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Research Article

Development of Novel Elastic Vesicle-Based Topical Formulation of Cetirizine Dihydrochloride for Treatment of Atopic Dermatitis

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Abstract. Cetirizine is a piperazine-derived second-generation antihistaminic drug recommended for treatment of pruritus associated with atopic dermatitis. The present investigation encompasses development of a nanosized novel elastic vesicle-based topical formulation of cetirizine dihydrochloride using combination of Phospholipon® 90G and edge activators with an aim to have targeted peripheral H_1 antihistaminic activity. The formulation was optimized with respect to phospholipid/drug/charge inducer ratio along with type and concentration of edge activator. The optimized formulation was found to be satisfactory with respect to stability, drug content, entrapment efficiency, pH, viscosity, vesicular size, spreadability, and morphological characteristics. The *ex vivo* permeation studies through mice skin were performed using Franz diffusion cell assembly. It was found that the mean cumulative percentage amount permeated in 8 h was almost twice (60.001±0.332) as compared to conventional cream (33.268±0.795) and aqueous solution of drug (32.616±0.969), suggesting better penetration and permeation of cetirizine from the novel vesicular delivery system. Further, therapeutic efficacy of optimized formulation was assessed against oxazolone-induced atopic dermatitis in mice. It was observed that the developed formulation was highly efficacious in reducing the itching score (4.75 itches per 20 min) compared to conventional cream (9.75 itches per 20 min) with profound reduction in dermal eosinophil count and erythema score. To conclude, a novel vesicular, dermally safe, and nontoxic topical formulation of cetirizine was successfully developed and may be used to treat atopic dermatitis after clinical investigation.

KEY WORDS: atopic dermatitis; cetirizine; elastic vesicles; oxazolone; topical.

INTRODUCTION

Atopic dermatitis (AD) is an inflammatory, chronically relapsing, pruritic skin condition with a predilection for the skin flexures. It is characterized by poorly defined erythema with edema, eczematous lesions, xerosis, and lichenification, along with elevated plasma levels of IgE, IL-4, IL-13, eosinophils, mast cells, *etc.* The conventional therapies include the use of emollients, minimizing contact with irritants, corticosteroids, immunosuppressants, antibiotics, and antihistamines. However, these therapies are often associated with certain drawbacks. For instance, the local and systemic side effects occur with topical corticosteroids, whereas the use of immunosuppressants can cause intense stinging, itching, or burning and the risk of developing skin infections. The prime indication for antihistamine therapy in AD is the treatment of pruritus mediated by histamine-activated H_1 receptors (1).

Cetirizine, the active carboxylic acid metabolite of hydroxyzine, is a potent second-generation antihistamine possessing anti-inflammatory properties and high specific affinity for histamine H_1 receptors (2). Studies have shown cetirizine to be effective in treatment of skin inflammatory conditions by reducing histamine, bradykinin, and allergen-induced wheal and flare reactions; decreasing monocyte and T-lymphocyte chemotaxis; reducing eosinophil responses; and decreasing intercellular adhesion molecule-1 expression on epithelial cells (2,3). The oral administration of cetirizine (used as cetirizine dihydrochloride, and referred to as cetirizine further) is commonly related to different side effects including sedation, ocular dryness, tiredness, and dry mouth (4). Therefore, the topical dosage forms for cetirizine could be expected to be a rational and effective tool for avoiding the oral side effects as well as for targeting the drug to inflamed skin. No topical formulation of cetirizine is available in the market till date. However, some patents and literature reports describe the use of gels (5) and conventional liposomes (6) as topical carriers for cetirizine.

In recent years, there has been an increasing interest in the development of novel elastic vesicular approaches (Transfersomes®) for effective dermal delivery of variety of hydrophobic and hydrophilic drugs. The extremely high flexibility of their membrane permits the elastic liposomes to squeeze themselves, even through pores much smaller than their own diameter under the influence of the transcutaneous hydration gradient. Elastic vesicles (EVs) can exert different functions after topical application. They can improve drug deposition within the skin at the site of action where the goal is to reduce systemic absorption and thus minimize side effects (7–9).

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The aim of the present work was to design and develop a novel topical delivery system of cetirizine using EVs to overcome the setbacks associated with conventional oral therapy and to provide targeted therapy with enhanced skin bioavailability. Also, efficacy was compared with cetirizine in aqueous solution, conventional cream base, and liposomes. The topical formulation is expected to effectively counteract the locally released histamine and other inflammatory mediators, so that the inflammatory symptoms of AD are reduced.

MATERIALS AND METHODS

Materials

Cetirizine dihydrochloride used in the study was a generous gift sample from Indswift ltd (Mohali, India). Phospholipon 90G was a gift sample from Phospholipids GmbH (Germany). Sodium deoxycholate was purchased from Himedia Laboratories Ltd. (Mumbai, India). Stearylamine was procured from Sigma Labs (USA). Carbopol 980 NF was received as a gift sample from Lubrizol Advanced Materials India Private Limited, Mumbai, India. Span 80, Tween 80, Cetyl alcohol, and Triethanolamine were procured from Loba Chemie Pvt Ltd. (Mumbai, India). Isopropyl myristate and glyceryl monostearate was purchased from Central Drug House (P) Ltd, New Delhi. All other reagents were of analytical grade.

Animals

Male Laca mice 8–9 weeks old weighing 30–35 g and female BALB/c mice weighing 25–27 g were obtained from Central Animal House, Panjab University and used for carrying out *ex vivo* permeation, histopathology, and pharmacodynamic studies. Ethical approval to perform these studies was obtained from Institutional Animal Ethics Committee, Panjab University and their guidelines were followed throughout the studies.

Preparation of EVs

EVs were prepared by thin film hydration method with slight modification (10). Accurately weighed quantities of drug (10 mg), phospholipid Phospholipon® 90 G, and edge activators (EAs) in various ratios as listed in Table I were dissolved in 10 mL of chloroform–methanol (1:0.5) in 250 mL round bottom flasks. The solvent mixture was evaporated at 50°C under reduced pressure at 100 rpm using rotary film evaporator (Buchi RE 121, Switzerland). After complete evaporation of the solvent, the flask was kept under vacuum for 24 h to allow complete removal of the residual solvent. The obtained thin lipid film was hydrated at 50±2°C with 10 mL of aqueous phase (phosphate buffer saline, pH 6.4) at 60 rpm using rotary film evaporator to obtain a homogenous suspension of EVs. The suspension was kept for 1 h at ambient temperature for complete hydration process (swelling of phospholipids) to get large multilamellar vesicles (LMLV). LMLV were probe sonicated for 10 min (Titanium probe, Ultrasonicator, India) to get smaller vesicles. The sonicated vesicles were extruded through a sandwich of 100 and 200 nm polycarbonate membranes (Millipore, USA) to get uniform-sized vesicles. During the hydration of lipid film, glass beads were added for complete removal of film and to obtain a homogenous suspension free from vesicular aggregates. The amount of phospholipid, drug, and stearylamine was investigated in different ratios to obtain the optimized ratio. Different EAs like sodium deoxycholate, Span 80, and Tween 80 in various ratios were investigated to select the best suitable EA in optimum ratio for preparation of cetirizine dihydrochloride-loaded EVs. Similarly, liposomes of cetirizine dihydrochloride were prepared using 10% cholesterol instead of Span 80 in the optimized formula (Table II).

Preparation of Vesicular Gel

In order to make the prepared vesicles rheologically favorable for topical application, they were incorporated in a secondary vehicle (1% Carbopol 980 NF, pregelled with triethanolamine) by gentle levigation.

Preparation of Conventional Cream

An oil/water cream was prepared by separately heating both oil phase and aqueous phase at 65°C. The oil phase consisted of light liquid paraffin (10% *w*/*w*), isopropyl myristate (12% *w*/*w*), cetyl alcohol (5% *w*/*w*), stearic acid (6% *w*/*w*), BHT (0.2% *w*/*w*), and glyceryl monostearate (5% *w*/*w*), while the aqueous phase consisted of drug (0.1% *w*/*w*), triethanolamine (1.6% *w*/*w*), propylene glycol (5% *w*/*w*), sodium metabisulfite (0.5% *w*/*w*), and water (q.s. 100% *w*/*w*). The aqueous phase was added to oil phase under continuous stirring. The resulting emulsion was allowed to cool down gradually under constant stirring to obtain a cream.

Vesicle Morphology, Size Analysis, and Zeta Potential

Transmission Electron Microscopy (Philips CM12 electron microscope, Eindhoven Netherlands) was used as a visualizing aid to study the morphology and structure of vesicles. Following the negative staining with a 1% aqueous solution of phosphotungstic acid, vesicles were dried on a microscopic carbon-coated grid and viewed under the microscope at a suitable magnification. Photomicrographs of the vesicles were taken at suitable magnification. Analysis of vesicle size and size distribution was carried out by dynamic light scattering technique using a computerized Malvern Zetasizer™.

Analysis of zeta potential and polydispersity index of the selected formulation was carried out on Delsa Nano Particle Analyser (Beckman Coulter, USA). Zeta potential measurements were done at 25°C and electric field strength of 23.2 V/cm.

Entrapment Efficiency

For determination of entrapment efficiency, the vesicles were kept overnight at 4°C followed by ultracentrifugation at 35,000 rpm for 3 h at 4°C (11,12). The supernatant was removed and the drug quantity was determined in both the sediment and the supernatant. The sediment was dissolved in methanol and with subsequent dilution, the amount of entrapped drug was determined spectrophotometrically at λ_{max} 232 nm using the formula:

$$Entrapment\ efficiency\ (\%\ EE) = (C_T - C_S/C_T) \times 100$$

Table I. Effect of Different Compositions of EVs on Various Optimization Parameters

Formulation code	Ratios of ingredients	No. of vesicles/mm^3×10^3	Percent transmittance	% Entrapment efficiency
Ratio of lipid/drug				
TF-1	5:1	53.75	81.8	44.775±1.131
TF-2	10:1	130.00	74.0	64.961±1.414
TF-3	15:1	237.50	68.0	76.048±1.008
TF-4	20:1	185.00	71.0	71.655±1.610
TF-5	25:1	147.50	73.4	66.949±0.958
Ratio of lipid/charge inducer (stearylamine)				
TF-6	15:0.5	150.00	70.7	68.864±0.172
TF-7	15:1.0	176.25	69.3	70.141±0.156
TF-8	15:1.5	233.75	68.1	75.143±0.231
TF-9	15:2	190.00	68.7	73.584±0.148
TF-10	15:2.5	176.25	69.3	70.204±0.249
Ratio of lipid/EA (Span 80)				
TF-11	95:05	172.50	72.0	69.457±1.531
TF-12	90:10	240.00	68.0	76.564±1.725
TF-13	85:15	217.50	72.2	69.457±1.146
TF-14	80:20	97.50	75.2	61.927±1.459
TF-15	75:25	83.75	78.1	59.812±1.794
Ratio of lipid/EA (Tween 80)				
TF-16	95:05	56.25	81.2	48.849±1.49
TF-17	90:10	85.00	78.1	59.594±1.14
TF-18	85:15	70.00	80.0	54.517±0.53
TF-19	80:20	50.00	82.0	44.872±0.77
TF-20	75:25	46.25	83.1	39.627±1.41
Ratio of lipid/EA (sodium deoxycholate)				
TF-21	95:05	106.25	74.80	61.737±1.06
TF-22	90:10	156.25	70.40	68.526±0.58
TF-23	85:15	90.00	76.00	60.594±0.69
TF-24	80:20	75.00	79.40	57.168±0.95
TF-25	75:25	67.50	81.30	52.028±0.69

Where C_T is the total amount of cetirizine detected both in supernatant and sediment and C_S is the amount of drug detected in supernatant.

Degree of Deformability

The deformability index of EVs was determined using mini-filtration technique and compared with liposomal formulation. In brief, the vesicular suspension was passed through polycarbonate filter of 50 nm. The vesicle size and size distribution measurement was monitored before and after filtration by dynamic light scattering measurements using Malvern Zetasizer™ 2000.

Spreadability

The spreadability of the developed EV gel was evaluated using Texture Analyzer™ equipment, equipped with a 5 kg load cell to determine different rheological properties of prepared vesicular gel *viz*., work of shear, force of gel extrusion, stickiness, and firmness (13).

Drug Leakage Studies

The formulated EVs were evaluated for drug retentive potential at three different temperature conditions *i.e.*, 4±2°C, 25±2°C, and 40±2°C for a period of 6 weeks. The EV formulations were kept in sealed glass vials and samples were

Table II. Optimized Formula for Preparation of Elastic Vesicles and Liposomes

S.no.	Components (mg)	Elastic vesicles[a]	Liposomes[a]
1	Phosphoplipon® 90 G	150	150
2	Cetirizine dihydrochloride	10	10
3	Stearylamine	15	15
4	Span 80	16.6	–
5	Cholesterol	–	16.6

[a] Hydrated with phosphate buffer saline (pH 6.4) 10 mL

withdrawn periodically. The initial percentage entrapment of the drug was considered as 100% and the subsequent samples were relatively analyzed for percentage entrapment of drug.

Preparation of Skin for Permeation Study

Male Laca mice used for permeation studies were sacrificed by inhalation of overdose of chloroform. Hair on the dorsal side of the animals was removed with electric clipper in the direction of tail to head without damaging the skin. The shaven part of skin was cut and hypodermis including blood vessels was removed using surgical blade no. 23. A preliminary wash of skin was done with normal saline. The skin was then checked carefully for any surface irregularities such as tiny holes or crevices before using it for permeation studies (14).

Ex Vivo Skin Permeation and Deposition Studies

The permeation of cetirizine from the vesicular formulations through hairless dorsal skin of Laca mice was determined using Franz diffusion cell assembly, with receptor compartment volume of 30 mL and effective diffusion area of 3.14 cm^2. The receptor cells containing phosphate buffer saline pH 6.4 as diffusion medium was constantly stirred with a magnetic stirrer and equilibrated at 37±1°C with the help of thermoregulated outer water jacket. Various formulations containing cetirizine, equivalent to 0.5 mg were applied onto the skin in the donor compartment. Aliquots of 2 mL were withdrawn through the sampling port at suitable time intervals and analyzed for drug concentration employing UV spectrophotometer at λ_{max} 232 nm after appropriate dilutions. Proper sink conditions were maintained by replacing with equal amounts of diffusion medium into the receptor cells.

At the end of the permeation experiments, the skin surface in the donor compartment was rinsed with methanol to remove excess drug from the surface. The receptor media was then replaced with 50% (*v*/*v*) methanol (9,15). The contents were allowed to stir for next 24 h followed by spectrophotometric determination for amount of drug retained in skin at λ_{max} 232 nm (16,17). Similar permeation and skin retention studies were performed using blank formulations, *i.e.*, without drug and absorbance values were subtracted from the test formulations to account for the effect of excipients. The cumulative amount permeated per unit area (in microgram per square centimeter), flux (in microgram per hour per square centimeter), and skin retention (in microgram per square centimeter) were calculated. All the experiments were performed in triplicate.

All data was statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's method. Results were quoted as significant where $P<0.05$.

Skin Sensitivity Studies and Histopathological Examination

Male Laca mice (25–30 g) were employed for these studies. The hair on the dorsal side of the animals was removed with electric clipper in the direction of tail to head without damaging the skin. One mouse was kept as control (untreated). EV gel was applied uniformly on the dorsal region and kept in contact for 4 h. After that, the animals were sacrificed by cervical dislocation method and exposed dorsal surface was cut. Then, each specimen was fixed in 10% buffered formalin; embedded in paraffin and microtomed. The sections were stained with hematoxylin and eosin. Finally, the specimens were observed under a high power light microscope and were evaluated for their integrity.

In Vivo Pharmacodynamic Evaluation

AD was induced on the skin of female BALB/c mice by topical treatment with oxazolone. Upon application of oxazolone to the skin of hairless mice in low doses for a period of 3 weeks, mice develop symptoms characteristic for AD including barrier dysfunction, secretion of IgE, epithelial cell hyperplasia, fibrosis, and infiltration of inflammatory cells into the dermis and epidermis and secretion of T_H2 cytokines (18). Animals were divided into four groups (A, B, C, and D) containing four animals per group (Table V). Ten microliter of oxazolone (5% *w*/*v* in ethanol) was topically applied daily for a week to animals of group B, C, and D. After 1 week, the oxazolone-treated groups were further treated topically with 60 μL oxazolone (0.1% *w*/*v* in ethanol) once every alternate day for an additional 2-week period (19). After half an hour of oxazolone application, cetirizine cream base formulation and cetirizine EV gel each containing 1.0 mg drug was applied daily on the back of animals of groups C and D, respectively. During this duration, the change in erythema score and number of itchings/scratchings were investigated. After completion of 3 weeks, animals were sacrificed and skin was removed. Excised skin specimens were fixed in 10% neutral formalin and embedded in paraffin. Five-micrometer-thick sections were prepared and stained with hematoxylin and eosin. The number of eosinophils and mast cells were counted under light microscope (×400 magnification) in 100×100 μm area (50 fields) and compared (20).

RESULTS

Preparation and Optimization of EVs

The cetirizine-loaded EVs were prepared by thin film hydration method. The effect of different variables (phospholipid/drug ratio, phospholipid/charge inducer ratio, and phospholipid/EA ratio) were studied on number of vesicles per cubic millimeter, percent transmittance, and percent entrapment efficiency during the optimization step (Table I).

Effect of Phospholipid/Drug Ratio

The formulation batch codes TF-1 to TF-5 represent the various ratios of phospholipid to drug (5:1–25:1). As per data depicted in Table I, the entrapment efficiency increases from 45 to 76% approximately for batches TF-1 to TF-3 with maximum entrapment shown by TF-3 having phospholipid to drug in ratio 15:1, followed by decrease in entrapment efficiency.

Effect of Phospholipid/Charge Inducer Ratio

Stearylamine was used as charge inducer to decrease the vesicular aggregation, thereby increasing the physical stability of vesicles. With increase in content of charge inducer from 0.5 to 1.5 as per batches TF-6 to TF-8, the percent entrapment

efficiency increases from 69 to 75% approximately followed by slight decrease as depicted in Table I.

Effect of Phospholipid/EA Ratio

EAs are usually single-chain surfactants, which when incorporated into the vesicular formulations destabilize the vesicles and enhance the elasticity of phospholipid bilayer by decreasing the interfacial tension (21). In this study, three different EAs namely Tween 80, Span 80, and sodium deoxycholate were investigated in five different ratios for their suitability to form novel EVs possessing requisite formulation characteristics.

The percentage entrapment efficiencies ranging from 40 to 60 and 52 to 69% were obtained with Tween 80 (TF-16 to TF-20) and sodium deoxycholate (TF-21 to TF-25), respectively. The entrapment efficiencies observed for Span 80 type of edge activator range from 60 to 77% approximately (TF-11 to TF-15), among which maximum entrapment of 76.564± 1.725 was shown by batch TF-12 containing 10% *w*/*w* of Span 80 followed by decrease at higher concentration (Table I).

Decrease in transmittance means increase in turbidity of suspension due to increase in vesicle count (in cubic millimeter) as shown by batch TF-12 (68% transmittance and 240 vesicles/mm^3; Table I). On the basis of entrapment efficiency, the vesicle count (in cubic millimeter) values and percent transmittance results, batches TF-3, TF-8, and TF-12 were found to be the best among the different batches formulated. Hence, the optimized formulation was prepared by using phospholipid and drug in 15:1 ratio, 1.5% stearylamine as charge inducer and 10% Span 80 as EV as depicted in Table II.

Vesicle Morphology, Size Distribution, and Zeta Potential

The average vesicle size was 139.7 nm along with a polydispersity index (PDI) of 0.247 (Fig. 1). The log size distribution curve showed normal distribution indicating homogenous size of observed vesicles and low PDI ascertains narrow variation in size distribution. As depicted by transmission electron microscopy of formulated vesicles (Fig. 2), the vesicles were rounded and evenly spaced. The zeta potential of the optimized EV formulation was found to be 12.73 mV indicating a fairly stable formulation.

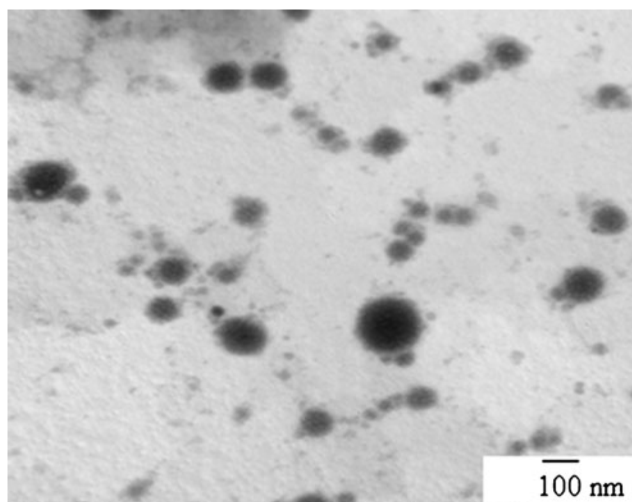

Fig. 2. Transmission electron microscopy of optimized CET 5 formulation (magnification, ×400,000)

Degree of Deformability

Degree of deformability of EVs and conventional liposomes was determined by passing them through vesicle ex-

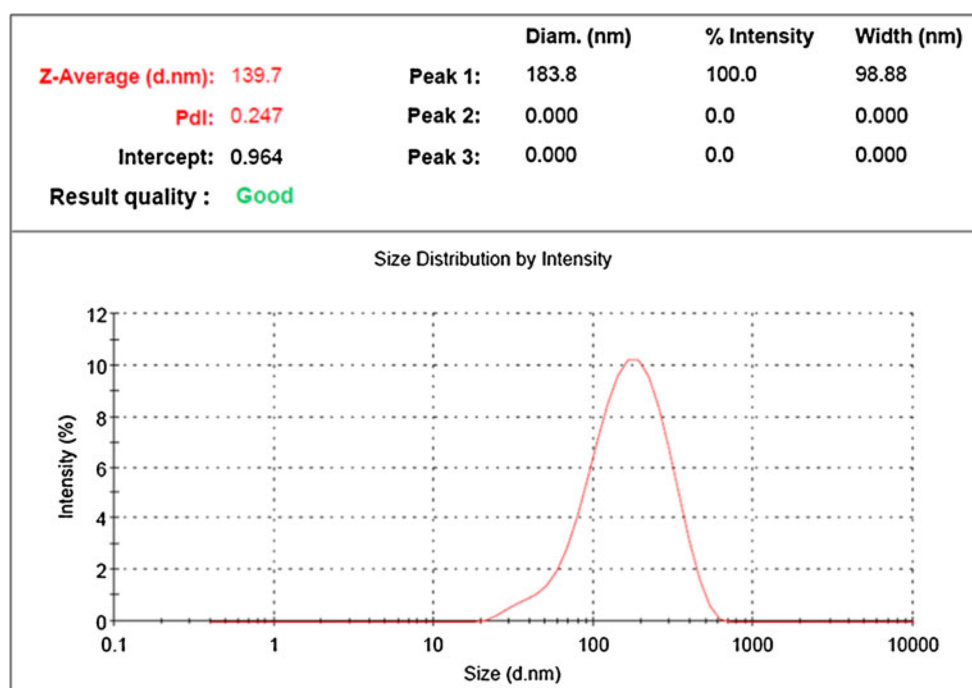

Fig. 1. Particle size distribution of optimized CET 5 formulation

Table III. Degree of Deformability of Vesicular Formulations

Formulations	Size before extrusion (nm)[a]	Size after extrusion (nm)[a]	% Deformability
Elastic vesicles	139.70±12.25	115.50±7.32	17.32
Liposomes	207.10±23.30	100.10±6.52	51.66

[a] Values represent mean±SD (n=3)

truder, and the vesicle sizes of EVs and liposomes before and after extrusion were determined. Table III depicts the size of vesicles before and after extrusion as well as the percent deformability. The EVs retained their size upon extrusion while the size of liposomes was reduced to almost half of their initial size. This showed that EVs are much more flexible as compared to liposomes.

Spreadability

Spreadability is an important aspect of a topical formulation and determines stickiness, ease of application, extrudability from the tubes, and overall consumer acceptance to a product. It was determined in terms of various spreadability parameters such as firmness (1.03 kg), work of shear (1.034 kg s^{-1}), work of adhesion (−0.233 kg s^{-1}), and stickiness (−0.789 kg).

Drug Leakage Studies

Results showed very less leakage of drug (~5%) for the EV suspension samples stored at lower temperatures and significantly higher leakage for those stored at higher temperatures (~15% at 25±2°C and ~21% at 40±2°C), suggesting that the refrigerated conditions seem to be the best storage conditions for the prepared systems.

Ex Vivo Drug Permeation Study

The *ex vivo* drug permeation studies indicated that percent permeation from EV suspension (CET 5; 60.001± 0.332%) and EV gel (CET 6; 50.143±0.914%) was significantly higher than liposomal suspension (CET 3; 38.118±0.867%), liposomal gel (CET 4; 34.983±0.741%), cream base (CET 2; 33.268±0.795%), and aqueous solution of drug (CET 1; 32.616±0.969%) in 8 h suggesting the superiority of EVs over liposome or other conventional formulations as depicted in Table IV.

As revealed in Table IV, the rate of permeation flux was observed to be high for cetirizine EV suspension, *i.e.*, CET 5; 12.846±0.593 μg/cm^2/h followed by EV gel (CET 6; 10.069± 0.177 μg/cm^2/h), liposomal suspension (CET 3; 8.671± 0.570 μg/cm^2/h), liposomal gel (CET 4; 7.469±0.190 μg/cm^2/h), conventional cream base (CET 2; 5.851±0.148 μg/cm^2/h), and aqueous solution of drug (CET 1; 5.776±0.300 μg/cm^2/h).

Skin Retention Study

Skin retention studies were carried out in order to gain an insight into the drug retention behavior of cetirizine within the skin layers, when formulated into different formulations. Table IV shows the skin retention values of different formulations of cetirizine, *i.e.*, EV gel (CET 6; 16.905%)>EV suspension (CET 5; 16.226%)>liposomal gel (CET 4; 7.611%)> liposomal suspension (CET 3; 6.199%)>conventional cream (CET 2; 3.669%)>aqueous solution (CET 1; 1.861%).

Histopathological Studies

The aim of these studies was to establish the dermal tolerance of the EV formulation. No change was observed in the anatomical structure and no pathological changes were found on EV gel-treated mouse skin in comparison to control-untreated mouse skin as depicted by Fig. 3. Thus, the results established the local safety of prepared formulations on mice skin.

In Vivo Pharmacodynamic Evaluation

AD with signs of acute erythema and pruritus was produced by oxazolone challenge on the back skin of mice (19). While a single oxazolone challenge to hairless mice skin produces an acute AD, 9-10 oxazolone challenges over a 3-week period provoked a persistent chronic dermatosis with evidence of moderate to severe pruritus. The itching/scratching shown by mice was noted for 20 min at the end of each week after application of cetirizine EV gel formulation (Fig. 4). The prepared formulation caused marked reduction in itching score with time. The EV-treated mice showed a decrease in itching score (14, 7.75, and 4.75

Table IV. Permeation Parameters of Various Cetirizine Formulations (n=3)

Formulation code	Mean cumulative amount permeated/area (mg/cm^2) in 8 h	Mean cumulative amount permeated (%) in 8 h	Permeation Flux (μg/h/cm^2) in 8 h	Mean % Skin retention per unit area (μg/cm^2) in 24 h
CET 1	0.052±0.001	32.616±0.969	5.776±0.300	1.861±0.148
CET 2	0.053±0.001	33.268±0.795	5.851±0.148	3.669±0.226
CET 3	0.060±0.001	38.118±0.867	8.671±0.570	6.199±0.352
CET 4	0.055±0.001	34.983±0.741	7.469±0.190	7.611±0.235
CET 5*	0.094±0.000*	60.001±0.332*	12.846±0.593*	16.226±0.078*
CET 6*	0.080±0.079*	50.143±0.914*	10.069±0.177*	16.905±0.330*

* $p<0.05$, significantly different from other formulations

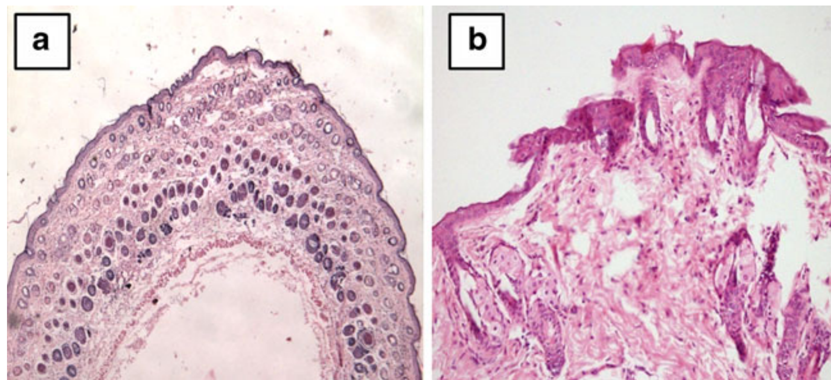

Fig. 3. Histopathological photographs showing absence of any histological and pathological changes. **a** Control untreated skin, **b** treated skin with EV gel (magnification, ×100)

itchings/20 min after 4, 5, and 6 weeks, respectively) as compared to conventional cream base-treated mice (38.75, 17.25, and 9.75 itching/20 min after 4, 5, and 6 weeks, respectively; Fig. 5). Erythema score also decreased subsequently from 3 (moderate to severe erythema) with oxazolone control to 0 (no erythema) with application of EV gel formulation and results of conventional cream base depicted a less decrease in erythema score, *i.e.*, 1 (slight erythema) as compared to EV gel as depicted by Table V.

Histological Analysis of Mast Cells and Eosinophils in the Skin Lesions

Samples of skin treated with blank Carbopol gel, oxazolone, cetirizine cream, and EV gel were fixed with paraformaldehyde and embedded in paraffin. They were sectioned and stained further with hematoxylin-eosin (H&E). The number of mast cells and eosinophils in the dermis stained by H&E, in a field of 100× 100 μm were counted under the microscope (Fig. 6). The cells in 50 fields (0.5 mm^2 in total) of the dermis were counted (20). The number of mast cells increased in oxazolone-treated mice as compared to control but on application of cetirizine EV gel formulation, there was no effect on mast cell count (Fig. 7). The number of eosinophils decreased drastically in EV formulation as compared to conventional cream.

DISCUSSION

AD is a common, inflammatory, chronically relapsing, and pruritic skin disorder for which there is little effective medication (22). Hence, there is a need of effective topical treatment using novel EVs of cetirizine dihydrochloride. The crucial feature of EV formulations in comparison to liposomes is extreme flexibility/elasticity of membrane which permits them to squeeze themselves even through pores much smaller than their own diameter. This high flexibility is achieved by combining phospholipids and surfactants (EAs) with sufficiently differ-

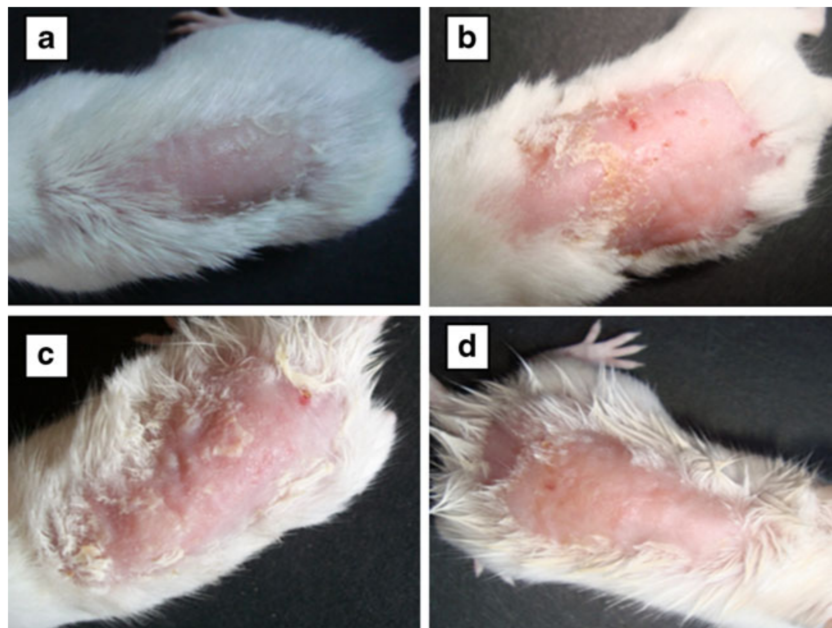

Fig. 4. *In vivo* pharmacodynamic evaluation (after 6 weeks); **a** blank Carbopol-treated mice, **b** oxazolone-treated mice, **c** conventional cetirizine cream-treated mice, and **d** cetirizine EV gel-treated mice

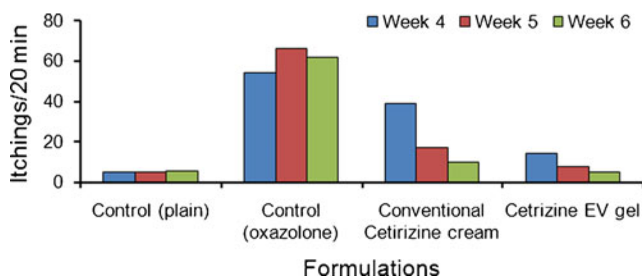

Fig. 5. Effect of different formulations of cetirizine on itching score in mice

ent packing characteristics into a single bilayer (9). EVs prepared by thin film hydration using various EAs were evaluated for percentage transmittance and entrapment efficiency. Entrapment efficiency is the percentage fraction of the total drug incorporated into the EVs; an initial increase in percent entrapment values with phospholipid concentration was observed (TF-1 to TF-3) and it may be due to the availability of increased accommodation for drug with the increased bilayer configuration of vesicles. The entrapment values reduced with higher phospholipid content (TF-4 and TF-5) probably because the fraction of lipid taking part in encapsulation was reduced upon increasing total lipid concentration. Similar results were reported in previous literature (23).

Stearylamine improves the stability of the formulation by inducing surface charge, an initial increase in drug entrapment could be attributed to increase in interlamellar distance between successive bilayers in the MLV structures due to repulsion caused by charge inducer (24) and thus, resulting in a greater overall entrapped volume. Further increase in content of charge inducer in the batches TF-9 and TF-10 did not increase the entrapment efficiency values due to limited solubility of drug in the lipid matrix.

Among the various EAs investigated, Span 80 (10%) was selected as it showed maximum entrapment efficiency. The decrease in entrapment efficiency values with increased concentration, *i.e.*, beyond 10% of EA may be due to possible coexistence of mixed micelles and vesicles at higher concentration of surfactant leads to lower drug entrapment in mixed micelles (9).

The positive value of zeta potential indicates a fairly stable formulation. The small difference in size of EVs as compared to liposomes after passage through vesicle extruder indicated that these vesicles change their shape and rupturing of vesicles was minimal as compared to liposomes. These results were in accordance with the previous literature (25,26).

The spreadability results showed that the optimized formulation was nonsticky in nature. It required less force and work of shear, which implied that it had acceptable spreadability, free from severe adhesion. During drug leakage studies, higher leakage of drug at higher temperatures may be attributed to increase in the fluidity of lipoid bilayers leading to defects in the membrane packing, due to gel to liquid transition behavior phenomenon, thus making it leaky. Hydrolysis of phospholipids and degradation of drug at elevated temperature condition may be accounted as other reasons responsible for greater loss of drug. Similar results of drug leakage at higher temperature were reported in previous literature (16).

The *ex vivo* permeation studies revealed that the vesicular systems solubilized the drug by accommodating it in lipid bilayers, and hence the permeability of drug through the skin was improved. The incorporation of vesicles in hydrocolloid, *i.e.*, Carbopol gel retarded drug release as compared to vesicular suspension as observed by the obtained permeation flux values due to slow diffusion across the gel network (17).

As evident from results of skin retention studies, vesicular systems (EVs and liposomes) have shown maximum drug retention in skin *vis-à-vis* nonvesicular systems (aqueous solution and conventional cream base). This may be attributed to the depot-forming characteristics of the vesicular systems. Further, the higher retention of drug in the skin with EVs *vis-à-vis* liposomal systems may be accounted to their elastic nature as compared to liposomes that not only help in the better penetration of drug across the skin but also form microdepots within the skin layers. Thus, it can be inferred that the prepared EV formulations could effectively make the drug molecules accessible within skin layers, retaining them within close vicinity of the target site (17).

The *in vivo* pharmacodynamic evaluation revealed marked reduction in itching/scratching score as well as erythema score upon EV gel application on oxazolone induced AD on mice skin as compared to conventional cream base. The observed effect was due to extremely small size and elasticity of the EVs due to which permeation through the skin layers increases. Also, EAs like Span 80 present in EVs formulations act as penetration enhancers.

As per histological studies, there was no effect on mast cell count in cetirizine EV gel-treated skin as it lack inhibitory

Table V. Treatment Groups Along with the Effect on Erythema Score

Group	Treatment (formulation)	Erythema score[a]		
		Week 4	Week 5	Week 6
A	Blank Carbopol gel	0	0	0
B	Oxazolone	3	3	3
C	Cetirizine cream base	2	1	1
D	Cetirizine EV gel	1	1	0

[a] Erythema score 0, 1, 2, 3, 4 represents no erythema, slight erythema (light pink), moderate erythema (dark pink), moderate to severe erythema (light red), severe erythema (dark red), respectively

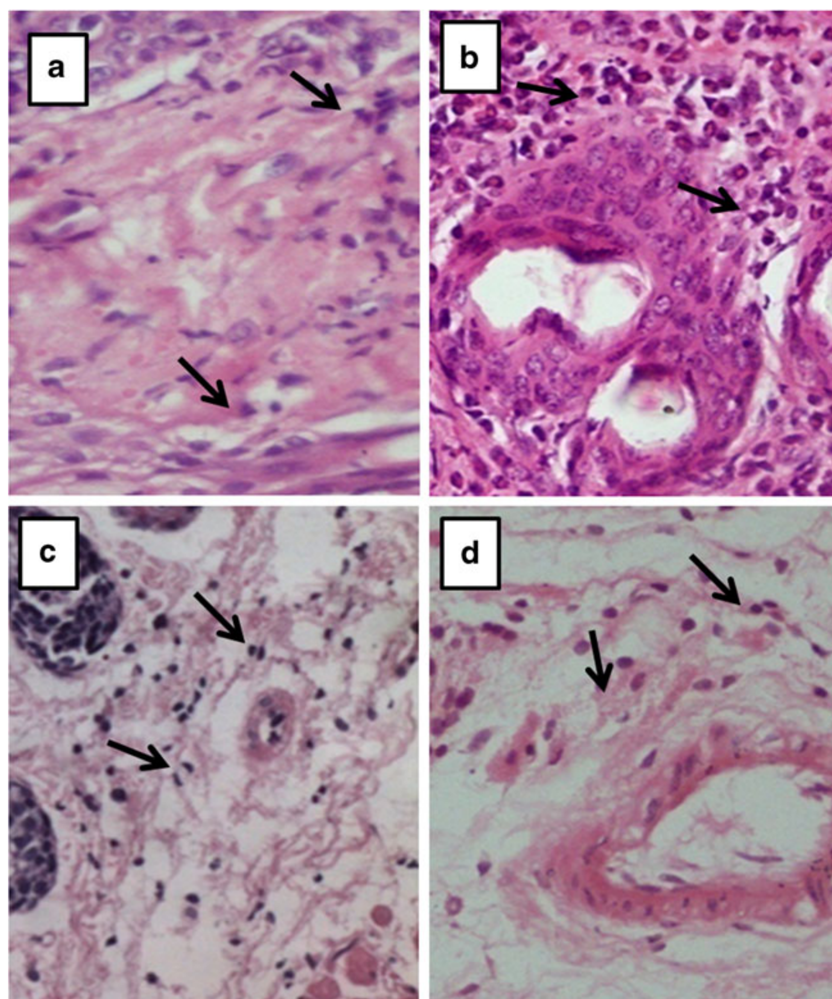

Fig. 6. Histological photographs of mice skin (after 6 weeks); **a** blank Carbopol-treated skin, **b** oxazolone-treated skin, **c** conventional cetirizine cream-treated skin, and **d** cetrizine EV gel-treated skin (*arrows* presence of eosinophils)

effect on mast cell production (3) and rather acts on histaminic receptors which are absent on mast cells.

The number of eosinophils decreased drastically in EV formulation compared to conventional cream due to the inhibitory effect of cetirizine on eotaxin, IL-4, RANTES, etc which are responsible for eosinophil recruitment in dermal tissue (27).

AD is characterized by decreased IFN-γ and increased IL-4, Ig-E, Th2 response, *etc.* Cetirizine increases the level of IFN-γ which results in reduction of Ig-E, eosinophil, and other mediators. Thus, improving the symptoms associated with AD.

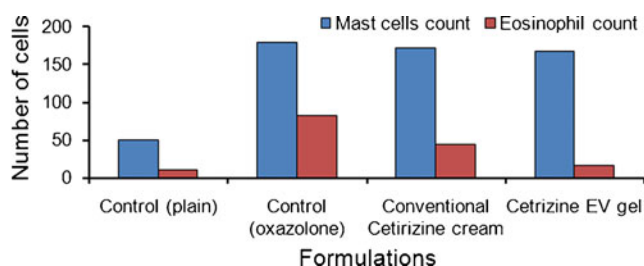

Fig. 7. Effect of different formulations of cetirizine on dermal mast and eosinophil count

CONCLUSION

An attempt was made to develop a dermal delivery system of cetirizine dihydrochloride, which can effectively deliver the drug in the skin and shorten the onset of action time for treatment of AD. The prepared topical formulation with optimal characteristics and histopathological safety was able to permeate the skin barrier effectively. The therapeutic efficacy of the optimized formulation showed improved performance during *in vivo* pharmacodynamic activity on oxazolone-induced AD. This topical formulation may counteract the locally released histamine and other inflammatory mediators by binding competitively to relevant receptors so that symptoms of AD may be largely reduced. In addition, topical application may moisturize the skin and reduce the irritation resulting from allergic condition. Further clinical studies are required to establish the efficacy and safety of this topical formulation on human skin.

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