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Topical delivery of doxepin using liposome containing cream: An emerging approach in enhancing skin retention

Ainaz Didevar Asl[1], Shahab Bohlooli[2], Masoomeh Dadkhah[3] and Leila Rezaie Shirmard[4]*

[1]School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran
[2]Department of Pharmacology, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran
[3]Pharmaceutical Sciences Research Centre, Ardabil University of Medical Sciences, Ardabil, Iran
[4]Department of Pharmaceutics, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran

Abstract: Conventional formulation of topical doxepin has similar antihistaminic effects as oral doxepin; however, its efficacy is limited due to poor localized effects on the skin. This study was designed to compare the *ex vivo* permeation and retention of two topical doxepin formulations; liposomal cream and plain cream. Methods: Doxepin-containing liposomes were prepared with the thin-film hydration method and assessed for size, size distribution, morphology, entrapment efficiency (EE%) and stability Using rat skin specimens in a Franz diffusion cell. Doxepin concentration in skin and receptor fluid was quantified by a validated HPLC method. The optimized liposomal formulation represented a uniform shape with narrow size distribution and an average diameter of 208.7±5.6nm. EE% of doxepin was 79±1.3 and the liposomes were stable at least for six weeks at 4°C. *Ex vivo* studies showed that while a significantly higher amount of doxepin has passed through the skin and entered the receptor compartment from conventional dosage form (47.06±2.5µg/cm2vs 11.20±0.6µg/cm2 for liposomal formulation), liposomal doxepin favoured accumulation in dermis and epidermis. These results suggest that the liposomal doxepin cream is an effective and easy-to-use formulation and may improve the cutaneous retention of doxepin, thus decreasing its systemic side effects.

Keywords: Doxepin, liposome, *ex vivo* study, atopic dermatitis.

INTRODUCTION

Atopic dermatitis (AD) is a chronic disease characterized by red, itchy and inflamed skin (Eyerich *et al*., 2019). It is a type of endogenous eczema that can be associated with other diseases like allergic rhinitis, food allergy and bronchial asthma. There are some main criterias cause AD like genetic and environment factors, epidermal barrier dysfunction, immune dysregulation and alteration of the cutaneous microflora (Patel *et al*., 2019). There are several dermatoses like nummular eczema, prurigo, pompholyx, seborrhoeic dermatitis and pityriasis alba that may be related to atopic dermatitis (Almutairi, 2019). AD is most often a constructional skin deformity associated with an abnormal immune response. Therefore, the best choice for treatment is dual management which targets both protection and healing (Darlenski *et al*., 2014). Protection can be accomplished by diminishing the dryness of the skin, mainly through the daily application of skin-hydrating agents such as mosturizer creams (Draelos *et al*., 2019; Patel *et al*., 2019) and healing is achieved by the administration of oral or topical therapeutic agents. Among many mediators of itching in AD, histamine has been the subject of much attention and has been thoroughly studied (Ständer and Luger, 2010). Histamine in the epidermis is derived from the keratinocytes and mast cells in the dermis, but it's role in the epidermis is yet to be fully discovered (Ashida *et al.*, 2001). Because AD is chronic and recurring, the patient needs long-term treatment, which may have to be continued for decades. Topical antihistaminic agents and steroids are currently used to treat atopic dermatitis; however, long term treatment with these drugs might lead to occurrence of side effects such as atrophy of the skin, secondary adrenocortical insufficiency, infection and other systemic side effects that might lead to poor patient compliance (Silverberg *et al*., 2016).

Although oral administration of doxepin as a H1 receptor antagonist can effectively relieve itching (Zabihi *et al*., 2017), many patients experience inter-individual variability in plasma level of doxepin and side effects such as drowsiness, dry mouth, tachycardia, urinary retention and blurred vision. One of the strategies to overcome these complications is the use of topical formulations to deliver doxepin directly into the skin (Sacha *et al*., 2019). Zonalon®, the topical cream of doxepin, was approved by FDA in 1994 for the treatment of pruritus in AD; still, its long-term use is not recommended since it may cause skin complications (Bonnel *et al*., 2003). Moreover, partial efficacy was observed due to limited penetration into the deeper layers of the skin. Since doxepin rapidly decreases itching, its concomitant use with corticosteroids enhances patient compliance and makes it possible to use less potent corticosteroids. Furthermore, this drug reduces the need for a long-term administration of corticosteroids and other drugs (Berberian *et al*., 1999).

**Corresponding author:* e-mail: lyla81@yahoo.com

As discussed earlier, conventional transport media such as creams and ointments cannot localize an optimal drug concentration to the skin and control the systemic exposure (De Leeuw *et al*., 2009). Thus, recent studies in dermatology have focused on new drug delivery approaches like using hydrogels, patches, nanoparticles and liposomes to improve the efficiency of formulations and the safety of treatment (Lotfipour *et al*., 2019). Liposomes are small spherical vesicles consisting of one or more phospholipid bilayers used to encapsulate a wide range of all types of therapeutic agents i.e., amphiphilic, hydrophilic and lipophilic agents and have a great potential to deliver them (Ghofrani *et al*., 2019; Patel *et al*., 2021). They are suitable for both small molecular weight and high molecular weight drugs delivery. Enhancement of stability while protecting encapsulated drugs from the external environment, is one of the considered advantages of liposomes (Witika *et al*., 2021). Generally, liposomes are biocompatible, completely biodegradable, non-toxic, flexible and non-immunogenic, thus can be considered a safe and ideal means for systemic and topical delivery of drugs (Salimi, 2018). It has been shown that liposomes can efficiently deliver active molecules to their site of action and reduce their adverse effects that may originate from systemic drugs absorption, in both *in vitro* and *in vivo* (Akbarzadeh *et al*., 2013; Ahmed *et al*., 2019). Liposomes are promising carriers for skin drug delivery due to their similarity to the endogenous skin lipids and their ability to hydrate the skin, making them useful for reducing skin burning and irritation (De Leeuw *et al*., 2009). This similarity between the liposomes instruction and the biological membrane makes an obvious enhancement of drug accumulation at the site of action as well (Ahmed *et al*., 2019). Besides, most of the drugs have poor penetration and are not able to reach the deeper layers of skin in traditional topical drug delivery; liposomes can penetrate into deeper layers of skin and accumulate in the epidermis. These features result in increased drug absorption into the skin, prolonged and sustained delivery of the drug and decreased systemic absorption (Jung *et al*., 2011; Patel *et al*., 2021).

According to the literature, previously studied topical liposomal formulations in AD have established an appropriate skin permeation (Jung *et al*., 2011). Therefore, we hypothesized that modifying the topical doxepin formulation by loading doxepin into liposomes would increase the residence time of doxepin inside the skin. Liposomes could reduce absorption of doxepin to systemic circulation and enhance accumulation of drug in site of action; so as a consequence systemic side effects are decreased against traditional formulation and patient uses less times of drug during treatment as well. Liposomes may overcome topical side effects of doxepin and will be used for a long-time treatment. Surprisingly, an invention a topical composition for the treatment of atopic disorder containing doxocepine hydrochloride as an active ingredient and excellent in skin permeation and deposition, is introduced for its usefulness as a therapeutic agent for treatment and moisturizing potential of atopic dermatitis (Patent No. KR100618033B1).

In the present study, doxepin-loaded liposomes were prepared by the thin film hydration method and their physicochemical properties were assessed. Liposomes were incorporated in o/w cream for topical application in order to diminish the dryness of the skin. A head-to-head comparison of the efficacy of the liposomal and conventional formulations to penetrate through the rat's abdominal skin was performed using Franz diffusion cell.

MATERIALS AND METHODS

Materials

Doxepin hydrochloride, Phosphatidylcholine (PC) and cholesterol were purchased from Sigma Aldrich, Germany. High pressure liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). High purity deionized water was obtained from a Milli-Q purification system (Millipore, MA, USA).

Preparation of liposomes

Doxepin-loaded liposomes were prepared using the thin-film hydration method (Peralta *et al*., 2018). Briefly, the lipid components consisting of cholesterol and PC with the ratio of 1:5 were dissolved in 6 ml chloroform and methanol (2:1 v/v) in a round bottom flask. Doxepin was dissolved in methanol (10% w/v) and added to the lipid mixture. Solvents were evaporated by a nitrogen flux for at least 20 min in a water bath at 40°C. The flask was placed in a desiccator under vacuum overnight to ensure the complete removal of solvents. Then, the dry lipid film was hydrated with 5ml of deionized water, using 10 g of glass beads. The flask was once again rotated at 100 rpm, for 1.5h at 50°C. The suspension was kept at 4°C for 24h to complete the lipid hydration. At this point, the liposomes could be visualized by an optical microscope. An ultrasonic homogenizer (BANDELIN SONOPULS HD 2070, UK) was used to sonicate liposomal dispersion for 15 minutes at 80% output power in an ice bath to generate nano-sized liposomes (Sætern *et al*., 2004). The liposome suspension was frozen and then lyophilized by freeze drier for 48h and kept in the refrigerator (Joseph, 2018).

Preparation of topical liposomal cream containing 1% doxepin and determination of content uniformity

Cetyl alcohol and vaseline were heated to 70-75°C as an oil phase. Purified water and tween 80 (2.8%) were heated to 75-80°C as an aqueous phase. The oily phase was then progressively added to the aqueous phase and mixed to generate oil in water cream. Liposomes

containing equivalent to 100mg of doxepin were incorporated in 10g of the plain cream by slow stirring until a homogenous cream was formed.

In order to check the uniformity of the content in both creams, samples were taken from different parts and the drug content of each sample was determined as follows: 0.1 g cream was dissolved in 1 ml PBS and stirred for 12 h to disrupt the cream structure. Doxepin was extracted from the mixture as described in Section "Entrapment Efficiency (EE %) and Loading Capacity (LC%) of Liposomal Doxepin." The solution was filtered by 0.45 µm polyamide filter and then analyzed by HPLC method. Doxepin content was calculated according to the following equation:

$$\text{Drug content}(\%) = \frac{\text{Amount of doxepin detected by HPLC}}{\text{Total amount of doxepin employed}} \times 100$$

Determination of size, size distribution and morphology of liposomal doxepin

The size and morphology of liposomes were characterized by dynamic light scattering (DLS), light microscopy and transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

A sample of primary suspension was analyzed under a light microscope to evaluate the shape and size distribution of the vesicles. After sonication, the suspension was diluted with purified water. Mean particle size and polydispersity index (PDI) of liposomes were determined by DLS (Nano-ZS, Malvern Panalytical and Malvern, UK). By depositing a drop of the diluted mixture on a carbon-coated copper grid, the morphology of liposomes was investigated using TEM (CM 30, Philips Eindhoven, The Netherlands). For SEM, the samples were left to dry overnight. The dehydrated samples were gold coated using Emitech K550® coater and visualized with SEM (LEO 1430VP, Germany and Britain).

Entrapment efficiency (EE%) and loading capacity (LC%) of liposomal doxepin

Doxepin-loaded liposomes were separated from non-entrapped drug using a two-step centrifugation method. At first, the samples were centrifuged at 5000 rpm at 4°C using a refrigerated centrifuge (SIGMA 3-30KHS, Germany) for 10 min in order to precipitate the liposomes. The supernatant was collected to analyze non-entrapped Doxepin content using HPLC system (YL 9100 HPLC system, Korea). Before injection, doxepin was extracted from the aqueous phase as follows: 1 ml of solution was mixed with 4 ml n-hexane and shaken vigorously for 10 min. Then, the upper phase, which contained n-hexane and doxepin, was separated and dried under nitrogen gas in a water bath at 40°C. In order to prepare the samples for injection, 100µl of acetonitrile was added, followed by centrifugation at 3000 rpm for 5 min.

A reverse-phase C18 column, 15×4.6 mm, 5 µm was used with a mobile phase consisting of 85% acetonitrile and 15% acetate buffer which was delivered at a flow rate of 1 ml/min. UV detector was set at 220 nm (Rahman *et al*., 2009).

The entrapment efficiency (EE%) and loading capacity (LC%) of the liposomes were calculated according to the following formulas (Hasanpouri *et al*., 2018).

$$\text{Entrapment efficiency(EE\%)} = \frac{\text{Total drug content - Drug content in supernatant}}{\text{Total drug content}} \times 100$$

$$\text{Loading Capacity (LC\%)} = \frac{\text{Total drug content Drug content in supernatant}}{\text{Total lipad}} \times 100$$

Stability studies

To investigate physical stability, liposomal dispersions were stored at 4°C and 25°C for 6 weeks. The average diameter of liposomes was measured every three days and EE% was analyzed weekly as two parameters of stability during the storage period.

Fourier transform infrared spectroscopy (FTIR)

To investigate doxepin interactions with lipids after encapsulation, the FTIR of lipids, doxepin, void liposome and doxepin-loaded liposome in powder form was carried out by Perkin-Elmer Spectrum 100. The above-mentioned powders were separately mixed with KBr and pressed by a manual Tablet Presser to prepare pellets. For each pellet, data was collected in the form of a spectrum extending from 400 to 4000 cm-1.

Ex vivo skin permeation

The abdominal skin of Wistar rat was used for *ex vivo* permeation studies using Franz diffusion cell based on a method explained in United States Pharmacopeia. The rats were supplied by Ardabil University of Medical Sciences (ARUMS) and all *ex vivo* experiments were conducted according to the university's guidelines and regulations.

For the purpose of this study, the hypodermis layer was removed and in case the skin samples were damaged during this procedure, they were discarded.

Permeation of doxepin from plain cream was compared to drug permeation from liposomal doxepin cream; therefore, our study was conducted with two samples: (1) liposomal doxepin cream and (2) doxepin plain cream.

The skin samples were placed in the donor compartment with 1.2 cm^2 effective area. The receptor chamber was filled with 12 ml isotonic phosphate buffer saline (PBS), pH=7.4, which was stirred at a rate of 1000rpm and its temperature was set at 37±1°C to ensure the surface layer temperature of 32°C. An appropriate amount of liposomal doxepin cream and doxepin plain cream equivalent to 1% doxepin was applied to the skin surface. Permeation studies were carried out for 30h and aliquots of 300 µl

Table 1: Stability of liposomes containing Doxepin, Entrapment Efficiency (EE%) of samples was measured over 6 weeks upon storage at 4°C and 25°C. The results are reported as mean±SD value from three independent samples.

Storage Temp (°C)	Storage period (weeks)						
	Initial	1st week	2d week	3rd week	4th week	5thweek	6th week
4±1°C	79±1.3	77±2.1	77±0.8	77±2.6	77±0.2	77±2.8	77±3.3
25±1°C	79±1.3	75±0.5	71±3.2	68±3.2	64±1.8	58±2.2	50±1.2

Table 2: Linear correlation coefficients (R^2) of zero order, first order, Higuchi and Korsmeyer-Peppas kinetic models calculated to determine the release pattern of doxepin from liposomal and plain formulations.

Kinetic model	Mechanism of release	Equation	Linear correlation coefficient (R^2)	
			Liposomal cream	Plain cream
Zero order	Constant rate of release	$M_0 - M_t = k_0 t$	0.964	0.943
Higuchi	Diffusion and permeability	$M_t = k_H \sqrt{t}$	0.867	0.854
First order	Diffusion (Fick's first law)	$lnM_0 - lnM_t = k_1 t$	0.923	0.988
korsmeyer-peppas	Diffusion (semi empirical model)	$Q_t/Q_\infty = K_P t^n$	0.883	0.898

were withdrawn at different time intervals from the receptor medium and replaced with the same volume of fresh medium to maintain sink condition. The maximum concentrations of doxepin observed in the receptor medium, 5µg/mL, were about 16% of the aqueous solubility of the drug (about 0.031mg/mL), thus meeting sink conditions.

For skin retention study, after taking the last samples, the skins were removed from the device and the remaining formulations on the skins were gently washed with PBS and wiped with tissue paper. Using medical tape, the SC was detached from the epidermis and dermis. The medical tape was placed on the skin and separated from it; this procedure was repeated 20 times. Furthermore, the remaining skins (epidermis and dermis) were mashed and homogenized. Doxepin extraction procedure was performed on all samples and the amount of doxepin was determined by HPLC.

Skin retention studies

After taking the last sample, the skins were removed from the devices and the remaining formulations on the surface of the skins were gently washed with PBS and wiped with tissue paper.

Using medical tape, the SC was detached from the epidermis and dermis. The medical tape was placed on the skin and separated from it 20 times. Besides, the remaining of the skins (epidermis and dermis) were mashed and homogenized. Doxepin extraction procedure was performed on all samples (see *Entrapment Efficiency (EE%) and Loading Capacity (LC%) of Liposomal Doxepin* for extraction method). Subsequently, drug content was quantified by the previously reported HPLC method (N. Rahman *et al*., 2009).

Mechanism of doxepin release

The *ex vivo* release data of doxepin release from the plain cream and liposomal cream were applied to some of the most popular kinetic models, including zero order, first order, Higuchi and korsmeyer-peppas mathematical models. These models and their equations are shown in table 2. Fitting the data on these models allows the formulator to have a better understanding of the release mechanism of the drug from the formulation and also predict the amount of released drug in different time intervals. The most accurate kinetic model for doxepin cutaneous permeability was determined according to the R^2 value that was calculated for each model. The model with an R^2 value closer to 1 can explain the mechanism of release more accurately.

Ethical approval

This current study was accepted by the Ethics Committee of the Ardabil University of Medical Sciences (Protocol No. IR.ARUMS.REC.1397.189).

STATISTICAL ANALYSIS

All of the studies were done in triplicate and the data are given as mean values±standard deviation. One-way ANOVA was used to analyze data to determine the statistical significance. In this study, the level of significance is taken as $p<0.05$.

RESULTS

Characterization of liposomes and liposomal/plain cream

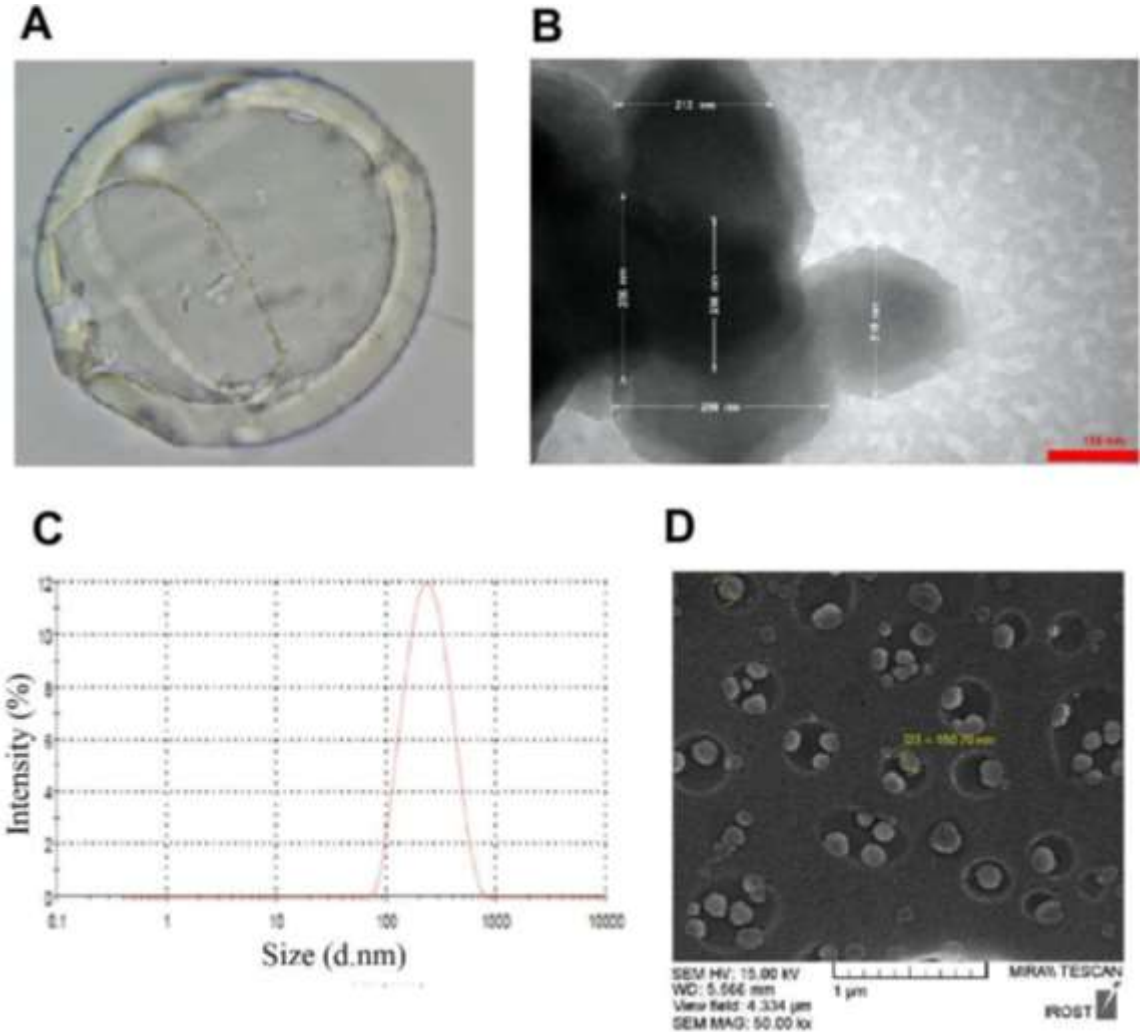

Fig. 1: A. light microscopy image of liposomes before sonication B.TEM of liposomal doxepin C. Distribution of liposomal doxepin size obtained from DLS D. SEM of liposomal doxepin.

The morphological characters of liposomal doxepin were investigated by TEM and light microscopy. At first, small vesicles were examined under a light microscope at 100x magnification (fig. 1A). Secondly, after sonication of liposomal dispersion, TEM image confirmed the presence of almost spherical and unilamellar liposomes (fig. 1B). DLS reported that the average particle size of the developed liposomes was 208.7±5.6nm and PDI value was 0.187 (fig. 1C). Low PDI values (<0.2) demonstrate homogeneity in particle size distribution (Verma *et al.*, 2003).

SEM images illustrate the formation of uniform liposomes by thin-film hydration method. According to HPLC data, there was no significant difference in the drug content between liposomal *doxepin* cream (98.62±3.21) and plain cream (98.98±2.19 and doxepin content of both formulations was uniform (P>0.05).

Entrapment efficiency (EE%) and loading capacity (LC%) of liposomal doxepin

EE% of doxepin liposomes, measured by the indirect method using HPLC, was calculated to be 79±1.3%. This high percentage of entrapment can be attributed to the hydrophobic nature of doxepin. Furthermore, the liposomes had a drug LC% value of around 79%.

Stability studies

The average diameter of freshly prepared liposomes was 208.7±5.6 nm with an EE% of 79±1.3%. Fig. 2 shows that storing the liposomes at 4°C during a period of 6 weeks does not significantly affect the size of liposomes and EE% remained nearly unchanged (table 1.). Nevertheless, there was a major change in size and EE% when liposomes were stored at 25°C ($p < 0.05$). In table 1, the changes in the EE% of liposomes stored at either 4°C or 25°C are presented. During the first week of the study, EE% decreased from 79% to 77% and then remained constant when kept at 4°C. For the samples kept at room temperature EE% underwent a gradual reduction during 6 weeks, reaching 50%. As shown in fig. 3, the

aggregation of liposomes at room temperature leads to the formation of larger liposomes. Moreover, it is supposed that a high temperature would cause drug leakage due to the higher lipid membrane fluidity of liposomes and hence decrease the EE% (Raeiszadeh *et al*., 2018).

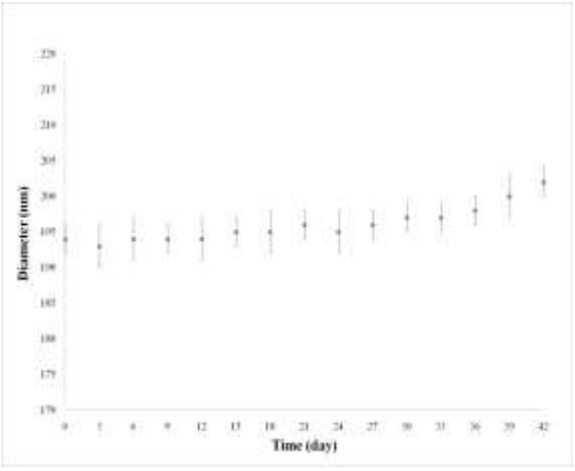

Fig. 2: Liposomal doxepin diameter as a function of time. DLS measurements were taken every three days after samples were prepared on day 0 and maintained at 4°C. The results are depicted as mean±SD from three independent samples.

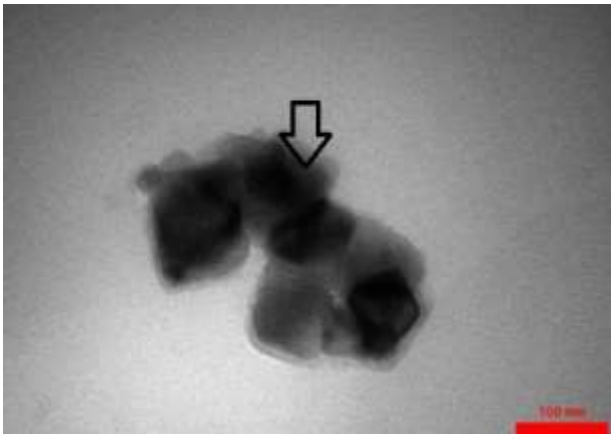

Fig. 3: TEM image of liposomal doxepin. The arrow indicates the aggregation of liposomes and formation of larger particles after 14 days of storage at 25°C.

Fourier transformed infrared spectroscopy (FTIR)

In the FTIR spectrum of doxepin (fig. 4), the absence of N-H stretch confirms the presence of a tertiary amine in the structure of doxepin. Furthermore, the peak at 1218 cm^{-1} is attributed to the stretch of the C-N bond. The absorptions at 1007cm^{-1} and 752cm^{-1}represent C-O-C and $(-CH_2-)_2$, respectively.

In void liposomes, a broad absorption at 3394 cm^{-1} corresponds to OH of the carboxylic group. Liposomal doxepin has the same characteristic peaks as liposome's spectrum; however, there is a slight difference in their wave numbers, which indicates the presence of doxepin inside liposomes. Moreover, since the drug and lipids did not interact to form a chemical bond, no new peak was observed in the spectrum.

Ex Vivo permeation and retention

Ex vivo permeation studies are considered suitable tools for predicting the performance of liposomal creams under *in vivo* conditions.

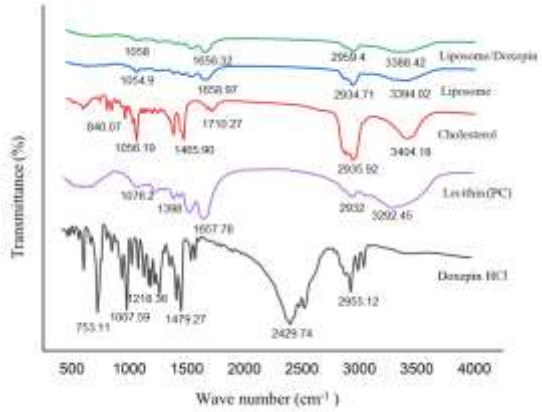

Fig. 4: FTIR spectra of doxepin, PC, cholesterol, blank liposome and doxepin containing liposome

After 30 h, the mean cumulative amount of drug permeated through the skin from the liposomal cream was 11.20±0.6 µg/cm^2, which was 4.2 times lower than the plain cream, indicating that the liposomes have significantly modified the topical delivery of doxepin compared to the plain cream (fig. 5).

Fig. 5: Cumulative amount of doxepin permeated through each cm^2 of rat's skin from liposomal and conventional formulations during 30h. Results represent mean ± SD for three experiments.

Over the 30h duration of the *ex vivo* study, a lower amount of drug was detected in the receptor compartment of the liposomal formulation. As shown in fig. 6, liposomal cream decreased the percentage of doxepin transported through the rat's skin compared to plain cream. Liposomal doxepin showed 6.3-fold higher retention when compared with plain cream in the skin after 30h ($p<0.05$). Liposomes, being similar to the structure of skin regarding their lipid composition, offer a better penetration to the SC compared to conventional dosage forms.

Mechanism of doxepin release through rat's skin

The most appropriate kinetic model for doxepin release from plain cream was determined to be the first-order (table 2), where the drug release is directly proportional to the amount of residual drug. In this model, the amount of drug released per unit of time declines continuously as the concentration of drug in the applied formulation decreases; therefore, products that represent this pattern of release cannot deliver the required amount of drug over time and are disfavored as topical formulations. On the other hand, liposomal doxepin fitted well into zero order kinetic model, indicating that the release has a constant rate and is independent of concentration. According to the literature, the formulations that follow this model are the best pharmaceutical forms for prolonged release, with a great potential for topical application (Santis *et al*., 2013; Yan *et al*., 2007).

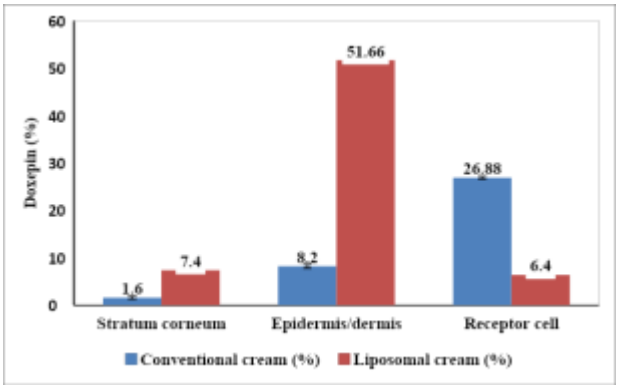

Fig. 6: Doxepin's accumulation in rat's skin (SC and epidermis+dermis) and receptor compartment from the liposomal and plain formulations as percentage of applied amount. ANOVA was used for statistical analysis, $P \leq 0.05$.

Liposomes increase the localization of therapeutic agents within the skin by depositing in both the epidermis and dermis, reducing systemic absorption of the drug. Therefore, liposomes are suitable drug carriers for the treatment of skin diseases. In this study, we prepared a liposomal cream of doxepin to compare the *ex vivo* permeation and retention of liposomal doxepin with the non-liposomal cream. Physicochemical properties, skin permeation and retention and stability of liposomal doxepin were investigated.

We established that the liposomal cream was able to increase the retention of doxepin inside the skin compared to the plain cream, according to numerous studies, the composition of liposomes has a significant impact on the extent of drug permeability (Honeywell-Nguyen *et al*., 2003). According to the literature, a wide range of lipids can be used for formulation of liposomes. Phosphatidylcholine is a commonly used phospholipid to make liposomes due to its amphipathic character; however, many other potential biological membrane lipids have been assessed. Cholesterol was also incorporated in the structure of the prepared liposomes since it is indicated that cholesterol concentrates the hydrophobic chain of the lipids in liposome bilayer and restricts lipid exchange, hence acts as an extra stabilizing agent (Ravar *et al.*, 2015).

In the case of atopic dermatitis, a chronic inflammatory skin disorder, the application of topical doxepin and steroids can lead to various systemic and cutaneous side effects. The development of a topical liposomal formulation can be a promising approach to increase the drug accumulation in the skin, reduce unnecessary systemic exposure and also minimize the side effects (Eichenfield *et al*., 2017; El-Maghraby *et al.*, 2008).

The main challenge for optimal delivery of therapeutics into the skin is the SC layer which has a "brick and mortar" structure. SC consists of corneocytes (the "bricks") and intercellular lipids (the "mortar"). It forms the outermost barrier layer of the skin and is considered to restrict drug's transport and limit the therapeutic effect of conventional topical dosage forms (McGrath & Uitto, 2008; Rahman *et al*., 2015). Topical liposomal formulations have a similar lipid bilayer structure of the SC, which facilitates their penetration to deep layers of skin (Carrer *et al.*, 2008). The retention of the liposomal drugs in the skin, which promotes a sustained release of drugs, may be attributed to molecular interactions, such as hydrogen bonding, van der Waals and π interactions between liposomes, drug and skin lipids (Gaede *et al.*, 2005).

According to previous studies, thin film hydration method produces homogenous liposomes with high entrapment efficiency (Kumar *et al.*, 2010). With reference to the related studies and also the data obtained from the pilot study, lipid composition and drug ratio were determined. As shown in fig. 1A, the initially prepared liposomes were quite large and could be visualized with a light microscope. The final nano-sized liposomes were prepared using an ultra sonicator probe. The TEM image of prepared liposomal doxepin presented in fig. 1B, displays non-aggregated and uni-lamellar liposomes that have a large internal aqueous space relative to the sphere diameter. These observations were in accordance with the results obtained from size analysis by DLS. It should be noted that the diameter of liposomes is slightly larger in TEM images than DLS (234±36.9 vs. 208.7±5.6). When a drop of suspension is placed on the carbon-covered copper grid, liposomes are adsorbed to the surface, explaining the discrepancy (De Leeuw *et al*., 2009). The stability of liposomes was evaluated by measuring their mean diameter and EE%. Size growth and decreased doxepin encapsulation at room temperature may be attributed to aggregation of liposomes into large particles and drug leakage, respectively. At higher temperatures, the EE% declines due to the disruption of the bilayer

structure, which makes the vesicle leakier to the encapsulated drug. This observation is reported in prior studies as well (Peralta *et al*., 2018). The prepared liposomes showed good physical stability at 4°C after more than 6 weeks of storage.

Permeation profiles of doxepin from the liposomal cream and plain cream are shown in fig. 5. Notably, we found that liposomal doxepin was able to penetrate the skin better than free doxepin (p=0.02). The effect of liposome was noticeable in doxepin retention in the epidermis and dermis, as illustrated in fig. 6, indicating that the liposomal cream can increase the amount of doxepin in the skin. Eroglu *et al*. reported that applying the liposomal betamethasone valerate gel to rat skin enhanced the betamethasone concentration in the skin and decreased the systemic absorption of betamethasone as compared with betamethasone a plain gel (Eroğlu *et al*., 2016). A similar effect was also demonstrated in the human skin using a liposome-based gel of hydrocortisone (Moldovan *et al.*, 2006).

DISCUSSION

Topical liposomal preparation can also moisturize the skin by contributing lipids to the SC and therefore be of great benefit in AD, where the moisturizing capacity of a formulation can play a crucial role in the treatment of the disease.

A desirable formulation for delivery of a drug into the skin, is the one that increases the penetration to the point where an optimum amount of the drug is accumulated in the tissue and stops where the drug is about to reach the systemic circulation. The improvement of the drug's penetration through skin layers with the use of liposomes is probably related to its ability to alter the organization of cutaneous lipids. Liposomes act as drug localizers in the skin by accumulating in the epidermis and thereby restricting the drug's movement towards deeper layers and reaching the systemic circulation, resulting in lower side effects and higher patient compliance (Honeywell-Nguyen *et al*., 2003; Verma *et al*., 2003).

The liposomal formulation investigated in the present work is desirable for topical delivery of doxepin. The improved skin retention of liposomal doxepin cream as compared to plain doxepin cream may be due to van der Waals and π interactions between lipid components of liposomes and SC. Therefore, the unique characteristics of liposomes allow for formation of a depot of doxepin in the skin and a sustained release of drug, which will not only decrease the dosing frequency but also prolong the time in which the applied dose of drug reaches the bloodstream and thus significantly reduce the occurrence of possible side effects. The liposomes were prepared by thin film hydration method and characterized in terms of size, doxepin encapsulation and stability during storage. Liposomal cream containing doxepin accomplished an acceptable accumulation of doxepin in rat's skin compared to doxepin cream formulation in the *ex vivo* study. Based on our observations and data, this liposomal delivery approach offers improved efficacy as compared with conventional formulations. However, the physical stability of the formulation imposes challenges on the development of commercial product liposomes in the treatment of skin disorders. The overall results show that loading of doxepin in liposomes can be a promising approach for the topical treatment of AD with reduced systemic side effects. Furthermore, the use of lipid nanocarriers improves the retention of drugs in the target tissue and has the side benefit of hydrating the skin. Future studies could be conducted to investigate the effect of this formulation in the treatment of AD.

CONCLUSION

The liposomes were prepared by thin film hydration method and characterized in terms of size, doxepin encapsulation, and stability during storage. Liposomal cream containing doxepin accomplished an acceptable accumulation of doxepin in rat's skin compared to doxepin cream formulation in ex vivo study. This delivery approach offers improved efficacy as compared with the conventional formulations. However, physical stability of the formulation imposes challenges on development of commercial product liposomes in the treatment of skin disorders. The overall results show that loading of doxepin in liposomes can be a promising approach for the topical treatment of AD with reduced systemic side effects. Furthermore, the use of lipid nano carriers improves retention of drug in the target tissue and has the additional benefit of hydrating the skin. Future studies could be conducted to investigate the effect of this formulation in treatment of AD.

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