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Original Article

Tolnaftate-Loaded Ethosomal Gel for Topical Delivery: Formulation and In Vitro Evaluation

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Abstract

Objectives Tolnaftate (TOL) is used as a topical antifungal agent but has poor skin penetration. Therefore, the present study is designed to formulate an ethosomal gel of TOL to enhance drug penetration through the skin.

Methods Using the cold method ethosomal formulations with different concentrations of ethanol and soy phosphatidylcholine (SPC) were formulated. The formulation of ethosomes was characterized by vesicle size, polydispersity index and zeta potential, % drug entrapment efficiency (EE), and scanning electron microscopy (SEM). The optimized ethosomal formula was incorporated in a 1% Carbopol hydrogel dosage. Evaluation of in vitro penetration of prepared gel was performed on goat skin using Franz diffusion cells and compared with conventional gel.

Result The ethosomal formulation EF5 showed the highest % EE (76.82%) with a small vesicle size of 210.1 nm and was selected as the optimized formulation. The SEM result shows that the vesicles were spherical, smooth, and in the 200-nm range. In vitro, permeability study shows that steady-state flux of TOL from ethosomal and conventional gels were 6.667 and 3.685 µg/cm/h, respectively ($p < 0.001$). It indicated that the flux of Ethosomal hydrogel (EHG) is 2.0-fold higher than conventional hydrogel (CHG); this may be due to the flexible nature of ethosomes and ethosomal core containing ethanol, which dissolves the lipid bilayer of skin and overcomes the barrier. In vitro, an antifungal study shows that the incorporation of TOL in ethosomal hydrogel retains their activity.

Conclusion From the results, it was concluded that TOL topical ethosomal gel for antifungal activity will possibly be a good choice.

Keywords
- tolnaftate
- ethosome
- hydrogel
- soy phosphatidylcholine

Introduction

Dermatological diseases, particularly fungal infections, are found to be problematic to treat because fungi grow slowly, and infections often occur in tissues that are poorly penetrated by antifungal agents. The two classes of superficial fungal infections are dermatomycoses and candidiasis. Skin, nail, and hair infections, known as dermatomycoses, are mainly caused by Trichophyton, Microsporum, and Epidermophyton species. In candidiasis, yeast-like organisms infect the mucous membrane of the mouth, vagina, or skin.[1] Therapy of fungal infections usually requires prolonged treatment. A growing number of topical and oral-acting agents are available for the treatment of these infections, such as fluconazole, ketoconazole, miconazole, clotrimazole, tolnaftate (TOL), terbinafine, nystatin, etc.

The oral antifungal agents have some disadvantages like headache, gastrointestinal disturbance, urticaria, diarrhea, nausea, anorexia, vomiting, and photosensitivity. Because of the good targeting ability of the drug in treating fungal infection and less systemic side effects, topical delivery is

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often preferred.in terms of therapeutic effect and bioavailability, conventional approaches such as gels, ointments, and creams are ineffective in treating cutaneous infections. Ethosomes have been used to overcome this problem, showing the best results in treating topical fungus.[2]

Ethosomal systems are vesicular systems composed mainly of phospholipids, ethanol, propylene glycol, and water. They are known mainly to deliver drugs to the outer layer of skin and were shown to improve permeation through the stratum corneum barrier. They also enhanced drug delivery and increased drug entrapment efficiency (EE). Efficient intracellular delivery of drugs from ethosomes can be achieved for lipophilic, hydrophilic, and amphiphilic drug molecules.[3,4] Ethosomes for topical delivery showed good results as novel carriers of drugs such as fluconazole,[5] piroxicam,[6] and flurbiprofen,[7] etc.

TOL is a synthetic thiocarbamate used as an antifungal agent for topical delivery. It acts by inhibiting the squalene epoxidase enzyme, which is important in the biosynthetic pathway of ergosterol, which acts as a constituent of the fungal membrane. TOL in cream or powder form requires a long therapy time due to the poor penetration property, thereby decreasing patient compliance.[8] Therefore, the present study is designed to formulate ethosomes loaded with TOL to enhance topical absorption of the drug and to get an acceptable hydrogel incorporated with optimized ethosomes as a topical product.

Materials and Methods

Materials

TOL was purchased from Yarrow Chem Product, Mumbai, Maharashtra, India. Soya lecithin was collected from Hi Media Laboratories, Mumbai, Maharashtra, India. Cholesterol was purchased from Merck, Mumbai, Maharashtra, India. Carbopol 934 and triethanolamine were obtained from Lobachemie, Mumbai, Maharashtra, India.

Methods

Formulation and Characterization of Ethosomes Loaded with TOL

Preparation of Ethosomes

TOL-loaded ethosomes were prepared using the cold method, as in the composition in ►**Table 1**.

The drug TOL, cholesterol, and soy phosphatidylcholine were dissolved in 3 mL of different ethanol concentrations by keeping on a magnetic stirrer. The above mixture was heated at 300°C, kept in a water bath. Propylene glycol and water were added at the last stirring stage at 700 revolutions per minute (rpm). For the formation of vesicles the mixture was stirred continuously for another 15 minutes.[9,10]

Characterization of TOL-Loaded Ethosomes

Vesicle Size, Vesicle Size Distribution, and Zeta Potential

Using a Zetasizer (Nano ZS, Malvern Instruments, United Kingdom) by dynamic light scattering, size characterizations of prepared vesicles were carried out. Zeta potential is the overall charge that any particle obtains in that particular medium, which is responsible for the stability of that formulation.[9]

Percentage Entrapment Efficiency

Measured 10 mL of prepared suspension was taken in a Tarsus 15-mL centrifuge tube and cold centrifuged at 12,000 rpm for 1 hour at 4°C. Then, sediments and supernatant were separated after centrifugation. Using the ultraviolet (UV) spectroscopic method at 257 nm, the concentration of TOL present in the supernatant was analyzed. The % EE was calculated using the following formula[9]:

$$\%\ \text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Amount of unetrapted drug}}{\text{Total amount of drug}} \times 100$$

Scanning Electron Microscopy

Scanning electron microscopy (SEM) is used to determine the structure of vesicles. With the help of Zeiss Sigma SEM, the morphology of optimized ethosomal formulation was determined. Here, on a clean glass stub a tiny drop of ethosomal suspension was mounted, and then suspension was air-dried and vesicles were coated with the Polaren E 5100 sputter coater, which was observed under SEM.

The formulation with small vesicle size, low polydispersity index (PDI), high zeta potential, and % EE was considered the optimized ethosome formulation, which is then incorporated into the hydrogel to improve retention in the skin when used as a topical application.

Table 1 Composition of TOL-loaded ethosome for 10 mL

Formulation code	Ethanol 3 mL (% W/W)	Soy phosphatidylcholine (mg)	Cholesterol (mg)	Drug (mg)	Propylene glycol (mL)	Water
EF1	20	300	25	150	1	q.s. 10 mL
EF2	30	300	25	150	1	q.s. 10 mL
EF3	40	300	25	150	1	q.s. 10 mL
EF4	20	400	25	150	1	q.s. 10 mL
EF5	30	400	25	150	1	q.s. 10 mL
EF6	40	400	25	150	1	q.s. 10 mL

Abbreviations: q.s., quantity sufficient; TOF, tolnaftate.

Table 2 Composition of various hydrogel formulations

Form code	Drug	Carbopol 934	Glycerine	Triethanolamine	Sodium benzoate	Distilled water
EHG	Ethosomal suspension equivalent to 100 mg drug	1%	0.2 mL	q.s.	20 mg	q.s. 10 g
CHG	100 mg drug	1%	0.2 mL	q.s.	20 mg	q.s. 10 g

Abbreviations: CHG, conventional hydrogel; EHG, ethosomal hydrogel; q.s., quantity sufficient.

Formulation and Characterization of Hydrogel Loaded with Optimized Ethosomal Formulation

Preparation of Hydrogel Loaded with Ethosomal

In the first step, Carbopol was soaked in sufficient distilled water. Then, the Carbopol solution was neutralized by slowly adding triethanolamine and continuously stirring until the gel was formed. In the end, sodium benzoate was added, which served as a preservative. As per ►**Table 2**, an optimized ethosome equivalent to 100 mg of the drug was incorporated into the gels.[7,11]

Characterization of Hydrogel Formulations

Drug Content Determination

Prepared hydrogel (100 mg) was dissolved in 10 mL of acetone and then diluted using pH 6.8 phosphate buffer. This solution was then analyzed at 239 nm using a UV spectrophotometer and taking buffer as blank to determine the drug in the ethosomal hydrogel.

pH Determination

Any formulation intended to be used on the skin must be nonirritating. This is ensured by measuring the pH of the prepared formulation. The pH meter is used to measure pH at ambient temperature.

Determination of Spreadability

The formulated hydrogel is tested for its spreadability after 48 hours. The spreading diameter of hydrogel between two glass slides after 1 minute gives the formulation the spreadability. In a premarked circle of 1 cm diameter on a glass slide, 500 mg of weighed hydrogel was placed on which another glass slide was placed. The hydrogel spreads as the weight added increases.[12] The diameter is noted to calculate the spreadability of the gel by using the following formula:

$$S = (M \times L)/T$$

where S is the spreadability, M is the weight tide on upper slide, L is the length of the glass slide, and T is the time taken to separate the slide completely from each other.

Measurement of Viscosity

The viscosity of the prepared formulations was determined using Brookfield DV-II+ Pro viscometer at temperature 32.0 ± 0.1°C at different angular velocities, using spindle number 4.[13]

In Vitro Drug Permeability Study of Hydrogel

Franz diffusion cell was used to carry out in vitro permeability studies. It has a donor compartment and a reservoir compartment. One end of the donor compartment is covered with goat ear skin. The skin of the goat ear was obtained from the butcher shop; after removing the hair from the skin, it was then placed in saline till it was used. On the dermal side of the membrane, 200 mg (equivalent to 2 mg of the drug) of the gel was placed. The reservoir compartment is filled with 30 mL of pH 7.4 phosphate buffer, stirred continuously with a magnetic bead at 50 rpm speed, maintaining 37 ± 0.5°C temperature. Sampling was done at a periodic interval of 720 minutes, and to maintain sink condition, fresh buffer solution was added back with each periodic interval. The sample was diluted using a phosphate buffer of pH 6.8, and absorbance was measured at 257 nm using a UV spectrophotometer. The cumulative amount of drug permeated per unit area (μg/cm^2) is calculated from the linear part of the slope obtained by plotting the steady state flux (J_{SS}) against time (h).[14] The permeability coefficient (K_P) through the goat skin was determined using the following equation:

$$\text{Permeability coefficient } (K_P) = J_{ss}/C$$

where C is the concentration of the drug in the gel.

Findings were analyzed by GraphPad software, using paired *t*-test.

Evaluation of In Vitro Antifungal Efficacy of Formulations

TOL is effective against *Candida albicans*, the most common cause of fungal infections on the skin. As a result, it was chosen as a fungal inoculum model for in vitro studies. Note that 100 μL of *C. albicans* fungal inoculums were seeded in Petri dishes containing 20 mL medium (Sabouraud dextrose agar). After drying the Petri dishes at room temperature for 15 minutes, 4 wells of 2-cm diameter were bored out of the agar plates. Each well was filled with 2 g of ethosomal hydrogel and conventional hydrogel formulations. A marketed cream of 1%, equivalent to 2 g of formulation, was used as a reference. *C. albicans* fungal plates were incubated for 2 days at 25°C, and the zone of inhibition was observed. The results were recorded by calculating the zone of inhibition surrounding the wells.[14]

Table 3 Physical appearance, vesicle size, PDI, zeta potential, and % EE of ethosomal formulations

Formulation code	Physical properties	Vesicle size ± SD (nm)	PDI ± SD	Zeta potential ± SD mV	% EE ± SD
EF1	Yellow colloidal	365.1 ± 3.8	0.272 ± 0.018	−31.17 ± 1.65	64.98 ± 0.91
EF2	Yellow colloidal	202.5 ± 5.2	0.214 ± 0.025	−34.08 ± 1.98	72.41 ± 1.21
EF3	Yellow colloidal	273.6 ± 4.8	0.263 ± 0.049	−36.11 ± 0.99	59.21 ± 1.09
EF4	Yellow colloidal	374.9 ± 6.4	0.202 ± 0.081	−30.34 ± 1.31	69.17 ± 1.33
EF5	Yellow colloidal	210.1 ± 6.4	0.201 ± 0.027	−33.51 ± 1.38	76.82 ± 1.04
EF6	Yellow colloidal	262.9 ± 4.7	0.281 ± 0.069	−35.31 ± 1.19	61.18 ± 1.19

Abbreviations: EE, entrapment efficiency; PDI, polydispersity index; SD, standard deviation.
Note: Mean ± SD ($n = 3$).

Results and Discussion

Formulation and Characterization of Ethosomes Loaded with Tolnaftate

TOL-loaded ethosomes were prepared using the cold method using different strengths of 20 to 40% alcohol and 3 to 6% soy phosphatidylcholine (SPC). Most of the formulations appeared yellow colloidal.

Vesicle Size, Vesicle Size Distribution, and Zeta Potential

The vesicle size range of the ethosome was found to be 202.5 to 374.9 nm, as shown in ►**Table 3**. Results show that as the concentration of alcohol increases, 20 to 30% of vesicle size decreases at both concentrations of SPC concentration. This may be due to the negative charge provided by ethanol for the surface of ethosomes, thereby preventing the aggregation of the vesicular system due to electrostatic repulsion, and the resultant shows reduced vesicle size. It may also be that high concentrations of ethanol cause interpenetration of the ethanol hydrocarbon chain, which leads to a reduction in vesicular membrane thickness and, hence, reduces vesicular size. Abdulbaqi et al also found similar results: as the concentration of ethanol increases, vesicle size decreases.[15] An increase in alcohol concentration from 30 to 40% increases vesicle size; greater alcohol levels may promote bilayer leakage, resulting in a modest increase in vesicular size and a substantial reduction in % entrapment efficacy. The phospholipid SPC concentration increased from 300 to 400 mg, and the vesicle size slightly increased due to increased phospholipid molecules in the vesicle bilayers where TOL is situated.

All the ethosome formulations had PDI of less than 0.3, showing a narrow vesicle distribution and good homogeneity. Due to the ethanol in the system, the vesicular charge was shifted from positive to negative, and the zeta potential values of all ethosome formulations were found to be above 25 mV. Zeta potential further increased negatively as the concentration of ethanol increased.

Percentage Entrapment Efficiency

►**Table 3** shows the influence of SPC and ethanol concentration on TOL EE in ethosomal formulation. As the ethanol concentration was raised from 20 to 30% v/v, there was an increase in the percentage of EE. However, at higher ethanol concentrations, the percentage of EE experienced a sharp decrease. This could be attributed to ethanol's ability to partially fluidize lipid bilayers, leading to the leakage of entrapped drug. The increase in SPC concentration from 300 to 400 mg % EE was increased, as shown in ►**Table 3**. It may be due to a stable ethosome bilayer forming at higher concentrations, which prevents drug leaching and thus increases % EE. The formulation with small vesicle size, low PDI, high zeta potential, and % EE was selected as the optimized ethosomal formulation. The formulation EF5, which was prepared with 30% ethanol and 400 mg SPC, showed the highest % EE (76.82%) with a small vesicle size of 210.1 nm and was selected as the optimized formulation. EF5 formulation was used for further studies.

Scanning Electron Microscopy

SEM was used to determine the surface morphology and vesicle shape, as shown in ►**Fig. 1**. The vesicles were uniform and spherical in shape, having smooth surfaces. SEM analysis also showed that the vesicle size was in the nano range.

Formulation and Characterization of Hydrogel Loaded with Optimized Ethosomal Formulation

Ethosomal gel was prepared using Carbopol 934 as the polymer, and the prepared gel was smooth and off-white.

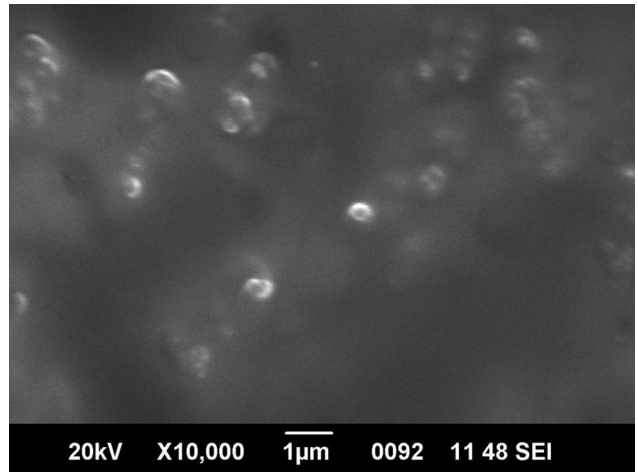

Fig. 1 Scanning electron microscopy (SEM) of optimized ethosomal vesicle (EF5).

Table 4 Characterization of hydrogel

Formulation code	pH	Viscosity (cps)	Spreadability (g/cm^2)	% Drug content
EHG	6.5 ± 0.46	558.7 ± 3.43	5.5 ± 0.83	95.6 ± 3.38
CHG	6.6 ± 0.39	498.6 ± 2.75	5.8 ± 0.67	92.6 ± 2.57

Abbreviation: EHG, ethosomal hydrogel.

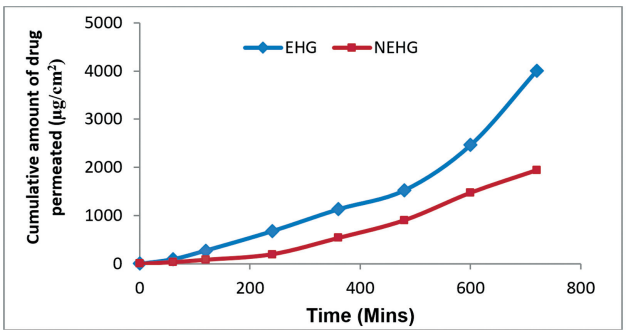

Fig. 2 Comparative in vitro drug permeability study of ethosomal hydrogel with a conventional hydrogel of tolnaftate (TOL).

It was characterized by measuring pH, viscosity, spreadability, and % drug content. The results are shown in ►**Table 4**.

Both formulations have pH values in the skin pH range, which is considered safe when applied to the skin. The drug content of the prepared formulation was found to be greater than 90%. This revealed a uniform distribution of drugs throughout the formulation and that drug loss during the hydrogel formulation was minimal. Ethosomal hydrogel (EHG) and conventional hydrogel (CHG) spreadability were 5.5 and 5.2 g/cm^2, respectively. This indicates that with little shear, the prepared gel can be spread easily, showing good spreadability. The ethosomal hydrogel had a higher viscosity than the nonethosomal hydrogel, possibly due to the presence of ethosomal vesicles that give the formulation its viscosity.

In Vitro Drug Permeability Study of Hydrogel

As shown in ►**Fig 2**, compared to conventional hydrogel, the ethosome hydrogel formulation showed a significant increase in permeation through goat skin. This was most likely due to the drug's rapid delivery from the ethosomal formulation to the stratum corneum and epidermal layers.

The total quantity of TOL delivered from EHG and CHG was 4010.83 and 1941.4 µg ($p < 0.0001$), respectively; this shows that the drug permeability was substantially higher in EHG than the CHG. Increased drug permeation indicates high vesicular penetration because of ethanol in the core, which solubilizes the lipid in the stratum corneum. ►**Table 5** shows the permeability parameter; the steady-state flux in the case of EHG was higher than the CGL. The steady-state flux of EHG and CHG was 6.667 and 3.685 µg/cm^2.min, respectively, whereas the permeability coefficient of EHG and CHG was shown to be 0.067×10^{-3} and 0.037×10^{-3} cm/min, respectively, after 720 minutes. The results showed that EHG had a 2.0-fold higher flux and permeability coefficient than CHG. The direct relationship of flux and permeability coefficient was revealed from the above result. These previous results could be attributed to ethanol content in the ethosomal core, which dissolves the skin's lipid and overcomes the skin's barrier properties. Peram et al[16] in vitro skin permeation studies proved the superiority of ethosomes over the traditional liposomes in terms of the amount of drug permeated and deposited in skin layers.

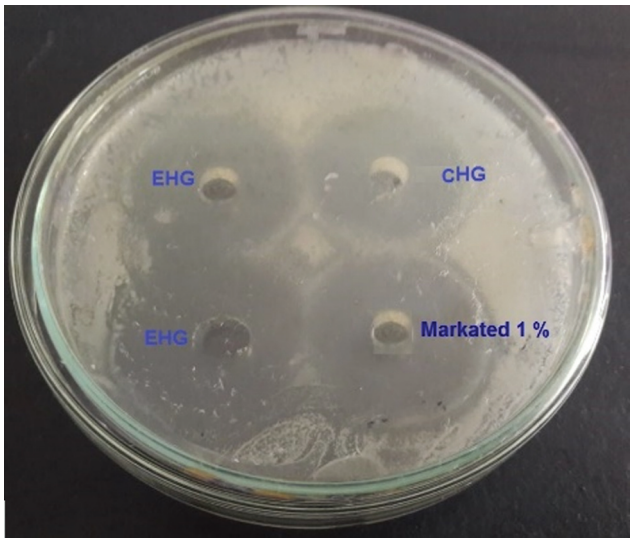

Fig. 3 Comparative in vitro antifungal study of hydrogels with the marked formulation.

In Vitro Antifungal Efficacy of Formulations

An in vitro antifungal study was performed against *C. albicans* using the cup plate method shown in ►**Fig. 3**. The zone of inhibition of both hydrogels was almost similar to that of

Table 5 Permeated amount of tolnaftate at 720 minutes, flux, lag time, and permeability coefficient

Formulation code	Permeated amount at 720 min (µg/cm^2)	Flux (µg/cm^2.min)	Lag time (T_g) (min)	Permeability constant (K_p) $\times 10^{-3}$ (cm/h)
EHG	4010.83	6.667	185.6	0.067
CHG	1941.40	3.685	205.7	0.037

Abbreviation: CHG, conventional hydrogel; EHG, ethosomal hydrogel.

the marketed product. This shows that the incorporation of the drug in ethosomal hydrogel does not decrease its activity.

Conclusion

TOL-loaded ethosome was successfully prepared and showed nanosized vesicle size and reasonable EE. In vitro, drug permeation studies through goat skin membrane showed more drug release from ethosomal hydrogel than nonethosomal hydrogel. Based on the findings, it is reasonable to conclude that the ethosomal system could be a promising drug carrier for topical TOL delivery.

Conflict of Interest

None declared.

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