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Effect of soy phosphatidyl choline on the bioavailability and nutritional health benefits of resveratrol

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ABSTRACT

Resveratrol has several nutritional health benefits including a cardioprotective effect. Although it has high oral absorption, but its rapid metabolism results in less systemic availability and restrict its efficacy. To overcome this, resveratrol complex with hydrogenated soy phosphatidyl choline (HSPC) was developed and its effect was evaluated on doxorubicin induced cardiotoxicity in rats. The cardioprotective activity of the resveratrol complex and free resveratrol (4 and 8 mg/kg) were evaluated by measuring various antioxidants and cardio protective enzymes. The complex significantly restored the activity of SOD, catalase, LDH and creatine kinase with respect to doxorubicin treated group ($P<0.05$ and <0.01). Plasma concentration of resveratrol obtained from the complex was higher than pure resveratrol. This study highlighted on the improved bioavailability and cardio protective health benefit of resveratrol by HSPC due to its sustained delivery and potential antioxidant property.

1. Introduction

Resveratrol (3,5,4-trihydroxystilbene) is a polyphenol found naturally in grapes (King, Joshua, & David, 2006). The 'French paradox' has been assigned for their consumption of red wine containing resveratrol responsible for cardiovascular benefit (Hung, Chen, Huang, Lee, & Su, 2000). Further its estrogenic activity helps to prevent bone loss in post-menopausal women (Sharma, Kulkarni, & Chopra, 2007). It has been found to inhibit initiation, promotion and progression of tumor *in vitro* and *in vivo* (Jang et al., 1997) Resveratrol has been shown inhibit production of PGE_2 in human peripheral blood leukocytes dose dependently (Richard, Porath, Radspieler, & Schwager, 2005), while significantly decreased the elevated levels of rat PGD_2 in *in-vivo* models (Martín, Villegas, La Casa, & Lastra, 2004). Further, it has anti-oxidant activity associated with its chemo-preventive and cardio protective activity (Lin & Tsai, 1999).

Soy phosphatidyl choline is an important carrier system for the drug molecules, giving required sustained/controlled release *in vivo* due to rapid clearance from the body and plays a major role in delivering the molecules with poor bioavailability. Several herbal medicines having potential therapeutic activities have very short half life and are excreted from our body accordingly. To enhance the therapeutic benefit of these phytoconstituents, several works have been reported from our laboratory. The activity of ellagic acid can be enhanced by its phospholipid complex (Venkatesh, Mukherjee, Maiti, & Mukherjee, 2009). Constituents like naringenin, quercetin, and curcumin, have potential therapeutic activity but poor oral absorption and faster elimination. It has been demonstrated that complex with HSPC enhances the bioactivity of these phytomolecules (Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007; Maiti et al., 2005). So the focus of this work was to develop a complex using soy phosphatidyl choline, so as to enhance the health benefits of resveratrol which has poor oral bioavailability.

The detection of resveratrol in humans, mice and rat plasma has been studied, but the study on its bioavailability is limited. In spite of an unusual high absorption, the oral bioavailability of pure resveratrol is considerably low due to its rapid and extensive metabolism, resulting in little unchanged resveratrol in the systemic circulation. A maximum of 2 μm metabolites were found in systemic circulation after a 25 mg of its oral dose (Walle, Hsieh, DeLegge, Oatis, & Walle, 2004). Therefore, to maintain the steady plasma concentration of resveratrol inside the body so as to exert its therapeutic activity for enough time, frequent administration of the molecule is required and it necessitates the demand for development of a dosage form, which can maintain the concentration of unchanged resveratrol in the blood for a longer period of time. The present study was designed to evaluate the bioavailability of the HSPC complex of resveratrol with respect to the pure resveratrol. Doxorubicin is one of the most effective anti-tumor agent whereas its use is limited due to its dose-dependent cardiotoxicity which causes lipid peroxidation and oxidative damage in the heart. Though numerous cardio protective agents have been

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investigated in the past against doxorubicin, almost none have stood against the test of time (Fajardo & Bernstein, 2007). Thus the present study was performed on the prepared resveratrol–HSPC complex which will certainly give an insight into the effect of HSPC on enhancement of bioavailability and hence the increase in cardio protective activity of resveratrol against doxorubicin induced cardiotoxicity.

2. Materials and methods

HSPC was procured from Lipoid, Ludwigshafen, Germany; resveratrol was generously gifted by Orchid Chemicals & Pharmaceuticals Ltd., Chennai, India. Creatine kinase and LDH kits were purchased from Span diagnostics Ltd., Kolkata, India. Glutathione, bovine serum albumin, tris base, nitroblue tetrazolium, and 5,5-dithiobis (2-nitrobenzoic acid) reagent were purchased from SRL chemicals, Mumbai, India. 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.

2.1. Preparation of resveratrol–phospholipid complex

The complex was prepared with resveratrol and HSPC at a molar ratio of 1:2 based on our earlier reported method from our laboratory (Venkatesh et al., 2009). Briefly, the resveratrol 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. Resultant clear solution was evaporated to 2–3 mL and 10 mL of n-hexane was added to it with continuous stirring. Resveratrol–HSPC complex was precipitated, was filtered and dried under vacuum to remove traces of solvents. Resultant resveratrol–HSPC complex (yield 92.5% w/w) was kept in an amber colored glass bottle flushed with nitrogen and stored at room temperature.

2.2. Determination of resveratrol content in the complex

Content of resveratrol in the complex was determined spectrophotometrically (Cecil CE 7200 Spectrophotometer). A calibration curve was obtained with aliquots of different concentration of resveratrol dissolved in required 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 308 nm to evaluate the concentration of resveratrol 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.

2.3. Characterization of the complex

2.3.1. High performance thin layer chromatography (HPTLC)

Resveratrol–HSPC complex and pure resveratrol were dissolved in methanol, spotted on the Silica Gel $60F_{254}$ pre-coated TLC plates and chromatogram was developed in chromatographic chambers using dichloromethane: methanol (4:1) as solvent system at a room temperature of 30 °C, at an angle of 70°. After development of chromatogram, the plates were scanned with the help of Camag TLC scanner 3 and the R_f values of the spots were recorded.

2.3.2. Ultra violet (UV) spectroscopy

UV–visible spectroscopic analysis was performed in a CECIL CE 7200 spectrophotometer to find the λ_{max} of the compound.

2.3.3. Infra red (IR) spectroscopy

The infrared absorption spectra of the samples were taken with Spectrum One FT-IR spectrometer (Perkin Elmer, USA). The spectra were recorded in the region of 4000 cm^{-1} to 400 cm^{-1}.

2.3.4. Nuclear magnetic resonance (NMR) spectroscopy

The ^{1}H NMR spectra of the resveratrol and its HSPC complex were taken with DPX-400 MHz spectrometer (Brucker, Switzerland) and the formation of the complex was characterized.

2.3.5. Scanning electron microscopy (SEM)

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).

2.4. Evaluation of resveratrol–HSPC complex

2.4.1. Animals

Male albino rats (Wistar strain) weighing 180–220 g, were used for this study. Animals were housed in groups of 6 in colony cages at an ambient temperature of 20–25 °C and 45–55% relative humidity with 12 h light/dark cycles. They had free access to pellet chow (Brook Bond, Lipton India) and water *ad libitum*. All rats were fasted overnight before the experiment. The experiment was performed with the ethical guidelines as provided by committee for the purpose of control and supervision of experiments on Animals (CPCSEA).

2.4.2. Cardio protective and antioxidant study

Rats were divided into six groups of six animals each as shown in the table below.

Group	1st week	2nd week
Group I	DW	DW
Group II	DW	DW + DOXO
Group III	RT 4 mg/kg/day	RT 4 mg/kg/day + DOXO
Group IV	RT 8 mg/kg/day	RT 8 mg/kg/day + DOXO
Group V	RT CPX-4 mg/kg/day	RT CPX-4 mg/kg/day + DOXO
Group VI	RT CPX-8 mg/kg/day	RT CPX-8 mg/kg/day + DOXO

DW	Distilled water with Tween 80 (1% v/v) p.o.
DOXO	Doxorubicin injection 4 mg/kg, i.p.
RT	Resveratrol as suspension in tween 80 (1% w/v) through enteral route
RT CPX	Resveratrol complex as suspension in tween 80 (1%v/v) through enteral route

2.4.3. Enzyme estimation

The rats were sacrificed immediately under anesthetic condition and the blood was aspirated from the left ventricle, centrifuged to collect the plasma. Total cholesterol (Wybenga, Pileggi, Dirstine, & Di Giorgio, 1970), triglycerides (Herbert, 1984), LDH (Teitz, 1976) and creatine kinase (Szasz & Gruber, 1978) were determined using the ready to use kits supplied by Span diagnostics Ltd., Kolkata, India. Heart was dissected out, 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) (Ellman, 1959), glutathione peroxidase (GPX) (Paglia & Valentine, 1967), superoxide dismutase (SOD) (Kakkar, Das, & Viswanathan, 1984) and catalase (CAT) (Beer & Seizer, 1952). Thiobarbituric acid reactive substances (TBARS) were also measured according to the method of Ohkawa, Hash, and Yagi (1979). Protein