Development and Antimicrobial Evaluation of Binary Ethosomal Topical Gel of Terbinafine Hydrochloride for the Treatment of Onychomycosis

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ABSTRACT

The objective of this investigation was to prepare and evaluate the binary ethosomal gel containing terbinafine HCl (TH) to treat onychomycosis. It was reported that binary ethosomes possessed good permeation and stability characteristics than ethosomes. Binary ethosomes of TH were prepared by film hydration method. Prepared binary ethosomes were evaluated for optimized system. Further, ex-vivo skin permeation studies were conducted with rat skin. The formulation of binary ethosomes was converted to gel by using carbopol 934 and evaluated for rheological properties. Antifungal testing of gel was done by cup plate method, using Candida albicans and zones of inhibition of growth were measured. In-vitro and ex-vivo diffusion studies indicated that formulation BE4 with greater amount of binary alcoholic phase, showed faster release and increased flux over others. SEM studies revealed that binary ethosomes were in spherical shape. Based on size, EE, drug release profile and antifungal studies, the BE4 formulation was selected for gel preparation. The prepared 0.5% binary ethosomal BE4 gel showed good content uniformity, pH 5.5, no skin irritation, good consistency and better rheological behaviour. Further, antifungal studies of BE4 gel indicated that gel had lower antifungal activity than plain BE4 formulation, probably due to gelling effect in agar diffusion studies. In conclusion, we developed a pharmaceutical gel containing binary ethosomes of BE4 having TH to provide anti-fungal effects useful for the treatment of onychomycosis.

KEYWORDS: Terbinafine HCl; Onychomycosis; Binary ethosomes; Topical gel; Cup plate method.

Introduction

Onychomycosis is a fungal infection of the toenails or fingernails. It mainly causes fingernails or toenails to thicken, discolor, disfigure and split. Half of all nail disorders are caused by onychomycosis, and it is the most common nail disease in adults. It is caused by three main classes of organisms: dermatophytes, including Epidermophyton, Microsporum, and Trichophyton species (fungi that infect hair, skin and nails and feed on nail tissue), yeasts, and non-dermatophyte molds (Boni, 1998).

Most of the medicines used to treat onychomycosis were not very effective because, they cannot penetrate the nail deep enough (Archan and Deepshikha, 2011). Hence, there is a need to develop an effective topical treatment that can increase the permeation of drug through stratum corneum, allow site-specific administration and minimize the systemic exposure of the therapeutic agent (Gyati et al., 2013). Therefore, colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions, liposomes, and other micelles like niosomes, transfersomes and ethosomes consisting of small particles of 10-400 nm diameter show great promise as drug delivery systems (Bhalaria et al., 2009; Patel and Bhargava, 2012).

Ethosomes are novel lipid carrier systems, and are the modified forms of liposomes containing high ethanol content, being developed by Touitou et al., 2000. Ethosomal drug delivery is non-invasive and delivers the drug to the deep skin layers or the systemic circulation (Vivek et al., 2012). They are reported to have better permeation (Jain et al., 2007).

Ehab and Mina, 2007 reported that the in vitro skin permeation studies of ethosomal gel showed much more efficiency in delivering salbutamol sulphate than liposomes. Zhang et al., 2012 compared the skin permeation of ethosomes, binary ethosomes, and transfersomes against liposomes under non-occlusive condition. The binary ethosomes showed most effective drug penetration through skin. Sarath et al., 2012 reported that the percentage drug released from ethosomes was nearly 26% greater than plain drug incorporated cream across cellulose membrane.
Ethosomes with high concentrations of ethanol probably make the vesicle membranes leaky. This leads to a decrease in entrapment of drug. Moreover, propylene glycol has been showed to have a stabilizing effect in formulations against aggregation of vesicles (Mancon et al., 2009). Therefore, to enhance the stability of ethosomes, binary ethosomes are formulated (Yan et al., 2010). Binary ethosomes contain binary alcohol phase (BAP, comprised of ethanol and propylene glycol) instead of a single ethanol phase. Binary ethosomes have an intact spherical shape and a lipid bilayer and can easily penetrate into deeper layers of the skin.

Terbinafine hydrochloride (TH), is an allylamine with fungicidal effect and has a broad spectrum of activity against a variety of yeasts and filamentous fungi such as Candida spp., Cryptococcus spp., Aspergillus spp., and dermatophytes (Newland and Rahman, 2009; Sakine and Ozgen, 2013). Liang QF et al., 2009, studied the anti fungal effect of terbinafine eye drops in management of filamentous mycotic keratitis Syed et al., 2016, prepared and evaluated the role of ethosomal formulation on skin permeation of TH. It had been reported that binary ethosomes have good stability characteristics as ethosomes and no binary ethosomal gels were prepared until now incorporating TH.

The main objective of present work is to develop an optimized formulation of terbinafine hydrochloride binary ethosomal gel, using appropriate gelling agent. Further, to study the ex-vivo permeation and antimicrobial effect of prepared binary ethosomal gel useful in the treatment of onychomycosis.

Materials and Methods

Reagents

Terbinafine HCl was a gift from M/s. Aurobindo Labs., Hyderabad, India. Soya phosphatidyl choline was obtained from Lipoid, Germany. Centrisart filters (molecular weight cut off 20,000) were purchased from Sartorius, Goettingen, Germany. Ethanol, chloroform, methanol, acetonitrile and dichloromethane were of HPLC grade (Merck, Hyderabad, India). Other chemicals used were of laboratory reagent grade.

Preparation of Binary Ethosomes (BE)

Binary ethosomes of TH were prepared by solvent evaporation followed by sonication method (Touitou et al., 2000). About 50 mg of soya phosphatidyl choline was dissolved in 5 mL of chloroform: methanol (3:1) in round bottom flask. Then 5 mg of drug was dissolved in 5 mL chloroform-methanol mixture and added to lipid phase. The organic solvent was removed using rotary vacuum evaporator above lipid transition temperature (65°C) for 1hr to form a thin lipid film on the wall of the flask. Finally, traces of solvent mixture were removed from deposited lipid film by leaving the contents under vacuum overnight. Hydration was done with different concentrations (20%, 30%, 40% and 50%) of hydro binary alcoholic (ethanol: propylene glycol in the ratio of 7:3) solutions for 1hr. After hydration the formulation was allowed to swell for 2hrs and later sonicated for 3 cycles of 5 min each with 5 min gap in between. Later the formulation was stored in a tightly closed container in the refrigerator.

Compositions of binary ethosomes are showed in Table 1.

### TABLE 1
Composition of binary ethosomes.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>BE1</th>
<th>BE2</th>
<th>BE3</th>
<th>BE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbinafine HCl (mg)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Soya phosphatidyl choline (mg)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Chloroform : Methanol (1:1) (mL)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ethanol (mL)</td>
<td>1.4</td>
<td>2.1</td>
<td>2.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Propylene glycol (mL)</td>
<td>0.6</td>
<td>0.9</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Distilled water (mL)</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Total (mL)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Preparation of Binary Ethosomal Gel using Carbopole 934

Accurately weighed 0.25 g of carbopol 934 was added to 50 mL of optimized (BE4) binary ethosomal formulation with stirring to give final concentration 0.5% (w/w) and kept aside for overnight for complete hydration of polymer chains. Next day, the gel was prepared by adding 0.5 mL of triethanol amine as a neutralizer and was characterized.

Preparation of Control gel

Accurately weighed 0.25g of carbopol 934 was added to a beaker containing 50 mL of distilled water containing 50 mg of drug and was kept aside for overnight for complete hydration of polymer. Next day, the gel was prepared by adding 0.5 mL of triethanol amine as a neutralizer and was characterized.

Measurement of particle size, PDI and zeta potential (ZP) of binary ethosomes

The mean size, PDI and zeta potential of binary ethosomes were measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer (Nano ZS90). The prepared binary ethosomes of 100 µL of was diluted to 5mL with double distilled water to get optimum Kilo Counts Per Second (KCPs) of 50-200 for measurements. Zeta potential of the diluted dispersion was measured using Malvern Zetasizer (Nano ZS90, UK). The surface charge on the drug particles and their mean ZP values were obtained from the instrument (Narendar and Kishan, 2016).

Determination of Entrapment Efficiency

The aqueous medium was separated by ultrafiltration using centrisart tubes (Sartorius, USA) which consisted of filter membrane (M.Wt. cut off 20,000 Da) at the base of the sample recovery chamber. About 2.5 mL of the formulation was placed in the outer chamber and sample recovery chamber was placed on top of the sample and centrifuged for 1 h at 4,000 rpm. The vesicles along with the encapsulated drug remained in the outer chamber and aqueous phase moved into the sample recovery chamber through filter membrane (Dudhipala and Veerabrahma, 2015). The amount of terbinafine hydrochloride in the aqueous phase was estimated by
UV-Vis spectrophotometer at $\lambda_{\text{max}}$ 222 nm (Basavaiah and Vamsi, 2016). About 0.1mL of supernatant was taken and unentrapped drug was calculated. About 0.1 mL of formulation was taken and the total drug was measured. To get the entrapped drug, drug in supernatant was subtracted from the total drug.

**Assay of Formulations**

Accurately, 0.1 mL of formulation was taken and vesicular suspension was analyzed by adding 0.9 mL of methanol. The final volume was made up to 10 mL with distilled water. The absorbance values of diluted samples were measured using UV Spectrophotometer and drug content was estimated (Dubey et al., 2010).

**In vitro Drug Release (Diffusion) Studies of Binary Ethosomes and Hydrogel**

Dialysis membrane having pore size of 2.4nm and molecular weight cut-off between 12,000 -14,000 was used for the release studies. Dialysis membrane was soaked overnight in double distilled water prior to the release studies. The *in vitro* drug release study was performed for all the formulations and prepared hydrogel under the following conditions:

**Conditions**

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Franz diffusion cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion medium</td>
<td>pH 6.4 phosphate buffer</td>
</tr>
<tr>
<td>Rotation speed</td>
<td>100 rpm</td>
</tr>
<tr>
<td>Sampling volume</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

The temperature of release medium was maintained at 37 ± 0.5°C. At predetermined time intervals (0.5 h, 1, 2, 4, 6, 8, 10, 12, and 24 h), about 1 mL of aliquot samples were collected and replenished with same volume of fresh medium. The aliquot samples were diluted appropriately and drug content was estimated using UV-Visible spectrophotometer.

**Physical Stability Studies**

Optimized TH loaded binary ethosomes were stored at room and refrigerated temperature for 60 days. The formulation subjected for stability study was stored in glass containers to avoid any sort of interactions. The samples were withdrawn periodically and measured for average size, zeta potential, PDI, EE and drug content. The number of samples estimated was in triplicate.

**Morphological Examination of Binary Ethosomes by Scanning Electron Microscope (SEM)**

Morphological characteristics were observed by SEM (Scanning Electron Microscope-Hitachi 200). The formulation was spread on circular aluminum stub pre-coated with silver glue (for enhancing conductivity to electrons) and dried in vacuum oven to form a dry film which was then observed under the scanning electron microscope in varying magnifications and micrographs were recorded under voltage of 5kV (Syed et al., 2016).

**pH Measurements of Prepared Gels**

The pH was determined using digital pH meter (Remi, Hyderabad, India) by dipping the glass electrode completely into gel system so as to cover the electrode. The electrode was just brought in contact with the surface of the gel in order to measure the surface pH of the gel.

**Viscosity Measurement of Gels**

The viscosities of formulated gels were determined using a Brookfield programmable DV-III Rheometer (cup and bob). The sample holder was filled with 100mg sample. The spindle (No. 40) was used to measure the viscosity of preparations. The sample was allowed to settle for 5 min prior to taking the readings and the results were noted.

**Stiffness Determination of Gels**

About 100 µg of gel was applied as thin layer on skin and spread with point finger. The resistance to spread was observed (qualitative test). Three different grades were given based on feeling expressed by volunteer as +, ++ and +++.

+ = highest stiffness, ++ = medium stiffness, +++ = lowest stiffness

**Skin Irritation of Gels**

Approximately 100 µg of gel was applied as thin layer on skin and observed for 60 min for possible irritation (reddening & itching) of skin. The skin irritation test was performed, for each gel on 3 human volunteers. The observations were noted (Dubey et al., 2007).
**Microbiological Methods**

Antimicrobial testing was done by cup plate method (Heatley, 1944). About 25 mL of sterilized YEPD medium (composed of 3 g of yeast extract, 10 g of peptone, 20 g of dextrose and 15g of agar/L) was poured into each sterilized petri plate and was allowed to solidify. To each petri plate, a drop of *Candida albicans* suspension was added and spread using sterile cotton swabs. Cups of 8 mm diameter were made using flame sterilized and cooled borer. The formulations of about 50 µL were added into each bore in appropriately labelled petri dishes, using sterile tip with micro pipette. The plates were then incubated at 30°C in invert position for 24 h after diffusion and zones of inhibition of growth were recorded.

**Results and Discussion**

**Measurement of Size, PDI and ZP**

TH loaded binary ethosomes were prepared (BE1-BE4) by sonication method as per composition in Table 1. The prepared formulations were characterized for size, PDI, ZP and the values are represented in Table 2. The size, PDI and ZP of the formulations ranged from 224.06±12.26 - 316.6±21.03 nm, 0.234±0.06 to 0.382±0.03 and -27.1±0.17 to -21.5±1.73 mV, respectively. From the results, initially the effect of ethanol concentration on size and PDI was studied. In all the formulations a trend was observed i.e., as the alcohol and propylene glycol percentage was increased, the size of binary ethosomes and PDI were decreased. The poly dispersity indices were below 0.4. The rank order of BE formulations based on particle size are: BE4<BE3<BE2<BE1. Previously, Verma et al., 2003 reported that, as the size of the vesicles was below 300 nm, more drug release/permeation took place in to the deep layers of the skin. Zeta potentials were gradually increased as the binary alcohol percentage increased. The charge of vesicles is an important parameter that can influence both stability and skin vesicle interaction. The magnitude of the ZP gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential, then they will tend to repel each other and there will be no tendency for the particles to come together (Narendar and Kishan, 2016).

**Entrapment Efficiency and Assay of BE Formulations**

Entrapment efficiency and drug content of the prepared BEs were estimated by spectrophotometry and results are showed in Table 2. The entrapment efficiency of terbinafine HCl was increased from BE1 to BE4 (70.57 ± 2.37 to 92.5 ± 1.04%). It indicated that as the percentage of binary alcoholic phase increased, the entrapment efficiency of terbinafine HCl was also increased. Therefore, the results indicated that entrapment efficiency had depended on ethanol and propylene glycol concentration of BE formulations. Total drug content in the BE formulations was determined and ranged in between 97.1±1.08 to 98.3±1.08%.

**In vitro and ex vivo Drug Release Studies of BE Formulations**

*In vitro* drug release through dialysis membrane and *ex vivo* drug permeation through rat skin were estimated and showed in Figure 1 and 2. From the results, formulation BE4 and BE3 showed relatively faster release when compared to formulation BE1 and BE2. About 45% of drug was released in 10h in case of BE4 and 38% in case of BE3 formulation, where as in between 25-30% was released in case of BE1 and BE2 formulations. This result indicated, that drug from BE4 formulation released faster over the others, probably due to increased permeability through dialysis membrane.

**TABLE 2**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>ZP (mV)</th>
<th>Assay (%)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BE1</td>
<td>316.6 ± 21.03</td>
<td>0.382 ± 0.03</td>
<td>-21.5 ± 1.73</td>
<td>97.81 ± 1.96</td>
<td>70.57 ± 2.37</td>
</tr>
<tr>
<td>BE2</td>
<td>294.8 ± 7.01</td>
<td>0.357 ± 0.08</td>
<td>-22.7 ± 1.63</td>
<td>97.54 ± 2.78</td>
<td>79.08 ± 1.26</td>
</tr>
<tr>
<td>BE3</td>
<td>241.6 ± 7.04</td>
<td>0.267 ± 0.03</td>
<td>-25.5 ± 2.22</td>
<td>97.1 ± 1.08</td>
<td>85.4 ± 0.89</td>
</tr>
<tr>
<td>BE4</td>
<td>224.06 ± 12.26</td>
<td>0.234 ± 0.06</td>
<td>-27.1 ± 0.17</td>
<td>98.3 ± 1.08</td>
<td>92.5 ± 1.04</td>
</tr>
</tbody>
</table>

**Fig. 1. In vitro drug release profiles of terbinafine HCl from binary ethosomal formulations (Mean±SD, n=3).**
All formulations showed more than 50% drug release in 12 h during ex-vivo release studies. BE4 showed 87% of drug release within 12 h, whereas BE1, BE2 and BE3 showed 58.5%, 67.9% and 75.7% of drug release, respectively. From BE1, drug was released slowly when compared to BE2, BE3, and BE4 in the first hour itself. The release rate was faster relatively as binary alcohol content in binary ethosomes was increased.

When compared to the permeation of terbinafine HCl from skin, to that of from dialysis membrane, the release through the dialysis membrane was slow. Drug from BE4 formulation was permeated relatively faster both in vitro and in ex-vivo studies when compared to other formulations. The order of release of drug from formulations both in vitro and ex-vivo was similar i.e., BE4 > BE3 > BE2 > BE1. The flux of the formulation ranged from BE1 to BE4 was 0.0097±0.0002 to 0.0156 ± 0.0001mg/cm².h. From BE1 to BE4, as the percentage of binary alcohol increased, the flux was increased. BE4 showed the maximum flux than other formulations.

From the physical characters, EE, in vitro drug release and ex vivo permeation studies, formulation BE4 was considered as optimized formulation. Further, BE4 formulation was added to hydrogel prepared by carbopol 934.

**Scanning Electron Microscope Studies**

The morphology of the binary ethosomes was studied by using Scanning Electron Microscope (SEM, Hitachi, Japan). BE4 is showing the spherical shape with variable sizes (Figure 3).

**Stability Studies**

The stability of optimized formulation (BE4) was determined by monitoring the particle size, PDI, ZP, assay and entrapment efficiency of TH after storage at refrigerated temperature and room temperature for a period of 2 months. About 9 vials with each containing 3 mL of formulation were stored at room temperature (30°C) and at refrigerated temperature (4°C) respectively. The results of the stability studies are shown in Table 3. Samples stored at room temperature showed slight increase in size, PDI and zeta potential when compared with the samples stored at refrigerated temperature and this indicated that the binary ethosomes were stable at refrigerated temperature. Entrapment efficiency is the integral part of stability of vesicles. According to the data, the %EE was decreased by 8.8% at room temperature and at refrigerator temperature it was 4.62% for ethosomal formulation. There was no significant change observed with the BE4 formulation in case of percent drug content.

![Fig. 2. Ex-vivo drug release profiles of terbinafine HCl from binary ethosome formulations (Mean ± SD, n = 3).](image1)

**Fig. 2.** Ex-vivo drug release profiles of terbinafine HCl from binary ethosome formulations (Mean ± SD, n = 3).

![Fig. 3. Scanning electron micrographic image (x1200) of BE4 formulation.](image2)

**Fig. 3.** Scanning electron micrographic image (x1200) of BE4 formulation.
TABLE 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial (0 days)</th>
<th>After 15 days</th>
<th>After 30 days</th>
<th>After 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4°C</td>
<td>30°C</td>
<td>4°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Size (nm)</td>
<td>210.6 ± 6.54</td>
<td>218.5 ± 3.13</td>
<td>263.3 ± 1.23</td>
<td>225.8 ± 7.59</td>
</tr>
<tr>
<td>PDI</td>
<td>0.206 ± 0.02</td>
<td>0.218 ± 0.02</td>
<td>0.220 ± 0.02</td>
<td>0.225 ± 0.02</td>
</tr>
<tr>
<td>ZP (mV)</td>
<td>-20 ± 2.6</td>
<td>-23 ± 3.28</td>
<td>-27.3 ± 1.25</td>
<td>-30 ± 2.59</td>
</tr>
<tr>
<td>EE (%)</td>
<td>93.7 ± 2.45</td>
<td>89.9 ± 2.98</td>
<td>88.3 ± 1.66</td>
<td>89.6 ± 1.37</td>
</tr>
<tr>
<td>Assay (%)</td>
<td>98.1 ± 1.13</td>
<td>97.8 ± 1.91</td>
<td>97.09 ± 2.26</td>
<td>97.8 ± 3.82</td>
</tr>
</tbody>
</table>

Antimicrobial Activity of Binary Ethosomal Formulations

This test was performed to know the in vitro performance of all the prepared binary ethosomal formulations. Required volume of formulation each containing 25 µg of drug was tested against Candida albicans. The zone of inhibition was observed for each formulation (Table 4). Anti-microbial testing was also performed for blank binary ethosomal formulations (i.e., without terbinafine HCl) to know the excipient-content influence. Blank formulations except BE1, showed a distinct zone of inhibition. Blank formulations also exhibited growth inhibition on C. albicans. This indicated that the alcohol content in BEs inhibited the growth. In general, the formulations containing drug (terbinafine HCl) showed more zone of inhibition than blank formulations. Terbinafine HCl loaded BE4 (with 50% BAP) showed maximum zone of inhibition when compared to BE1, BE2, and BE3.

TABLE 4.

Antimicrobial activity of drug loaded binary ethosomal formulations in comparison to blank BE formulations (Mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean diameter of zone of inhibition for drug loaded binary ethosomes (mm)</th>
<th>Mean diameter of zone of inhibition for blank binary ethosomes (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BE1</td>
<td>16 ± 1</td>
<td>--</td>
</tr>
<tr>
<td>BE2</td>
<td>17.6 ± 0.57</td>
<td>11 ± 0.5</td>
</tr>
<tr>
<td>BE3</td>
<td>16.6 ± 0.57</td>
<td>13 ± 0.86</td>
</tr>
<tr>
<td>BE4</td>
<td>20 ± 0</td>
<td>14 ± 0.62</td>
</tr>
</tbody>
</table>

Evaluation of Topical Gels

The formulation BE4 was converted into a topical gel by using carbopol 934. Control gel was prepared by adding carbopol 934 and drug to the water. The objective was to get an optimized gel formulation for topical application. The gels were tested for the physical parameters such as pH, viscosity, stiffness, skin irritation and ex-vivo drug release studies and in vitro antimicrobial activity. The results of the gels were showed in Table 5. The pH of gel formulations was 5.4 to 5.5 which lies in normal pH range of skin (5.5-6.5) and had not produced any skin irritation when applied to skin. The preparation was viscous and stiff and has low flowability. The assay of the gels was found to be more than 95%.

**In vitro and ex vivo Release Studies of BE Hydrogel Formulations**

The in vitro drug release and ex-vivo permeation studies through rat skin were calculated and represented in Figure 4. The drug permeation was relatively faster when compared to the release through dialysis membrane.

TABLE 5

Physical evaluation of gels.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Viscosity (Cp)</th>
<th>pH</th>
<th>Stiffness</th>
<th>Skin irritation</th>
<th>Assay (%)</th>
<th>Flux (µg/cm² h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BE4 Gel</td>
<td>5.85</td>
<td>5.5</td>
<td>++</td>
<td>-</td>
<td>96.5</td>
<td>0.0116 ± 0.0004</td>
</tr>
<tr>
<td>Control Gel</td>
<td>6.99</td>
<td>5.43</td>
<td>++</td>
<td>-</td>
<td>97.3</td>
<td>0.0088 ± 0.0004</td>
</tr>
</tbody>
</table>

**Fig. 4.** Comparison of in vitro (A) and ex vivo (B) skin permeation of terbinafine HCl from BE4 gel and control gel.
The drug, terbinafine HCl from BE4 gel was released faster when compared to that of control gel. More than 50% of drug was released from BE4 gel in 10 h. Flux values were calculated and was found to be 0.0088 ± 0.0004 and 0.0116 ± 0.0004 mg/cm² h for control gel and BE4 gel formulation respectively. Presence of ethanol and propylene glycol probably influenced the faster skin permeation of terbinafine HCl in BE4 gel.

When the flux values of gels and BE4 formulation (0.0156 ± 0.0016 mg/cm² h) were compared, the gels exhibited less value. This clearly indicated that the gels, to some extent retarded the permeation across skin in ex-vivo study. However, when compared with the flux of control gel, BE4 gel showed high flux. It clearly indicated that binary ethosomes (BE4) increased the skin permeability of the drug.

**Antimicrobial Activity of Hydrogel Formulations**

Both drug loaded BE4, blank BE4 gel and control gel were tested for their *in vitro* antimicrobial activity against *Candida albicans*. The antimicrobial activity of different formulations and hydrogel against *Candida albicans* were showed in Figure 5. Each gel and its formulation were tested against *Candida albicans*. Each formulation containing amount of drug equal to 25μg was loaded into respective bore. BE4 formulation and drug solution showed more zone of inhibition, whereas gels showed less zone of inhibition. This difference is probably due to release barrier of drugs from gel matrix into surrounding agar (Table 6). Further, drug loaded BE4 gel showed high zone of inhibition than control gel and gel with empty BE4 vesicles.

**TABLE 6**  
Comparison of antimicrobial performance (zone of inhibition) of prepared formulations (Mean±SD, n=9).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug solution</td>
<td>16.5 ± 0.5</td>
</tr>
<tr>
<td>Control gel</td>
<td>10.6 ± 0.28</td>
</tr>
<tr>
<td>Gel with empty BE4 vesicles</td>
<td>10 ± 0.00</td>
</tr>
<tr>
<td>Drug loaded BE4 gel</td>
<td>11.5 ± 0.28</td>
</tr>
</tbody>
</table>

**Conclusions**

In conclusion, we developed binary ethosomal gels containing terbinafine HCl. Optimal BE4 gel was prepared, characterized and tested for its antimicrobial activities and skin permeation tests, to provide evidence for the functioning of gel in-vivo. Drug loaded BE4 gel showed a good antimicrobial activity than control gel and gel with empty BE4 vesicles. The BE4 gel is non-irritating and has sufficient stiffness. In future, BE4 gel could be used for detecting the clinical efficiency against onychomycosis in patients.

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**Declaration of Interest**

The authors report no conflicts of interest. The authors are alone responsible for the content and writing of this paper.

**References**


Shruthi et al: Development and Antimicrobial Evaluation of Binary Ethosomal Topical Gel of Terbinafine


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