EVALUATION OF ANTIFUNGAL ACTIVITY OF CLOTRIMAZOLE MICROSPHERES WITH DIFFERENT POLYMERS AGAINST CANDIDA ALBICANS

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ABSTRACT

Background and Aim: Numerous controlled drug delivery systems have been used in management of fungal infections nowadays. One such approach is using microspheres as carrier for drugs. The aim of this study is to compare the antifungal activity of clotrimazole microspheres prepared with natural and synthetic polymers against candida albicans at different concentrations. 

Materials and method: Clotrimazole microspheres are prepared by using different polymers like hydroxy propyl methyl cellulose (HPMC), ethyl cellulose (EC), Poly lactic glycolic acid (50:50) (PLGA) and chitosan. Clotrimazole microspheres with all the polymers and clotrimazole pure powder were taken at different concentrations (1.5, 1, 0.5 % w/v) and tested against candida albicans by agar diffusion well punch method at 24 hours and 48 hours. Results: Clotrimazole pure powder (Cp) showed maximum mean inhibitory diameter (MID) of 36 ± 1.74mm at 1% w/v concentration at the end of 24 hours followed by clotrimazole microsphere with chitosan polymer (CMC) showed 20 ± 1.71mm at 1% concentration. Microsphere forms prepared with other polymers like HPMC,
EC and PLGA showed less MID compared to chitosan. After 48 hours MID of CMC was increased to 26.3 ± 1.13mm, whereas the MID of Cp did not change after 48 hours. **Conclusion:** Though the MID of CMC was less compared to Cp at 24 hours, it increased after 48 hours compared to Cp where there was no change, suggesting that the drug is diffusing slowly from the microsphere indicating that it can be used as controllable drug delivery system.

**KEYWORDS:** Microsphere, Polymers, Mean inhibitory diameter, Chitosan.

**INTRODUCTION**
A well designed sustained drug delivery system should address some of the problems associated with conventional therapeutic modalities. At the same time it should enhance the therapeutic efficacy of the given drug. Maximum therapeutic efficacy is obtained when the drug is delivered at the target site in optimum concentration with least amount of toxicity and minimal adverse effects. Numerous approaches are present to deliver the therapeutic drug to the target site in a controlled release fashion, one such promising approach is use of microspheres as carrier of drugs. Microspheres received a lot of attention not only for prolonged release of drug but also for targeting of anticancer drugs. [1]

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000 µm). Microspheres are sometimes referred to as micro particles. [1-2] They can be prepared from various natural and synthetic polymers, in which the drug is dispersed throughout the microsphere matrix. [3]

Candida infections receive increasing attention, presumably due to increased prevalence worldwide. The recognition that candida is an important pathogen has led to many laboratory studies. [4] Oral candidiasis is one of the most common opportunistic fungal infections, most commonly present in immune compromised patients. Though other species of candida have been isolated, candida albicans remains the major pathogen of oral candidiasis. [4, 5, 6] with the rising frequency of fungal infections, as well as increase of resistance to antifungal agents, it is imperative that clinical applicable antifungal activity or susceptibility testing should be available. [7] The present study is undertaken to evaluate the antifungal efficacy of clotrimazole microspheres prepared by using different polymers and compare the activity with pure clotrimazole against candida albicans microbiologically.
MATERIALS AND METHOD
The present microbiological study was done in Microbiology department of Kamineni Institute of Medical Sciences, Telangana state. Clotrimazole microspheres were prepared by modified wet granulation technique using different polymers like HPMC, EC, PLGA (50:50) and chitosan.

Microbiological study
Clotrimazole pure powder and clotrimazole microspheres with HPMC, EC, PLGA and Chitosan polymers were assayed for antifungal activity against candida albicans at various concentrations 0.5, 1, and 1.5%.

A sabouraud’s dextrose agar medium was prepared and poured into a sterile plate. Standard American type culture collection (ATCC) candida albicans strain 24433 was collected and incubated in prepared sabouraud’s dextrose agar medium and were stored at 4°C for sterility. After ensuring sterility, inoculum of the strain was prepared with peptone water and the turbidity of candida albicans inoculum was adjusted to 0.5 McFarland’s standard.

A total of 225 wells with a diameter of 6mm and 4 mm depth were punched into the agar plates with a sterile punch. Each form of clotrimazole at each concentration consisted of five plates of agar media with three punches in a plate with 15 wells in each form. Among 225 wells, 45 wells were filled with clotrimazole pure powder (Cp) at 0.5, 1 and 1.5% w/v with 15 wells at each concentration. 45 wells were filled with clotrimazole microsphere with HPMC polymer (CMH) at 0.5, 1, 1.5% w/v with 15 wells at each concentration. Next 45 wells were filled with clotrimazole microsphere with Ethyl cellulose polymer (CME) at 0.5, 1, 1.5% w/v with 15 wells at each concentration. Another 45 wells were filled with clotrimazole microsphere with PLGA (50:50) polymer (CMPLG) at 0.5, 1, 1.5% w/v with 15 wells at each concentration. Remaining 45 wells were filled with clotrimazole microsphere with Chitosan polymer (CMC) at 0.5, 1, 1.5% w/v with 15 wells at each concentration.

Plates were incubated at 37°C and mean inhibitory zone (MIZ) was measured in millimeters (mm) after 24 and 48 hours using a transparent ruler.

Results obtained in this study were calculated by using one way ANOVA and post HOC tests for comparing among all the five forms of clotrimazole.
RESULTS
Cp at 1% w/v has shown the maximum mean inhibitory diameter of 36 ± 1.74mm (mean ± SD) at 24 hours compared to all other forms of clotrimazole followed by CMC with 20 ± 1.71mm, CME with 17 ± 1.90mm, CMH with 13 ± 1.61mm, CMPLG 11 ± 1.24mm. At 48 hours Cp maintained almost the same MID whereas CMC MID has shown statistically significant increase to 26.1 ± 3.27mm compared to pure and other microsphere forms, CME with 20.7 ± 1.75mm, CMH with 14 ± 1.61mm, CMPLG with 13 ± 1.61mm (Table 1).

1% concentration of clotrimazole was found to be more efficient in inhibiting *C. albicans* compared to 0.5 and 1.5% concentration in both pure and microsphere forms.

Table 1: showing the (mean ± S.D) MID of pure and microsphere forms of clotrimazole with different polymers in mm at 24 and 48 hours.

<table>
<thead>
<tr>
<th></th>
<th>0.5%</th>
<th>1%</th>
<th>1.5%</th>
<th>0.5%</th>
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<tbody>
<tr>
<td>Cp</td>
<td>27.2 ± 2.40</td>
<td>36 ± 1.74</td>
<td>36.7 ± 1.78</td>
<td>27.2 ± 2.40</td>
<td>36 ± 1.74</td>
<td>36.7 ± 1.78</td>
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<tr>
<td>CMH</td>
<td>7.36 ± 2.27</td>
<td>13 ± 1.61</td>
<td>13.8 ± 1.38</td>
<td>8.16 ± 2.06</td>
<td>14 ± 1.61</td>
<td>14 ± 1.54</td>
</tr>
<tr>
<td>CME</td>
<td>11.1 ± 1.46</td>
<td>17 ± 1.90</td>
<td>17.3 ± 2.28</td>
<td>11.5 ± 1.66</td>
<td>20.7 ± 1.75</td>
<td>20.8 ± 2.56</td>
</tr>
<tr>
<td>CMPL</td>
<td>7 ± 0.98</td>
<td>11 ± 1.24</td>
<td>11.7 ± 1.31</td>
<td>8.04 ± 1.61</td>
<td>13 ± 1.61</td>
<td>13.3 ± 1.93</td>
</tr>
<tr>
<td>CMC</td>
<td>6 ± 1.30</td>
<td>20 ± 1.71</td>
<td>20.7 ± 1.75</td>
<td>6.24 ± 1.55</td>
<td>26.1 ± 3.27</td>
<td>26.3 ± 3.43</td>
</tr>
</tbody>
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MID – Mean inhibitory diameter; Cp – pure clotrimazole powder, CMH – clotrimazole microsphere with HPMC polymer, CME- clotrimazole microsphere with Ethyl cellulose; CMPL- clotrimazole microsphere with poly lactic glycolic acid polymer; CMC - clotrimazole microsphere with Chitosan polymer

DISCUSSION
In the past few decades, a worldwide increase in the incidence of fungal infections has been observed. The majority of clinically used antifungals have various drawbacks in terms of toxicity, efficacy and their frequent use has led to the emergence of resistant strains.[7] The challenge has been to develop other strategies for the treatment of candidiasis, and other fungal diseases considering the increase in opportunistic fungal infections.[8] There are many drugs for the treatment of fungal diseases; however there are a limited number of efficacious antifungal drugs.[9] Development of controlled drug delivery system is necessary to overcome such problems.

Clotrimazole is a synthetic imidazole having a broad spectrum of fungicidal activity, being effective against both dermatophytes and yeast like fungi.[10] has a very stable chemical
structure and is not inactivated by heat, light or acid and is not water soluble.\textsuperscript{[11]} The present study prepared clotrimazole microspheres using different polymers for sustained drug delivery and tested the anti-candida activity of microspheres at different concentrations of pure and microsphere forms of clotrimazole.

This study results suggested that Cp showed maximum MID at 1% concentration, compared to CMH, CME, CMPL, and CMC at 24 hours. Among microspheres with different polymers CMC showed maximum MID at 1% concentration at 24 hours.

At 48 hours, the MID of microsphere forms with all polymers increased compared to pure form, where there was no increase in MID at all concentrations (0.5, 1, 1.5%). Among microspheres, CMC exhibited maximum mean MID of $26.1 \pm 3.27$ mm at 1% concentration suggesting that 1% clotrimazole is effective in both pure and microsphere forms. The increase in MID of microsphere forms at 48 hours compared to pure form suggests that the drug is leaching slowly from the microsphere.

Clotrimazole at 1.5% concentration in both pure and microsphere forms showed very negligible increase in MID compared to 1% and it was statistically not significant. This is similar with the study of Pingo et al.\textsuperscript{[12]} who demonstrated that clotrimazole at 1% concentration by weight was effective in inhibiting the fungal growth. Increasing the concentration of the clotrimazole did not increase the degree of fungal inhibition.

In conclusion, clotrimazole microsphere with chitosan polymer exhibited better antifungal activity compared to other polymer made microspheres. Though pure form is exhibiting more antifungal activity at 24 hours, at the end of 48 hours microsphere showed increase in antifungal activity almost similar to pure form, suggesting that the drug is released slowly from the microsphere form and it can be used as sustained drug release system.

**Conflicts of interest:** Nil

**REFERENCES**


