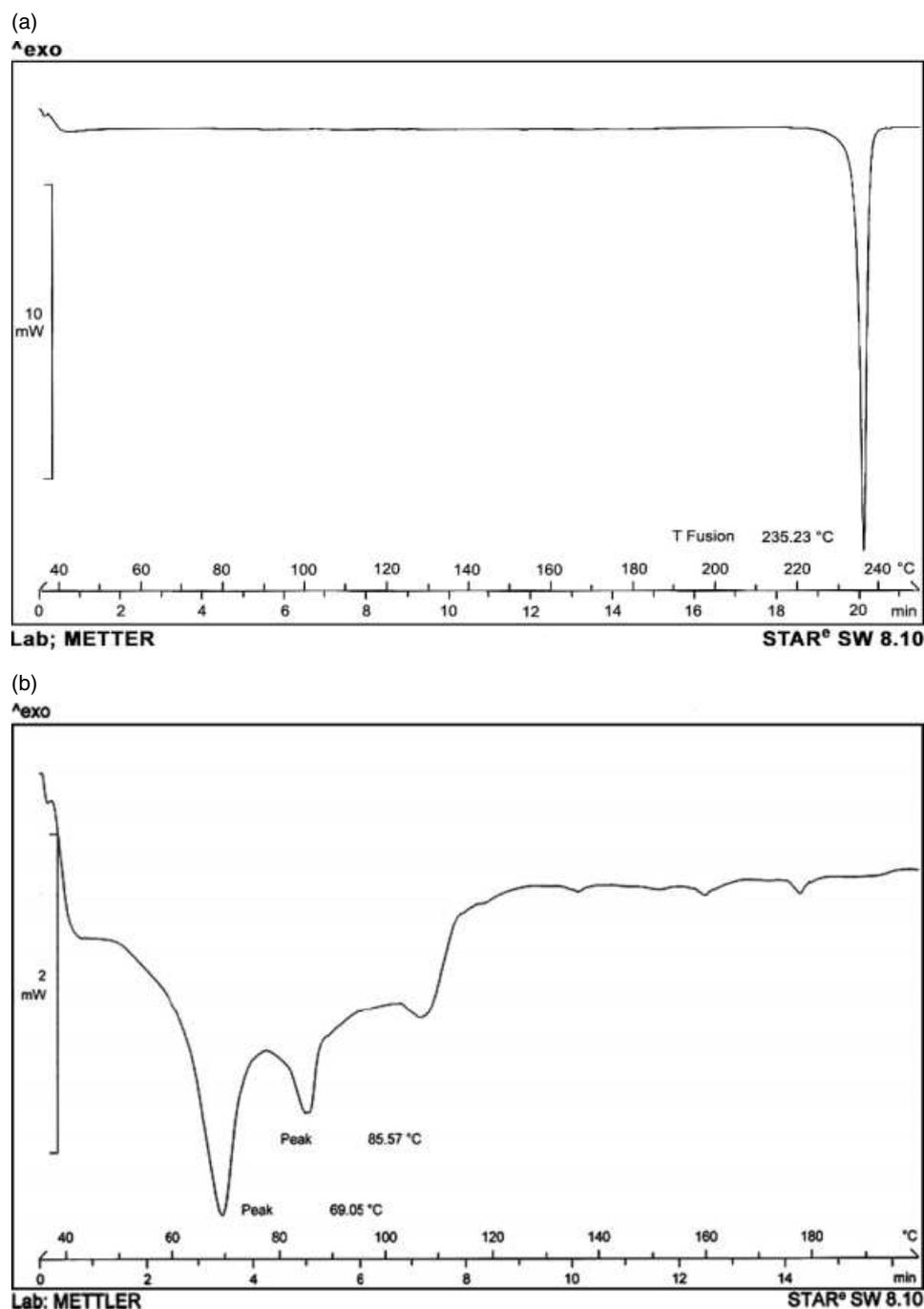


Figure 2. DSC thermograms showing peak transition onset temperatures of (a) andrographolide and (b) andrographolide complex.

development the plates were air dried and observed under UV light for the appearance of spots. The plates were scanned and the R_f values of the spots were recorded.

Hepatoprotective activity

Animals

Male albino rats (Wistar strain) weighing 180–200 g, purchased from a local supplier, were used for this study. The 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 a 12/12 h light/dark cycle. They had free access to pellet chow (Brook Bond, Lipton India) and water. Experimentation on the

animals was performed in accordance with the guidelines of the institutional animal ethical committee.

Dosage

The rats were divided into six groups of ten animals each. Group I animals received only distilled water with 0.3 g kg⁻¹ carboxymethyl cellulose (CMC) for 7 days and served as normal. Group II animals were administered distilled water with 0.3 g kg⁻¹ CMC for 7 days and an equal mixture of CCl₄ and olive oil (50% v/v, 1 mL kg⁻¹ i.p.) on day 7. Group III and V animals received andrographolide in distilled water with 0.3 g kg⁻¹ CMC at a dose of 25 and 50 mg kg⁻¹ respectively for 7 days and an equal mixture

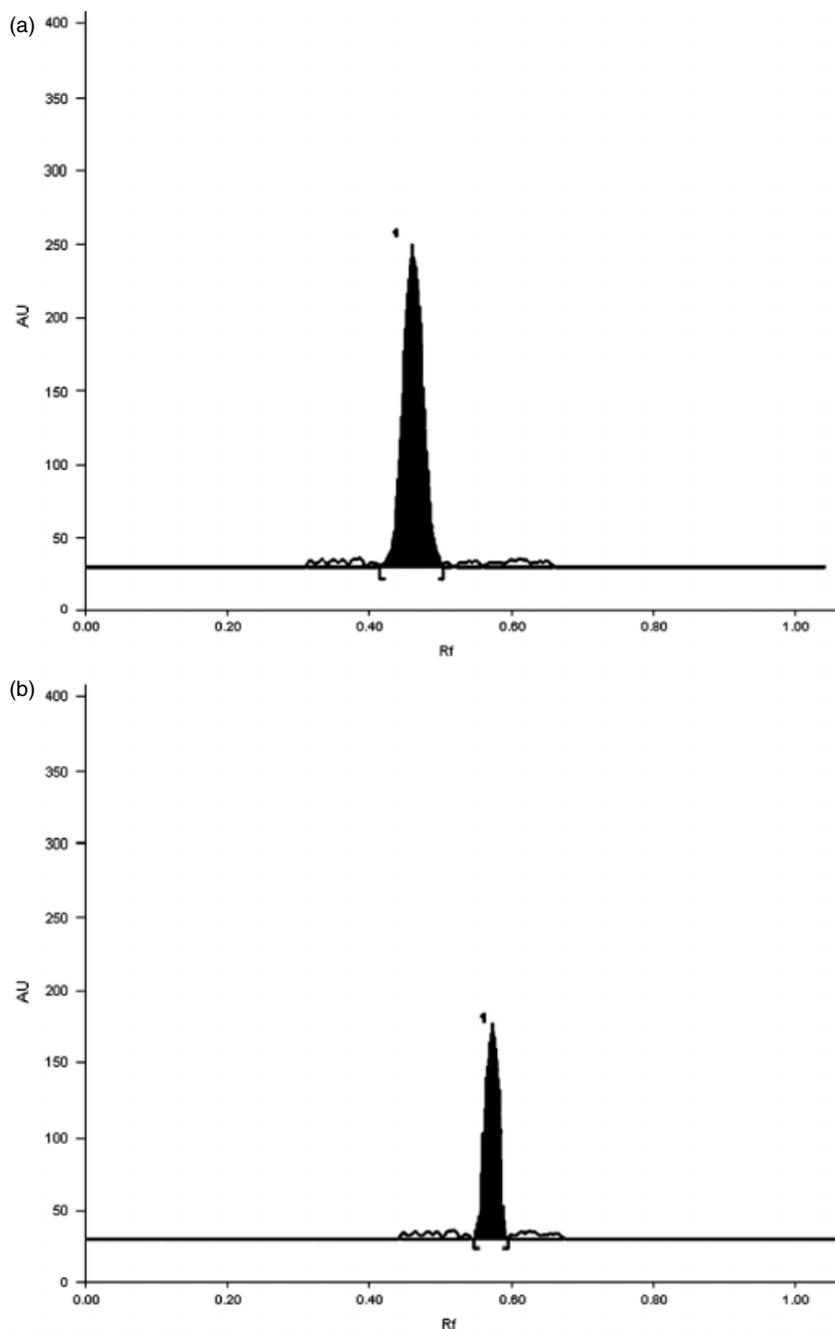


Figure 3. HPTLC fingerprints of (a) andrographolide and (b) andrographolide complex.

of CCl₄ and olive oil (50% v/v, 1 mL kg⁻¹ i.p.) on day 7. Group IV and VI animals were treated with andrographolide complex at a dose equivalent to 25 and 50 mg kg⁻¹ andrographolide respectively for 7 days and an equal mixture of CCl₄ and olive oil (50% v/v, 1 mL kg⁻¹ i.p.) on day 7.

Liver function tests and enzyme estimation

Animals were fasted for 24 h after intoxication and then anaesthetised with ether prior to collection of blood from the retro-orbital plexus on day 8. Serum was separated by centrifugation at 2000 × g for 10 min at 4 °C. Liver function tests were performed by measuring the levels of serum enzymes: aspartate aminotransferase (AST) and alanine amino-

transferase (ALT) by the method of Reitman and Frankel,³⁶ alkaline phosphatase (ALP) by the method of Kind and King³⁷ and lactate dehydrogenase (LDH) by the method of Wroblewski and LaDue.³⁸ The amounts of total and direct bilirubin were measured by the method of Malloy and Erelly.³⁹ Total protein (TP) was estimated according to the method of Lowry et al.⁴⁰ All determinations were carried out on days 8–10 post-administration.

Estimation of andrographolide in rat plasma

Male albino Wistar rats (150–200 g) were divided into two groups (n = 6 per group and time point). Group 1 animals received andrographolide in distilled water with 0.3 g kg⁻¹ CMC orally at a

Table 1. Rat serum levels of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)

Treatment group	LDH (U L ⁻¹)			ALT (U L ⁻¹)			AST (U L ⁻¹)			ALP (U L ⁻¹)		
	Day 8	Day 9	Day 10	Day 8	Day 9	Day 10	Day 8	Day 9	Day 10	Day 8	Day 9	Day 10
Group I	229.40 ± 27.29**	235.70 ± 25.10**	238.30 ± 24.51**	40.67 ± 1.33**	39.50 ± 1.60**	39.17 ± 2.01**	37.17 ± 1.92**	37.33 ± 1.20**	37.33 ± 1.21**	134.40 ± 3.65**	134.20 ± 3.64**	134.30 ± 3.65**
Group II	620.30 ± 44.30	631.00 ± 44.82	625.70 ± 45.65	85.83 ± 1.85	83.00 ± 2.38	80.00 ± 2.31	126.20 ± 2.60	110.90 ± 3.80	116.30 ± 3.85	195.80 ± 3.75	190.20 ± 2.87	193.50 ± 2.28
Group III	430.21 ± 15.75**	428.15 ± 12.5**	435.15 ± 17.2**	53.25 ± 1.32**	54.15 ± 1.52**	56.20 ± 1.32**	50.31 ± 1.42**	50.15 ± 1.22**	52.19 ± 1.24**	174.05 ± 2.01**	174.00 ± 1.95**	174.90 ± 1.72**
Group IV	436.60 ± 20.18**	420.40 ± 20.1**	372.80 ± 12.87**	58.88 ± 1.61**	48.07 ± 1.04**	43.45 ± 1.31**	54.77 ± 1.90**	47.33 ± 1.94**	43.17 ± 1.55**	177.00 ± 2.63**	162.20 ± 2.13**	151.50 ± 2.21**
Group V	410.20 ± 26.67**	424.50 ± 21.19**	413.20 ± 17.7**	47.45 ± 1.14**	48.73 ± 0.64**	48.20 ± 1.51**	42.80 ± 1.12**	44.55 ± 2.94**	45.63 ± 1.53**	166.10 ± 1.56**	160.70 ± 1.46**	165.20 ± 4.97**
Group VI	428.00 ± 23.93**	392.80 ± 22.24**	347.60 ± 9.95**	49.57 ± 2.19**	42.87 ± 0.89**	39.68 ± 1.28**	48.37 ± 2.95**	39.33 ± 1.90**	36.07 ± 2.18**	176.30 ± 3.31**	156.60 ± 3.06**	134.90 ± 2.64**

Values are mean ± SEM (n = 10). * P < 0.05; ** P < 0.01 (significant with respect to CCl₄-treated group II).

Table 2. Rat serum levels of total protein, direct bilirubin and total bilirubin

Treatment group	Total protein (mg mL ⁻¹)			Direct bilirubin (mg dL ⁻¹)			Total bilirubin (mg dL ⁻¹)		
	Day 8	Day 9	Day 10	Day 8	Day 9	Day 10	Day 8	Day 9	Day 10
Group I	6.81 ± 0.17**	6.88 ± 0.51**	6.86 ± 0.31**	0.22 ± 0.01**	0.22 ± 0.01**	0.22 ± 0.01**	0.66 ± 0.03**	0.65 ± 0.02**	0.64 ± 0.01**
Group II	4.22 ± 0.33	4.34 ± 0.34	4.44 ± 0.33	0.92 ± 0.01	0.92 ± 0.04	0.88 ± 0.06	1.13 ± 0.02	1.05 ± 0.03	0.98 ± 0.03
Group III	5.62 ± 0.41**	5.64 ± 0.29**	5.41 ± 0.15**	0.66 ± 0.02**	0.64 ± 0.02**	0.69 ± 0.01**	0.83 ± 0.01**	0.82 ± 0.03**	0.85 ± 0.02**
Group IV	5.56 ± 0.30**	5.92 ± 0.07**	6.14 ± 0.15**	0.71 ± 0.01**	0.64 ± 0.02**	0.54 ± 0.02**	0.87 ± 0.02**	0.73 ± 0.01**	0.65 ± 0.02**
Group V	5.67 ± 0.29**	5.85 ± 0.15**	5.72 ± 0.13**	0.59 ± 0.03**	0.57 ± 0.02**	0.59 ± 0.04**	0.80 ± 0.01**	0.78 ± 0.03**	0.76 ± 0.03**
Group VI	5.61 ± 0.14**	6.07 ± 0.01**	6.46 ± 0.25**	0.70 ± 0.01**	0.58 ± 0.01**	0.43 ± 0.01**	0.84 ± 0.02**	0.69 ± 0.01**	0.63 ± 0.03**

Values are mean ± SEM (n = 10). * P < 0.05; ** P < 0.01 (significant with respect to CCl₄-treated group II).

dose of 25 mg kg⁻¹ body weight, while group 2 animals received andrographolide complex orally at a dose equivalent to 25 mg kg⁻¹ andrographolide. Blood samples were collected 0.5 h post-administration from the jugular vein of all groups of animals under ether anaesthesia at different time points and kept in separate centrifuge tubes containing 0.38 g kg⁻¹ trisodium citrate solution (9:1 v/v). The blood was allowed to clot at room temperature for about 1 h and then centrifuged at 3000 X g for 10 min. The plasma was separated and kept at -20 °C prior to analysis.

The concentration of andrographolide in rat plasma was determined by high-performance liquid chromatography (HPLC) using the method of Panossian *et al.*⁴¹ with slight modification. An HPLC system (Shimadzu, LC-10AT, Kyoto, Japan) equipped with a UV-visible spectrophotometric detector and a data processor was used, together with a LiChrospher RP 18 column (5 mm, 125 mm × 4 mm; Merck, Darmstadt, Germany). The mobile phase consisted of methanol/water (60:40 v/v) and elution was carried out at a flow rate of 0.7 mL min⁻¹. A wavelength of 229 nm was used for detection. The injection volume was 20 µL and the relative retention time of andrographolide with respect to the internal standard propyl-*p*-hydroxybenzoate was 0.515.

Extraction of andrographolide from plasma and preparation of sample

A 1 mL aliquot of blood plasma was mixed with 160 µL of internal standard solution (propyl-*p*-hydroxybenzoate in methanol, 0.1 mg mL⁻¹) and 3.25 mL of acetone and vortexed. Proteins precipitated at 4 °C for 5 min were removed by centrifugation at 5000 X g for 15 min. The supernatant was vacuum evaporated to dryness and the residue was dissolved in 80 µL of methanol, centrifuged and subjected to HPLC.

Preparation of standard solution

A 10 mg quantity of andrographolide was dissolved in methanol in a 10 mL volumetric flask. The solution was then serially diluted with methanol to a final concentration of 0.1 µg mL⁻¹.

Validation of extraction and quantification method

Andrographolide from rat plasma was separated completely by the extraction process, and standard curves ranging from 0.05 to 10 µg mL⁻¹ were linear ($r = 0.9875$). The minimum detection level of andrographolide was 25 ng mL⁻¹. Validation of the method for extraction and quantification of andrographolide from rat plasma was done by performing recovery rate experiments. Three concentration ranges, high, middle and low, were selected and extraction as well as quantification of andrographolide from those regions was carried out as described earlier. Recovery rates of andrographolide from high, middle and low concentration ranges were 87.25, 85.23 and 84.50% respectively. Inter-day relative standard deviation (RSDs) were 3.27, 4.54 and 4.02% respectively and intra-day RSDs were 4.16, 3.12 and 3.98% respectively.

Pharmacokinetic parameters

The main pharmacokinetic parameters of andrographolide in free form and as a complex were determined using WinNonlin 4.1 (Pharsight Corp., Mountain View, CA, USA). Maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) were obtained directly from the concentration-time curve. Area under the concentration-time curve (AUC_{0-t_n} and $AUC_{0-\infty}$), elimination half-life ($t_{1/2,el}$), elimination rate constant (K_{el}), clearance (cl)

and volume of distribution (V_d) were also determined. Relative bioavailability (F) was calculated using the formula

$$F = \frac{\text{total amount of drug absorbed from complex } (A_{max,complex})}{\text{total amount of drug absorbed from andrographolide } (A_{max,andrographolide})} \times 100 = \frac{[(V_d \times K_{el} \times AUC_{0-\infty})_{complex}]}{[(V_d \times K_{el} \times AUC_{0-\infty})_{andrographolide}] \times 100}$$

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). For the hepatoprotective activity study, statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad Prism 3 (GraphPad Software, Inc., La Jolla, CA, USA). P values <0.05 were considered significant. For the plasma concentration study, animal data were analysed by Student's t test. Again, P values <0.05 were considered significant.

RESULTS

Generation of andrographolide herbosome

The microscopic view of the complex showed a vesicular structure (Fig. 1). The vesicles consist of phospholipid, while andrographolide is present in the lipid bilayer in intercalated form. DSC is a fast and reliable method for screening drug-excipient compatibility and provides maximum information about the possible interactions. In DSC an interaction is indicated 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. Figures 2(a) and 2(b) show the DSC thermograms of andrographolide and andrographolide complex respectively. A sharp peak with a maximum at 235.23 °C appeared in the DSC thermogram of andrographolide (Fig. 2(a)), but no additional endothermic or exothermic peaks were observed. In the DSC thermogram of andrographolide complex the peak at 235.23 °C was absent, but two new peaks with maxima at 69.05 and 85.57 °C appeared (Fig. 2(b)). Figures 3(a) and 3(b) show the HPTLC chromatograms of andrographolide and andrographolide complex respectively. It was observed that andrographolide had an R_f value of 0.46, whereas andrographolide complex had an R_f value of 0.56. From the above observations the generation of a phospholipid complex of andrographolide was confirmed through different instrumental analytical techniques.

Enhanced hepatoprotection of andrographolide herbosome

The hepatoprotective activities of andrographolide and andrographolide complex at different doses are shown in Tables 1 and 2.

Effect of treatment on levels of AST and ALT

Hepatic damage induced by CCl₄ caused a significant ($P < 0.01$) increase in the levels of AST and ALT compared with normal animals. Oral administration of andrographolide reduced the elevated levels of these marker enzymes, but the effect did not persist for very long. In contrast, andrographolide complex not only reduced the elevated enzyme levels but also produced a prolonged effect (Table 1).

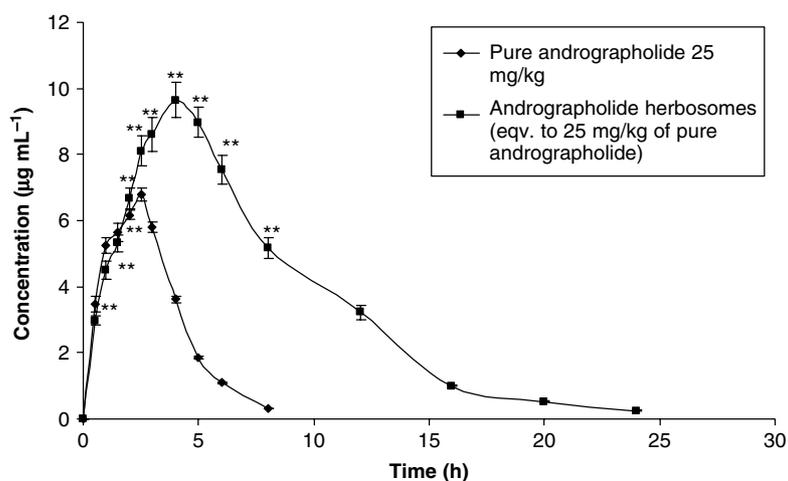


Figure 4. Plasma concentration of andrographolide in rats after oral administration of 25 mg kg⁻¹ andrographolide (◆) and andrographolide complex equivalent to 25 mg kg⁻¹ andrographolide (■). Values are mean ± SEM (*n* = 6 per group and time point). ***P* < 0.001 (significant with respect to andrographolide-treated group).

Table 3. Pharmacokinetic parameters of andrographolide and andrographolide complex in rats

Pharmacokinetic parameter	Group 1 (25 mg kg ⁻¹ andrographolide p.o.)	Group 2 (andrographolide complex equivalent to 25 mg kg ⁻¹ andrographolide p.o.)
<i>C</i> _{max} (µg mL ⁻¹)	6.79 ± 0.54	9.64 ± 0.72
<i>T</i> _{max} (h)	2.50	4.0
AUC _{0–tn} (µg mL ⁻¹ h)	26.24 ± 1.23	85.50 ± 2.77
AUC _{0–∞} (µg mL ⁻¹ h)	26.74 ± 1.42	87.30 ± 2.35
<i>t</i> _{1/2,el} (h)	1.20 ± 0.05	4.01 ± 0.12
<i>K</i> _{el} (h ⁻¹)	0.57 ± 0.04	0.21 ± 0.01
<i>cl</i> (L h ⁻¹)	0.17 ± 0.01	0.055 ± 0.003
<i>V</i> _d (L)	0.30 ± 0.02	0.26 ± 0.02

Values are mean ± SEM (*n* = 6).

Effect of treatment on levels of ALP and LDH

CCl₄ intoxication resulted in a significant (*P* < 0.01) increase in the levels of ALP and LDH compared with normal animals. Pretreatment with andrographolide reduced the elevated levels of these marker enzymes in a dose-dependent manner. Andrographolide complex also produced a significant (*P* < 0.01) ameliorative effect that persisted for a longer time (Table 1).

Effect of treatment on levels of direct and total bilirubin

As seen in Table 2, administration of CCl₄ caused a significant (*P* < 0.01) increase in the serum levels of direct and total bilirubin compared with normal animals. Andrographolide at the tested doses significantly (*P* < 0.05 and *P* < 0.01 respectively) reduced the elevated bilirubin levels compared with CCl₄-treated rats. Andrographolide complex also reduced the elevated bilirubin levels, and the effect persisted for a longer time.

Effect of treatment on levels of total protein

The TP level was significantly reduced in CCl₄-treated animals compared with normal animals. Treatment with andrographolide

increased the depleted protein level, but the effect did not persist for very long. In contrast, andrographolide complex not only increased the depleted protein level but also produced a prolonged effect (Table 2).

Increased and prolonged plasma concentration of andrographolide

Figure 4 shows the results of the plasma concentration study of andrographolide and andrographolide complex in rats. A peak plasma concentration of 6.79 µg mL⁻¹ was attained within 2.5 h when andrographolide was administered. However, in the case of andrographolide complex the peak concentration (9.64 µg mL⁻¹) appeared at 4 h. The higher concentration was also maintained for a longer period of time before decreasing. Thus the phospholipid enhanced the plasma concentration of andrographolide in a significant manner and the effect persisted for a longer period of time.

Pharmacokinetic parameters

Table 3 shows the main pharmacokinetic parameters of andrographolide and andrographolide complex in rats. *C*_{max} and *T*_{max} were increased in the case of the complex. The elimination half-life of andrographolide was increased when it was in phospholipid-complexed form, and eventually the clearance of the molecule in this form was also lowered. The phospholipid complex persisted for a longer period of time in the rat body, with a higher relative bioavailability of 104.24%.

DISCUSSION AND CONCLUSION

Andrographis paniculata is a well-known plant used as a health food in traditional systems of India and China. Andrographolide is a chemical constituent obtained from *A. paniculata* and has multiple pharmacological activities, including hepatoprotective activity.³² It has been shown to be active against paracetamol-induced toxicity in an *ex vivo* preparation of isolated rat hepatocytes.²⁶ Despite its well-known hepatoprotective activity, andrographolide has several bioavailability constraints. Although it is rapidly absorbed into the blood following oral administration, its rapid clearance lowers its elimination half-life. Also, the high plasma

protein binding of andrographolide decreases its bioavailability. These drawbacks necessitate the development of some novel compound at the same dosage as the conventional form that can maintain the concentration of andrographolide in blood for a longer period of time. Phospholipids play a major role in drug delivery technology. There are numerous advantages of phospholipids in addition to their solubilising property when considering them for a carrier system. Since andrographolide shows high plasma protein binding and has a lower elimination half-life as well as mean residence time,⁴¹ an attempt was made in the present study to formulate a novel dosage form of this molecule (andrographolide herbosome) to overcome these shortcomings. The resulting phospholipid (DSPC)–andrographolide complex resulted in better hepatoprotection of rat liver injury caused by CCl₄ intoxication.

Andrographolide herbosome was prepared by a simple and reproducible method. The physicochemical investigations showed that andrographolide formed a complex with DSPC. The complex produced hepatoprotective activity for a longer time and normalised adverse conditions of rat liver more efficiently than the free drug or its extract. The results even showed that the complex equivalent to 25 mg kg⁻¹ andrographolide exerted a similar effect to 50 mg kg⁻¹ andrographolide in the long term. The effect produced by andrographolide herbosome may be due to a combination of the sustained release property of the complex and the protective effect of DSPC on andrographolide. Complexation plays a major role in sustaining andrographolide release from the herbosome, which is evident from the experimental results. Thus the formulation can be helpful in reducing the amount and frequency of administered dosage by virtue of its sustained release property. The results obtained from the *in vitro* release study also pointed towards the sustained release property of the complex and indicated the possibility of its longer retention inside the body and thus prolonged duration of action. Based on the above observations, it can be concluded that complexation of a phospholipid with andrographolide may solve the problem of rapid clearance and lower elimination half-life associated with andrographolide.

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