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DESIGN AND CHARACTERIZATION OF DIOSMIN-CYCLODEXTRIN COMPLEX AS A NOVEL TRANSDERMAL GEL

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ABSTRACT

Topical application of flavonoid has attracted interest due to their high vasoprotective and anti-inflammatory activities. The unfavorable physicochemical properties of diosmin (DSN) restrict its topical application. The impact of DSN/Cyclodextrins (CDs) complex formation on the improvement of the dissolution and permeation behavior of DSN has not so far been investigated. This study investigated the potential of DSN/HP-β-CD complex formation to enhance topical delivery and improve skin penetration of DSN. Physicochemical studies (Differential scanning colorimetric (DSC), infrared spectroscopy (IR), and x-ray diffractrometry) indicate successful complex formation at 1:2 molar ratios (MR) of DSN:DSN/HP-β-CD. Dissolution study indicates significant improvement of the dissolution rate of DSN from the of DSN:DSN/HP-β-CD complex compare to physical mixture (PM) or pure DSN. The highest release rates were found with gel containing inclusion complex compared to the gels containing pure DSN and its PM. Clinical study indicates pronounced effects of gel containing DSN/ HP-β-CD complex on the paw edema compared to pure DSN or PM. In conclusion, transdermal gel containing DSN/ HP-β-CD complex could be used as a new topical delivery system to improve DSN dissolution and permeation.

KEY WORDS: **Transdermal gel, Cyclodextrins, Diosmin, topical formulations, solubility and permeability**

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INTRODUCTION

Diosmin (DSN) is a phytochemical, mostly found in citrus fruits[3]. DSN is widely used as a natural supplement for the treatment of hemorrhoids, lymphedema, chronic venous insufficiency, and varicose veins, on account of their vasoprotective activity[4-6]. However, a possible percutaneous administration would be interesting, the permeability of DSN considered the most problem for its skin penetration due to its unfavorable physicochemical properties[7]. There is a strong link between permeability, solubility and fraction of drug absorbed in humans[8], only solubilized drug molecules can be absorbed by the cellular membranes to subsequently reach the site of drug action. Nevertheless, impact of enhancing dissolution and permeation characteristics of DSN has not so far been investigated. The cyclodextrins (CDs) have a wide range of pharmaceutical applications to improve the dissolution rate, bioavailability and stability of drug molecules [9].The novel applications of CDs are expected to solve the problems associated with the delivery of many novel drugs through different delivery routes. The pharmaceutical applications of CDs in different areas may be attributed to their ability to affect drug absorption; indirectly, by improvement in the drug physicochemical properties, and/or directly, by influencing the biomembrane permeability [10-12]. On account of the potential use of HP-β-CD as suitable drug carriers in drug delivery systems with emphasis on the recent developments as penetration enhancer. The study aimed to enhance the dissolution and percutaneous absorption of DSN through novel formulation of gel containing DSN/HP-β-CD. The prepared DSN/HP-β-CD gel formulations were intended to be applied in vivo in an animal model.

MATERIALS AND METHODS

Material

Diosmin (DSN) was kindly gifted from Sedico Pharmaceutical Company (Cairo, Egypt). Hydroxypropyl- β-Cyclodextrins(HP-β-CD) and pluroinc acid F-127 (PF-127) were purchased from Sigma (Sigma-Aldrich Inc., St. Louis, Missouri, USA).Semi-permeable dialysis cellulose membrane(avg. flat width 33 mm (1.3 inch.),Sigma Aldrich chemical co., (USA).

Methods

. Preparation of the Physical Mixture (PM)

Accurately weighed amount of DSN and HP-β-CD (1:1 and 1:2)molar ratio (MR)were passed through sieve and then thoroughly blended for 15 min.

Preparation of Solid Complex

Based on our preliminary work, it has been found that the drug content of the prepared complex indicate that the ratio of DSN to HP-β-CD was 1:2 MR. Therefore, the prepared binary systems of DSN and HP-β-CD were at 1:2 MR but 1:1 MR was used for comparison only in the dissolution and diffusion studies. The neutralization method was used for the preparation of DSN/HP-β-CD solid complex. DSN was precisely weighed and added to 25 ml of 1 N sodium hydroxide solution, into this solution HP-β-CD was added (1:1 and 1:2 MR). The mixture was stirred with a magnetic stirrer for 1 hour at room temperature until a transparent solution was obtained. 25 ml of 1 N hydrochloric acid was dripped into the solution and agitated for 3 hours. The formed precipitate was separated by filtration, then dried at room temperature [13-14].

Preparation of DSN and its Loaded HP-β-CD System Transdermal gel

Transdermal gel formulations were prepared according to cold method(15). Solution of the thermo sensitive polymer PF127 (20 % w/v) was prepared by addition of the polymer to distilled water with stirring overnight at 4°C to obtain aclear solution.DSN, DSN/HP-β-CD PM and DSN/HP-β-CD complex-loaded gel formulations were prepared by their dispersion or dissolution in the cold PF127 solution with gentle mixing. Each formulation contained DSN(5 mg/ml).

Physico-chemical Characterization

X-ray Powder Diffraction (XRD)

Diffractometer (UNISANTIS, GERMANY) was used for measurements of the studied samples. The operating conditions were: voltage 45 kV; current 0.8 mA; scanning speed 1/min. The results were recorded over a range of 5–60° (2θ) using the Cu-target X-ray tube and Xe-filled detector.

Differential Scanning Calorimetry (DSC) Analysis

DSC- Shimadzu 60 with TDA trend line software was used to study the powdered samples of DSN; HP-β-CD; theirbinary systems. 8-10 mg samples were heated at a scanning rate of 10°C/min under dry air flow (100 ml/min) between 50°C and 300°C.

FT-IR Spectroscopy

FTIR spectroscopy (Perkin Elmer, NY, USA) was carried out using KBrdsc method. Samples, 1-2 mg (pure DSN, pure HP-β-CD and DSN/HP-β-CD binary systems) were mixed separately with KBr. Using 8400S model instrument the powder blends were scanned over a wave number range of 500 to 4000 cm^{-1}.

In Vitro Dissolution Study

Using USP XXVI rotating Paddle method, the dissolution studies of the prepared binary systems of DSN with HP-β-CD were carried out and compared with pure DSN powder. The dissolution media 500ml of orthophosphate buffer pH 12 or phosphate buffer pH 7.4was continuously stirred at 100 rpm at 37± 0.5°C. Samples each contains 50 mg DSN were added to the stirred dissolution media. The samples (5 ml) were withdrawn at time intervals (0.25, 0.5, 0.75, 1, 3, 4 and 6hr)and analyzed spectrophotometrically at 346 nm, the dissolution media were compensated with 5 ml of plain dissolution medium. Experiments were run in triplicates.

In Vitro Diffusion Study

The amount released of DSN from HP-β-CD complex embedded in thermo sensitive gel was estimated and compared with free DSN and it's PM with HP-β-CD. Accurately weighted amount of the prepared gel containing 5 mg of DSN or its equivalent weight of the PM and the inclusion complex were putted in two open

ended tube (1 cm in diameter) with cellophane membrane fixed on one end. The dialysis membrane was kept in the release medium overnight before dialysis to ensure wetting of the membrane. The tube was immersed in the release media to allow the membrane to be in touch with the surface. The receptor compartment was filled with 50 mloforthophosphate buffer pH 12or phosphate buffer pH 7.4. The receptor solution was maintained at 37±0.1°C and 100 rpm for 6hr in a thermostatic shaker water bath before beginning the experiment. Aliquots (3 ml) were withdrawn at regular interval of 1 h for a period of 8 h and replaced with equal volume of fresh medium equilibrated at 37±0.1°C. All the samples were analyzed spectrophotometrically at 346 nm. Experiments were run in triplicates([16]

InVivo Study

The formulated transdermal gels of DSN were topically investigated for anti-inflammatory effect based on carrageenan induced rat paw edema in rats [1]. Animals were allowed to free access to feed and water before the experiment. Nine male albino rats each 200 g of approximately the same age were assigned into three groups, each group containing three rats. Each group topically treated with 0.5 g gel containing 2.5mg DSN. The drug was in the form of; pure DSN, DSN/HP-β-CD PM and inclusion complex (1:2 MR) for first, second and third groups respectively. On the surface of the right hind paw the gels were applied, then immediately covered by thin vinyl sheet and gauze. After 1 h, the covers were removed and 0.1 ml freshly prepared saline suspension containing 1% carrageenan was subcutaneously injected into both the right (treated) and the left (untreated) hind paw using insulin disposable syringe. After carrageenan injection right and left paw thickness was measured; immediately, after 1h, 2h, 3h and 4h using a caliper. The paw thickness was recorded at different time points. The percentage inhibition in paw edema was calculated for each group [2]. Animals were treated through the standard regulations and guidelines of Minia University for the care and use of Laboratory Animals. *The statistical analysis of data was carried out using ANOVA-test at a level of significance of $p < 0.05$.*

RESULTS AND DISCUSSION

X-ray Diffractograms

The X-ray diffraction patterns of DSN, HP-β-CD and their binary systems are shown in Figure 1. DSN diffractograms showed a high intensity peaks at 12, 16, 20, 22, 24 and 25 2θ indicating the crystallinity nature of the drug. Conversely, HP-β-CD diffractograms indicate its amorphousness. The main peaks of DSN diffractograms appear nearly at the same 2θ of DSN/HP-β-CD PM with less intensity due to the dilution by 1:2 M ratio. On the other hand, a complete disappearance of the characteristics peaks of DSN from the diffractograms of DSN/HP-β-CD coprecipitate indicating change in the crystallinity state of the drug from the crystalline form to the amorphous form upon inclusion withHP-β-CD cavity.

DSC Thermograms

DSN, HP-β-CD and DSN/HP-β-CD blends thermograms are presented in Figure(2). DSN thermogram exhibited sharp endothermic peak at 285◦C corresponding to its melting point. HP-β-CD thermogram showed broad endothermic peaks at 70 to 120◦C, corresponding to evaporation of water molecules from CDs cavity. The thermogram of DSN/HP-β-CD PM showed no shift for the peaks of both DSN and HP-β-CD. On the other hand, the thermogram of DSN/HP-β-CD coprecipitate showed a complete disappearance of the melting peak of DSN. This finding may be due to the possibility of complete inclusion of DSN into the DSN/HP-β-CD cavity and formation of DSN/HP-β-CD true complex [17]

FTIR Spectroscopy

FTIR spectra were carried out for further investigation of complex formation and the results are presented in Figure(3). The spectra of the function groups of the DSN and DSN/HP-β-CD showed overlapping. Therefore, the fingers print bands of DSN in the region of 400–1300 cm^{-1}were used as indicator in this study. The results showed that the same finger print bands of DSN were observed with its PM with HP-β-CD but with less intensity. The relative decrease in their intensities may be due to the mixing of DSN and HP-β-CD in 1:2 MR. On the other hand, the finger print bands of DSN in the region of 400–1300 cm^{-1} were clearly disappeared from their complex spectra probably due to the restriction of bending and stretching vibrations of DSN due to the CDs cavity[18].

In Vitro Dissolution Study

The poor solubility of DSN makes its dissolution test a very difficult task. Garner, R.C., et al[19] reported the presence suitable amount of sodium hydroxide in the dissolution medium was necessary to perform a suitable dissolution profile of DSN andachieve sink conditions. Sodium orthophosphate buffer (pH 12) was the only buffer system that was reported for DSN dis-solution medium due to the highest solubilization capacity of DSN [16].Therefore, it was selected as the dissolution medium achieving sink conditions in our study. On the other hand, the suitability of non-sink conditions (phosphate buffer 7.4) to reflect in vivo conditions of poorly soluble drugs was reported.Ghazal, H.S., et al.[20]they performed a comparative dissolution study for poorly soluble drug (itraconazole)in non-sink media. So, phosphate buffer pH 7.4 a non-sink dissolution medium versus orthophosphate buffer pH 12 a sink-conditions was utilized in this work to investigateDSN dissolution. The dissolution profiles of DSN and DSN/CDs binary systems powder under sink conditions and non-sink conditions are shown in Figs 4 and 5, respectively. Results showedpronounced improvement of DSN dissolution in both sink (Fig 4) and non-sink conditions (Fig 5) from all binary systems compared to crude drug. The dissolution rates for two solid complexes (1:1 and 1:2 M ratios) were greater than PM and pureDSN. The enhancement of dissolution profiles of DSN from its PM (1:2 MR) during the first 15 min followed by a slower ratein both buffer media was observed. The dissolution behavior of DSN in the PM is in agreement with the physicochemical

results. Two factors may attributed to the enhancement of DSN dissolution rate in the PM; (1) improvement of drug wettability and/or solubility due to the carrier local solubilization actions on the layer surrounding the drug particles (2) ‘‘in situ’’ formation of readily soluble complexes in the dissolution medium [21]. Figures (4 and 5), revealed the dissolution profiles for the inclusion complexes were significantly different from those for non-complexedDSN. Moreover, the dissolution of DSN was dependent on the pH of the dissolution medium and the MR of the drug to CDs. In sink condition, the percentage of dissolved DSN was 33%, while, 67% for 1:1 MR, and above 88% for 1:2 MR within 15 min. Thus, there was a 2–2.7 folds increase in the DSN solubility upon complexation. However, in non-sink conditiononly 3% of the DSN was dissolved during the same period. Thus, there were a 14 and28.3 fold increases in the percentage of DSN dissolved after 15 min upon complexation with CDs at 1:1 and 1:2 MR, respectively. The enhancement of the dissolution efficiency may attributed to; (1) carriers surfactant-like action due to the exterior surface wettability, which could decrease the interfacial tension between poorly soluble drugs and the dissolution medium; (2) formation of complex which increase the DSN solubility; and (3) theamorphous state following complexation as confirmed by XRD studies[22].We can conclude that the effect of the polymer ratio in the system was more evident on DSN dissolution behaviors. The 1:2 MR inclusion complexes showed a similar performance in dissolution studies. This may be indicated that a true complex of 1:2 MR was formed between the DSN and HP-β-CD.

In Vitro Diffusion Study

Figures (6 and 7)showed the in vitro release profiles of DSN gel formulations loaded with DSN/DSN/HP-β-CD complex, PM and pure DSN in orthophosphate and phosphate buffers, respectively. The presence of DSN or DSN/DSN/HP-β-CD complex in the gel form resulted in a slow release profile. However, the dissolution rate of pluronicgel is the controlling factor in drug release. The gel medium interface is eroding at a constant rate and concentration of the drug at the surface is remaining relatively constant. Therefore, drug diffuse out at a constant rate[23].On the other hand, the release from the DSN/CDs complex is a competitive process controlled by diffusion of the drug by the components in the dissolution media[24].These two factors may have contributed to control the release of DSN from the gel within a period of 6 h. At the same time, there is no difference in the release profiles of DSN/DSN/HP-β-CD complex under sink and non-sink conditions in case of 1:2 MR. These results indicated that a true complex was formed between the DSN and DSN/HP-β-CD.

In Vivo Study

Figure8showed the inhibitory effect of DSN, its PM and complex with CD loaded gels on the carrageenan-induced paw edema after 4 h of rat paws treated topically. It clear that the inhibitory effect was in the order; complex > DSN > PM. Moreover, the developed gel containing DSN/HP-β-CD complex loaded pluronic gel showed a significant ($p < 0.05$) inhibition of rat paw edema after 4 h of application, the percentage of inhibitionwas51.0%, 42.0% and 88.5%for DSN, PM and inclusion complex respectively (Figure 8and Table 1). This has indicated an in vivo anti-inflammatory activity enhancement of the drug in its complex form. The inhibitory effects of DSN/HP-β-CD complex gel on the induced paw edema may be attributed to its superior skin permeation. There are many possible mechanisms that could explain the ability of the formulation to modulate the transfer of DSN across skin: (1) The CDs increases the total amount of dissolved drug molecules on the surface of the skin and this increases the concentration of the drug over the aqueous diffusion layer leadings to rapid drug delivery to the membrane surface. The DSN release from the HP-β-CD complex is more rapid than the release of DSN molecules through the aqueous diffusion layer; (2) CDs will disrupt the hydrogen-bonded water network in the aqueous diffusion layer. This could facilitate penetration of DSN/HP-β-CD complexes through the aqueous diffusion layer and improve drug availability at the membrane surface. (3) complexation of drug molecules with CDs could prevent them from interacting with molecules in the aqueous diffusion layer, increasing their overall delivery rate to the membrane surface[25].(4) CDs are able to interact with some lipophilic components of the skin and cornea[26]. Reported pure aqueous buffer solutions of HP-β-CD have been shown to be able to extract lipids from the stratum corneum. Other possible mechanisms of CDs action have not yet been elucidated.

Figure (1)
X-ray patterns of (A) DSN, (B) HP-β-CDs, (C) DSN/HP-β-CDs PM and (D) DSN/HP-β-CD complex.

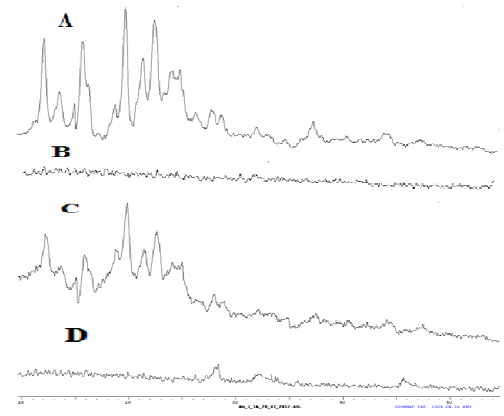

Figure (2)
DSC thermograms of (A) DSN, (B) HP-β-CDs, (C) DSN/HP-β-CDs PM and (D) DSN/HP-β-CD complex

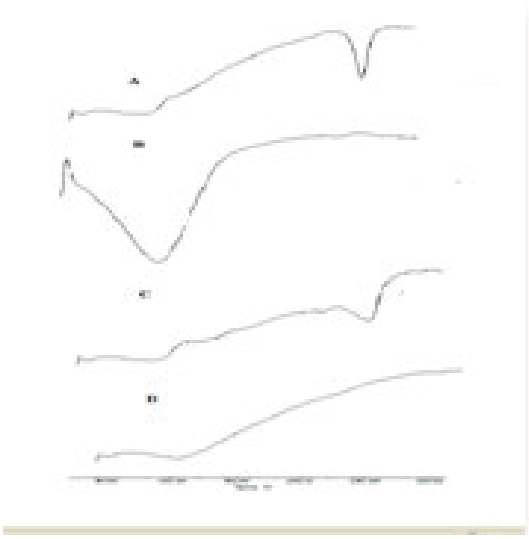

Figure (3)
FT-IR spectra of (A) DSN, (B) HP-β-CDs, (C) DSN/HP-β-CDs PM and (D) DSN/HP-β-CD complex

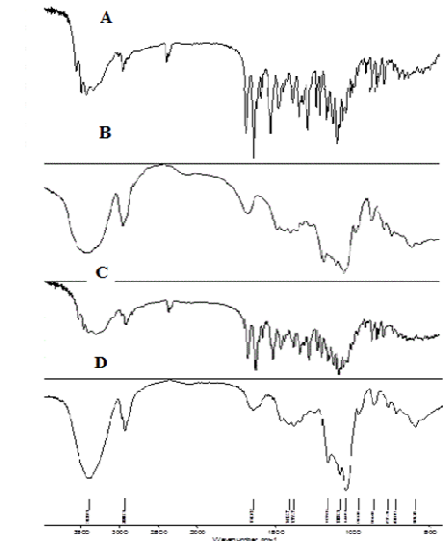

Figure (4)
Cumulative percentage of drug dissolved in orthophosphate buffer pH 12

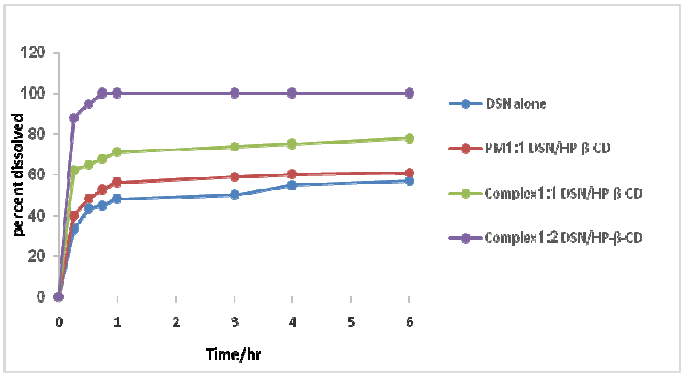

Figure (5)
Cumulative percentage of drugdissolved in phosphate buffer pH 7.4.

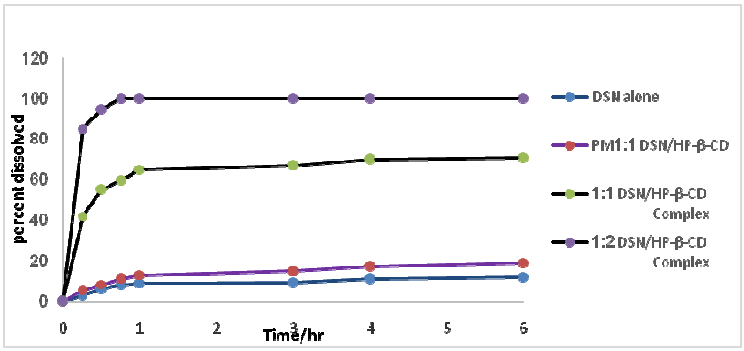

Figure (6)
Cumulative percentage of drug released from the formulated gels in orthophosphate buffer pH 12.

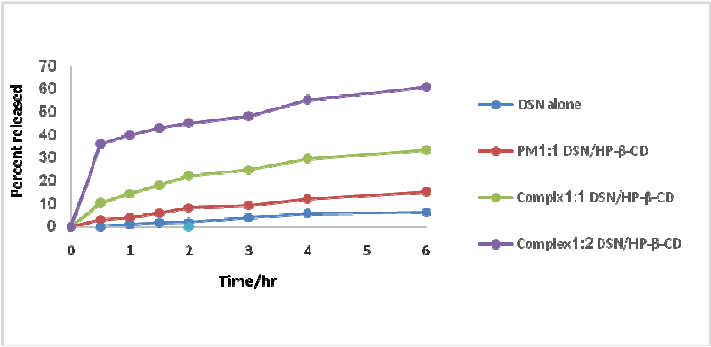

Figure (7)
Cumulative percentage of drug releasedfrom the formulated gels in phosphate buffer pH 7.4.

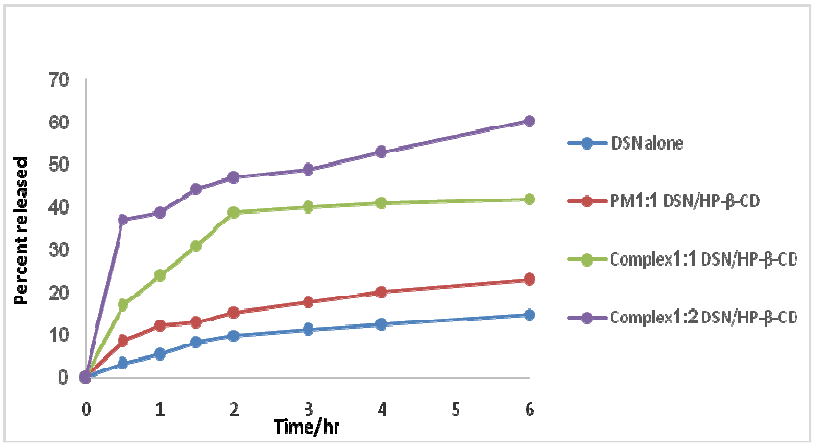

Figure (8)
Inhibitory effect of DSN, PM and complex with HP-β-CD on the carrageenan-induced paw edema.After 4 h of topically treated.

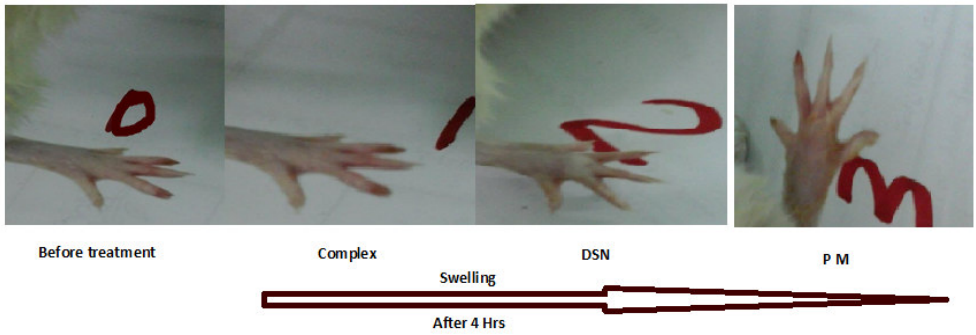

Table (1)
Effect of topical application of DSN transdermal gels on paw edema inhibition

Formulation	1 h		2 h		3 h		4 h	
	TE (mm)	PI %	TE (mm)	PI %	TE (mm)	PI %	TE (mm)	PI %
DSN	5.89 (0.01)*	5.50	5.52 (0.02)	24.0	5.33 (0.03)	33.5	4.98 (0.06)	51.0
DSN/CD PM (1:2 MR)	5.99 (0.01)	0.50	5.61 (0.04)	19.5	5.49 (0.02)	25.5	5.16 (0.03)	42.0
DSN/CD Complex (1:2 MR)	5.42 (0.02)	29.0	5.03 (0.04)	48.5	4.70 (0.03)	65.0	4.23 (0.03)	88.5

Values are mean ± SD (n = 3). TE: edema thickness; PI: percent inhibition

CONCLUSION

Complexion of DSN with HP-β-CD was a successful process for enhancing the solubility, dissolution rates and penetration of the DSN. The extent of dissolution enhancement was dependent mainly on the molar ratio of DSN to DSN/HP-β-CD indicating a true complex was formed at 1:2 MR. Moreover, formulation of DSN/HP-β-CD complex loaded transdermalgel is considered a new successful topical formulation of DSN. In future, the study will needs further test on humans for a clinical applications.

REFERENCES

1. Halici Z, Dengiz GO, Odabasoglu F, Suleyman H, Cadirci E, Halici M. Amiodarone has anti-inflammatory and anti-oxidative properties: an experimental study in rats with carrageenan-induced paw edema. Eur J Pharmacol. 2007;566(1):215-21.
2. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 guide for the care and use of laboratory animals. ILAR journal. 1997;38(1):41-8.
3. Frydman A, Weisshaus O, Bar-Peled M, Huhman DV, Sumner LW, Marin FR, et al. Citrus fruit bitter flavors: isolation and functional characterization of the gene Cm1,2RhaT encoding a 1,2 rhamnosyltransferase, a key enzyme in the biosynthesis of the bitter flavonoids of citrus. The Plant journal : for cell and molecular biology. 2004;40(1):88-100.
4. Labrid C. A lymphatic function of Daflon 500 mg. International angiology : a journal of the International Union of Angiology. 1995;14(3 Suppl 1):36-8.
5. Manthey JA. Biological properties of flavonoids pertaining to inflammation. Microcirculation (New York, NY : 1994). 2000;7(6 Pt 2):S29-34.
6. Smith PC. Daflon 500 mg and venous leg ulcer: new results from a meta-analysis. Angiology. 2005;56 Suppl 1:S33-9.
7. Majumdar S, Srirangam R. Solubility, Stability, Physicochemical Characteristics and In Vitro Ocular Tissue Permeability of Hesperidin: a Natural Bioflavonoid. Pharm Res. 2009;26(5):1217-25
8. Pade V, Stavchansky S. Link between drug absorption solubility and permeability measurements in Caco-2 cells. Journal of pharmaceutical sciences. 1998;87(12):1604-7.
9. Tiwari G, Tiwari R, Rai AK. Cyclodextrins in delivery systems: Applications. Journal of Pharmacy and Bioallied Sciences. 2010;2(2):72-9.
10. Chouhan P, Saini TR. Hydroxypropyl-β-cyclodextrin: A Novel Transungual Permeation Enhancer for Development of Topical Drug Delivery System for Onychomycosis. J Drug Del. 2014;2014:7.
11. Yan Y, Xing J, Xu W, Zhao G, Dong K, Zhang L, et al. Hydroxypropyl-beta-cyclodextrin grafted polyethyleneimine used as transdermal penetration enhancer of diclofenac sodium. Int J Pharm. 2014;474(1-2):182-92.

12. Loftsson T. Drug permeation through biomembranes: cyclodextrins and the unstirred water layer. Die Pharmazie. 2012;67(5):363-70.
13. Jantarat C, Sirathanarun P, Ratanapongsai S, Watcharakan P, Sunyapong S, Wadu A. Curcumin-Hydroxypropyl-β-Cyclodextrin Inclusion Complex Preparation Methods: Effect of Common Solvent Evaporation, Freeze Drying, and pH Shift on Solubility and Stability of Curcumin. Trop J Pharm Res. 2014;13(8):1215-23.
14. Lo Y, Hsu C, Tsai T, Cham T. Comparison of the Solubility and Dissolution Rate of Gliclazide-beta-cyclodextrin Inclusion Complexes Prepared by Liquid/liquid Extraction and Neutralization. Journal of Food and Drug Analysis. 2006;14(3):230.
15. Kim Y-T, Shin B-K, Garripelli VK, Kim J-K, Davaa E, Jo S, et al. A thermosensitive vaginal gel formulation with HPγCD for the pH-dependent release and solubilization of amphotericin B. Eur J Pharm Sci. 2010;41(2):399-406.
16. Freag MS, Elnaggar YSR, Abdallah OY. Lyophilized phytosomal nanocarriers as platforms for enhanced diosmin delivery: optimization and ex vivo permeation. International Journal of Nanomedicine. 2013;8:2385-97.
17. Giordano F, Novak C, Moyano JR. Thermal analysis of cyclodextrins and their inclusion compounds. Thermochimica Acta. 2001;380(2):123-51.
18. Anwer MK, Jamil S, Ansari MJ, Al-Shdefat R, Ali BE, Ganaie MA, et al. Water soluble binary and ternary complexes of diosmin with β-cyclodextrin: Spectroscopic characterization, release studies and anti-oxidant activity. J Mol Liq. 2014;199(0):35-41..
19. Garner RC, Garner JV, Gregory S, Whattam M, Calam A, Leong D. Comparison of the absorption of micronized (Daflon 500® mg) and nonmicronized 14C-diosmin tablets after oral administration to healthy volunteers by accelerator mass spectrometry and liquid scintillation counting. J Pharm Sci.. 2002;91(1):32-40.
20. Ghazal HS, Dyas AM, Ford JL, Hutcheon GA. In vitro evaluation of the dissolution behaviour of itraconazole in bio-relevant media. Int J Pharm.. 2009;366(1-2):117-23.
21. Williams HD, Trevaskis NL, Charman SA, Shanker RM, Charman WN, Pouton CW, et al. Strategies to Address Low Drug Solubility in Discovery and Development. Pharmacological Reviews. 2013;65(1):315-499..
22. Hussein AK, Ibrahim MA, Amin MA, Ahmed OAA, Afouna MI. Improved in vitro dissolution parameters and in vivo hypolipidemic efficiency of atorvastatin calcium through the formation of hydrophilic inclusion complex with cyclodextrins. Drug Development Research. 2011;72(5):379-90.
23. Anderson BC, Pandit NK, Mallapragada SK. Understanding drug release from poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) gels. J controlled rel. 2001;70(1):157-67..
24. Bilensoy E, Çırpanlı Y, Şen M, Doğan AL, Çalış S. Thermosensitive mucoadhesive gel formulation loaded with 5-Fu: cyclodextrin complex for HPV-induced cervical cancer. J Incl Phenom Macrocycl Chem. 2007;57(1-4):363-70.
25. Loftsson T, Vogensen SB, Brewster ME, Konráðsdóttir F. Effects of cyclodextrins on drug delivery through biological membranes. Journal of pharmaceutical sciences. 2007;96(10):2532-46.
26. Legendre JY, Rault I, Petit A, Luijten W, Demuynck I, Horvath S, et al. Effects of β-cyclodextrins on skin: implications for the transdermal delivery of piribedil and a novel cognition enhancing-drug, S-9977. Eur J Pharm Sci. 1995;3(6):311-22.