J Incl Phenom Macrocycl Chem (2012) 73:377–386
DOI 10.1007/s10847-011-0074-8

ORIGINAL ARTICLE

Phyto-phospholipid complex of catechin in value added herbal drug delivery

Ajay Semalty · Mona Semalty · Devendra Singh · M. S. M. Rawat

Received: 23 April 2011 / Accepted: 24 October 2011 / Published online: 11 November 2011

Abstract Catechin (an anti-inflammatory, antioxidant, antitumour, and hepatoprotective bioflavonoid) is poorly absorbed across the GIT because it has multiple ring molecules that are too large to be absorbed by simple diffusion. It typically has poor miscibility with oils and other lipids which limit their ability to pass across the lipid rich outer membranes of enterocytes of small intestine. Thus catechin–phospholipid complex were prepared to improve its absorption by imparting an environment of improved lipophilicity. The phospholipid complexes of catechin were prepared with phosphatidylcholine in presence of dichloromethane by conventional solvent evaporation technique. Pharmacosomes thus prepared were evaluated for solubility, drug content, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X ray powder diffraction (XRPD), in vitro dissolution study and in vitro antioxidant activity. Prepared phospholipid complex showed high drug content (99.40%. w/w) and improved lipid solubility (0.79–1.97 mg/mL). FTIR, NMR, DSC and XRPD data confirmed the formation of phospholipid complex. Unlike the free catechin, catechin complex showed a sustained release over the 24 h of study. Catechin-phospholipid complex showed slightly better antioxidant activity than that of catechin at all dose levels. Thus it can be concluded that the phospholipid complex of catechin may be of potential use for improving absorption of catechin across the lipidic biological barriers in gastrointestinal tract. It was concluded that the complexation with phospholipids did not interfere with the biological activities. This herbal drug delivery system can pave the way for large molecules to pass through the lipophilic biological membrane (by the virtue of their amphiphilic nature) and get absorbed into the systemic circulation.

Keywords Phospholipid complex · Catechin · Solubility · In vitro dissolution · Phytosome

A. Semalty (✉) · M. Semalty
Department of Pharmaceutical Sciences, H.N.B. Garhwal University, Srinagar (Garhwal), INDIA
e-mail: semaltyajay@gmail.com

D. Singh · M. S. M. Rawat
Department of Chemistry, H.N.B. Garhwal University, Srinagar (Garhwal), INDIA

Introduction

Catechins, a group of similarly structured polyphenolic phytochemicals of chemical and medicinal value come under pharmacognostic classification as 'flavonols'. Catechins (the examples include catechin, epicatechin, epigallocatechin, gallocatechin, epigallocatechin gallate etc.) are poorly absorbed through the gastrointestinal and [1, 2]. Heating catechins past their point of decomposition release pyrocatechol, which explains the common origin of the names of these compounds. They demonstrated very strong antioxidant activity both in vitro and in vivo conditions. Our current study deals with investigations on catechin, one of the flavonoids belonging to catechins family of phytochemicals. Catechin was first isolated from plant extract catechu, *Acacia catechu* – Leguminosae from which it derives its name. Catechin and epicatechin are epimers with (−)- epicatechin and (+)-catechin are optical isomers found in nature. Naturally, catechin exists as a mixture of (−)-epicatechin and (+)-catechin (Fig. 1).

Catechin has demonstrated several pharmacological benefits. A study reported that catechin prevented the formation of acute gastric lesions by 80% by series of

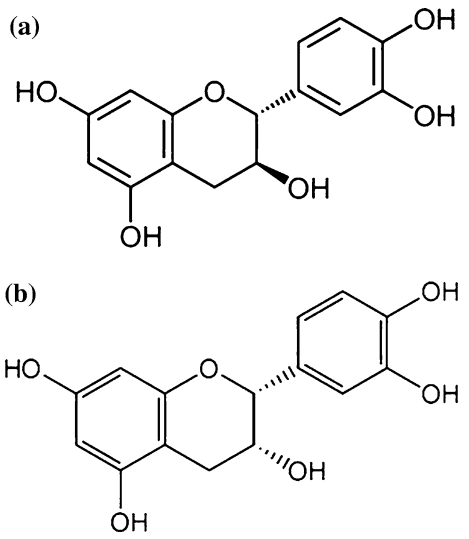

Fig. 1 Chemical Structure of catechin (**a**) and epicatechin (**b**)

experiments lasting half a year. As the drug had low toxicity in humans, it was recommended for clinical trials [3].

It was proved that catechin at a dose of 36 or 72 mg per rat administered intraperitoneally substantially inhibited adhesion formation which was induced by gentle scraping [4]. In a study, the efficacy of catechin to inhibit intestinal tumor formation and suppression of focal adhesion kinase activation in the Min/+ mouse was reported [5]. Administration of (+)-catechin in an AIN – 76A diet at doses of 0.1 and 1% decreased the intestinal tumor number by 75 and 71%, respectively.

Kalender et al. investigated the effect of catechin and vitamin E on reducing toxic effects of Idarubicin (anthracycline antibiotic used in treating acute leukemia) in rats. It was showed that catechin significantly reduced Idarubicin induced carditoxicity in rats [1]. Takano et al. studied the protective effect of (+)-catechin against 5-fluorouracil induced myelosuppression in mice [6]. Intraperitoneally injected (+)-catechin (1–10 mg/kg per day) accelerated the recovery of number of white blood cells and platelets against the hematotoxicity produced by 5-fluorouracil in mice. These findings suggested that (+)-catechin selectively enhances the recovery of population of granulocytes reduced by 5- flurouracil in mice. Similarly, the catechin demonstrated benefit in chronic fatigue syndrome [7], prevention of tamoxifen induced biochemical perturbations in mice [8] and hepatoprotection [9, 10].

Like other flavonoids, catechin is poorly absorbed across the GIT because it has multiple ring molecules that are too large to be absorbed by simple diffusion. It has poor miscibility with oils and other lipids which limit their ability to pass across the lipid rich outer membranes of enterocytes of small intestine. Catechin consists of two benzene rings (A and B) and a pyran ring (called C – ring), which might be the cause for poor oral absorption [2].

Phospholipids play a major role in drug delivery technology. It is an important carrier for those drug molecules which require sustained/controlled release in vivo due to faster elimination from the body. These amphiphilic drug-lipid complexes (termed as pharmacosomes or phytosomes), are stable and more bioavailable drug delivery systems with low interfacial tension between the system and the GI fluid thereby facilitating membrane, tissue, or cell wall transfer, in the organism [11].

The problem of poor absorption of catechin can be overcome by preparing phospholipid complexes of it. These complexes or phytosomes may improve their absorption by imparting an environment of improved lipophilicity and also sustaining the release (in dissolution). Thus catechin–phospholipid complex was evaluated for various physicochemical investigations like drug content, chemical interaction (FTIR and NMR), phase transition behavior (DSC), crystallinity (XRPD), surface morphology (SEM) and in vitro dissolution study in comparison with pure catechin. The in vitro antioxidant activity of the complex was also evaluated to observe any probable adverse effect of the process of complexation on the antioxidant activity of catechin.

Materials and methods

Materials

(+)-Catechin hydrate was purchased from Sigma Aldrich Mumbai. Soya phosphatidylcholine (LIPOID S-80) was obtained as a gift sample from LIPOID GmbH Germany. Butylated hydroxyanisole (BHA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from E. Merck Mumbai and Himedia Mumbai respectively. All other chemicals were of analytical grade.

Methods

Catechin-PC complex was prepared by taking catechin with an equimolar concentration of PC. The equimolar concentration of PC and catechin were placed in a 100 mL round bottom flask and refluxed in dichloromethane for 3 h. On concentrating the solution to 5–10 mL, 30 mL of n-hexane was added to get the phospholipid complex as a precipitate followed by filtration. The precipitate was collected and placed in vacuum desiccators.

Drug content

To determine the drug content in the complex, complex equivalent to 100 mg were weighed and added in 100 mL

of methanol taken in a 100 mL volumetric flask. The volumetric flask was stirred continuously for 24 h on a magnetic stirrer. Dilutions were made suitably and measured for the drug content UV spectrophotometrically (Lambda25 Perkin Elmer UV/Visible Spectrophotometer) at 278 nm in methanol.

Solubility

To determine the change in solubility due to complexation, solubility of drug and the complex was determined in water and n-octanol by shake flask method as described in our previous study [12].

Scanning electron microscopy (SEM)

To detect the surface morphology of the prepared complex, SEM of complex was performed at UGC-DAE consortium Indore and IIT Roorkee by Scanning Electron Microscope (JEOL JSM 5600).

Fourier transform infrared spectroscopy (FT-IR)

FTIR spectra for the various powders were obtained on a Perkin Elmer FTIR spectrometer (Perkin Elmer Life and Analytical Sciences, MA, USA) in the transmission mode with the wave number region 4,000–500 cm^{-1}. KBr pellets were prepared by gently mixing 1 mg sample powder with 100 mg KBr.

Differential scanning calorimetry (DSC)

Thermograms of catechin, phosphatidylcholine (80%) and the naringenin-PC complex were recorded using a differential scanning calorimeter (2,910 Modulated DSC V4.4E, TA Instruments, US) in Department of Chemistry, University of Delhi. The thermal behavior was studied by heating 2.0 ± 0.2 mg of each individual sample in a covered sample pan under nitrogen gas flow. The investigations were carried out over the temperature range 25–250 °C with a heating rate of 10 °C min^{-1}.

X-ray powder diffractometry (XRPD)

The crystalline state of drugs in the different samples was evaluated with X-ray powder diffraction. Diffraction patterns were obtained on a Bruker Axs- D8 Discover Powder X-ray diffractometer (Germany). The X-ray generator was operated at 40 kV tube voltages and 40 mA of tube current, using the Ka lines of copper as the radiation source. The scanning angle ranged from 1 to 60 ° of 2θ in step scan mode (step width 1 °/min). Drug, phosphatidylcholine (80%), and drug-PC complex were analyzed with X-ray diffractions.

Nuclear magnetic resonance (NMR)

Proton NMR of drug, phospholipids and their complexes were recorded on Bruker 400 MHz NMR (Germany), using TMS as internal standard.

Dissolution study

In vitro dissolution studies for drug complex as well as plain drug were performed in triplicate in a USP XXIII six station dissolution test apparatus (Veego Model No.6DR, India) at 100 rpm and at 37 °C. An accurately weighed amount of the complex equivalent to 100 mg of drug was put into 900 mL distilled water (media). Samples (3 mL each) of dissolution fluid were withdrawn at different intervals and replaced with the equal volume of fresh media, to maintain sink conditions. Withdrawn samples were filtered (through a 0.45 mm membrane filter) and diluted suitably and then analyzed spectrophotometrically.

In vitro antioxidant activity

In vitro antioxidant activity was determined in terms of DPPH radical scavenging activity. The free radical scavenging activity of catechin and its complex was measured and compared with the activity of butylated hydroxy anisol (BHA) for radical-scavenging ability using the stable radical DPPH [13]. The free-radical scavenging activities of catechin, its complex and BHA (used as a standard) were measured by decrease in the absorbance of methanol solution of DPPH.

0.1 mM solution of DPPH in methanol was prepared and 1.5 mL of this solution was added to 3.5 mL of extract solution in water at different concentrations (20–100 μg/mL). Thirty minutes later, the absorbance was measured at 515 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged } (\%) = [(A_{cont} - A_{test})/\ A_{cont}] \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Statistical analysis

Results were expressed as mean values and standard deviations (± SD).

Results and discussion

In the present experiment catechin–phospholipid complex were prepared by a simple and reproducible method.

Content (percent loading) of catechin in the complex, as estimated by UV spectrophotometry (at 278 nm in methanol), was found to be 99.40%. (w/w). Phyto-phospholipid complexes or Phytosomes of herbal drugs have been reported to sow a high percentage of drug loading in various previous studies [2, 11, 12]. Like pharmacosomes of synthetic drugs, complexation showed a good percent loading of the drug which might be a vital aspect for feasibility of the clinical or therapeutic delivery of drug.

Solubility of the catechin complex was found to be much higher (in water and n octanol) than the catechin. Table 1 provides the solubility data. Catechin is poorly miscible with lipids but the complex of catechin with the phospholipids increased the solubility of catechin in n-octanol significantly from 0.79 to 1.97 mg/mL. However, there was a slight increase in water solubility also (from 2.63 to 2.79 mg/mL). This increase in lipophilic nature of catechin may result in improved bioavailability due to increase in permeability of the molecule across the lipid rich biomembrane. The further permeation studies of the complex with suitable biomembranes may validate the results. In the next phase of research the permeation studies shall be conducted.

Scanning Electron Micrographs of the complex are shown in Fig. 2. Unlike the needle shaped structure of catechin, their phospholipid complexes were found to be slightly spherical or irregular shaped with rough surface morphology. Complexes were found to be as free flowing particles. The average particle size of phospholipid complex was found to be 50 μm.

The formation of the complex can be confirmed by the FT-IR spectroscopy comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. FTIR spectra showed the changes in peaks in complexes and positions from that of Catechin (Fig. 2a) and PC (Fig. 2b). FTIR spectra showed the changes in peaks in complexes and positions from that of catechin and PC. FT-IR spectra of complex were significantly different from that of components and that of physical mixtures (Fig. 3).

Catechin (Fig. 3a) showed the characteristic IR (KBr) peaks at 3343.28.01, 2932.04, 1628.67 and 1605.59 cm^{-1}. PC (Fig. 3b)showed a broad peak at 3437.05, and sharp peaks at 2917.93 and 2849.91 several sharp peaks below 1800 cm^{-1} (1739.34, 1651.85, 1468.79 etc.) while the complex (Fig. 3c) showed a very broad peak at 3282.91 cm^{-1} (which shows that some interaction has occurred at hydroxyl group). On the other hand the physical mixture (Fig. 3d) of PC and catechin showed broad peak just like PC at 3371.27 cm^{-1} while it shows all the sharp peaks at about the same positions as that of catechin.

DSC studies

In order to substantiate the association of Catechin with PC, DSC analysis was performed on Catechin, PC, and the Catechin -PC complex. The results of the DSC test confirmed the association of Catechin and PC in the complex as both peaks representing Catechin and PC changed position (Fig. 4). Phospholipids (Fig. 4b) showed two major peaks at 83.21 °C and 107.90 °C and a small peak at 64.45 °C. The first one peak of phospholipids is mild peak (at 64.45 °C), which is probably due to the hot movement of phospholipids polar head group. The second (83.21 °C) peak 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. This melting might have

Table 1 Solubility study of catechin and its complex

Drug	Aqueous solubility (mg/mL)[a]	n-Octanol solubility (mg/ml)[a]
Catechin	2.63 ± 0.003	0.79 ± 0.012
Catechin -PC complex	2.79 ± 0.019	1.97 ± 0.026

[a] Data expressed as mean values and standard deviations (± SD); $n = 3$

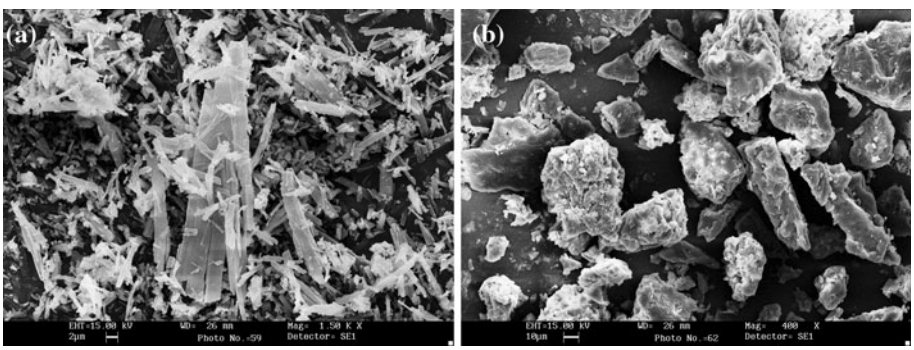

Fig. 2 SEM of catechin (**a**) and catechin phospholipid complex (**b**)

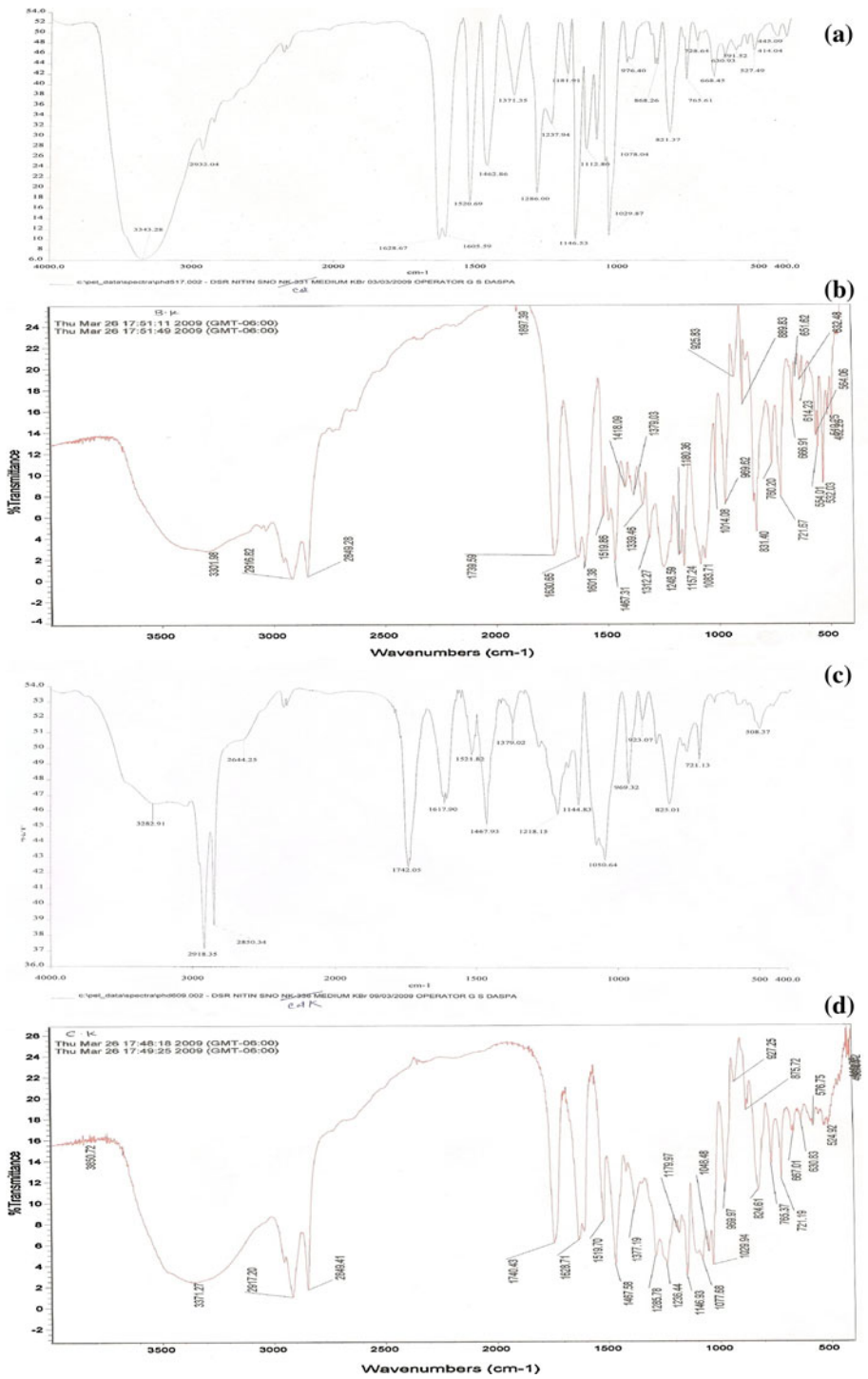

Fig. 3 FTIR spectra of Catechin (**a**); phosphatidylcholine or PC (**b**); Catechin -phosphatidylcholine complex (**c**); and physical mixture of Catechin with PC (**d**)

occurred in two phases which subsequently gave another peak (107.90 °C) which is relatively less sharp. Catechin (Fig. 4a) showed four sharp endothermic peaks at 108.48, 147.26, 174.0, 292.67 °C. On the other hand catechin-PC complex (Fig. 4c) showed a single sharp peak at 54.01 °C, which is different from the peaks of the individual components of the complex. Moreover the onset temperature is 49.33 °C only. It is evident that the original peaks of catechin and phospholipids disappear from the thermogram of the complex and the phase transition temperature is lower than that of phospholipids. It has been reported that reduction in melting point and enthalpy accounts for reduced crystallinity of the drug [14]. The DSC data of the prepared complex showed a noticeable reduction in the enthalpy as well as the melting point in comparison with catechin and PC.

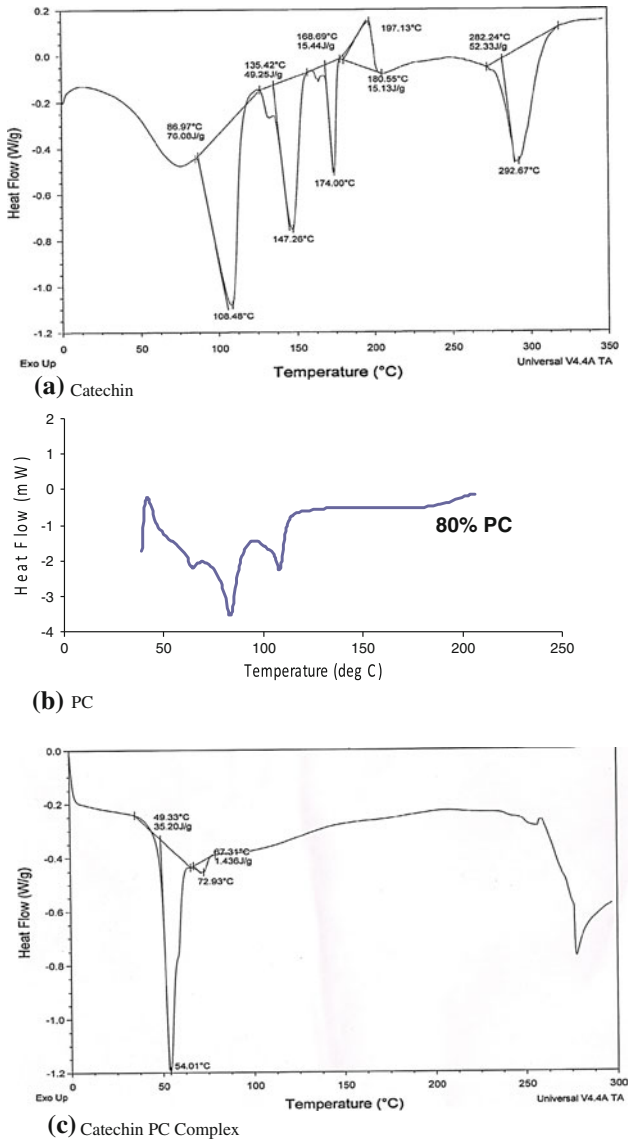

Fig. 4 DSC Thermograms of Catechin, PC and Catechin-PC complex

These DSC data are well supported by the results of DSC thermograms of the phospholipid complexes of some phytoconstituents like naringenin, silybin, puerarin, curcumin and gallic acid [12, 15–19]. In all these studies the thermogram of the complex also exhibited a single peak which was different from the peak of phytoconstituents and phospholipids.

This interaction may be due to hydrophobic interaction and/or hydrogen bonding. The –OH groups of the phenol rings of catechin may be involved in hydrogen bonding whereas the aromatic rings may be involved in hydrophobic interaction. As a result, the major sharp peaks of phospholipids disappear and lower the phase transition temperature.

X-Ray powder diffraction study

The XRPD of Catechin -phospholipid complex (Fig. 5) revealed a broad peak similar to PC. It suggested that the Catechin in phospholipid complex was either in amorphous form or molecularly dispersed.

These results were well supported by the previous studies done with the phospholipid complexes of naringenin, puerarin, insulin, diclofenac and aceclofenac [12, 20–24]. The disappearance of Catechin's crystalline diffraction peaks confirmed the formation of phospholipid complex. Unlike liposomes, bonding between drug and the phospholipids in development of pharmcosomes (drug-phospholipid complex), might have resulted into the significant change of its X-ray diffraction.

NMR

Proton NMR of Catechin: δ 2.4 (dd, 1H, H-4 equatorial); δ 2.78 (dd, 1H, H-4, axial); δ 3.86 (m, 1H, H-3); δ 4.47 (d, 2H, H-2); δ 5.7 (d, 1H, H-6); δ 5.9 (d, 1H, H-8); δ 6.6 (dd, 1H, H-6); δ 6.68 (dd, 1H, H-5′); δ 6.7 (d, 1H, H-2′); δ 3.88 (s, 1H, 3 -OH); δ 8.5 (s, 1H, 5 -OH);. δ 8.6 (s, 1H, 7 -OH); δ 8.2 (s, 1H, 3′ -OH); δ 8.04 (s, 1H, 4′ -OH).

The phospholipid complex with catechin (Fig. 6), revealed similar H-NMR except the proton of phenolic –OH, which shifted downfield. The phenolic protons assigned for 5 -OH at δ 8.5; 7 –OH at δ 8.6; 3′ –OH at δ 8.2; and 4′ –OH at δ 8.04 are shifted to δ 8.8, 8.9, 8.7 and 8.48, respectively in the complex, indicating that the interaction of phospholipid with phenolic –OH of catechin. The downfield of protons in the phospholipid complex have also been reported and well supported by various previous studies [12, 25].

Dissolution study

To study the effect of complexation on dissolution profile of catechin, dissolution study of catechin–phospholipid complex and pure (free) catechin was done in water. The catechin–phospholipid complex showed better dissolution profile than the catechin (i.e. the un complexed or plain/free catechin) (Fig. 7). Unlike the free catechin, catechin complex showed a sustained release over the 24 h of study. Due to high water solubility free catechin goes into the media with in 1 h of the in vitro drug release study. The slow or sustained release pattern of the complex may be due to increased lipophilicity (or n-octanol solubility) of the complex. However, catechin is released almost completely (97.5%) from the phytosomes at the end of 24 h. Therefore, it can be concluded that the phospholipid complexes can serve as value added herbal drug delivery systems which not only have more lipid solubility (for

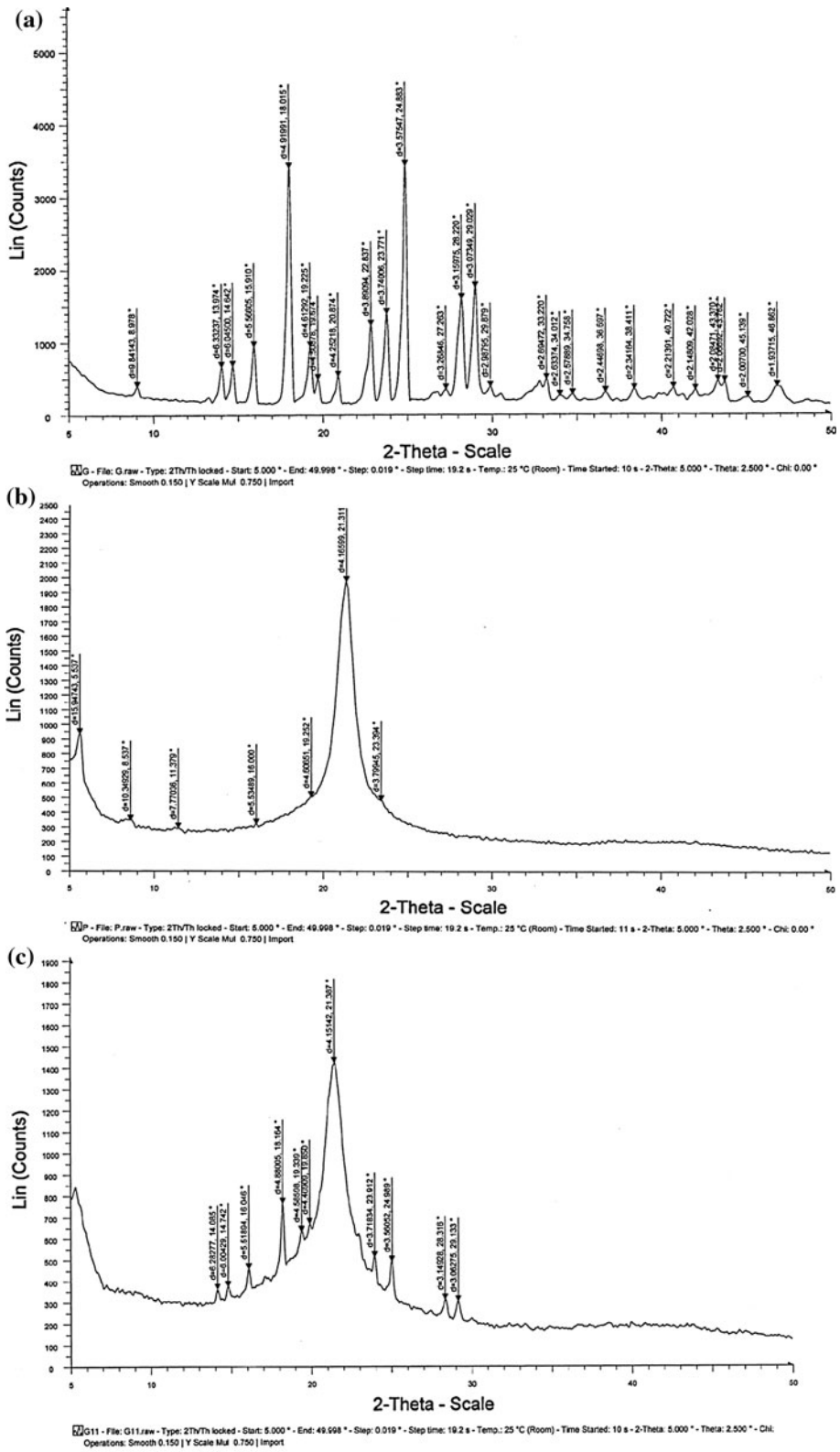

Fig. 5 High resolution X-ray diffraction (HRXRD) study of Catechin (**a**), Phospholipid (**b**) and Catechin-Phospholipid complex (**c**)

improved permeation) but also have the sustained release effect. The phospholipids based carrier drug delivery systems of catechin, when prepared in the form of liposomes, also sowed the similar release (sustained) study [26].

In vitro antioxidant activity

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable

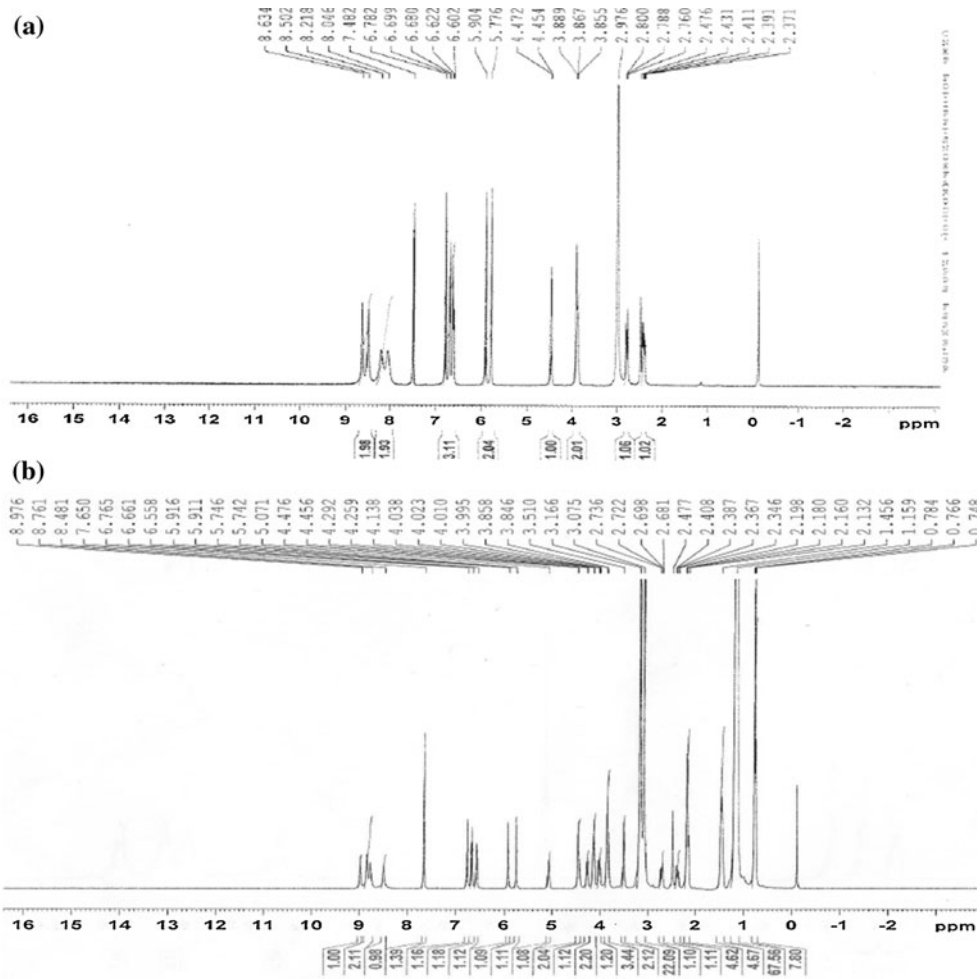

Fig. 6 ^{1}H NMR of **a** catechin (in DMSO) and **b** catechin-phospholipid complex (in DMSO)

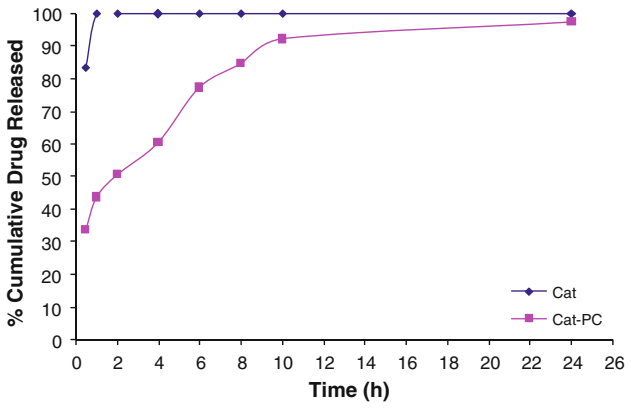

Fig. 7 Dissolution study of catechin (Cat) and catechin – phospholipid complex (Cat-PC)

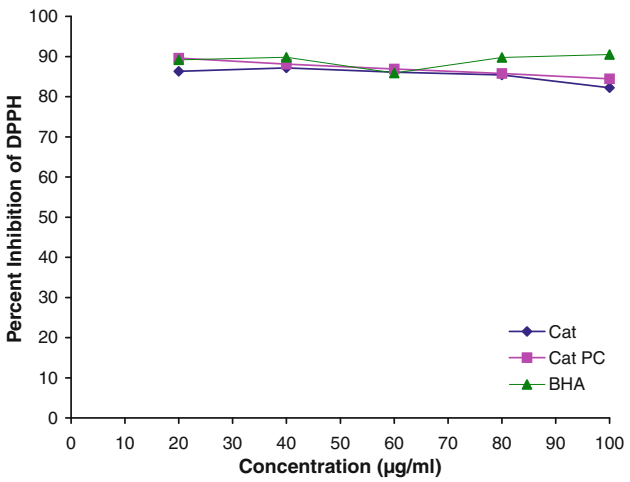

Fig. 8 Effect of concentration on in vitro free radical scavenging activity of catechin and its complex

diamagnetic molecule [27]. Methanolic DPPH (0.1 mM) solution gives a violet color which shows λ_{max} at 515 nm. When an antioxidant is mixed with this solution, the concentration of the stable free radical 2, 2-diphenyl-1- picryl-hydrazyl or DPPH is reduced which can be detected by the decrease in the optical absorbance of DPPH at 515 nm.

Catechin showed excellent antioxidant activity at all dose levels. Catechin-phospholipid complex showed slightly better antioxidant activity than that of catechin at all dose levels (Fig. 8). At 100 µg/mL, catechin and its complex showed 82.22 and 84.47% scavenging activity

against 90.53% of BHA after 30 min. Therefore, the antioxidant activity of the catechin remains unchanged even after the complex formation and it can be concluded that the process of complexation did not adversely affected the antioxidant activity of catechin. Various studies revealed that the phospholipid complexes of polyphenolic phytoconstituents show the improved bioavailability and hence the better therapeutic effects [28].

Conclusions

In the present study, catechin–phospholipid complex were prepared by a simple and reproducible method and evaluated for various physicochemical parameters. The physicochemical investigations showed that catechin formed complex with phospholipids with better lipid solubility, The dissolution study confirmed its sustained release profile and lipopophilic behaviour. The FTIR, ^{1}H NMR, DSC and XRPD studies confirmed the formation of the complex. Moreover, the complexation did not interfere with the biological activities of catechin. Thus it can be concluded that the phospholipid complex of catechin may be of potential use for improving absorption of catechin across the lipidic biological barriers in gastrointestinal tract and for sustaining the release. With the sustained release profile, it may be possible to get a sustained action with smaller dose. This value added herbal drug delivery system can pave the way for large molecules to pass through the lipophilic biological membrane (by the virtue of their amphiphilic nature) and get absorbed into the systemic circulation.

Acknowledgments Authors acknowledge the grant provided by the University Grant Commission, Govt. of India for the research work. Authors are also thankful to LIPOID GmbH Germany for providing the gift sample of phosphatidylcholine for the research work. Facilities provided by the IIT Roorkee, NMR Laboratory, Faculty of Pharmacy Jamia Hamdard, New Delhi and Department of Chemistry, University of Delhi are thankfully acknowledged.

References

1. Kalender, S., Kalender, Y., Ates, A., Yel, M., Olcay, E., Candan, S.: Protective role of antioxidant vitamin E and catechin on idarubicin induced cardiotixicity in rats. Bra. J. Med. Bio. Res. **34**, 1379–1387 (2002)
2. Kid, P., Head, K.: A review of the bioavailability and clinical efficacy of milk thistle phytosome: a Silybin–phosphatidylcholine complex. Alt. Med. Rev. **11**, 12–15 (2005)
3. Reimann, H.J., Lorenz, W., Fisher, M., Meyer, H.J., Schmal, A., Frolich, R.: Histamine & acute haermorrhagic lesions in rat gastric mucosa; prevention of stress ulcer formation by (+)-catechin, an inhibitor of specific histidine decarboxylase in vitro. Inflammation Res. **7**(1), 69–73 (1977)
4. Rivkind, A.I., Marshood, M., Durst, A.L., Becker, Y.: Cianidanol ([+]–cyanidanol-3) prevents the development of abdominal adhesions in rats. Arch. Surgery. **118**(12), 14–17 (1983)
5. Weyant, M.J., Carothers, A.M., Bertagnolli, M.M., Dannerberg, A.J.: [+]–catechin inhibits intestinal tumor formation and suppresses focal adhesion kinase activation in the Min/+ mouse. Cancer Res. **61**, 118–125 (2001)
6. Takano, F., Tanaka, T., Aoi, J., Yahagi, N., Fushiya, S.: Protective effect of (+) -catechin against 5-fluorouracil induced myelosuppression in mice. Toxicology. **201**(1–3), 133–142 (2004). doi:10.1016/j.tox.2004.04.009
7. Singal, S.K., Tirkey, N., Chopra, K.: Green tea extract and catechin ameliorate chronic fatigue induced oxidative stress in mice. J. Medicinal Food. **8**(1), 47–52 (2005). doi:10.1089/jmf.2005.8.47
8. Parvaz, S., Jabassum, H., Rehman, H., Benarjee, B., Athar, M., Raisuddin, S.: Catechin prevents tamoxifen induced oxidative stress and biochemical perturbations in mice. Toxicology. **225**(2–3), 109–118 (2006). doi:10.1016/j.tox.2006.05.009
9. Siegers, C.P., Volpel, M., Scheel, G., Younes, M.: Effect of dithiocarb & catechin against CCl4-alcohol induced liver fibrosis. Agents Actions. **12**(5), 743–748 (1982). doi:10.1007/BF01965096
10. Varga, M., Buris, L.: Some morphometric evidence of hepatoprotective effects of (+) cyanidanol -3. Pharmacol. Biochem. Behav. **33**(3), 523–526 (1989). doi:10.1016/0091-3057(89)90380-8
11. Semalty, A., Semalty, M., Rawat, B.S., Singh, D., Rawat, M.S.M.: Pharmacosomes: the lipid based novel drug delivery system. Expert. Opin. Drug Deliv. **6**, 599–612 (2009). doi:10.1517/17425240902967607
12. Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M.: Preparation and characterization of phospholipid complexes of naringenin for effective drug delivery. J. Incl. Phenom. Macrocycl. Chem. **67**, 253–260 (2010). doi:10.1007/s10847-009-9705-8
13. Blois, M.S.: Antioxidant determination by the use of a stable free radical. Nature **181**, 1199–1200 (1958). doi:10.1038/1811199a0
14. Paradkar, A.R., Pawar, A.P., Chordiya, J.K., Patil, V.B., Ketkar, A.R.: Spherical crystallization of celecoxib. Drug Dev. Ind. Pharm. **28**, 1213–1220 (2002). doi:10.1081/DDC-120015354
15. Yanyu, X., Yunmei, S., Zhipeng, C., Quineng, P.: The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. Int. J. Pharm. **307**, 77–82 (2006). doi:10.1016/j.ijpharm.2005.10.001
16. Li, Y., Yang, D.J., Chen, S.L., Chen, S.B., Chan, A.S.C.: Comparative physicochemical characterization of phospholipids complex of puerarin formulated by conventional and supercritical methods. Pharm. Res. **25**, 563–577 (2007). doi:10.1007/s11095-007-9418-x
17. Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P., Mukherjee, P.K.: Curcumin-phospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats. Int. J. Pharm. **330**, 155–163 (2007). doi:10.1016/j.ijpharm.2006.09.025
18. Kumar, M., Ahuja, M., Sharma, S.K.: Hepatoprotective study of curcumin-soya lecithin complex. Sci. Pharm. **76**, 761–774 (2008). doi:10.3797/scipharm.0808-09
19. Singh, D., Rawat, M.S.M., Semalty, A., Semalty, M.: Gallic acid-phospholipid complex: drug incorporation and physicochemical characterization. Lett. Drug Design Discov. 8, 2011, (BSP/LDDD/E-Pub/00114) In press
20. Shi, K., Cui, F., Yu, Y., Zhang, L., Tao, A., Cun, D.: Preparation and characterization of a novel insulin phospholipid complex. Asian J. Pharm. Sci. **1**(3–4), 168–174 (2006)
21. Cui, F., Shi, K., Zhang, L., Tao, A., Kawashima, Y.: Biodegradable nanoparticles loaded with insulin-phospholipid complex for oral delivery : preparation, in vitro characterization and in vivo evaluation. J. Control. Release. **114**, 242–250 (2006). doi:10.1016/j.jconrel.2006.05.013
22. Yoo, H.S., Park, T.G.: Biodegradable nanoparticles containing protein-fatty acid complex for oral delivery of salmon calcitonin. J. Pharm. Sci. **93**, 488–495 (2003)
23. Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M.: Development and physicochemical evaluation of pharmacosomes of

diclofenac. Acta Pharma. **59**, 335–344 (2009). doi:10.2478/v10007-009-0023-x
24. Semalty, A., Semalty, M., Rawat, B.S., Singh, D., Rawat, M.S.M.: Development and evaluation of pharmacosomes of aceclofenac. Indian J. Pharm. Sci. **72**, 571–575 (2010). doi:10.4103/0250-474X.78522
25. Bombardelli, E., Curri, S.B., Gariboldi, P.: Cosmetic utilization of complexes of Panax ginseng saponins with phospholipids in PHYTOSOME® form. Fitoterapia. **60**(S1), 55–70 (1989)
26. Jithan, A., Ganji, S.: Formulation, excipient properties and pharmacological activities of catechin liposomes. Phcog. Mag. **4**(13), 121–126 (2008)
27. Soares, J.R., Dins, T.C.P., Cunha, A.P.: Antioxidant activity of some extracts of *Thymus zygis*. Free Rad. Res. **26**, 469–478 (1997). doi:10.3109/10715769709084484
28. Semalty, A., Semalty, M., Rawat, M.S.M., Federico, F.: Supramolecular phospholipids-polyphenolics interactions: the PHYTOSOME® strategy to improve the bioavailability of phytochemicals. Fitoterapia **81**, 306–314 (2010). doi:10.1016/j.fitote.2009.11.001