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Cheng Zeng

a) Xinjiang Institute of Materia Medica, Urumqi 830004, P. R. China

b) Institute of Traditional Chinese Medicine, Xinjiang Medical University, Urumqi 830054, P. R. China

Wen Jiang

Department of Pharmacy, The Sixth Affiliated Hospital of Xinjiang Medical University, Urumqi 830002, P. R. China

Xin-chun Wang

College of Pharmacy, Shihezi University, Shihezi 832002, P. R. China

Mei-e Tan

College of Pharmacy, Shihezi University, Shihezi 832002, P. R. China

Jian-guo Xing

Xinjiang Institute of Materia Medica, Urumqi 830004, P. R. China

Total flavonoids extract from *Dracocephalum moldavica* Composite phospholipid liposomes: Preparation, *in vitro* drug release and permeability of Caco-2 cell

Cheng Zeng, Wen Jiang, Xin-chun Wang, Mei-e Tan, Jian-guo Xing

Abstract

To prepare Composite Phospholipid Liposome (CPL) of Total Flavonoids Extract from *Dracocephalum moldavica* (TFDM), determine its physicochemical properties, investigate its *in-vitro* release and increase the bioavailability of TFDMCPL. The CPL was prepared by ammonium sulfate transmembrane gradients. The CPL and TFDM were separated by Sephadex-G50 chromatography. The concentration of TFDM in the CPL were detected by HPLC, then the entrapment efficiency (EE) was evaluated. And the shape, particle size, zeta potential, drug release *in vitro* were investigated, differential scanning calorimetry (DSC) techniques was employed to investigate molecular interaction in TFDMCPL and study the transcellular transportation and cytotoxicity of TFDMCPL using Caco-2 cell model. The EE of TFDM was 84.17±2.2%, mean size of CPL was 136.2±3.7 nm, polymey disperse index (PDI) was 0.158±0.015 and zeta potential was -19.8±1.2 mV. TFDMCPL were found to enhance the release of drugs more effectively than TFDM based on the *in vitro* model and CPL were found to enhance the permeability of TFDM more effectively than the TFDM alone based on the *in vitro* model of the epithelial barrier. The ammonium sulfate transmembrane gradient is suitable for preparing TFDMCPL. This HPLC method was accurate and sensitive, and can be applied to the determination of TFDMCPL. And TFDMCPL have potential to be used to improve the bioavailability of poorly soluble drugs after oral administration.

Keywords: Total flavonoids extract from *Dracocephalum moldavica*; composite phospholipid liposome; entrapment efficiency; *in vitro* release; differential scanning calorimetry; Caco-2 cell.

1. Introduction

Dracocephalum moldavica L (DM) is an annual herb in Labiatae family, which is used as a traditional Uygur medicine for centuries in the name of Baeranjiboya^[1]. DM possesses important medicinal values against the following diseases: Bronchitis, hypertension, hepatitis, dizziness, biliary tract infections, and other diseases. DM is mainly produced in several provinces of northeast, northwest, and north of China; Southeastern Xinjiang has abundant resources^[2]. DM has a long history to be recorded in many classical books on Uygur medicines. A portion of minority population in Xinjiang region uses this herb alongside tea, and the civil society uses this as a Uygur medicine to treat coronary heart disease, cold nervous headache, colds, and bronchitis etc., It is also used to improve the quality of blood^[3]. Flavonoids as the main active ingredient in the DM. The current study shows that main component of total flavonoids in DM (TFDM) is tilianin^[4] (Fig 1).

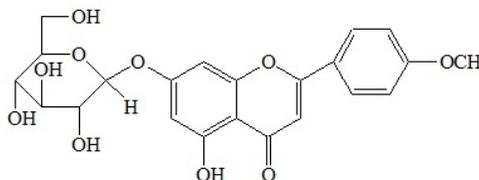


Fig 1: Chemical structure of tilianin

Correspondence**Jian-guo Xing**

Xinjiang Institute of Materia Medica, Urumqi 830004, P. R. China.

TFDM has low solubility and low permeability. So one way is the formulation of poorly water soluble and permeable drugs with lipids containing formulations such as Composite phospholipid liposomes (CPL) to improve the absorption profile of drug [5]. CPL were made by mixtures of different phospholipids (soybean lecithin, SPC and Hydrogenated soya phosphatide, HSPC) [6]. CPL can be prepared to have many useful properties, by varying the type of phospholipid used to make CPL and by attaching some molecules to the surface of liposomes. They have been used for oral drug delivery to improve the oral bioavailability of some drugs that are not easily absorbed [7, 8, 9].

The *in vitro* characterization of intestinal transport can be made by different approaches such as intestinal cell lines, everted gut sacs and isolated perfused organs [10, 11]. Caco-2 cells likewise maintain the capacity to form polarized cell monolayers *in vitro* once confluence has been reached in culture [12]. The Caco-2 cell line was initially introduced as an experimental tool for studying intestinal transport mechanisms [13]. Subsequently, the use of this cell model was extended to the monitoring of drug intestinal permeability and for predicting the oral absorption of drug substances [14]. Subsequent evaluations have shown the expressed transporter profile of Caco-2 cells to be qualitatively similar to that of the human small intestine [15].

The aim of this study is to be prepared by ammonium sulfate transmembrane gradients method. The mean particle size, PDI, zeta potential, EE, morphology, Stability, DSC and *in-vitro* release of TFDMCPL were investigated. In addition, the cytotoxicity and cellular transport of TFDMCPL were evaluated in Caco-2 cell culture model. We expect that novel nano-formulation carrier systems will have the potential to be applied in novel drug delivery and clinical therapy to improve oral bioavailability and increase efficacy of TFDM.

2. Materials and methods

2.1 Materials

TFDM (Xinjiang Just Pharmaceutical Company, China); Soybean phospholipid (SPC, purity: 96%, lipoid company, German); Hydrogenated soya phosphatide (HSPC, purity: 98%, Shanghai Advanced Vehicle Technology Pharmaceutical Ltd, China); Cholesterol (Hui Xing Biochemical Reagent Co. Ltd, China); Tiliain (purity: 98%, Xinjiang Pharmaceutical Research Institute); Sephadex G-50 (Beijing Pharmacia Company, China), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) (Thermo Fisher Scientific, China). Caco-2 cells were purchased from Cell Resource Center of Peking Union Medical College (Beijing, China). Other chemicals and reagents used were chromatographic or analytical grade.

2.2 Equipments

SPD-10Avp type HPLC (Shimadzu Corporation, Japan); Temperature control heating magnetic stirrer (IKA®-Werke GmbH & Co.KG, Germany); JY 92-IIN ultrasonic cell grinder (Ningbo scientz biotechnology Polytron Technologies Inc, China); Nano S90 laser particle size analyzer (Malvern Instruments Ltd, England); 12-well plates with polyester membrane inserts (12 mm diameter, pore size 0.4µm,

Transwell, Costar Corporation, Beijing, China); Millicell-ERS volttohmmeter (Millipore Corporation, China).

2.3 Preparation of TFDMCPL

Ammonium sulfate gradient method was used to prepare the CPL of blank. Briefly, SPC, HSPC and Cholesterol were dissolved with the solution of ethanol. The mixture was completely homogenized through using the ultrasound, then was injected into the Ammonium sulfate solution (0.2 mol/L) and was stirred for 30 min by magnetic stirrer and reclaimed. The mixture was sonicated for 5 min by probe sonication for 1 min cycles (1 s working and 2 s rest) at 400W. Finally, the resulting mixture was placed in the dialysis bag (molecular weight cut off 8000-14,000) and the receptor compartment was filled with 10 times Phosphate Buffered Saline (PBS, pH=7.4) of volume for 8h.

Phosphate Buffered Saline (PBS, pH=7.4) contained TFDM (0.2 mg /mL) was injected into CPL of blank and was stirred for 20 min by magnetic stirrer. The resulting CPL suspension was extruded through filtration membrane with 0.22 µm pore size.

2.4 Characterization of TFDMCPL

Particle size distribution and the zeta potentials of the CPL were determined by laser diffractometry using a Malvern NanoZS90. EE was determined by via gel-filtration method with SephadexG-50 column and total flavonoids extract from DM encapsulation was determined following solubilization of vesicles in ethanol and analyzed by a validated HPLC method (r^2 : 0.9998). The quantitative determination of total flavonoids extract from DM was performed using HPLC. The system consisted of a UV detector, C18 column- SPD-10Avp (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of 0.5% methanol (solvent A) and acetonitrile (solvent B) (80:20, v/v). The flow rate was 1 mL/min and with an injection volume of 10 µL. The column temperature was kept at 35 °C. The detection wavelength was set at 324 nm.

2.5 Transmission Electron Microscopy (TEM)

The morphologies of TFDMCPL and CPL of blank were observed using a transmission electron microscopy (TEM). After dilution with distilled water, the samples were negatively stained with 2% (w/v) phosphotungstic acid for observation.

2.6 CPL stability study

Empty CPL and TFDMCPL (approximately 50 mL) were stored in plastic centrifuge tube (50 mL) at 4 °C in the dark for 6 months. The vesicle mean particle size, PDI and EE were determined at t_0 and after 6 months.

2.7 DSC

The samples (TFDM, physical mixture, blank-CPL and TFDMCPL) were loaded individually into a standard aluminum sample pan and hermetically sealed after weighed, and which were scanned from 20 °C to 500 °C with a heating rate of temperature change was 5 °C/min and a delay of 5 min was set up between sequential scans to allow thermal equilibration on a differential scanning calorimeter DSC-70 (Shimadzu, JAPAN).

2.8 *In vitro* Release of TFDMCPL

The studies of in-vitro release were performed by the dialysis bag method. First of all, the dialysis bag (molecular weight cut off 8000-14,000) was soaked in distilled water for 12 h before use. Then, 10 mL of TFDM or a sample of TFDMCPL (0.1mg/mL) was placed in the dialysis bag and the receptor compartment was filled with 100 mL of phosphate buffer (pH 7.4) at 37 °C with gentle agitation (30 rpm). 1.0 mL of the dissolution medium was withdrawn from the receptor compartment at intervals of 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 h and replaced with the same volume of fresh dialysis medium. Finally, the concentration of TFDM was determined by HPLC. All analyses were performed in triplicate to allow proper statistical analysis.

2.9 Cell cultures

The human colon carcinoma Caco-2 cells were maintained in 75 cm² flasks to which 12mL of Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) non-essential amino acids, 1% (v/v) L-glutamine. The cells were incubated at 37 °C in an atmosphere with 95% relative humidity and a CO₂ flow of 5%. The medium was changed every 2-3 days. When the cell monolayer reached 80% confluence, the cells were detached with a solution of trypsin (0.25g/L) and EDTA (ethylene diamine tetraacetic acid, 0.22 g/L), and reseeded at a density of 1-1.5 × 10⁴ cells/cm². The assays were performed with cultures between passages 32 and 43.

The transport assays were carried out in 12-well plates with polyester membrane inserts (12 mm diameter, pore size 0.4µm). In this system the cells are seeded onto the porous membrane of the insert that separates the well into two compartments: apical (upper) and basolateral (lower). Cell were seeded (8 × 10⁴ cells/cm²) onto the inserts, with the addition of 0.5 mL of DMEM to the apical chamber and 1.5 mL of DMEM to the basolateral chamber. The cells were incubated at 37 °C, with 5%CO₂ and 95% relative humidity, with a change of medium every 2-3 days until cell differentiation was reached (18-20 days post-seeding). In order to evaluate the evolution of the monolayers during cell differentiation in the two-compartment system, the transepithelial electrical resistance (TEER) was measured by a voltohmmeter. The cell monolayer was considered completely formed when the resistance reached in the range of 400-600 ohm·cm², cell monolayer was used for transport studies. Wells with TEER value of >400 oh m·cm².

2.10 Evaluation of cytotoxicity

The Caco-2 cells used for the Cell Counting Kit – 8 (CCK-8) assay were seeded at a density of 2 × 10⁴ cells/well in 96-well cell culture plates and were pre-incubated for 24 h before sample treatment. Next, the cells were treated with CPL at various lipid concentrations from 10 to 600 µg/mL in serum-free medium (pH 7.4) for 24, 48, 72 h. The samples were removed, the cells were incubated with 100 µl of 10% (v/v) CCK-8 and 90% (v/v) MEM for 4 h. The relative cell viability (%) was calculated based on absorbance at 450 nm using a microplate reader. The viabilities of the non-treated control cells were normalized to 100%, six experimental sets were carried out, and each set was performed in sextuplicate.

$$\% \text{Relative cell viability} = (\text{OD}_{450, \text{ sample}} - \text{OD}_{450, \text{ blank}}) / (\text{OD}_{450, \text{ control}} - \text{OD}_{450, \text{ blank}}) \times 100$$

Where OD is the optical density at an absorbance of 450 nm.

2.11 Permeability Studies

TFDM permeation studies were carried out with Caco-2 cells. Culture medium was replaced from each well by Hank's Balanced Salt Solutions (HBSS) in the apical and basolateral side of the well and cell monolayers were subsequently equilibrated for 30 min at 37 °C. TFDM solution and TFDMCPL were respectively added to the apical side of the monolayer. The wells were then placed on a shaker at 30 rpm and 37 °C for 2 hours. After that the samples from the basolateral side was analyzed by a validated HPLC method (r² > 0.9992).

Apparent permeability co-efficient (cm/s) was calculated by the following equation.

$$P_{\text{app}} = (dC/dt) \cdot 1 / (AC_0)$$

Where, dC/dt = rate of drug permeation (µg/s), A = Surface area of the insert (cell monolayer) (1.12cm²), C₀ = Initial concentration of drug in the apical side (µg/mL). Data reported were arithmetic mean values ± standard deviation (mean ± SD). The statistical significance of the differences was performed using an analysis of variance (ANOVA) test and a p value <0.05 or 0.01 was considered significant.

3. Results and discussion

3.1 Drug-loaded CPL mean size, zeta potential and EE

Table 1 presents the mean size and the zeta potential of the CPL suspension with and without TFDM.

The addition of the drug increased slightly the vesicle size and PDI (respectively, 106.4 and 136.2 nm, 0.124 and 0.158 without and with TFDM) (Fig.2). This increase of the mean size could be explained by the entrapment of the total flavonoids extract from DM in the vesicles bilayers.

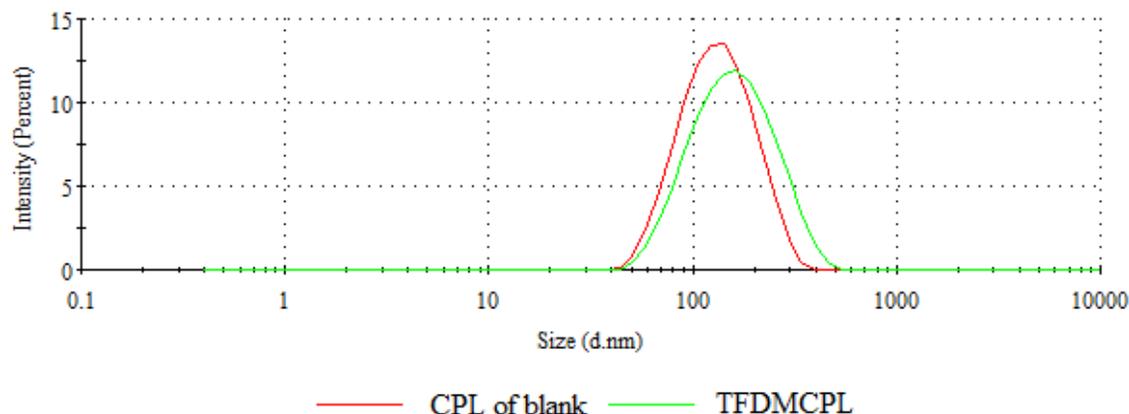
The drug-loaded CPL suspension had an upper zeta potential compared to drug-free formulation. Indeed, the zeta potential was -26.7 mV for the drug-free CPL suspension and became -19.8 mV for the drug-loaded CPL suspension. Zeta potential measurements give information about the surface properties of the carrier and therefore can be useful to determine the type of the association between the active substance and the carrier (whether the drug is encapsulated in the body or simply adsorbed on the surface).

In our study the negative surface charge was further shielded in the presence of the drug, suggesting that at least a part of the association was surface-adsorption and the rest was incorporated within the lipidic matrix. These zeta potential data allowed predicting a very good stability of the preparations (a negative zeta potential higher than 15 mV was sufficient to prevent vesicle coalescence).

The desirable therapeutic effect of CPL as drug carriers can be achieved if they are loaded with a sufficient amount of an active compound. Therefore, suitable entrapment efficiencies of drugs are required. In our study, a relatively higher encapsulation efficacy with more than 32% in comparison to traditional liposomes. The high encapsulation efficiency was believed to be due to the high lipophilicity of total flavonoids extract from DM and therefore its good solubility in phospholipids.

Table 1 Drug-free and drug-loaded mean size and zeta potential.

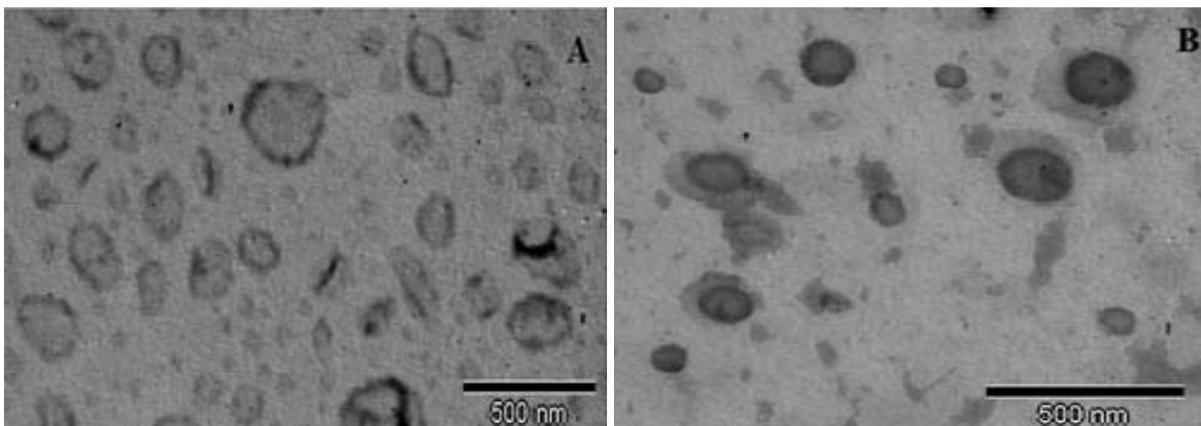
Batch	Mean size \pm S.D. ^a (nm)	Zeta potential \pm S.D. ^a (mV)
Drug-free CPL ^b	106.4 \pm 2.4	-26.7 \pm 0.8
Drug loaded CPL ^b	136.2 \pm 3.7	-19.8 \pm 1.2

^a standard deviation (n=3).^b the mean of 3 batches.**Fig 2.** The particle size and PDI of CPL of Blank and TFDMCPL

3.2 Surface morphology study by transmission electron microscope (TEM)

As shown in Fig.3, the morphological investigation using transmission electron microscopy revealed nanometric sized

and spherical shaped CPL. According to TEM micrographs, CPL ranged in size from 100 to 220 nm correlating well with measurement obtained. Vesicle membranes were composed of phospholipids bilayers.

**Fig 3:** Transmission electron microscope images of morphology of A (CPL of Blank) and B (CPL of total flavonoids extract from DM)

3.3 Stability study of CPL

The mean particle size, PDI of CPL and the EE of TFDMCPL were examined after 6 months of storage at 4 °C. An increase of size of empty CPL from 106.4 \pm 2.4 nm to 148.3 \pm 5.6 nm and PDI from 0.127 \pm 0.012 to 0.227 \pm 0.021 and size of TFDMCPL from 136.2 \pm 3.7 nm to 216.7 \pm 3.7 nm and PDI from 0.158 \pm 0.015 to 0.285 \pm 0.021 were observed. However, the EE of TFDM determined after 6 months of storage at 4 °C were identical to those obtained at t_0 suggesting that CPL retain the TFDM constituent during the storage.

3.4 DSC analysis

Based on DSC experiment, in Fig 4 (A), DSC thermogram of TFDM showed an obvious endothermic peak (transition peak) at 122.8 °C. Physical mixture was composed of SPC, HSPC,

cholesterol and TFDM at a ratio of 1:1:1:1. From the Fig 4 (B), it shown that a melting point around 69 °C, 211.9 °C, 396.1 °C, and the DSC thermogram is significantly different from TFDM (Fig 4 A), blank-CPL (Fig 4 C) and TFDMCPL (Fig 4 D). In Fig 4 (C), a major endothermic peak at 123.2 °C and 237.3 °C, respectively, it indicated that a possible interaction of lipid (SPC and HSPC) and cholesterol during the process of forming the different of lipid bilayer, and compare to DSC thermogram of TFDMCPL (Fig 4 D), it shown that a shift of endothermic peak of 30-40 °C. The TFDMCPL had a substantial change (shifted peak of TFDM) in the DSC thermogram, it supposed that the peak shifted because of the blank-CPL and TFDM strongly interacted with each other (the hydrocarbon chains of the lipid and TFDM) and also might contribute to improve EE.

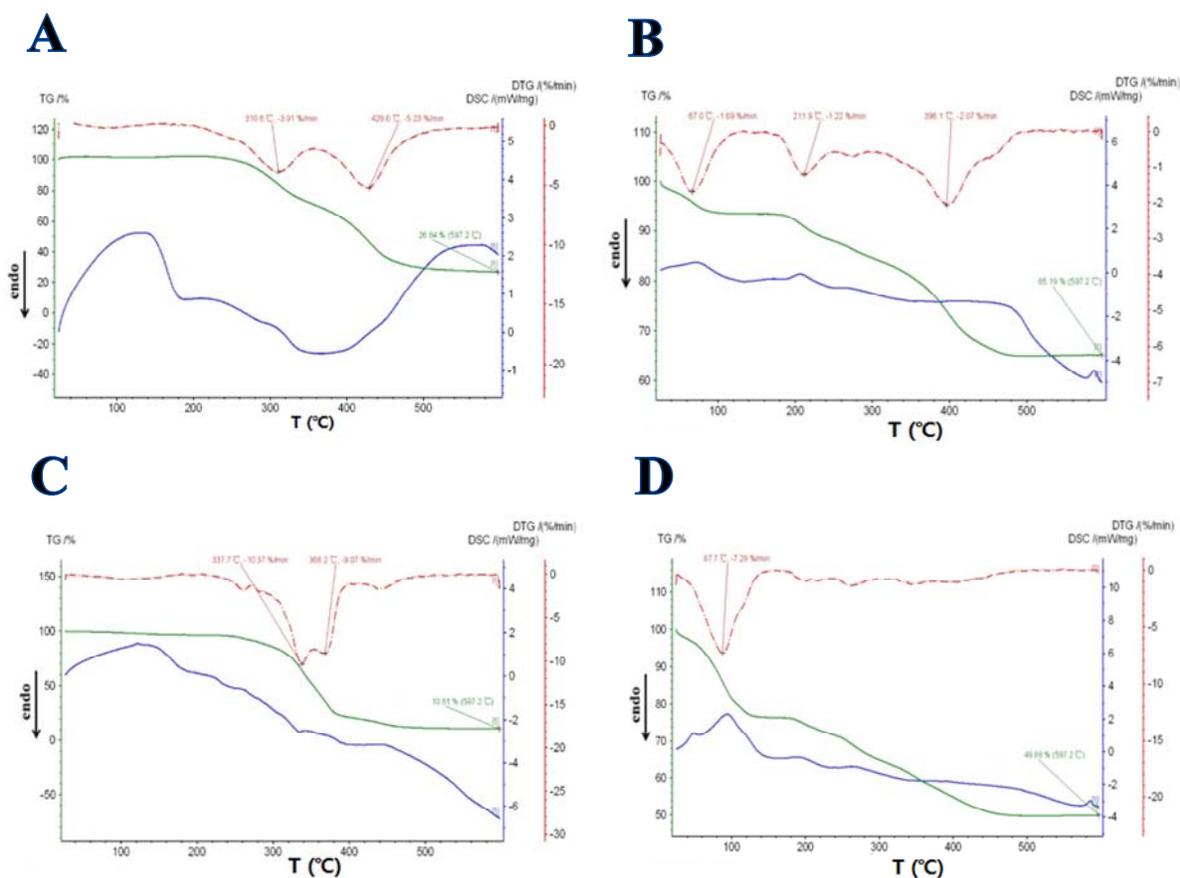


Fig 4. DSC analysis of TFDM (A), physical mixture (B), blank-CPL (C) and TFDMCPL (D)

3.5 *In vitro* Release of TFDM- CPL

The amount of drug released from CPL was drafted as a function of time. The cumulative amount of TFDM in the receptor over 24 h was charted after administration of TFDMCPL as shown in Fig.5. It was obvious that the drug release from CPL lifted significantly. When comparing the results which were consistent with the general summary that composite phospholipid liposomal entrapment of drugs enhances their release.

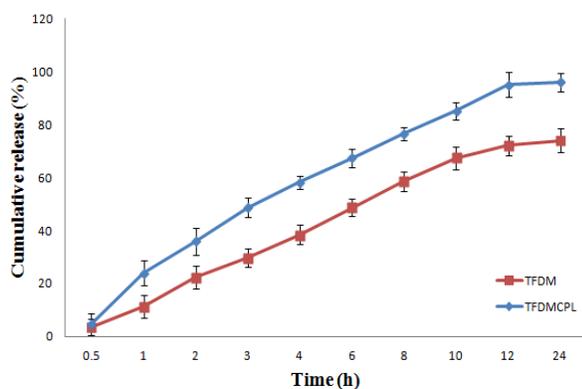


Fig 5: The release of TFDM and TFDMCPL (n=3)

3.6 Cytotoxicity

The viability of Caco-2 cells in the presence of CPL of blank were evaluated using a standard CCK-8 assay. Cell viability was calculated as percent of control cells. As shown in Fig.6,

CPL showed little or no reduction in cell viability over a wide concentration range from 10 to 600 $\mu\text{g/mL}$. The cell viability was approximately 100% even at a high concentration (600 $\mu\text{g/mL}$) of CPL.

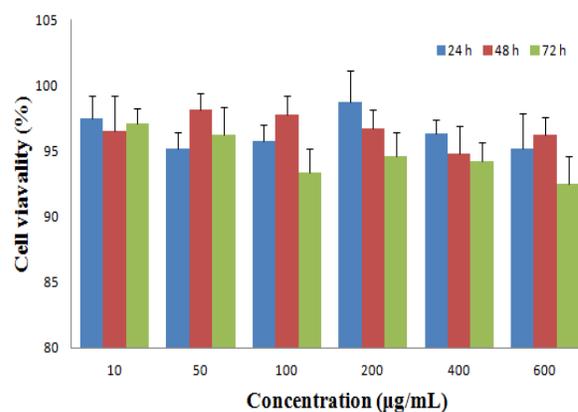


Fig 6: Cytotoxicity of CPL of blank

3.7 Transport experiments in the Caco-2 cell culture model

Caco-2 cell culture model has become the standard screening system to estimate the intestinal permeability of drug candidates. Within the framework of the Biopharmaceutics Classification System (BCS), the rate of mass transfer of a compound across the Caco-2 monolayer can even be considered to allow a waiver for *in vivo* bioequivalence studies.

The apical to basolateral permeability of TFDM alone or formulation are shown in Fig.7. Permeability coefficient (P_{app}) of TFDMCPL is significantly higher ($p < 0.05$) than those of TFDM alone. As can be seen from Fig.7, a 6 fold increase on TFDM permeability was obtained for CPL when compared TFDM alone. It can be concluded that absorption enhancing effect was due to composite phospholipid liposomal formulation. The absorption enhancing effect of CPL was also found in several other papers due to its biocompatible accessories and different lipids were fixed (SPC and HSPC), which will coexist in two immiscible phases and create several discontinuous regions to greatly increase solubility of difficult soluble drugs.

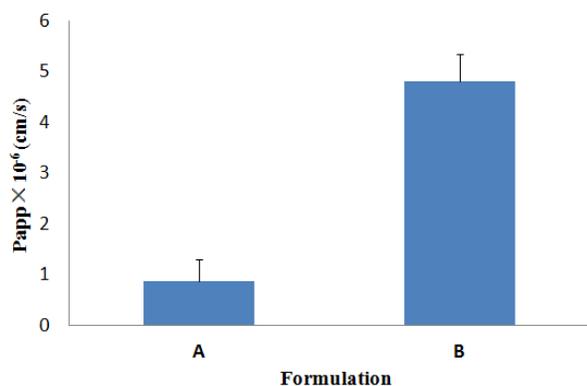


Fig 7. Permeability values of A (TFDMCPL), B (TFDM alone)

4. Conclusion

TFDM which is a poorly water soluble and low oral bioavailability compounds were successfully encapsulated in the CPL formulations. They encapsulate TFDM constituents with high EE values. According to the physicochemical properties and drug in-vitro release of TFDMCPL, which with high EE, small size, well suited PDI and the final CPL was able to potentially promote releasing of TFDM.

According to the Permeability Studies, the permeation enhancer effect observed for the CPL make them attractive candidates that could be effective to improve bioavailability of TFDM after oral administration. They were also stable after 6 months of storage at 4 °C.

5. References

- Bindu MB, Kusum B, Banji D. Novel strategies for poorly water soluble drugs. *Int. J. Pharm. Sci. Rev. Res.* 2010; 4(1):76-78.
- Chen J, SU X, Cai BC, Wang W, Qi Y. Determination of Content and Encapsulation Efficiency of Liposomes Containing Total Alkaloids from Seed of *Strychnos nux-vomica* L. *Traditional Chinese Drug Research and Clinical pharmacology*, 2009; 3(20):249-252.
- Hermida LG, Roig A, Bregni C, Sabés-Xamaní M, Barnadas-Rodríguez R. Preparation and characterization of iron-containing liposomes: their effect on soluble iron uptaken by Caco-2 cells. *J Liposome Res.* 2011; 21(3):203-212.
- Jiang J, Yuan X, Wang T, Chen H, Zhao H, Yan X, *et al.* Antioxidative and cardioprotective effects of total flavonoids extracted from *Dracocephalum moldavica* L. against acute ischemia/reperfusion-induced myocardial injury in isolated rat heart. *Cardiovasc Toxicol*, 2014; 14(1):74-82.
- Jiang W, He CH, Xing JG. Preparation technology of *Dracocephalum moldavica* total flavonoids pellets and its release mechanism. *Chinese Traditional and Herbal Drugs*, 2013; 44(21):26-29.
- Kan P, Wang AJ, Chen WK, Tsao CW. Liposome for incorporating large amounts of hydrophobic substances. *Unite States Patent (20040126886 A1)* 2004.
- Li L, Chen J, Cai BC. HPLC Fingerprint of Novel Liposomes Containing Total Alkaloids from Semen *Strychni*. *Traditional Chinese Drug Research and Clinical pharmacology*, 2010; 21(6):637-639.
- Liapunova PN, Salo ND, Sergienko TA. Anatomical study of the Moldavian dragon's head herb (*Dracocephalum moldavica* L). *Farmatsiia*, 1975; 24(5):15-20.
- Ma J, Guan R, Shen H, Lu F, Xiao C, Liu M *et al.* Comparison of anticancer activity between lactoferrin nanoliposome and lactoferrin in Caco-2 cells *in vitro*. *Food Chem Toxicol*, 2013; 59(1):72-77.
- Maimaitiyiming D, Hu G, Aikemu A, Hui SW, Zhang X. The treatment of Uyghur medicine *Dracocephalum moldavica* L on chronic mountain sickness rat model. *Pharmacoqn Mag*, 2014; 10(40):477-482.
- Martínez-Vázquez M, Estrada-Reyes R, Martínez-Laurraquío A, López-Rubalcava C, Heinze G. Neuropharmacological study of *Dracocephalum moldavica* L. (Lamiaceae) in mice: sedative effect and chemical analysis of an aqueous extract. *J Ethnopharmacol.* 2012; 141(3):908-917.
- Qu YQ, Chen J, Lin AH, Hou T, Cai BC. Study on Antitumor Activity of Novel Liposomes Containing Total Alkaloids from Seed of *Strychnos nux-vomica*. *Chinese Journal of Experimental Traditional Medical Formulae.* 2012; 3(18):143-145.
- Singh RS, Michel D, Das U, Dimmock JR, Alcorn J. Cytotoxic 1, 5-diaryl-3-oxo-1,5-pentadienes: an assessment and comparison of membrane permeability using Caco-2 and MDCK monolayers. *Bioorg Med Chem Lett*, 2014; 24(22):199-202.
- Vázquez M, Devesa V, Vélez D. Characterization of the intestinal absorption of inorganic mercury in Caco-2 cells. *Toxicol in vitro*, 2015; 29(1):93-102.
- Zeng C, Huang W, He CH, Xing JG. Research progress of composite phospholipids liposomes. *J Int Pharm Res.* 2015; 42(1):8-12.
- Zheng Y. Editorial : Novel formulation strategies for poorly water-soluble drugs and herbal medicines. *Curr Pharm Des*, 2014; 20(3):301-302.