doi.org/10.36721/PJPS.2020.33.1.REG.033-040.1

Preparation, evaluation and pharmacokinetics of diosmin herbosome in beagle dogs

Imene Adouani[1], Anas Sarwar Qureshi[2]* and Tai-Jun Hang[3]
[1]Department of Pharmacy, Ferhat Abbas University Setif-1, Setif, Algeria
[2]Department of Anatomy, University of Agriculture, Faisalabad, Pakistan
[3]Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, PR China

Abstract: Diosmin is one of the most widely used phlebotonic drugs, but its poor bioavailability has restricted its usage. The aim of this study was to formulate a complex Diosmin with phospholipids (75% in PC, in 1:2 molar ratios) and to evaluate for solubility, drug content, X-ray diffraction (XRD), differential scanning calorimetry (DSC) and *in vitro* dissolution study. Further to test the bioavailability of both the complex and Alvenor® in beagle dogs and compare pharmacokinetic parameters. Diosmin herbosome was found to be more soluble than both pure diosmin and Alvenor®. The complex contained 71.94% drug content. DSC thermograms and XRD also proved the claim of the complexation. The dissolution profile of diosmin herbosome and Alvenor® in water-ethanol medium showed an increase of the dissolution for diosmin herbosome. Comparison of plasma concentration and main pharmacokinetic parameters of diosmin herbosome treated and Alvenor® treated dogs showed a higher Cmax for the complex with longer elimination half-life. The complexation of diosmin with phospholipids can be potentially used in enhancing the absorption and solubility, consequently increasing the bioavailability of the drug.

Keywords: Bioavailability, enhancement, diosmin, herbosome, dogs.

INTRODUCTION

There is an extensive body of literature examining therapeutic activities of diosmin (Bedada and Boga, 2017; Bogucka-Kocka *et al*., 2013; Filho *et al*., 2018; Queenthy *et al*., 2018). One of its main actions is the treatment of venous diseases (Hnatek, 2015). It is also used in the management of hemorrhoids (Giannini *et al*., 2015) and to reduce swelling after surgeries. Diosmin has an effect against heart problems and blood pressure (Queenthy and John, 2013; Senthamizhselvan *et al*., 2014), diabetes (Jain *et al*., 2014), along with several types of cancer (Lewinska *et al*., 2015; Naso *et al*., 2016). In addition, it has potential anti-inflammatory (Imam *et al*., 2015) and antioxidant (Naso *et al*., 2016) actions. Despite this demonstrated efficacy, its poor solubility along with poor oral bioavailability compromise the potential for therapeutic uses. Diosmin is completely degraded by the intestinal bacteria into its aglycom diosmetin (Silvestro *et al*., 2013), which is systemically absorbed (Walle, 2004). Diosmetin is then rapidly glucuronic-conjugated in the rat and provides an explanation for the low bioavailability of diosmin.

In order to fully utilize the potential of this agent, it is necessary to improve its bioavailability. Therefore, phospholipids are used to enhance the bioavailability of some drugs by modifying their rate of release, improving their solubility and facilitating their permeation (Amit *et al*., 2013). The phospholipids complex increases the absorption of active ingredients when topically applied on the skin (Tsai *et al*., 2015) and improves systemic bioavailability when orally administered. Pharmacokinetic studies in different animals like rats and dogs as well as in humans have shown increased bioavailability (Gupta and Dixit, 2011; Semalty *et al*., 2012). In a comparative study, the curcumin absorption was higher for Meriva® as compared to the unformulated curcumin (Zhang *et al*., 2013). At the same dose, the action of the 18ß-glycyrrhetinic acid phytosomes was found to be greater and to last longer than 18ß-glycyrrhetic acid alone. Similar is the case with many others such like; ginkgoselect phytosome and green select phytosome (Maryana *et al*., 2016). This means that the phospholipids complex does not only increases the active ingredient tolerability and absorption but also improves its efficacy (Mishra *et al*., 2012).

In this study, we seek to prepare a complex of diosmin with phospholipids to overcome its poor bioavailability along with enhancing its efficiency under low doses.

MATERIALS AND METHODS

Biological and chemical materials

The materials included; raw diosmin, soya phosphatidylcholine (PC), commercially available diosmin (Alvenor®), dichloromethane, methanol, dioxane, n-octanol, microcrystalline cellulose, magnesium stearate, talc, silicon dioxide, emcosoy, croscarmellose sodium and cornstarch.

*Corresponding author: e-mail: anas-sarwar@uaf.edu.pk

Preparation of diosmin-pc complex and physical mixture

To optimize the preparation technique in terms of solubility and drug content, different kinds of phospholipids (PHOSPHOLIPON® 50, LIPOID® S100, PHOSPHOLIPON® 85G and LIPOID® S75) were tested. The complex was tried for two different molar ratios (1:1 and 1: 2) of the drug to phospholipids. In addition; other conditions such as the reaction time and temperature were also investigated.

Finally, the complex was prepared using LIPOID® S 75 in 1:2 molar ratios as it was found optimum for complex formation. The PC was first dissolved in 10 ml of methanol and then was added to 90 ml of diosmin dissolved in dioxane. This mixture was first refluxed for 2 h then, evaporated to small volume under vacuum at 40°C in a rotary vacuum evaporator (Shanghai Biochemical Equipment®, R-202). The residue was re-dissolved in dichloromethane and was stirred for 1 h. The solvent was evaporated afterward, the collected residues were invested in the vacuum desiccators overnight and then were characterized.

Physical mixtures were prepared manually by mixing the appropriate quantities of diosmin and phospholipids in a mortar for 30min.

Preparation of dosage forms

Due to waxy nature of the prepared complex, its formulation to oral dosage forms possibly faced a lot of obstacles. A direct volumetric filling process can be applied, even if the apparently low density of the herbosome seems to limit the maximum amount of powder that can be filled into a capsule. To modify the stickiness of the final complex and monitor the release time, many excipients were tested.

Pre-formulation Studies

Drug content and entrapment efficiency

A quantity of complex was dissolved in an appropriate volume of water. The solution was subjected to ultracentrifugation (15800r/min for 20min). The supernatant and pellet were separated in two different tubes. Dilutions were made suitably and measured at 254 nm using UV-2401 PC UV/Visible recording spectrophotometer (Shimadzu, Japan). The test was prepared in triplicate.

Solubility

Alvenor®, physical mixture and pure diosmin, complex were dissolved, both in water and n-octanol, in order to determine any variation in the solubility of these compounds due to complex formation. An excess of diosmin was added to 5ml of each solvent. The solutions were put on continuous stirring for 24 h, thereafter, centrifuged for 20 min. the suitable delusions were made and the drug content was measured at 254 nm. The test was prepared in triplicate.

In vitro dissolution study

In vitro dissolution studies for herbosome capsules and Alvenor® were performed in triplicate using a ZRS-8G (Bio-Equip®) six station dissolution test apparatus. The paddle method was set at 120 rpm, 37°C and 1000mL of water/ ethanol mixture (9:1). The dissolution fluid was sampled at different intervals and an equal amount of new media was added. These samples were then filtered and their suitable dilutions were made which were then studied at 254 nm.

Evaluation and characterization

Differential Scanning Calorimetry Analysis

Thermograms of pure diosmin, PC, herbosome and the physical mixture were recorded using a differential scanning calorimeter (NETZSCH®, DSC 204). Each sample was covered under the pan, having a uniform gas nitrogen flow for its thermal behaviour studies. At a constant rate of 10°C per minute, the samples were heated from the range of temperature, 25-300°C, with gradual heating.

X-ray powder diffraction analysis

The crystalline state of pure diosmin, PC, herbosome and the physical mixture were evaluated with XRPD. D8 Advance Powder X-ray diffractometer (Bruker®) was employed to obtain the diffraction patterns.

The X-ray generator that receives a tube voltage of 40KV, tube current of 40MA and Ka lines of copper was employed generate the radiations. Meanwhile, the angle was set in step scan mode at 2 θ ranged at 3-40°C of temperature.

Bioavailability study and pharmacokinetics in beagle dogs

The enhanced bioavailability potential of the herbosome was tested in eight beagles (8.6-10.7kg), ramdomly placed into two groups. Each group received either herbosome capsules or Alvenor® tablets, having equivalent quantities of diosmin (450mg). After a period of washout, the groups were switched; this enabled the data collection on the same set of dogs. Two milliliters of blood was collected in heparin tubes after 30 min, 1, 2, 4, 5, 6, 7, 8, 10, 12, 24, 36 and 48 h of oral administration. After centrifugation, plasma samples were collected and kept at -20°C. The guidelines for health and care for the experimentation on animals were followed in true spirits as recommended by the National Institutes of Health.

Sample preparation and lc-ms-ms analysis

First, a mixture of 125µl of NaOAc (1 M, pH 5.0) and 0.4 ml of thawed plasma was incubated with 200µl of B-glucuronidase type H-1 (Sigma-Aldrich, St. Louis, MO,

USA) in a constant temperature shaker SHA-B (China) at 37°C and 100r/min for 18h. Then, 40µl of internal standard (3-Methylflavone-8-carboxylic acid), 1ml of NaCl (0.9%), and 200µl of HCl (1 M) were successively added and the mixture was agitated for 3s. Finally, 4 ml methyl tetra butyl ether/isopropanol mixture (1:1) was put and the mixture was again stirred again for 3 min. After thorough centrifugation, the mixture was heated to evaporate the organic phase under vacuum at 37°C. The resultant mixtures were again re-formed in 150µl of mobile phase and were again centrifuged (10min, 4000 rpm).

Samples of 20µl were analyzed using a Phenomenex Gemini C18 column (150 × 4.6 mm, Phenomenex®, Torrance, CA, USA) on a Finnigan TSQ Quantum Ultra AM triple-quadruple tandem mass spectrometer (Thermo Electron Corporation®, San Jose, CA, USA) having a source of electrospray ionization in its negative mode that is monitered at a wavelength of 288 nm. A mixture of acetonitrile: water-0.1% formic acid (55:45) was used to separate the mixture a 5min. Pure diosmin and its dilutions, prepared in a solution mixture of methanol and DMSO, were subjected to generate the standard curves. The spikes were observed on standard curves with internal standard. The same were prepared for the extracted and reconstituted for plasma samples and studied. For the sake of acquisition of the data and instrument control, Xcalibur™ workstation software (Version 1.4 SR1, 2003, Thermo Scientific™) was employed. The validation of the technique used was done by the addition of know different amounts of diosmin to the blank plasma of the dogs. The resulting concentrations of diosmin were 0.1, 0.2, 0.5, 1.0, 2, 5, 10, 15, 30 and 40 ng ml^{-1}. In order to test the precision and accuracy as well as linearity of the technique, analytical process was conducted on the dilutions.

STATISTICAL ANALYSIS

The data obtained was analyzed by Analysis of variance (ANOVA) technique using SPSS version 22 statistical computer software at 5% probability.

RESULTS

Preparation of the complex and dosage forms

Results have shown that the molar ratio and time of reaction played an important influence. The temperature has no or small effect on the quality of the prepared complex. Phospholipon 50 has been shown to be the best-used phospholipid giving high solubility and high drug content but it has been avoided in our preparation due to the big quantity needed to prepare 1:2 diosmin phospholipids complex because phospholipon 50 contains just 45% of PC which makes its preparation to dosage forms impossible. Finally, the best herbosome was prepared using lipoid S75 in the molar ratio 1:2 at 40°C refluxed first in a mixture of dioxane/methanol (7/3) for 2 h and then in dichloromethane for 1 h.

To prepare a tablet of the complex with a dose of 450 mg in diosmin, 70 % of excipients were used to minimize the stickiness of the complex and made it suitable for compression. On the other hand, direct compression is avoided due to the high unitary dose. The characteristics of the complex make its formulation into tablet impossible. To overcome this problem, an attempt was made to prepare hard gelatine capsules with just little quantity of excipients. To optimize the characteristics of the complex, many batches were prepared using different excipients in different concentrations. The results showed that the batch prepared using 1% croscarmellose, 10% MCC, 2% silicon dioxide and 1% magnesium stearate showed a quick disintegration within just 1 min.

Differential scanning calorimetry

The results of the DSC test confirmed the association of diosmin and PC in the complex as both peaks representing diosmin and PC changed position (fig. 1). Thermal analysis of the drug alone demonstrated characteristic thermograms with three endothermic peaks at 51.9°C, 101.1°C and 275.4°C. The thermograms of diosmin-PC complex showed a disappearance of the diosmin peak and appearance of new endothermic peaks at low onset temperatures. There was also change in the shape of the peak.

X-ray powder diffraction

In order to assess the possible variations in the morphology of the diosmin crystal pertaining to the polymorphic transition and to investigate the diosmin-PC complex in the solid state, we carried out XRPD analysis. fig. 2 shows the powder XRD patterns of pure diosmin, PC, and their complex. The diffraction pattern of diosmin powder diffraction exhibited a fractional sharp crystalline peaks, which is a peculiar feature of a molecules that has undergone crystallinity.

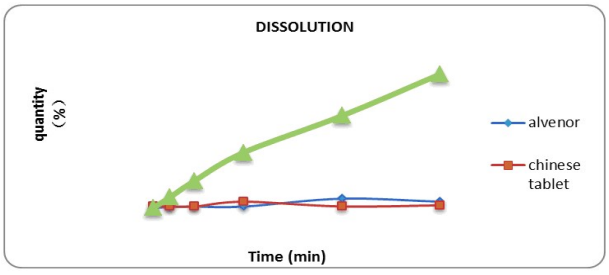

Fig. 3: Dissolution profile of diosmin-phospholipds phytosome capsules and micronized diosmin tablets

Drug content and solubility

The complex showed the same absorption spectra as pure diosmin confirming the theory of physical complexation. This complexation did not lead to the formation of a new

Table 1: Solubility comparison among various preparations (n=3).

Drug	Solubility in aqueous layer (in µg/ mL)
Pure diosmin	1.82 ±0.16
Alvenor®	3.92 ±0.04
Diosmin tablet	2.63±0.05
Diosmin-phospholipids complex	18.29± 0.13
Physical mixture	10.54±0.06

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

Table 2: The pharmacokinetic parameters of diomin complex and Alvenor® following a single dose of 450 mg (mean±SD, n = 8).

Dose	Diosmin Complex	Alvenor®
Cmax (ng/mL)	0.71±0.62	0.47±0.44
Tmax (h)	4.5±5.2	13.3±15.7
MRT0-72 (h)	86.57±82.40	67.66±28.89
t1/2 (h)	55.24±60.21	39.69±20.22

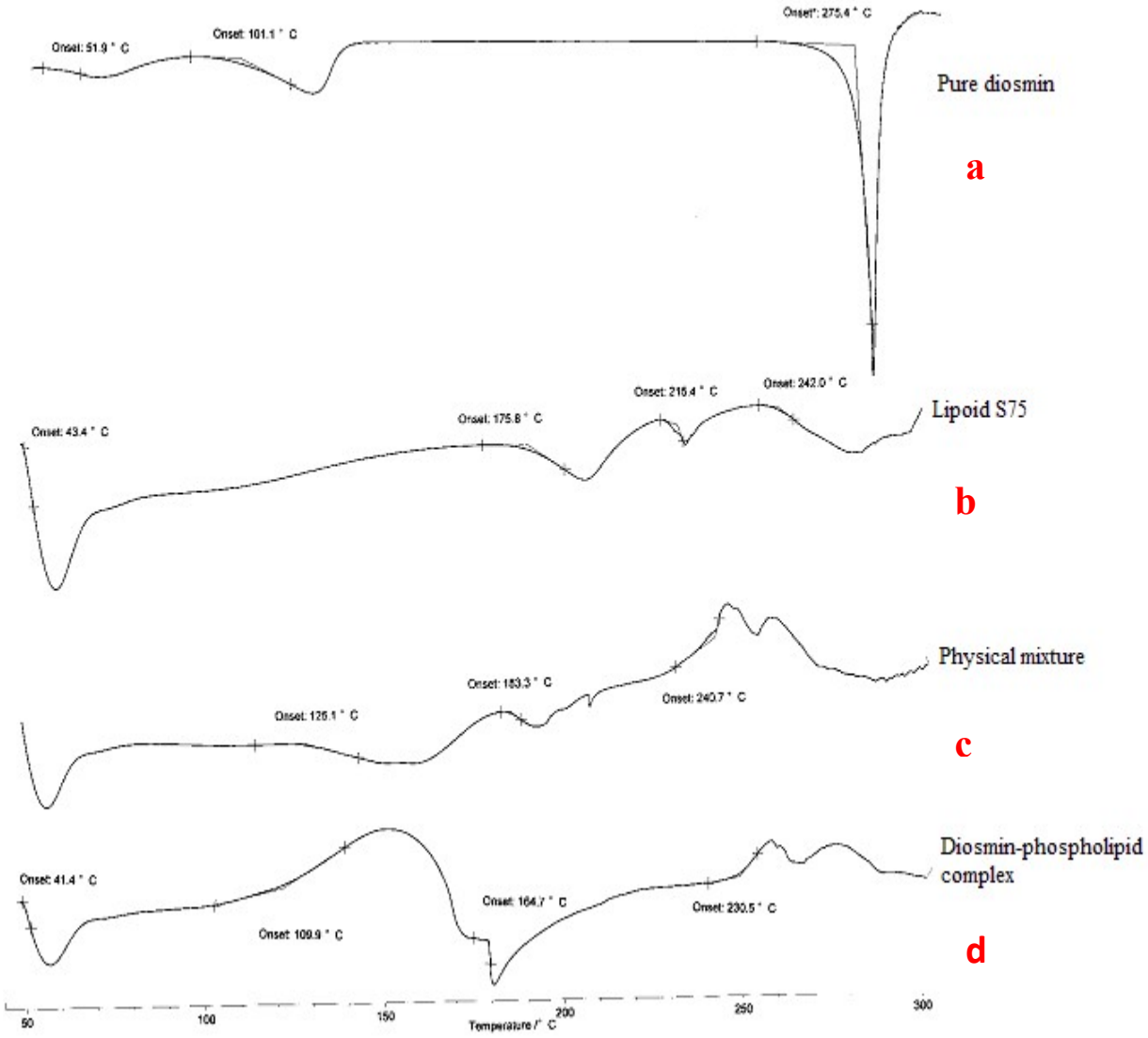

Fig. 1: DSC thermograms of Pure diosmin, Lipoid s75, Physical mixture and Diosmin-phospholipid complex

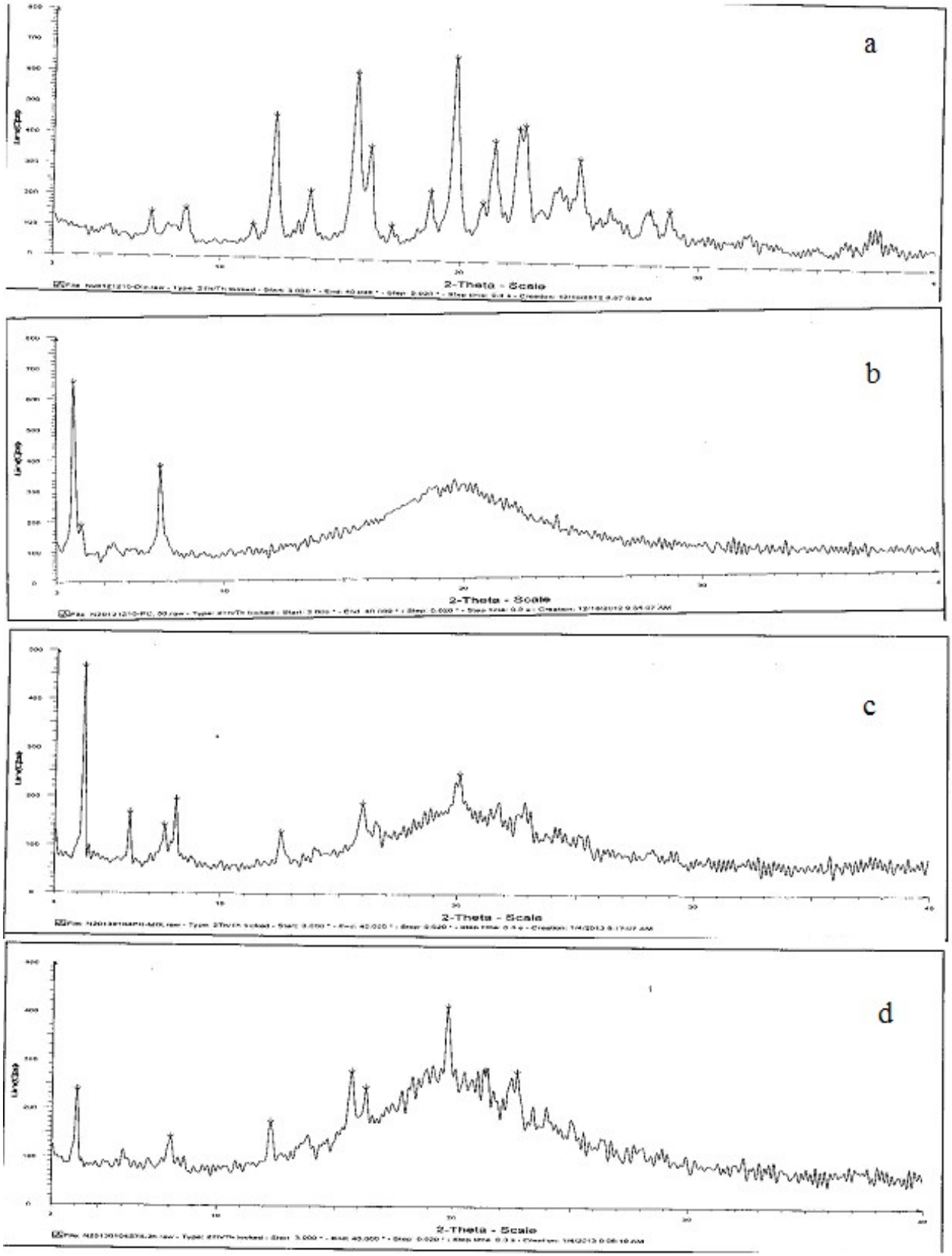

Fig. 2: X-Ray Powder Diffraction (XRPD) study of (a) Diosmin (b) PC (c) Diosmin complex (d) Physical mixture

compound. Diosmin is just enrolled by phospholipids through non-covalent bonds. The content of diosmin in the complex was 71.94 % (w/w). Herbosomes showed a high percentage of drug loading that makes its delivery clinically feasible.

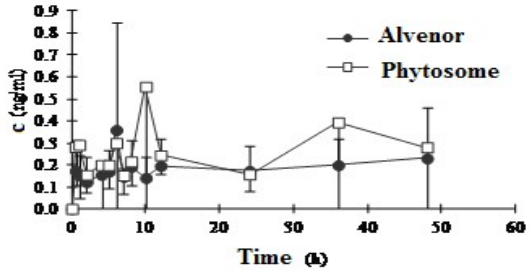

Fig. 4: Mean logarithmic plasma concentration-time curves of Phytosome (diosmin complex) and Alvenor® at a dosage 450 mg of single administration (n=8)

The solubility of the complex was found to be much higher than the pure diosmin and also Alvenor® (table 1).

In vitro dissolution study

The dissolution of the complex was better than Alvenor® (fig 3). A number of factors may contribute towards the dissolution of the solid like; wettability, particle size, crystal habit, surface energies and area. The wetting and dispersion action of the phospholipids contribute towards the higher solubalization of the drug. (Kim *et al*, 2013; Semalty *et al*, 2012). Which is why the complex exhibited a better dissolution profile.

Bioavailability study

The best linear fit and least-squares residuals for the calibration curves over the concentration range of 0.1 to 15ng.ml^{-1}were achieved with a weighing factor of 1/x, giving a typical equation of the calibration curves as R=0.9247 C+0.1145 (r=0.999), where C is the concentration of diosmin and R is the peak area ratio of diosmin to Internal Standard (IS). The low, middle and high concentration results were 111.34, 95.59 and 116.52%, respectively, that were achieved by the method of recoveries. The main pharmacokinetic parameters calculated by the non-compartment model are shown in table 2 and the mean plasma concentration-time curves are as shown in fig 4. By examining the abovementioned profile, it is noted that Cmax depicted an average value of 0.71ng.ml-1 when the complex was orally administered when Tmax was 4.5 min. however, in case of Alvenor® the values of Cmax and Tmax averaged at 0.47ng.ml-1 and 13.3min respectively, after its oral administration. All these parameter values were recorded by using Bapp procedure (Bioavailability Program Package, Nanjing, PR China).

DISCUSSION

The present study was undertaken to study the formulate a Diosmin-phospholipds preparation and study the pharmacokinetics of this complex in the Begal dogs and in vitro dissolution behavior. The results were compared with Alvenor®. In this study, the endothermic peak observed at 275.4°C by differential scanning calorimetry is attributed to the melting point of diosmin, however, Freag *et al*, (2013) recorded this endothermic peak at 291.5°C. The degree of purity of the diosmin seems to be reason behind the variability in the melting points. Pure phospholipids exhibited endothermic peaks at 43.4°C, 175.8°C, 215.4°C and 242.0°C. The first mild peak is probably due to the slightly hot raise in temperature that lead to the vigorous movements of polar heads of PC. Similar mild peak was also reported by Semalty *et al* (2012). The second and third peaks represent the melting of PC in two phases during which transition of phase occurred from gel to liquid crystalline. Gupta and Dixit (2010) recorded two peaks at 113.29°C and 185.37°C of phosphatidyl choline. Freag *et al* (2013) observed a single peak at 165.4°C. The difference of this may be attributed to different in type of phospholipids used in both studies. Further, it was found that the physical mixtures reflected endothermic peaks at 125.1°C and 183.3°C and an exothermic peak at 240.7°C. There is also a difference with the experiments of Freag *et al* (2013) that may be attributed to the percentage purity of diosmin, type of PC and their resulting complex.

The changes in the thermograms of diosmin-PC complex are attributed to some interactions like hydrogen bonding or van der Waals forces or combination of both may exist between diosmin and phospholipids as reported by Xu *et al* (2009). After combining the dosmin and PC, the hydro-carbon chain of phospholipid could rotate freely and envelops the polarity parts of phospholipid molecules that made the sequence, reduced between aliphatic hydroge-carbon chis of PC, drew the second endothermic peak of PC to remove and reduced the temperature required for transition of phase. Maiti *et al* (2007) reported that drug dissolution occurs in the molten phospholipids and leads to formation of complexes to some extent. The dissolution increases with the increase in temperature. This explains the phenomenon of disappearance of individual peaks and appearance of new ones. Our DSC findings are well supported by the Maiti *et al* (2007); Li *et al* (2007) and Semalty *et al* (2010[a]).

The XRPD of diosmin-PC complex revealed peaks similar to PC indicating that the diosmin in phospholipids complex was either molecularly dispersed or in amorphous form (Semalty *et al*, 2012). The formation of the complex is confirmed from the disappearance of the diffractional peaks of diosmin. The few remaining peaks were due to free diosmin. Our findings supports the

previous reports by Cui *et al*, (2006); Semalty *et al*, (2009[a]; 2010[b]; 2012); Shi *et al*, (2006) and Yoo and Park (2003).

Regarding the solubility, recording of vesicle formation in the media and amorphous nature of the complex explains the phenomenon of increased solubilization. The wetting and dispersion action of the phospholipids and their amphiphilic surfactant characteristics, the solubility of the drug could be increased. Gupta and Dixit (2011) explained this phenomenon indicating the amorphous nature of the complex.

From the bioavailability results, it is quite evident that phospholipids do not metabolize as fast as Alvenor® does. Alvenor® does not stay as long as the complex. This is why complex shows a notably higher bioavailability. These data show that a herbosome complex of PC and diosmin markedly enhances bioavailability in dogs. This is because of increased membrane permeability of the complex as membrane is a bilayered lipid moiety which facilitates the lipid particles across it and hence better biological effects (Semalty *et al.*, 2009[b] and 2010[c]).

CONCLUSION

In the present study, diosmin herbosome (1:2 molar ratio) was successfully prepared using a simple solvent evaporation method. DSC and XRD curves showed that drug and phospholipids combined and formed a complex with higher solubility and better dissolution profile compared to the physical mixture, pure diosmin and also Alvenor®. Studies on beagle dogs showed an increase of Cmax with sustained release and longer time of elimination. The complexation of diosmin with soy bean phospholipids increased its solubility, thus has remarkably enhanced its bioavailability.

REFERENCES

Amit P, Tanwar YS and Rakesh S (2013). Phytosome: Phytolipid Drug Delivery System for Improving Bioavailability of Herbal Drug. *J. Pharm. Sci. Biosci. Res*., **3**(2): 51-57.

Bedada SK and Boga PK (2017). Influence of diosmin on the metabolism and disposition of carbamazepine in healthy subjects. *Xenobiotica*., **47**(10): 879-884.

Bogucka-Kocka A, Wozniak M, Feldo M, Kocki J and Szewczyk K (2013). Diosmin-isolation techniques, determination in plant material and pharmaceutical formulations, and clinical use. *Nat. Prod. Commun.,* **8**(4): 545-550.

Cui F, Shi K, Zhang L, Tao A and Kawashima Y (2006). Biodegradable nanoparticles loaded with insulin-phospholipid complex for oral delivery: Preparation, *in vitro* characterization and *in vivo* evaluation. *J. Control. Release*, **114**(2): 242-250.

Filho LFS, Menezes PP, Santana DVS, Lima BS, Saravanan S, Almeida GKM, Filho JERM, Santos MMB, Araújo AAAS and de-Oliveira ED (2018). Effect of pulsed therapeutic ultrasound and diosmin on skeletal muscle oxidative parameters. *Ultrasound Med. Biol*., **44**(2): 359-367.

Freag MS, Yosra SRE and Ossama YA (2013). Lyophilized phytosomal nanocarriers as platforms for enhanced diosmin delivery: Optimization. *Int. J. Nanomedicine,* **8**: 2385-97.

Giannini I, Amato A, Basso L, Tricomi N, Marranci M, Pecorella G, Tafuri S, Pennisi D and Altomare DF (2015). Flavonoids mixture (diosmin, troxerutin, hesperidin) in the treatment of acute hemorrhoidal disease: a prospective, randomized, triple-blind, controlled trial. *Tech. Coloproctol.,* **19**(6): 339-345.

Gupta NK and Dixit VK (2011). Bioavailability enhancement of curcumin by complexation with phosphatidylcholine. *J. Pharm. Sci*. **100**(5): 1987-1995.

Hnatek L (2015). Therapeutic potential of micronized purified flavonoid fraction (MPFF) of diosmin and hesperidin in treatment of chronic venous disorder. *Vnitr Lek*., **61**(9): 807-814.

Imam F, Al-Harbi N, Al-Harbi M, Ansari M, Zoheir K, Iqbal M, Anwer M, Al-Hoshani A, Attia S and Ahmad S (2015). Diosmin downregulates the expression of T cell receptors, pro-inflammatory cytokines and NF-κB activation against LPS-induced acute lung injury in mice. *Pharmacol. Res.*, **102**(6): 111.

Jain D, Bansal MK, Dalvi R, Upganlawar A and Somani R (2014). Protective effect of diosmin against diabetic neuropathy in experimental rats. *J. Integr. Med*., **12(1)**: 35-41.

Kim H, Kim Y and Lee J (2013). Liposomal formulations for enhanced lymphatic drug delivery. *Asian Journal of Pharmaceutical Sciences,* 8(2): 96-103.

Li Y, Yang DJ, Chen SL, Chen SB and Chan ASC (2007). Comparative physicochemical characterization of phospholipids complex of puerarin formulated by conventional and supercritical methods. *Pharm. Res*., **25**(3): 563-577.

Lewinska A, Siwak J, Rzeszutek I and Wnuk M (2015). Diosmin induces genotoxicity and apoptosis in DU145 prostate cancer cell line. *Toxicol. Vitr*. **29**(3): 417-425.

Maiti K, Mukherjee K, Gantait A, Saha BP and Mukherjee PK (2007). Curcuminphospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. *Int. J. Pharm*., **330**(1-2): 155-163.

Maryana W, Rachmawati H and Mudhakir D (2016). Formation of phytosome containing silymarin using thin layer-hydration technique aimed for oral delivery. *Materials Today Proceedings,* **3**(3): 855-866.

Mishra N, Yadav NP, Meher JG and Sinha P (2012). Phyto-vesicles: Conduit between conventional and novel drug delivery system. *Asian Pac. J. Trop. Biomed*., **2**(3): 1727-1734.

Naso L, Martnez VR, Lezama L, Salado C, Valcarcel M, Ferrer EG and Williams PAM (2016). Antioxidant, anticancer activities and mechanistic studies of the flavone glycoside diosmin and its oxidovanadium (IV) complex. Interactions with bovine serum albumin. *Bioorganic. Med. Chem*., **24**(18): 4108-4119.

Queenthy SS and John B (2013). Diosmin exhibits anti-hyperlipidemic effects in isoproterenol induced myocardial infarcted rats. *Eur. J. Pharmacol*., **718**(1-3): 213-218.

Queenthy SS, Prince PSM and John B (2018). Diosmin prevents Isoproterenol-induced heart *mitochondrial* oxidative stress in rats. *Cardiovasc. Toxicol*., **18**(2): 120-130.

Semalty A, Semalty M, Singh D and Rawat MSM (2009[a]). Development and physicochemical evaluation of pharmacosomes of diclofenac. *Acta. Pharma*., **59**(3): 335-344.

Semalty A, Semalty M, Rawat BS, Singh D and Rawat MSM (2009[b]). Pharmacosomes: The lipid based novel drug delivery system. *Expert. Opin. Drug Deliv*. **6**(6): 599-612.

Semalty A, Semalty M, Singh D and Rawat MSM (2010[a]). Preparation and characterization of phospholipid complexes of naringenin for effective drug delivery. *J. Incl. Phenom.* **67**(3): 253-260.

Semalty A, Semalty M, Rawat BS, Singh D and Rawat MSM (2010[b]). Development and evaluation of pharmacosomes of aceclofenac. *Indian J. Pharm. Sci*. **72**(5): 571-575.

Semalty A, Semalty M, Rawat MSM and Federico F (2010[c]). Supramolecular phospholipids-polyphenolics interactions: The phytosome strategy to improve the bioavailability of phytochemicals. *Fitoterapia*, **81**(5): 306-314.

Semalty A, Semalty M, Singh D and Rawat MSM, (2012). Phyto-phospholipid complex of catechin in value added herbal drug delivery. *J. Incl. Phenom. Macrocycl. Chem*. **73**(1-4): 377-386.

Senthamizhselvan O, Manivannan J, Silambarasan T and Raja B (2014). Diosmin pretreatment improves cardiac function and suppresses oxidative stress in rat heart after ischemia/reperfusion. *Eur. J. Pharmacol*. **736**: 131-137.

Shi K, Cui F, Yu Y, Zhang L, Tao A and Cun D (2006). Preparation and characterization of a novel insulin phospholipid complex. *Asian J. Pharm. Sci*., **114**(2): 168-174.

Silvestro L, Tarcomnicu I, Dulea C, Attili NRBN, Ciuca, V, Peru D and Rizea SS (2013). Confirmation of diosmetin 3-O-glucuronide as a major metabolite of diosmin in humans, using micro-liquid-chromatography-mass spectrometry and ion mobility mass spectrometry. *Anal. Bioanal. Chem*. **405**(25): 8295-8310.

Tsai MJ, Huang YB, Fang JW, Fu YS, Wu PC (2015) Preparation and Characterization of Naringenin-Loaded Elastic Liposomes for Topical Application. PLoS ONE 10(7): e0131026. doi:10.1371/journal. pone.0131026

Walle T (2004). Absorption and metabolism of flavonoids. *Free Radic. Biol. Med*., **36**(7): 829-837.

Xu K, Liu B, Ma Y, Du J, Li G, Gao H, Zhang Y and Ning Z (2009). Physicochemical properties and antioxidant activities of luteolin-phospholipid complex. *Molecules*. **14**(9): 3486-3493.

Yoo HS and Park TG (2003). Biodegradable nanoparticles containing protein-fatty acid complex for oral delivery of salmon calcitonin. *J. Pharm. Sci*. **93**(2): 488-495.

Zhang J, Tang, Q, Xu X and Li N (2013). Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery. *Int. J. Pharm*. **448**(1): 168-174.