TOPICAL EMULGEL OF TOLNAFTATE WITH PENETRATION ENHANCER: DEVELOPMENT, CHARACTERISATION AND ANTIFUNGAL ACTIVITY

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Abstract

Tolnaftate, an antifungal agent, has poor solubility and scarce permeability. The main objective of the study was to formulate and evaluate emulgel of Tolnaftate for improved topical drug delivery. Emulgel was formulated by preparing Tolnaftate emulsion and incorporated into Carbopol-940 gel base with two different penetration enhancers i.e Eucalyptus oil (EO) and Transcutol at concentration of 1%, 3% and 5% w/w separately. The formulations were evaluated for physic-chemical properties, in vitro drug release, ex vivo diffusion studies and antifungal activity against Candida albicans. The satisfactory physicochemical parameters were exhibited by optimised emulgel formulation containing 5% EO (Batch-F7). Further, it showed 88.09% ± 0.24 in vitro drug release and 74.87% ± 0.73 ex vivo diffusion at the end of 8 hours which were considerably higher than emulgel without penetration enhancer. When assessed for antifungal activity, the zone of inhibition of Batch-F7 was significantly improved as compared to pure drug, emulgel without EO. This may be attributed to increased penetration of emulgel in fungi cells in presence of EO which translated into efficient antifungal activity. Therefore, it can be concluded that Tolnaftate emulgel was successfully prepared with simple and commercially feasible manufacturing process and can serve as potential topical delivery of Tolnaftate.

Introduction

Superficial fungal infections are amongst the most common skin diseases, affecting millions globally. These infections, which occur in both healthy and immune-compromised population, are caused by dermatophytes, yeasts and non-dermatophyte moulds. Dermatophytes, specifically Trichophyton, Epidermophyton and Microsporum species, and yeasts such as Candida albicans are responsible for most superficial fungal infections. Infections resulting from yeasts such as Candida are becoming an increasing concern, and constitute almost 10-15% of nosocomial infection cases (1, 2).

Topical drug delivery has been the mainstay in the treatment of local fungal infections, and has been an attractive option to treat these infections mainly due to localized effect and circumvention of side effects associated with oral therapy (3,4). However, topical route is one of the most challenging route as several pharmaceutical and biological factors determine the possibility and extent to which a drug can be delivered, and thus transport of drugs through topical route remains a persistent challenge. Most of the drugs are hydrophobic belonging to either biopharmaceutical classification system (BCS) Class II or class IV. Moreover, BCS class IV drugs pose more of a hurdle when considered delivering topically, due to poor permeability in addition to its poor solubility. Several formulation approaches have been developed to address these issues in topical delivery (4-6).

Gels are a relatively newer class of drug delivery systems constituting large amounts of aqueous or hydro alcoholic liquid in a complex network of solid particles, which may include either inorganic substances such as aluminiumsalts or organic polymers belonging to natural or synthetic origin (6). They embrace a higher aqueous component that assists in dissolution of drugs, and facilitate easy drug release through the vehicle that is essentially a liquid,
compared with the ointment or cream base (7, 8). Despite many advantages associated with gels, major drawback remains, which is its ability to deliver hydrophobic drugs. To overcome this limitation, emulgels have been emerged as an effective delivery system aiding the incorporation of hydrophobic drugs in gel dosage forms. When gels and emulsions are utilized in combination, the dosage forms are referred as emulgels (7-9). Emulgels for dermatological use offer an edge over the other counterparts due to some unique features such as being transparent, thixotropic, greaseless, easily spreadable and removable, non-staining, and pleasing appearance (8-11).

Tolnaftate (TNF) is a synthetic thiocarbamate used as an anti-fungal agent, and a BCS class IV drug. Accumulating body of evidence suggests that TNF was found to be active by topical application and inactive by the oral and intra-peritoneal routes. Tolnaftate is available in the market in different topical dosage forms like cream, powder, spray and liquid aerosol. (12, 13).

Also, several investigations on formulation development of TNF have been reported with an emphasis on novel delivery mainly vesicular systems such as liposomes, solid-lipid nanoparticles, nanostructured lipid carriers, etc. (14-16). However, formulation of topical delivery with improved skin penetration using simple and industrially scalable process has not been reported in the prior literature.

In this investigation, the attempt has been made to develop an emulgel formulation of TNF to enhance its solubility and further permeability for topical administration using Carbopol 940 as gelling agent and two types of penetration enhancers, i.e., Eucalyptus oil and Transcutol. The optimized formulation was further characterized for physicochemical parameters, in vitro drug release, ex vivo diffusion studies and its antifungal activity.

Materials and methods

Material
Tolnaftate was obtained as a gift sample from Arati drugs limited, Mumbai, India. Carbopol 940 and Transcutol (TC) were received from Lubrizol India Pvt Ltd and Gattefosse India Pvt Ltd respectively as a gift sample. All other ingredients used were of analytical grade.

Preparation of Tolnaftate Emulgel
Preliminary trials were carried out for selection of gelling agent and various polymers like Carbopol 980, HPMC K15, HPMC K 100 and Carbopol 934 were studied. Based on consistency, the Carbopol 940 was selected as a gelling agent for further studies. Carbopol 940 was dispersed in purified water with mechanical stirrer at a moderate speed followed by the pH adjustment in the range of 6 to 6.5 using triethanolamine. The oil phase was prepared by dissolving Span 80 in Light liquid paraffin while the aqueous phase was prepared by dissolving Tween 80 in purified water. Methyl paraben and Propyl paraben were dissolved in propylene glycol and TNF was dissolved in ethanol separately, and both solutions were added to the aqueous phase. The oily and aqueous phases were separately heated between 70° to 80°C. The oily phase was then added to the aqueous phase with continuous stirring followed by cooling to room temperature. The emulgel was prepared by adding gel and emulsion in 1:1 weight ratio and mix uniformly with magnetic stirrer. Further, penetration enhancers namely, Transcutol and Eucalyptus oil were incorporated in the above emulgel formulation during the polymer dispersion step at concentrations of 1%, 3% and 5% as shown in Table 1.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolnaftate</td>
<td>1.0</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>1.0</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>0.9</td>
</tr>
<tr>
<td>Tween 80</td>
<td>1.0</td>
</tr>
<tr>
<td>Light liquid paraffin</td>
<td>8.0</td>
</tr>
<tr>
<td>Span 80</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table 1: Formulation trials for Tolnaftate Emulgel
Evaluation of Tolnaftate Emulgel

Clarity and Grittiness
The evaluation of the developed formulations for clarity observed visually with naked eye. Smears of gels were prepared on glass slides and observed under a compound microscope for the presence of any insoluble particles or grittiness (17).

\[ \text{pH} \]
The pH of the sample was measured by a digital pH meter (model PICO+, LAB INDIA) at room temperature. The electrode was dipped in gel for 10 seconds, and the value was read on the digital interface (17).

Extrudability
It is a common empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow. In the present study, the method adopted for extrudability is based upon the quantity of emulgel and quantity extruded from lacquered aluminium collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of emulgel in 10 seconds. More the quantity extruded, better is extrudability (18, 20).

Rheological study
The viscosity of formulated gel and emulgel was determined using Brookfield viscometer with spindle no. 18 at 100 rpm at 25±0.5°C (19).

Drug content
The drug content of TNF in emulgel was estimated by dissolving 1000 mg of emulgel in 25 ml methanol with sonication for 15mins. The solution was then filtered through a membrane filter (0.45μm) and analysed using UV Visible spectrophotometer (Shimadzu Corporation, Japan) at 257 nm wavelength with suitable dilution.

In vitro diffusion study
In vitro diffusion study of different batches of emulgel was carried out by modified Franz diffusion cell having 20 ml volume. Dialysis membrane having (molecular weight cut off range 12000–14000 kDa, HiMedia Laboratories Pvt. Ltd, Mumbai, India) was used as diffusion membrane. Ethanol: water (2:1) was used as a diffusion medium due to complete insolubility of TNF in most of the buffers. The dialysis membrane was soaked in diffusion medium for 12 hrs prior to the experiment. The temperature was maintained at 37°C± 0.5°C throughout study. The gel equivalent to 2 mg of TNF was placed in the donor chamber and aliquots were periodically withdrawn from the receptor compartment till 8 hours and the same volume was replaced with the fresh diffusion medium. The samples were filtered through a whatman filter paper, and drug content was determined after suitable dilution by a UV spectrophotometer at 257 nm. The cumulative amount of drug released was calculated at each time point and graph of % drug release vs. time was plotted (20). To understand mechanism of drug release, the dissolution data was processed using PCP disso V3 software (Bharati Vidyapeeth College of Pharmacy, Pune, India), where zero order, first order, Higuchi’s model and Korsmeyer-Peppas models were applied. The best fitted equation yielding the highest regression coefficient for an emulgel formulation was worked out to elucidate the mechanism of drug release (21, 22).
Ex vivo diffusion study

Ex vivo diffusion study of the emulgel was carried out by modified Franz diffusion cell with same procedure given in section in vitro diffusion studies except rat skin was used as diffusion membrane. Freshly excised rat skin was soaked in ethanol: water (2:1) for 1 hour prior to study and then mounted on cells (23).

Antifungal activity

Antifungal activity of TNF, emulgel formulations (with and without EO) and marketed cream was carried out against Candida albicans by agar diffusion method. Candida albicans (ATCC 10231) strains were cultivated on the soybean casein digest agar medium and used for testing the antifungal activity of the formulations. TNF was dissolved in methanol to prepare solutions (60, 80, 100, 125 and 150ppm). All formulations were also dissolved in methanol and diluted to 100ppm concentration. Similarly, dilutions were made for marketed Tolnaftate cream (Tinaderm) in methanol. Sterile Sabroud dextrose agar was poured in the sterilised plates and then C. albicans suspension (100 μl) was added. The plates were agitated carefully for uniform distribution of the test organism in the agar plates and were further allowed to solidify. The each plate was bored for 6 holes in the medium with cork borer, each 1 cm in diameter. Methanolic drug solution (100 ppm), the emulgel batches-F4 and F7 (diluted to 100 ppm) and marketed preparation (diluted to 100 ppm) was filled in above plate. The plates were incubated for 5 days at a temperature of 25°C, and the diameter of the zone of inhibition at the end of 5 days was measured. The entire operation was carried out under aseptic conditions and the mean inhibition zone from three plates was calculated (24).

Results and discussion

The objective of the present study was to design and characterize an effective topical drug delivery of Tolnaftate, a BCS Class IV drug. Thus a unique approach of formulating hydrophobic drugs, emulgel, where emulsions could be easily incorporated into gels was attempted. The solubility of hydrophobic drugs can be improved by incorporating them into emulsion form, which further helps in enhancing their skin permeability (25). Emulgel also provides sustained drug release effect attributing to the presence of polymers in the formulation.

Physicochemical characterization of Tolnaftate Emulgel

The results of physicochemical parameters i.e pH, viscosity, extrudability and drug content of all batches are shown in Table 2. All trials of emulgel were found to be homogeneous and smears were transparent without grittiness or presence of any particles. The pH values of emulgel batches were found in the range of 6.0-6.4 which is similar to skin pH. Viscosity is an important parameter for characterizing the emulgels as it affects the extrudability and release of drug. The viscosity range observed for all formulation was 12,000-13000 Cps. The extrusion of the gel from the tube is important during its application and in patient acceptance. The extrudability of all formulations was found to be satisfactory. The percentage drug content of all formulations was found to be satisfactory and in the range of 93.76-101.13%.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Extrudability</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
</tr>
<tr>
<td>Grittiness</td>
<td>Non gritty</td>
<td>Non gritty</td>
<td>Non gritty</td>
<td>Non gritty</td>
<td>Non gritty</td>
<td>Non gritty</td>
<td>Non gritty</td>
</tr>
<tr>
<td>Viscosity (Cps)</td>
<td>12,000</td>
<td>12,560</td>
<td>13,000</td>
<td>12,300</td>
<td>12,300</td>
<td>12,300</td>
<td>12,652</td>
</tr>
<tr>
<td>Assay (%)</td>
<td>96.47</td>
<td>93.76</td>
<td>101.13</td>
<td>97.12</td>
<td>95.12</td>
<td>95.04</td>
<td>98.32</td>
</tr>
</tbody>
</table>

In vitro drug release

The in vitro drug release profiles of emulgels and emulgels batches with EO and TC are shown in Figure 1. The drug release through the emulgel formulation without penetration enhancer (Batch-F0) was slower and could reach up to 35.5% at the end of 8 hrs. Emulgels batches with 1%, 3% and 5% TC showed 41.63 %, 53.68 % and 63.95 %
drug release respectively which were higher than batch without penetration enhancers. The emulgel trials with EO exhibited a higher drug release in all the studied concentrations than those with TC. Maximum drug release was observed with 5% EO which was 88.12% at the end of 8 hrs. Thus, considering a remarkable rise in drug release from an emulgel containing 5% EO and 5% TC were considered in further ex vivo studies. Drug release kinetics data of the emulgels is given in Table 3, indicated that the optimized gel containing 5% EO followed a zero order drug release from the gel. 

### Table 3: Drug release kinetic analysis of Tolnaftate emulgels

<table>
<thead>
<tr>
<th>Pharmacokinetic model</th>
<th>F0</th>
<th>F4</th>
<th>F7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>0.89127</td>
<td>0.98532</td>
<td>0.97027</td>
</tr>
<tr>
<td>First Order</td>
<td>0.86632</td>
<td>0.9514</td>
<td>0.93313</td>
</tr>
<tr>
<td>Higuchi</td>
<td>0.78992</td>
<td>0.96661</td>
<td>0.9911</td>
</tr>
<tr>
<td>Korsemeyer Peppas</td>
<td>0.97591</td>
<td>0.92878</td>
<td>0.89669</td>
</tr>
<tr>
<td>Hixon-Crowell</td>
<td>0.87486</td>
<td>0.97518</td>
<td>0.94768</td>
</tr>
</tbody>
</table>

**Figure 1: In vitro drug release profiles of Tolnaftate Emulgel batches**

**Ex vivo diffusion study**

*Ex vivo* drug diffusion data followed the similar pattern of *in vitro* drug release study as shown in Figure 2 and 75.52% and 63.9% drug was diffused in 8 hours from emulgels batch with 5% EO and TC respectively. Whereas the *ex vivo* drug release from emulgel without penetration enhancer was 35.5%. The prominent difference between batches with or without penetration enhancer indicated the necessity of penetration enhancer in emulgel of TNF for better efficiency. Moreover, EO has been reported as an effective skin penetration enhancer, activity being attributed to 1,8-cineole, a monoterpenic cyclic ether which has an ability to enhance penetration of both lipophilic and hydrophilic compounds (28). Terpenes, including 1,8-cineole, bind to the stratum corneum and are said to enhance lipophilic drug penetration by improving the partition coefficient and hydrophilic drug penetration by facilitating diffusion. Additionally, 1,8-cineole has been reported to boost skin penetration by disrupting intercellular lipids in the subcutaneous and to change membrane fluidity at the low concentration. Also, emulgel formulation helps in improving the solubility followed by extended drug release which was particularly important for fungal infection.
Antifungal activity
As the drug solution yielded a median concentration of 100 ppm for exhibiting the zone of inhibition for the antifungal activity, emulgel equivalent to 100 ppm of TNF was selected for the study. It was observed that 100 ppm of the pure TNF produced a zone of inhibition of around 1.5 cm, whereas the emulgel formulated using 5% eucalyptus oil (F7), produced a zone of inhibition of about 2.5 cm. Moreover, the zone of inhibition produced by F7-batch of emulgel was comparable with that of the marketed Tolnaftate cream (Tinaderm) as depicted in Table 4. More importantly, the emulgel formulated with 5% eucalyptus oil showed twice the zone of inhibition as compared to the drug solution. This may be attributed to incorporation of eucalyptus oil in the emulgels which has facilitated the penetration across the diffusion membranes as well as through the fungal cell wall translating into efficient antifungal activity. (28,29)

Table 4: Zone of inhibition for Antimicrobial Assay

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>Concentration (ppm)</th>
<th>Zone of Inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>100</td>
<td>1.5±0.23</td>
</tr>
<tr>
<td>Emulgel without penetration enhancer (F0)</td>
<td>100</td>
<td>1.94±0.18</td>
</tr>
<tr>
<td>Emulgel with EO (F7)</td>
<td>100</td>
<td>2.5±0.34</td>
</tr>
<tr>
<td>Marketed cream</td>
<td>100</td>
<td>2.53±0.07</td>
</tr>
<tr>
<td>Blank control</td>
<td>0</td>
<td>No zone</td>
</tr>
</tbody>
</table>

Conclusion
Tolnaftate was successfully formulated in an emulgel drug delivery with eucalyptus oil as penetration enhancers with simple, commercial feasible manufacturing process. Emulgel formulation with 5% eucalyptus oil has revealed improved in vitro drug release and ex vivo diffusion. Moreover, the formulation led to enhanced in vitro antifungal activity, plausibly owing to improved penetration and extended drug release. However, in vivo investigation in relevant animal models may provide further insights into the efficiency of this drug delivery system and its relevance, which inevitably warrants further research.

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**Author Bibliography**

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