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Enhanced oral bioavailability and prophylactic effects on oxidative stress and hepatic damage of an oil solution containing a rosmarinic acid–phospholipid complex

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ABSTRACT

Rosmarinic acid (RA) is an important naturally occurring polyphenol from plants of the mint family with many health-promoting benefits, but these can be influenced by its poor bioavailability. In this study, the bioavailability and bioefficacy of RA as RA–phospholipid complex (RA–PLC) and as RA–PLC oil solution (RA–PLC–ME) were evaluated. Evaluations in Caco-2 cell monolayers *in vitro* revealed that the membrane permeability of RA–PLC and RA–PLC–ME was significantly increased ($p < 0.05$) over that of unformulated RA. Pharmacokinetic studies additionally revealed that RA–PLC and RA–PLC–ME were 1.2- and 2.9-fold more bioavailable than unformulated RA, respectively. Evaluation of bioefficacy *in vivo* demonstrated that oral administration of RA–PLC–ME produced a greater reduction in the serum levels of AST and ALT ($p < 0.05$, $p < 0.01$), and markedly increased antioxidative properties ($p < 0.05$), compared with unformulated RA. This study indicated that PLC–ME could act as an effective delivery device for RA to enhance oral bioavailability and bioefficacy.

Abbreviations: RA, rosmarinic acid; PLC, phospholipid complex; RA–PLC, rosmarinic acid–phospholipid complex; RA–PLC–ME, rosmarinic acid–phospholipid complex oil solution; PM, the physical mixture of rosmarinic acid and the phospholipid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; DSC, differential scanning calorimetry; PXRD, powder X-ray diffractometry; FTIR, Fourier transform infrared spectroscopy; P, *n*-octanol/water partition coefficient; P_{app}, the apparent permeability coefficient; C_{max}, maximum plasm concentration; T_{max}, peak time; $T_{1/2}$, elimination half-life; AUC_{0-t} and $AUC_{0-\infty}$, the area under the plasma concentration versus time curve (from zero to the last sampling time or from zero to infinity)

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Chemical compounds: Rosmarinic acid (PubChem CID: 5281792); Silibinin (PubChem CID: 5213); Ethanol (PubChem CID: 702); Methanol (PubChem CID: 887); Formic acid (PubChem CID: 284); Ethyl acetate (PubChem CID: 8857); Acetone (PubChem CID: 180); Carbon tetrachloride (PubChem CID: 5943); Trichloromethane (PubChem CID: 6212); Acetonitrile (PubChem CID: 6342); Acetic acid (PubChem CID: 176).

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1. Introduction

Functional foods are a vivid field of research seeking to alleviate or prevent modern morbidity, such as depressive disorder, coronary heart disease and diabetes. In recent years, there has been an upsurge of interest in rational design and product engineering to incorporate more bioactive compounds and functional food ingredients into our diet, to maximize the advantages of food processing and enhance food's contribution to human health exceeding its benefits (Benshitrit, Levi, Tal, Shimoni, & Lesmes, 2012).

Rosmarinic acid (RA), a phenolic compound, is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid commonly found in various plants from the mint family (Lamiaceae), such as *Perilla frutescens*, *Rosmarinus officinalis*, *Prunella vulgaris*, *Ocimum basilicum* and other crops (Kondo, Omri, Han, & Isoda, 2015; Lee & Scagel, 2010). These plants are usually used as raw materials in beverage or food flavour sources.

It has been reported that RA exhibits various biological activities, such as antioxidant (Domitrović et al., 2013; Gao, Wei, Zhao, Xiao, & Zheng, 2005), anti-inflammatory (Chu et al., 2012; Jiang et al., 2009), and anticancer (Moon, Kim, Lee, Choi, & Kim, 2010; Scheckel, Degner, & Romagnolo, 2008) effects. Besides, RA is also potentially valuable for the improvement of diabetes mellitus and obesity by inhibiting digestive enzymes, such as α-amylase and α-glucosidase (Zhu et al., 2014). Furthermore, RA has several neuronal benefits like anti-amyotrophic lateral sclerosis (ALS) (Shimojo, Kosaka, Noda, Shimizu, & Shirasawa, 2010), anti-Alzheimer's disease (AD) (Alkam, Nitta, Mizoguchi, Itoh, & Nabeshima, 2007), improving effect on experimental Parkinson's disease model (Wang et al., 2012b), nootropic effect, and anti-depressive benefit (Ito et al., 2008; Sasaki, Han, Shimozono, Villareal, & Isoda, 2013). However, the high water solubility of RA results in its poor permeation across intestinal epithelial cells and low oral bioavailability, which restricts its application as a functional food (Konishi, Hitomi, Yoshida, & Yoshioka, 2005; Konishi, Hitomi, & Yoshioka, 2004).

To improve RA's bioavailability, a limited number of pharmaceutical approaches have been investigated, including orally administered solid lipid nanoparticles (Campos, Madureira, Gomes, Sarmento, & Pintado, 2014) and chitosan nanoparticles (Da Silva et al., 2014), among others. However, low encapsulation efficacy and side-effects from the solubilizing or encapsulating excipients still limit its use.

The phospholipid complex (PLC) has emerged as one of the most successful methods for improving the bioavailability and bioefficacy of poorly absorbed plant constituents. This phospholipid complexation technique, which incorporates the phospholipid molecules containing phosphatidylcholine in their structure, to form complexes with plant constituents by hydrogen bonds or van der Waals forces, improves oil/water partition coefficients and membrane permeability, and hence increases their systemic bioavailability (Loguercio et al., 2012; Raju, Reddy, & Reddy, 2011). Most importantly, the phospholipids are important components of cell membranes, which make possible to penetrate the cell membrane and enter the cytoplasm of living mammalian cells without disturbing the cellular lipid bilayer. Many studies have depicted that constituents combined with phospholipids would be beneficial to increasing oral bioavailability and enhancing biological effect of drugs (Ma, Chen, Sun, Tong, & Zhang, 2014; Prasanna et al., 2013; Singh, Bhatt, Gill, & Suresh, 2013; Xiao, Song, Chen, & Ping, 2006; Yue, Yuan, Li, Yang, & Zhu, 2010).

In addition, it has been reported that medium-chain triacylglycerol (MCT) preferred in lipid-based formulations was used to enhance the bioavailability of constituents (Dahan & Hoffman, 2007; Han et al., 2009). Its characteristic structure and low toxicity also make it extensively used as oil phase in emulsion and other oral formulations (Yang, Decker, Xiao, & McClements, 2015). Among these MCT, Masester® E6000, caprylic/capric triacylglycerol, can be rapidly digested in the gastrointestinal tract after being emulsified by endogenous emulsifier such as bile and then hydrolysed by pancreatic lipase. The lipolysis products containing fatty acids and monoacylglycerol can not only be absorbed directly by themselves but also enhance other plant constituents' membrane permeability (Fernandez et al., 2007; Ljusberg-Wahren, Nielsen, Brogard, Troedsson, & Mullertz, 2005). Combining PLC with an oil solution is a potential further approach to improve penetration through lipoidal plasma membranes and increase the oral bioavailability of plant constituents. However, to the best of our knowledge, no data have been published to date on improving the oral bioavailability and the function of RA on its formulation with PLC or as an oil solution.

In the present studies, novel rosmarinic acid–phospholipid complexes (RA–PLC) were prepared by solvent evaporation and verified by differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD) and Fourier transform infrared (FTIR) spectroscopy. Additionally, *n*-octanol/water partition coefficients (*P*) were determined. Subsequently, an oil solution of RA–PLC (RA–PLC–ME) was obtained by dissolving RA–PLC in Masester® E6000 with the aim of further enhancing its oral bioavailability. Intestinal cellular permeability was examined using Caco-2 cell monolayers *in vitro*. An evaluation of RA–PLC and/or RA–PLC–ME's oral bioavailability and ability to prevent oxidative stress and hepatic damage induced by carbon tetrachloride in rodents was also undertaken.

2. Materials and methods

2.1. *Materials*

RA was purchased from Nanjing Zelang Medicine Co. Ltd. (Nanjing, China) with a purity of 98%. The phospholipid, namely soybean lecithin, containing 70–97% phosphatidylcholine, was kindly supplied by Shanghai Tai-wei Pharmaceutical Co. Ltd. (Shanghai, China). Silybin was employed as an internal standard (*I.S.*) and purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Masester® E6000 was purchased from Easydo Food Ingredient Co. Ltd. (Guangzhou, China). Bifendate pills were purchased from Beijing Union Pharmaceutical Factory (Beijing, China). The diagnostic kits for assaying the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), thiobarbituric acid reactive substances (TBARS), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering