ORIGINAL ARTICLE

Evaluation of the Antimycotic activity of Terconazole proniosomal Gel

1Rehab Bahy*,  2Doaa A. Helal
1Department of Microbiology and Immunology, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt.
ORCID ID: 0000-0002-2868-928X
2Department of Pharmaceutics, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt
ORCID ID: 0000-0002-1222-8112

ABSTRACT

Background: Terconazole (TCZ) counted is an antifungal drug with a broad spectrum of activity against Candida species. TCZ has been shown to be more active than imidazoles.

Objectives: The aim of this research was to assure the potential of proniosomal gel for the topical delivery of terconazole, and to detect its MIC against Candida albicans comparing it with the reported MIC of terconazole cream 0.8%. Methodology: The coacervation phase separation process was used to develop Terconazole-loaded proniosomal gels. The particle size (PS) and entrapment efficiency percentage of the produced proniosomal gels were assessed and in-vitro drug release. The particle size varies from (17.06–48.1 nm), range of EE% is (83.41%–95.66%). F4 (span 60, tween 80 (1:1), and cholesterol) have the highest EE% (95.66%), was tested by Transmission electron microscopy (TEM) and a zeta potential (ZP) test were performed. Results: The outcomes revealed a nanosized vesicle of terconazole. Proniosomal gel MIC was (32µg/ml) lower than the reported MIC of market terconazole cream 0.8% (128µg/ml). Conclusion: Proniosomal gel achieved the desired sustained topical effect. There was no significant change in EE percent, PS, or ZP in physical stability investigation.

INTRODUCTION

Candidiasis is a mycotic infection induced by Candida albicans, the most serious public health threat of all mycoses now. The vaginal mucosa, skin, oropharynx, bronchi, lungs, and gastrointestinal tract are the most typically affected areas. Candidiasis comes in acute and chronic forms, and it can be superficial or severe. Some antifungal agents, particularly those used against Candida albicans, are ineffective against some fungus species. Inability to respond to antymycotic therapy could be induced by the strains intrinsic resistance or acquired during treatment. Developing an antymycotic drug with lowest possible Candida resistance, or perhaps designing a controlled release approach capable of boosting the therapeutic efficiency of antifungal drugs, is one of current challenges.

Terconazole (TCZ) counted as a triazole antifungal drug with a broad spectrum of activity. TCZ has been discovered to be one of the most effective azoles against Candida species, and its principal mechanism of action was similar to that of imidazoles, in that it inhibits the fungus cytochrome P-450. TCZ, on the other hand, has been shown to be more active than imidazoles.

Hence TCZ is weakly soluble in water and to improve the efficacy of poorly soluble medicines, various ways were used to integrate them into drug delivery systems. Poorly soluble medicines may be embedded into lipid carriers with nanometric dimensions to improve their solubility and bioavailability. TCZ has been encapsulated in a several drug delivery systems, including proniosomal gel bilosomes, and self-nanoemulsifying systems, overcoming its low solubility.

Vesiclefoterconazoles such as niosomes, liposomes, and proniosomes have diverse compared to conventional dosage forms, there are a number of advantages. Vesicles can entrap both hydrophilic as well as lipophilic medicines in the aqueous layer or in the inner lipid membrane. Proniosomal are structurally analogous to the bilayer liposome and noisomembut more stable. Niosomes’ physical stability difficulties are reduced, and storage, transportation, distribution, and handling are easily accomplished. The aim of this work is to assure the potential of proniosomal gel for the topical delivery of terconazole, and to detect its MIC against Candida albicans comparing it with the reported MIC of terconazole cream 0.8%.

METHODOLOGY

Materials:
Terconazole was supplied as a free sample from Pharco, Egypt. Sorbitan monoesters (Span 20, 60) and Polysorbates (Tween 80), and Cholesterol were used to integrate them into drug delivery systems.
kindly supplied by the Egyptian (EPICO), Egypt. All additional chemicals were purchased from El-Nasr Company Co. Egypt, and were of analytical quality.

**Preparation of terconazoleproniosomal gel**

The method of coacervation phase separation was used for preparation of proniosomal formula using different nonionic surfactants with different ratios of the surfactant: cholesterol. Different formulations with different compositions were shown in (Table 1). The surfactant and cholesterol were combined with 0.5 mL absolute ethanol in a beaker, and then 0.8% of Terconazole was added. The beaker was then covered to prevent solvent loss and heated in a water bath (55°C–60°C) about 5 minutes while shaking until the solvent was completely dissolved.0.15 ml of distilled water (55°C–60°C) was applied and allowed to warm in the water bath for 5 minutes, yielding a translucent solution. The dispersion was turned to gel by allowing the fluid to cool.

**Table1: Composition of different terconazole proniosomal formulations using different nonionic surfactants with different ratios of surfactant: cholesterol.**

<table>
<thead>
<tr>
<th>Code</th>
<th>Span20 (%)</th>
<th>Span60 (%)</th>
<th>Tween80 (%)</th>
<th>Cholesterol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>90</td>
<td>-</td>
<td>-</td>
<td>70</td>
</tr>
<tr>
<td>F2</td>
<td>-</td>
<td>90</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>F3</td>
<td>45</td>
<td>-</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>F4</td>
<td>-</td>
<td>45</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>F5</td>
<td>70</td>
<td>-</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>F6</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>F7</td>
<td>35</td>
<td>-</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>F8</td>
<td>-</td>
<td>35</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>F9</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>F10</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>F11</td>
<td>25</td>
<td>-</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>F12</td>
<td>-</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

Each formula contains 0.8% terconazole.

**Evaluation of the prepared Proniosomal gels formulations:**

**pH measurement:**

A digital pH meter was used to determine gel pH of (3310, Jenway, UK). After dissolving 0.1g of gel in 10ml of distilled water, the electrode was dipped into the gel and reading was taken. Triplicate determinations for each formulation were done, mean ±SD was deduced x12.

**Polydisperse (PS), Polydispersity Index (PDI), and Zeta potential analysis (Q) of terconazole proniosomes:**

The **particlesize (PS), Polydispersity Index (PDI), and Zeta potential analysis (Q) of terconazole proniosomes:**

Polydisperse index (PDI) was identified for assessing size of particle size distribution and the homogeneity of the proniosomal vesicles. Zeta potential was also determined to confirm the stability of proniosomes. Each investigation was resolved in triplicate.

**Determination of entrapment efficiency (EE%):**

In glass tube, 0.5 g of gel was placed, along with 10 ml phosphate buffer (pH 5.5). In a sonicator, this aqueous solution was sonicated, followed by centrifugation at 15,000 rpm at 20°C for 30 min (Ultracentrifuge, Eppendorf, Germany). Supernatant was collected and tested for unentrapped terconazole content particles using an ultraviolet (UV) spectrophotometer (Shimadzu UV spectrophotometer, Japan) at λmax 227 nm.

\[
EE\% = \frac{\text{Totalamountofdrug} - \text{Unentrappeddrug}}{\text{Totalamount of drug}} \times 100
\]

**In-Vitro drug release study:**

Membrane diffusion technique was used to measure the release of terconazole from various proniosomal gels. The 20 mg of terconazole proniosomal gel was kept in a glass tube with a diameter of 2.5 cm and length of 10 cm that had previously been covered with a presoaked cellulose membrane has a molecular weight cutoff of 12,000Daltons. The lower end of the gel tube has just brushed the surface of the diffusion medium, which is 100 ml phosphate buffer (pH 5.5) at 37°C with a magnetic stirrer agitated at 100 rpm. Aliquots of three-milliliter sample were taken at a different time interval (0, 0.5, 1, 2, 4, 6, 8 and 12 h) and replaced with equal volume to keep the constant volume of the receptor’s phase. Using a UV spectrophotometer, the drug concentration in the collected samples was determined at max 227 nm. The formulas with the highest EE percent and the maximum in vitro drug release were candidates for further investigation.

**Transmission electron microscopy (TEM)**

The optimized formula will be visualized using a transmission electron microscope (TEM, Jeol – JXA-840A – Electron Microscope – Japan). Prior to analysis, the sample was diluted with dist. water 10 times. A drop of resulting suspension was dropped on a copper grid that had been film-coated, generating a thin liquid film. Which were then stained negatively using a phosphotungstic acid solution containing 2% (w/v) phosphotungstic acid. After air drying, stained films were photographed by transmission electron microscopy.

**Stability studies**

Physical stability for the optimised formula was tested by storing it in well-closed bottles in the refrigerator three months at 4°C1°C. Physical appearance, percent EE, PS, and ZP were all considered in the stability study.
Percentages of EE, PS, and ZP were tracked versus storage time.

**In vitro evaluating antifungal activity**

The antifungal activity was investigated for the selected formulation, which demonstrated the highest EE percent and extended drug release, was evaluated using *Candida albicans* strain ATCC 10231.

**Preparation of standard inoculums**

The yeast from a 24-hour culture on Sabouraud dextrose agar was used to make the inoculum, which was suspended in a sterile saline solution (0.85%). Using a spectrophotometer set to 530nm, the turbidity of the culture was adjusted to match the final concentration of a 0.5 McFarland standard (0.5–2.510³). At 37°C, the bacteria were grown in nutritional agar broth.

**Preparation of drug dilutions**

The chosen formula was diluted with the blank solution of saline solution (0.85%) to meet the final concentration of 256, 128, 64, 32, 16, 8, and 4 µg/ml. Normal saline was used as a blank.

**Determination of Minimum Inhibitory Concentration (MIC) using Agar Well diffusion method**

The MIC of Terconazole proniosomal gel against *C. albicans* strain were determined by the agar well diffusion method where 20 ml of Sabouraud dextrose agar were melted, cooled to 55°C and then inoculated with 1ml of the organism suspension. The inoculated agar was poured into a 15 cm diameter assay plate and set aside to cool on a flat surface. Eight wells, each 10 mm diameter, were cut out of the agar once the media had solidified, and 200 µl of each seven drug concentrations and blank solution were placed into each well, three plates were used for triplicate repetition and incubated at 35°C for 24 hours. Results were analyzed using the same criteria for fluconazole NCCLS disc diffusion breakpoints were used for the interpretative breakpoints.

**Statistical analysis:**

One-way analysis of variances was used to establish significance of difference between groups in statistical analysis; P 0.05 was considered as statistically significant.

**RESULTS**

As indicated in Table 2, pH of proniosomal formulations was within the physiological range of topical administration, ranging from 5.5 to 6.9.

### Table 2: Physical appearance, pH, particle size (PS), polydispersity index (PDI), Zeta potential analysis (ζ) and the entrapment efficiency percentage of terconazole in different proniosomal gels.

<table>
<thead>
<tr>
<th>Code</th>
<th>Physical appearance</th>
<th>pH</th>
<th>Particle Size±SD (nm)</th>
<th>PDI±SD</th>
<th>Zeta potential analysis (ζ)</th>
<th>EE%±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Viscous gel</td>
<td>5.5</td>
<td>33.47±1.07</td>
<td>0.23±0.023</td>
<td>-22.1</td>
<td>89.64±0.98</td>
</tr>
<tr>
<td>F2</td>
<td>White creamy gels</td>
<td>5.6</td>
<td>19.80±1.10</td>
<td>0.10±0.003</td>
<td>-24</td>
<td>91.37±1.09</td>
</tr>
<tr>
<td>F3</td>
<td>Pale yellow creamy gels</td>
<td>6.2</td>
<td>30.10±1.63</td>
<td>0.27±0.150</td>
<td>-19.6</td>
<td>85.43±1.99</td>
</tr>
<tr>
<td>F4</td>
<td>White creamy gels</td>
<td>6.0</td>
<td>23.41±0.51</td>
<td>0.16±0.050</td>
<td>-30</td>
<td>95.66±1.24</td>
</tr>
<tr>
<td>F5</td>
<td>Pale yellow viscous gel</td>
<td>5.6</td>
<td>31.20±0.67</td>
<td>0.22±0.055</td>
<td>-21.6</td>
<td>87.56±1.94</td>
</tr>
<tr>
<td>F6</td>
<td>White creamy gels</td>
<td>6.2</td>
<td>20.40±0.94</td>
<td>0.20±0.013</td>
<td>-23.9</td>
<td>88.24±1.02</td>
</tr>
<tr>
<td>F7</td>
<td>Pale yellow viscous gel</td>
<td>6.1</td>
<td>30.52±0.57</td>
<td>0.13±0.021</td>
<td>-22.9</td>
<td>90.21±1.32</td>
</tr>
<tr>
<td>F8</td>
<td>White creamy gels</td>
<td>5.5</td>
<td>28.45±0.74</td>
<td>0.26±0.028</td>
<td>-27.3</td>
<td>83.14±1.07</td>
</tr>
<tr>
<td>F9</td>
<td>Pale yellow viscous gel</td>
<td>5.9</td>
<td>33.54±0.41</td>
<td>0.22±0.019</td>
<td>-20.6</td>
<td>87.74±1.54</td>
</tr>
<tr>
<td>F10</td>
<td>White creamy gels</td>
<td>5.9</td>
<td>29.20±0.67</td>
<td>0.28±0.150</td>
<td>-23.6</td>
<td>86.24±1.02</td>
</tr>
<tr>
<td>F11</td>
<td>Pale yellow viscous gels</td>
<td>6.9</td>
<td>30.40±0.94</td>
<td>0.17±0.060</td>
<td>-23.9</td>
<td>88.21±1.32</td>
</tr>
<tr>
<td>F12</td>
<td>White creamy gels</td>
<td>6.4</td>
<td>32.52±0.57</td>
<td>0.23±0.053</td>
<td>-21.9</td>
<td>81.14±1.07</td>
</tr>
</tbody>
</table>

*SD: Standard deviation

The Particle size of the prepared proniosomal gel was found to be in the nanosize range (19.80–33.54nm). The vesicles were distinct and separated without aggregation [Figure2]. It was observed that vesicles prepared of span 60 show the smallest size than vesicle prepared of span 20.

The entrapment efficiency percent of terconazole formulations are clarified in Table 2. F4 (span 60: tween 80: cholesterol) and F2 (span 60: cholesterol) resulted in the highest entrapment efficiency(95.66% and 91.37%, respectively).

Figure 1 showed the rate of terconazole release from certain proniosomal gel having EE% greater than 88% (F4, F2, F7, F1, F6, and F11: 95.66, 91.37, 90.21, 89.64, 88.24, and 88.21% respectively). At P< 0.05, the proniosomal gel formulation (F4) had the highest EE percent and the longest drug release, making it the best formula. It’s possible that the slower release of terconazole from proniosomal prepared formulations
compared to a manufactured control gel (1.5 percent HPMC containing 0.8 percent w/w terconazole) is due to drug encapsulation in vesicles, which allows for a longer drug release rate. The proniosomal gel (F4) that was selected revealed. At P 0.001, the selected proniosomal gel (F4) demonstrated a significantly longer drug release of 50.2 percent 5.25 percent after 6 hours, compared to 95.11 percent 2.11 percent for the control.

Figure 2 showed that the optimized proniosomal formula (F4) was nanosized, smooth, nanovesicles with sharp edges and homogeneous nanovesicles.

The selected optimum formulation was subjected to a stability test. It was kept at 4°C 1°C for 3 months and the PS of the vesicles, ZP, and percent EE were measured.

The physical look remained unchanged. Furthermore, the EE percent, PS, and ZP of the terconazole proniosomal gel did not alter significantly at P > 0.05 [Table 3].

Table 3: Particle size, zeta potential, and percentage entrapment efficiency determined for the selected terconazole proniosome formulation when fresh and after storage in the refrigerator for 3 months at 4°C

<table>
<thead>
<tr>
<th>Form</th>
<th>PS (nm)±SD</th>
<th>ZP (ζ)</th>
<th>EE% ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>At1 Month</td>
<td>At3 Months</td>
</tr>
<tr>
<td>F4</td>
<td>23.41 ±0.51</td>
<td>24.51 ±1.51</td>
<td>26.14 ±1.64</td>
</tr>
</tbody>
</table>
The susceptibility results were analyzed for the proniosomal formula to detect its MIC using the same criteria for fluconazole. The diameters of the clear zone of inhibition of growth were illustrated in Table 4 and interpretation of results according to NCCLS criteria (document M60), where zone of inhibition ≥19 mm is susceptible, 18—13 mm susceptible dose dependent and ≤12 is resistance.

### DISCUSSION

Spans are lipophilic surfactant, it forms smaller vesicles to minimize the surface free energy. By increasing ZP the vesicle repulsion has improved, preventing reaggregation and ensuring the system's electrical stability.

Span60 has the greatest phase transition temp (50°C) and entrapment effectiveness percent. Hydration temperature which used to form niosomes should normally be higher than the system's gel–liquid phase transition temperature, resulting in less leaky niosomes with high entrapment efficiency. The lower the surfactant's HLB, meaning better drug entrapment efficiency also stability, as seen in proniosomes made using span 60.

The vesicular structure, which is made up of span 60, tween 80, and cholesterol and is thought to represent a significant barrier to drug diffusion and release. The period required for the hydration of proniosomes into niosome vesicles before initiating the release of the medication over the cellophane membrane is referred to as the delayed release of proniosomal gel formulations.

Because of its high phase transition temperature and low permeability, the non-significant change in size and EE percent in the span 60 surfactant-based formulation can be explained. This research demonstrated proniosomal gel's exceptional stability and appropriateness for topical terconazole administration.

The MIC of proniosomal Terconazole against C. albicans was found to be 32 µg/ml which considered to be equivalent to predetermined and published data of market terconazole cream 0.8% which had MIC of 128 µg/ml against C. albicans.

### CONCLUSION

The coacervation phase separation approach would be used to successfully prepare a terconazole-loaded proniosomal gel. The entrapment effectiveness of terconazole in the produced proniosomal gel ranged from 95.6 to 83.1 and PSs were in the nanosize range (19.80–33.54nm). TEM reveals the formation of smooth and spherical vesicles after hydration.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

### Acknowledgments

The authors are grateful to Faculty of Pharmacy, Fayoum University for the assistance in providing all the used materials and equipment. Also, authors are grateful for Pharco, Egypt for supplementing Terconazole raw material.

### REFERENCES


15. Clinical and Laboratory Standards Institute, C, CLSI supplement M60, in Performance standards for antifungal susceptibility testing of yeasts2017, Wayne PA.


