Olive Oil Based Organogels for Effective Topical Delivery of Fluconazole: \textit{In-vitro} Antifungal Study

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\textbf{Authors' contributions}

This work was carried out in collaboration among all authors. Author MMA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MMA, FF and AB managed the analyses of the study. Author MMA managed the literature searches. All authors read and approved the final manuscript.

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\textbf{ABSTRACT}

The objective of the study was to formulate olive oil based organogels for the topical application of fluconazole (FLZ), to ensure the efficient delivery of the drug deeper in to the skin layers.


\textbf{Results:} The results of evaluated parameters ensure the stability and effectiveness of the prepared olive oil based organogels. \textit{In-vitro} diffusion studied reflects decrease in drug release with increase in surfactant concentration due to increase in viscosity. Moreover, ex-vivo permeation studies revealed that the permeation of FLZ was enhanced for optimized formulations (F6) as compared to the marketed gel formulation. Further, the optimized formulation exhibits the broad zone of inhibition against fungal strains in comparison to control and marketed product during \textit{in-vitro} antifungal study.

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Conclusion: The olive oil based organogels formulation shown the enhanced permeation of FLZ from organogel network structure with good antifungal activity as compared to the marketed formulation. Henceforth, the FLZ organogel formulations could be used topically for the effective treatment of fungal infection.

Keywords: Fluconazole; olive oil; organogels; In-vitro antifungal activity; ex-vivo permeation.

1. INTRODUCTION

The prevalence of fungal infections has been increased globally, around three hundred million people affected by superficial fungal diseases and twenty million humans survive with high morbidity. Fungal infection reduces immune of the patients and its most vulnerable disease especially in developed and underdeveloped countries. There are many antifungal marketed formulations available as topical creams or ointment. Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different types of topical antifungal chemical moieties used in treatment of a variety of dermatological skin infections. Most of the antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions and sprays, which have several limitations such as low skin penetration, poor spread ability and poor drug release from the formulations [1].

Fluconazole (FLZ) is Triazole derivative, practically insoluble in water has the antifungal activity with a broad-spectrum activity against pathogenic fungi. It may act by interfering with permeability by inhibiting the fungal cytochrome P450 enzyme 14α-demethylase enzyme responsible for the synthesis of ergosterol the main sterol in the fungal cell membrane. It is topically active and only rarely administered parenterally due to its extensive first-pass effect and toxicity. Oral administration of FLZ causes nausea, vomiting, diarrhea. FLZ universally used in treatment of several systemic fungal infections including candidiasis [2]. The efficiency of the topical antifungal treatment depends on the penetration of drugs through the target tissue. Conventional topical formulations failed to deliver the drug deeper into the skin layers by crossing the epidermal horny layer, therefor the fungal infection relapses with a cycle of 2-3 months. In this context, organogels may play a major role for enhanced penetration of drugs deeper into the skin.

Organogel defined as the cross-linked soft-matrix incorporated with large amount of organic/lipophilic solvent inside its matrix. Organogels soft-matrix systems are applied for topical delivery systems, when the active agent is oil soluble or if we need the sustained release of the drug into the deep skin layers [3]. In the present study, olive oil used as oil phase and sorbitan monostearate (SMS) act an organogelator to produce organogel [4]. Olive oil based organogels could be formulated to enhance the release and to provide more sustained topical antifungal effect. Olive oil have huge beneficial effects on human health due to its antioxidant properties [5].Olive oil contains oleic acid as a major component with excellent antimicrobial and antifungal properties with enhanced permeation property for several topical drugs.

The objective of the study was to develop the olive oil based organogels for the effective topical delivery of fluconazole. Prepared organogels evaluated for permeation enhancement and efficiciveness against the pathogenic fungal strains.

2. MATERIALS AND METHODS

Olive oil and Span 60 obtained from Merck Specialties. FLZ obtained as a gift sample from Al-Jazira Pharmaceuticals., Riyadh, Saudi Arabia. All other reagents and chemicals used were analytical grade.

2.1 Preparation of Olive Oil Based Organogels

Olive oil based organogels were prepared by hot-melt technique [6]. Formulations were prepared by varying percentage concentration of olive oil and organogelator, nine formulations were prepared by maintaining constant FLZ concentration, composition shown in Table 1. The required quantity of sorbitan monostearate (Span 60) was taken in the beaker and heated at 60°C on a thermostatically controlled magnetic stirrer (Model: RT touch series, Thermo Fischer Scientific, USA) at 400 rpm. FLZ dispersed in olive oil and added to the heated SMS with
continuous stirring until the homogeneous dispersion achieved. The formulations were stored at room temperature (RT) for 24 hours and subjected for macroscopic and other evaluations.

**Table 1. Preparation of organogels by hot-melts technique**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>SMS</th>
<th>OO up to</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>F2</td>
<td>1</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>F3</td>
<td>1</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>F4</td>
<td>1</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>F5</td>
<td>1</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>F6</td>
<td>1</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>F7</td>
<td>1</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>F8</td>
<td>1</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>F9</td>
<td>1</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

*SMS: Sorbitan monostearate
** OO: Olive oil

2.2 Evaluations of Olive Oil Based Organogels

2.2.1 Macroscopic evaluation

The prepared organogels were observed physically for their appearance, texture, and color.

2.3 Determination of Gelation Temperature

The prepared organogels observed for changes in physical state on exposing to the incremental heat and the temperature at which the gels transformed to solution state was noted as gelation temperature [3]. The organogels of about 10 g enclosed in the transparent screw cap tubes. The tube was placed in the water bath, kept on the thermostatically controlled hot plate (Model: Labwise, Thomas scientific, USA) with increment in the temperature of 1°C per minute. The temperature at which gel-sol transition taking place was noted which was observed by inverting the tube at 180° angle [7]. The experiment was performed in triplicate to observe the reproducibility of gelation temperature and the mean values with standard deviation were recorded for each formulation.

2.4 Drug Content

The organogels formulations equivalent 10 mg drug dissolved in methanol (100 mL) stirred for 2 hours, and then tested for drug content estimation. The sample pre-filtered by 0.45 μm syringe filters (Millx-HV syringe unit, Merck, USA) and diluted with methanol as required analyze by UV-visible spectrophotometer (Shimadzu, Japan) at 270 nm absorption maxima.

2.5 pH Determination

The pH of all the formulations assessed using pH meter (Edge pH series, Hanna instruments) previously calibrated with standard pH 4.0 and 7.0 buffer solutions. The glass electrode immersed into the organogel and pH values for all the formulations recorded in triplicate.

2.6 Spreadibility Studies

The spreadibility test performed by in-house fabricated tester based on the Muller and Moulter [8]. One gm of organogels placed between the two 5x20 cm glass plates. The sample under investigation was sandwiched between two glass plates and a bar of 50.00 gm was placed on the top plate for 40 s. The initial and final spreading diameters recorded and percentage of spreadibility was calculated by using Eqs.1.

\[
\% \text{Spreadibility} = \frac{D_2 - D_1}{D_1} \tag{1}
\]

Where; D1 represent initial diameter of gel before load, and D2 final diameter of gel after load.

2.7 Viscosity Measurement

Viscosities of organogels were measured by spindle S63 at 50 rpm using viscometer (Model: DV2T, Brookfield, USA) by maintaining constant temperature of 25°C. [7] The gel samples were allowed to settle over 15 min before the viscosity measurement.

2.8 In-vitro Drug Permeation and Ex-vivo Flux Studies

In-vitro drug permeation study were performed using the Franz diffusion cell apparatus (EMFDC-6, USA) consisting of donor and acceptor chamber separated by Wister rat abdominal skin (shaved) as semipermeable membrane [9,10]. The donor chamber added with 1 gm organogel, acceptor chamber filled with skin pH 6.4-phosphate buffer. The accepter solution stirred at 50 rpm and kept at 37±0.5°C. Aliquots (1 mL) from acceptor chamber withdrawn at predetermined time intervals and replaced with the same volume of fresh phosphate buffer (pH 6.4) to maintain the sink
conditions. Cumulative % drug released was analyzed by UV- spectrophotometry at $\lambda_{\text{max}}$ 270nm. Furthermore, the flux and permeability coefficient were calculated by cumulative drug permeated per unit area Vs time, slope of which represents flux, permeability coefficient and enhancement ratio was determined by using following equations [11,12].

$$P = \frac{J_{ss}}{C_0}$$  \hspace{1cm} (2)

$$ER = \frac{J_{ss} \text{ of FLZ organogels}}{J_{ss} \text{ of Marked product}}$$  \hspace{1cm} (3)

Where $P_{\text{app}}$ represents apparent permeability coefficient, $C_0$ is concentration of FLZ in donor compartment, $J_{ss}$ designated for steady-state flux and $ER$ entitled for enhancement ratio respectively.

2.9 Antifungal Studies of FLZ Organogels

Antifungal activity of optimized organogel formulation was evaluated by cup-plate method using Sabouraud dextrose agar media. Selected organisms smeared on solidified media then the formulation filled in 1 cm diameter cup bored in the medium. Optimized formulation (F6), pure drug FLZ, control (formulation without drug) and marketed FLZ product were assessed for their effectiveness against the selected fungi strains (Candida albicans, and Aspergillus Niger). The zone of organisms’ growth inhibition inspected after incubation of petri-plates at controlled temperature 25°C for about three days [12].

2.10 Accelerated Thermal Stability Studies

The accelerated stability studies were performed for 2 months by thermos-cycling method. F6 optimized organogel subjected to repeated freeze thaw cycles at 20°C and 65°C using incubator for 15 minutes. The study was repeated for 5 freeze thaw cycles, following which physical parameters examined and diffusion study was performed the effects of temperature during storage conditions was presented by using similarity index of diffusion study for before and after thermal exposure. Similarity factor $f_2$ was calculated by Moore, et al equation [13,14].

$$f_2=50+\log \left[\left(1+(Rt-Tt)^{1/n}\right)-0.5\right]$$ \hspace{1cm} (4)

Where: $n$ is time points number, $R_t$ and $T_t$ are diffusion value of test and reference organogels at predetermined time interval.

3. RESULTS

3.1 Macroscopically Evaluation

Macroscopic / Physical properties of all F1-F9 formulations were satisfactory without any grittiness, prepared organogels were homogenous, smooth, transparent/translucent and yellowish white color.

3.2 Determination of Gelation Temperature

Sol-gel transition of organogels shown in Fig.1 represents cessation of organogel flow upon inversion of tube due to sol-gel transition. The results showed in Table -2 determines the temperature required for phase transitions, least temperature noted for F1 and maximum was for F9, increase in the concentration of SMS leading to resistant to flow and ensures the compatibility and effectiveness of the prepared olive oil based organogels. Temperature of gel-sol transition was ranged from 50±02°C to 65±01°C.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>pH</th>
<th>Spreadibility (g.s/cm)</th>
<th>Viscosity (cps)</th>
<th>Drug content (%)</th>
<th>T gel-sol (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>6.8±01</td>
<td>18</td>
<td>345</td>
<td>99.98±0.21</td>
<td>50±02</td>
</tr>
<tr>
<td>F2</td>
<td>6.3±03</td>
<td>12</td>
<td>750</td>
<td>99.65±0.25</td>
<td>52±03</td>
</tr>
<tr>
<td>F3</td>
<td>6.4±05</td>
<td>8</td>
<td>1020</td>
<td>99.45±0.45</td>
<td>54±04</td>
</tr>
<tr>
<td>F4</td>
<td>6.0±01</td>
<td>6</td>
<td>1200</td>
<td>99.45±0.21</td>
<td>55±01</td>
</tr>
<tr>
<td>F5</td>
<td>6.4±08</td>
<td>5</td>
<td>1700</td>
<td>99.56±0.23</td>
<td>58±03</td>
</tr>
<tr>
<td>F6</td>
<td>6.0±02</td>
<td>5</td>
<td>1850</td>
<td>99.14±0.14</td>
<td>60±04</td>
</tr>
<tr>
<td>F7</td>
<td>6.6±01</td>
<td>3</td>
<td>1915</td>
<td>99.25±0.31</td>
<td>62±02</td>
</tr>
<tr>
<td>F8</td>
<td>6.0±09</td>
<td>3</td>
<td>2035</td>
<td>99.13±0.78</td>
<td>64±01</td>
</tr>
<tr>
<td>F9</td>
<td>5.4±08</td>
<td>2</td>
<td>2200</td>
<td>99.34±0.54</td>
<td>65±01</td>
</tr>
</tbody>
</table>

All the experiments were done in triplicate
3.3 pH Determination

The pH ranged from 5.4±0.8 to 6.8±0.1 indicates the skin compatibility of all the nine formulations.

3.4 Drug Content

The drug content uniformity was within the desirable limits of (+/- 5%) as per pharmacopeial limits for all the prepared formulations. Quantitative spectrophotometric analysis showed the % drug content in all formulation was ranged from 99.13±0.78 to 99.98±0.21.

3.5 Spreadibility Studies

The results of spreadibility of organogels were presented in Table 2, ranged from 2-18 g.s/cm and the results shown that the spreadibility decreased significantly with the increase of olive oil concentrations.

3.6 Viscosity Measurement

The rheological study showed range of 345-2200 cps viscosity for formulations F1 to F9 respectively. F1-F3 formulation were less viscous as compared to F6-F9.

3.7 In-vitro Drug Diffusion and Ex-vivo Permeability- Flux Studies

The results of in-vitro drug diffusion studies were presented in Fig. 2. The results of F6 revealed that maximum in-vitro cumulative amount ≥93.46± 25% diffused in 8 h. Formulation F6 composed of 1% FLZ, 60% SMS and upto 100% OO (olive oil), further increase in GA concentration (> 60% of SMS) decreases the drug release. From the in-vitro drug diffusion studies it was found that the permeation of FLZ is more for F6 formulation as compared to the marketed FLZ gel formulation. Ex-vivo permeability- flux studies was performed to evaluate drug permeability of FLZ through the wistar albino rats skin. The permeation study revealed the enhanced permeation of F6 organogel of FLZ. The cumulative FLZ permeated across the skin was calculated and presented in Table-3. The ex-vivo data, showed the parameters that confirms effective delivery of FLZ by the F6 organogels as compared to the marketed fluconazole topical gel. The cumulative amount of FLZ permeated from marketed gel to F6 organogel was found to be 2100± 24 µg and 4400±38 µg, respectively. The Enhancement ratio was above 1 for F6 and indicates enhanced permeability compared to marketed gel formulation.

3.8 Antifungal Studies of FLZ Organogels

The antifungal activity of FLZ from its different formulations compared with marketed gel formulation on different fungal stains were reported in Table 4. The order of inhibition was found to be pure drug, control, marketed and optimized F6 formulation. F6 organogels found to be more effective against C. albicans, and A. Niger.

3.9 Accelerated Thermal Stability Studies

Accelerated thermal stability studies was performed in different freeze thaw cycles at different temperatures. The optimized formulation F6 was then assessed for physicalparameters and diffusion study under the same conditions as
mentioned in the diffusion study section. The results of cumulative drug released before and after stability test was compared using similarity index. The \( f_2 \) value was found to be 51.91 similarity index from the drug profiles of reference (before freeze thaw cycles) and release profile after freeze thaw cycles of test showed in Fig. 3. Based on the \( f_2 \) value which was more than 50 the F6 said to be a stable formulation and doesn't showed any significant environmental impact on the drug release.

**Table 3. In-vitro permeation studies**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>CAP (g)</th>
<th>( P_{app} \left(10^{-6} \text{ cm/sec}\right) )</th>
<th>Slope</th>
<th>Enhancement ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marketed</td>
<td>2100</td>
<td>5.23</td>
<td>160</td>
<td>-</td>
</tr>
<tr>
<td>F6</td>
<td>4550</td>
<td>32.41</td>
<td>250</td>
<td>1.56</td>
</tr>
</tbody>
</table>

*All the experiments were done in triplicate*

![Fig. 2. Cumulative % fluconazole released vs time profile of diffusion studies](image)

![Fig. 3. Cumulative % fluconazole released vs time profile of stability similarity studies](image)
Table 4. Comparative in-vitro-antifungal studies in selected strains

<table>
<thead>
<tr>
<th>Fungal Strain</th>
<th>Zone of inhibition (mm)</th>
<th>Pure drug</th>
<th>Control</th>
<th>Mkd gel</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>10±1.23</td>
<td>11±1.54</td>
<td>16±1.34</td>
<td>20±1.56</td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>12±1.36</td>
<td>14±2.65</td>
<td>18±2.43</td>
<td>19±2.4</td>
<td></td>
</tr>
</tbody>
</table>

4. DISCUSSION

Macroscopic / Physical properties of all F1-F9 formulations were satisfactory without any grittiness and lack of homogeneity. The sol-gel assessment revealed that transition temperature increased significantly with the decrease of olive oil proportion. The results of spreadibility showed the structural integrity of organogels after the application of load with optimum gel spreadibility. F6 organogel has the property to smeared over the affected skin. Viscosity of formulation increased with the decrease of olive oil concentration. The consistency of organogels increased with increasing concentration of SMS. The in-vitro drug release graph and permeation studies exposed SMS acts as permeation enhancer as well as gelation agent (GA). Increase in GA concentration decreases the drug permeation, which might be due to the extensive formation of network like structure of gel with high viscosity. The Enhancement ratio for F6 and indicates enhanced permeability as compared to marketed gel formulation. The antifungal activity measurement results revealed that the F6 organogel formulation has maximum inhibitory activity against the fungi strains (Candida albicans and Aspergillus Niger) as compared to marketed gel formulation. The results of antifungal activity support and in agreement with the results obtained from the in-vitro permeation studies. Similarity factor f2 value closer to 100 and more than 50 said to be similar as per FDA guidelines. Therefore F6 formulation found to be stable without any significant variation in the diffusion results at different storage conditions.

5. CONCLUSION

Fluconazole an imidazole derivative found to be effective against the pathogenic fungal strains and used to treat superficial mycoses. Organogels composed of organic/lipophilic component and organogelator Sorbitan monostearate (SMS) increases the drug permeability of drug. Olive oil also acts as antifungal and biocompatible with skin. Olive oil based organogels could be biocompatible, enhanced permeability, may goes deeper into the skin layer to eradicate fungi in comparison to marketed product and stable for 2 months. Henceforth F6 organogel found optimized and could be used for the effective treatment of superficial fungal infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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