Formulation and *In Vitro* Evaluation of Fluconazole Loaded Nanoemulgel Against *Candida albicans*

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**Abstract**
Fungal infections have grown as a threat to the health of human beings over the past few years. In healthy state the human body houses many commensal fungal species, *Candida albicans* being one of them. In human beings who are healthy, the fungus is present in the body without causing any disease. However, the same fungus has potential to proliferate and produce a disease under diverse conditions. As per reports, presence of Candida species accounts for the most prevalent infection in surgical patients who are critically ill. A number of therapeutics have been used for treating candidal infections. Among them, Fluconazole is reported to be effective. Fluconazole is available commercially either as tablets or for IV administration for the treatment of infections occurring by Candida infestation. However, both the dosage forms formulated suffer from certain disadvantages. In order to overcome their drawbacks, in the present study, Fluconazole loaded nanoemulsion based gel was formulated and its efficacy evaluated as an attractive alternative treatment option for Candida infections. *In vitro* efficacy of Fluconazole loaded nanoemulgel, placebo formulation, fluconazole gel and control (pure fluconazole) against *Candida albicans* (MTCC NO: 227) was compared using cup-plate method microbiological assay method. The
nanoemulgel was formulated by addition of carbopol 934P (1% w/w) in optimized Fluconazole loaded nanoemulsion. A significantly higher (p≤0.05) zone of inhibition was observed for Fluconazole nanoemulgel (35±1.1 mm) in comparison to fluconazole gel (15±1.2 mm), placebo formulation (9±1.6 mm) and control (9.8±1.2 mm) after 24 hours. Further, after 72 hours, the Fluconazole nanoemulgel exhibited zone of inhibition (34.9±1.6 mm) while the other three formulations (fluconazole gel, placebo formulation and pure fluconazole) did not exhibit any zone of inhibition. It was concluded that Fluconazole loaded nanoemulgel formulation due to its nanometricsize was effective in inhibiting the growth of Candida albicans more efficiently as compared to all the other formulations evaluated.

**Keywords:** Fluconazole, nanoemulgel, C. albicans

**INTRODUCTION**

The past few decades have witnessed a substantial increase in the advent of fungal infections affecting human beings. Despite the fact that fungal infections constitute a serious threat to human health, they have been underrated [1,2]. Fungal infestations account for approximately 15% of infections occurring in human beings [3]. Candida species are the most common fungi causing disease in human, C. albicans and C. glabrata being first and second in frequency of isolation, respectively [4]. Candida species account for 70–90% of all fungal infestations which are invasive [3] and is also reported to be the commonest pathogen causing fungal infections in majority of the clinical setups [5]. The morphological features of C. albicans have an important effect on several aspects of infection as well as on recognition of the host [6].

*C. albicans* can either cause superficial infections (examples include oral and skin candidiasis) or can result in systemic infections which can also be a serious threat to life. Since, *C. albicans* is present in 75% of the surface of mucosa even in healthy individuals; its outgrowth needs to be recognized in order to prevent the fungal disease [7].

Fluconazole, an imidazole derivative is reported to be effective against most of the pathogenic fungi present [8]. Among the triazoles, it is one of the most frequently prescribed drugs [9]. The drug not only exhibits good tolerance, but is also safe for the treatment of several infections due to low toxicity associated with its use [10]. It is currently reported to be the most preferred drug for treating AIDS patients with oropharyngeal candidiasis [11,12].

Fluconazole, an oral bis-triazole synthetic compound acts as an antifungal agent by inhibition of the cytochrome P450-dependent 14 alphademethylation step in production of ergosterol, which results in alterations in many membrane-associated cell functions.

The marketed formulations of the drug include tablets and IV formulation [13]. No local drug delivery system for application to oral mucosa for the treatment candidiasis is available commercially. The oral administration of the drug, although a convenient and patient compliant method of delivery of the drug, is associated with many side
effects, which include gastric distress, abdominal pain, first pass metabolism etc. Besides this, the drug is a BCS class II drug with low solubility (< 1 mg/ml) [14]. Therefore, in order to improve solubility, enhance penetration and reduce systemic side effects, there is necessity of the development of a nanoformulation of fluconazole for local application.

In the present study, Fluconazole loaded nanoemulgel was formulated for local delivery of the drug and its in vitro efficacy against Candida albicans was evaluated using cup plate method microbiological assay method and the results were compared with those obtained from placebo formulation, fluconazole gel and control (pure fluconazole). The treatment of fungal infections with oral fluconazolenanoemulgel gel would have several advantages including, drug targeting to the infection site, reduced potential for occurrence of side effects associated with the oral use of the drug, improved efficacy of treatment by improving its solubility and high patient compliance etc [15].

Materials and Methods
Nanoemulsion and Nanoemulgel components
Fluconazole was obtained as a gift sample from Ramson Remedies (Amritsar, India), Clove oil and Carbopol 934P were purchased from Sigma Aldrich Pvt. Ltd (Bangalore, India). Tween 20 was procured from Central Drug House, (New Delhi, India). All other chemicals/reagents were of analytical grade and purchased from Merck (Mumbai, India) and S.D. Fine Chem. (Mumbai, India).

Strain, growth media and culture conditions
Candida albicans (MTCC No. 227) was purchased from IMTECH (Institute of microbial technology, Chandigarh). Candida albicans was grown in suspension of YME (Yeast Malt Extract) and kept in a B.O.D incubator shaker. During the experiments, the viable cell concentrations present in suspension were checked by viable counts.

Formulation of Fluconazole loaded nanoemulsion based gel and placebo formulation using carbopol 934 P
The formulation of Fluconazole loaded nanoemulsion based gel (nanoemulgel) was prepared by using aqueous phase titration method. The formulation was prepared by adding gelling agent (carbopol 934 P) to the clove oil based fluconazole nanoemulsion formulation. The placebo formulation was also formulated by the same method but the drug was excluded.

In vitro antifungal activity
The study was performed as per the method reported by Maebashi et al 1995 and Vijayaet al 2014. 50 µl suspension was taken from the Candida albicans suspension (1x10^7 cfu/ml) and spread aseptically on plates of Sabouraud dextrose agar (SDA) using sterile cotton swab. After each application, the plates were rotated at 60° angle followed by pressing of the swab around agar surface edge. The plates were then dried with the lid closed at room temperature. After the plates dried, using sterile core borer, four wells (3mm diameter each) were punched into the agar medium and filled with 1 gram each of Fluconazole loaded nanoemulgel, fluconazole gel (0.5%w/w of
fluconazole), placebo formulation and pure fluconazole suspension respectively. Further, in order to allow the uniform diffusion of the drug, plates refrigerated for 2 hours. The plates were then incubated at 37°C, after which zone of inhibition around the four wells were recorded and compared [16,17].

**Results**

The zones of inhibition formed around the formulations on the S.D.A plate were measured. A zone of inhibition of 35±1.1 mm, 34.9 ± 1.4 and 34.9 ± 1.6 was observed for Fluconazole loaded nanoemulgel at the time interval of 24 hrs, 48 hrs and 72 hrs respectively. Fluconazole gel exhibited zone of inhibition of 15 ± 1.2; 8.2 ±1.3 ;00 ±1.2 at time interval of 24 hrs, 48 hrs and 72 hrs respectively. Placebo formulation demonstrated a zone of inhibition of 09 ± 1.6; 00 ± 1.3&00 ± 1.8 at 24 hrs, 48 hrs and 72 hrs time interval respectively; while control formulation exhibited zone of inhibition of 9.8 ± 1.2; 5.6 ± 1.5 and 00 ± 1.7 respectively at a similar time interval. The results of the study were expressed as mean± standard deviation (S.D). The Graph Pad Instat 3 software using two tailed paired t-test was used for the statistical analysis of the data obtained. Values at p≤0.05 were considered significant.

**Discussion**

**Preparation of Fluconazole loaded nanoemulgel and placebo formulation using carbopol 934P**

The fluconazole loaded nanoemulsion was formulated by aqueous titration method. Reports in literature suggest the antimicrobial potential of extracts and oils of aromatic plants and spices, in inhibiting the growth of microorganisms which are pathogenic [18,19].

Essential oils are composed of a mixture of volatile, natural as well as aromatic compounds produced by aromatic plants and are very commonly used in traditional medicine [20]. According to the number of isoprene units present, the essential oils are classified into two major types: monoterpenes and sesquiterpenes [21]. The use of essential oils is preferred as antimicrobial agents, since they have activity against a variety of microorganisms, besides having reduced side effects as compared to other chemically synthesized antimicrobial agents [22].

Among the various essential oils used as antimicrobial agent, Clove oil has been extensively explored by researchers due to its reduced side effects as well due to the presence of high content of essential oil (eugenol) [23]. Clove oil has been reported to inhibit the growth of yeasts, moulds and bacteria. The oil acts by altering the permeability of phospholipids present in the cell wall, thereby, inhibiting the growth of bacteria and different types of yeast [24]. The oil has also been reported to inhibit the growth of *Candida albicans* [25].

Based on the above reports, in the present study, for the formulation of nanoemulsion of fluconazole, clove oil was chosen as the oil phase. To the optimized nanoemulsion formulation, carbopel 934P was added as the gelling agent to produce the nanoemulgel formulation.
In vitro anti-fungal activity

Evaluation of the in vitro anti-fungal activity was done by using cup and plate microbiological assay. The zones of inhibition formed around the formulations on the S.D.A plate were measured. A significantly higher (p≤0.05) inhibition zone was observed for Fluconazole loaded nanoemulgel (35±1.1 mm) as compared to fluconazole gel formulation (0.5%w/w of fluconazole) (15±1.2 mm), placebo formulation (9±1.6) and control (9.8±1.2 mm) after 24 hours. The larger inhibition zone for Fluconazole nanoemulgel could be attributed to the nanosize of the drug present in the gel, which caused its greater SDA diffusion, thereby resulting in its greater penetration through fungal cell walls resulting in fungicidal activity. Besides this, the clove oil which is also reported to be a fungicidal agent due to its ability to change permeability of phospholipids present in the fungal cell membrane also could have contributed to enhanced zone of inhibition of fluconazole loaded nanoemulgel formulations as compared to other formulations evaluated. Further, even after 72 h, the Fluconazole loaded nanoemulgel exhibited zone of inhibition (34.9±1.6 mm) unlike the other three formulations fluconazole gel, placebo formulation and pure fluconazole which did not exhibit any zone of inhibition. From the results of cup-plate method microbiological assay method, it was concluded that the formulated fluconazole loaded nanoemulgel formulation exhibited fungicidal activity as compared to fluconazole gel, placebo formulation and pure fluconazole which exhibited fungistatic activity. The results are given in table 1, table 2 and figure 1.

Table 1: Zone of inhibition for different formulations against Candida albicans strain

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Formulation(s)</th>
<th>Zone of Inhibition (mm) mean ± S.D (n=3)</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td>MTCC No. 227</td>
<td>FluconazoleNanoemulgel</td>
<td>35 ± 1.1</td>
<td>34.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Fluconazole gel</td>
<td>15 ± 1.2</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Placebo formulation</td>
<td>09 ± 1.6</td>
<td>00 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Control (Pure Fluconazole)</td>
<td>9.8 ± 1.2</td>
<td>5.6 ± 1.5</td>
</tr>
</tbody>
</table>

Figure 1: Comparison of zones of inhibition for different formulations during in vitro anti-fungal activity against Candida albicans (MTCC No: 227)
Table 2: Observations of Zones of Inhibition for different formulations evaluated against strain of *Candida albicans* at different time intervals

<table>
<thead>
<tr>
<th>Incubation Time (hrs)</th>
<th>Observations of Zone of Inhibition for different Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Candida albicans (MTCC No. 227)</td>
</tr>
<tr>
<td>24</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>48</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>72</td>
<td><img src="image3.png" alt="Image" /></td>
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Where, FNG = Fluconazole nanoemulgel, FG = Fluconazole Gel, F = Pure Fluconazole Suspension, P= Placebo formulation
Conclusion: The zone of inhibition for Fluconazole loaded nanoemulgel (35 ± 1.1 mm) was found to be significantly higher (p≤0.05) as compared to fluconazole gel (15 ± 1.2 mm), placebo formulation(9±1.6 mm) and control (9.8± 1.2 mm) after 24 hours. Further, even after 72 h, the Fluconazole loaded nanoemulgel exhibited zone of inhibition (34.9± 1.6 mm) unlike the other three formulations ( fluconazolegel, placebo formulation and pure fluconazole) which didnot exhibit any zone of inhibition. From the results of cup-plate method microbiological assay method, it was concluded that the formulated fluconazole loaded nanoemulgel formulation exhibitedfungicidal activity as compared to the fluconazole gel, placebo and pure fluconazole which exhibitedfungistatic activity. The results prove that the formulated fluconazole loaded nanoemulsion has significantly higher antifungal activity against Candida albicans and therefore could serve as an attractive and potential option for the treatment of oral candidiasis.

Conflict of Interest: Nil

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References


