Comparison of Difference Between Fluconazole and Silver Nanoparticles in Antimicrobial Effect on Fluconazole-Resistant Candida Albicans Strains

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1. Background

In recent years, morbidity and mortality are increased significantly by severe fungal infections (1). Candida species have been one of the most common pathogens responsible for fungal infections, which cause hospital-acquired sepsis with annually mortality rate of up to 40% (2). Opportunistic fungi cause fungal infections, especially in vulnerable people with special conditions such as pregnancy or HIV-positive and immune-compromised patients who need intensive treatment with broad-spectrum antibiotics (3-5). Nowadays most of the available effective antifungal agents are based on polyenes (amphotericin B), echinocandins (caspofungin, micafungin, and anidulafungin) and triazoles (fluconazole, itraconazole, voriconazole, and posaconazole) (6, 7). However, scientists are looking for new natural and inorganic antimicrobial agents. The recent research on metal nanoparticles showed that silver nanoparticles (nanosilver) exhibits lower toxicity to mammalian cells and higher toxicity to microorganisms.

2. Objectives

Regarding comparison of difference between antimicrobial effect of nanosilver and some antibiotic agents on Candida albicans, we compared the effect of nanosilver with fluconazole and their combination on collected fluconazole-resistant and fluconazole-sensitive C. albicans.
3. Materials and Methods

In this experimental study, 18 fluconazole-resistant *C. albicans* isolated from the patient’s blood at the Shiraz University of Medical Sciences, Shiraz, Iran, two fluconazole-resistant *C. albicans* isolated from the patient’s blood at the Mycology Laboratory of Tehran University, Tehran, Iran, and one standard sample (ATCC10261) were used. We studied effect of fluconazole, nanosilver, and their combination on these *C. albicans* samples. The yeasts were identified using conventional mycological procedures.

In first stage, to identifying the fluconazole minimal inhibitory concentration (MIC) we used Clinical and Laboratory Standards Institute (CLSI) (M27-A3) protocol “Broth micro dilution”, which included:

1. Preparation of fluconazole stock solution (2048 µg/mL; potency, 999 µg/mL) (Pars Daru, 99.95% potency): Briefly, 21.5 µg of fluconazole was dissolved in 500 µL of absolute ethanol, and 10 mL of distilled water was added. The stocks were stored in -20°C.

2. Preparation of RPMI 1640 Medium: Briefly, 1.73 g MOPS [3- (N-morpholino propane sulfonic acid)] was dissolved in 50 mL of RPMI1640 Medium after filtration and was stored in 4°C.

3. Preparation some yeast suspension: After 24 hours incubation at 35°C, five *C. albicans* colonies in PDA (Potato Dextrose Agar) medium were mixed in 5 mL of physiology serum. After 15 second of vortex, they were diluted by RPMI-1640 medium to 1/100 and after that 1/20; therefore, this suspension had 0.5 × 10³ to 2.5 × 10³ cells.

4. Preparation of serial dilution of fluconazole: Chosen plate had 12 wells; we added 1 mL of RPMI to each one and then added 1 mL of fluconazole stock (2048 µg/mL) to first well. After turning up and down, we infused 1 mL of this well to second well and went on to tenth well. Therefore, tenth well had 0.5 µg/mL of fluconazole. Eleven well had just 1 mL of RPMI (Negative control) and twelfth well as a positive control had 1 mL of RPMI and 100 µL of fungi suspension.

5. Identification of drug sensitivity tests: We added 100 µL of yeast suspension to each well except negative control, and incubated them at 35°C for 48 hours.

In second stage, we used this protocol to identify the nanosilver’s MIC:

1. Preparation of Nanosilver stock solution (64 µg/mL): Selected plate had nine wells. We added 1 mL of RPMI to each one and then added 1 mL of nanosilver stock solution (64 µg/mL) to first well. After turning it up and down, we infused 1 mL of this well to second well and went on to seventh well. Therefore, seventh well had 0.5 µg/mL of Nanosilver stock solution. Eighth well had just 1 mL of RPMI (Negative control) and ninth well, as a positive control, had 1 mL of RPMI and 100 µL of fungi suspension.

2. Preparation serial dilution of Nanosilver: Selected plate had nine wells. We added 1 mL of RPMI to each one and then added 1 mL of nanosilver stock solution (64 µg/mL) to first well. After turning it up and down, we infused 1 mL of this well to second well and went on to seventh well. Therefore, seventh well had 0.5 µg/mL of Nanosilver stock solution. Eighth well had just 1 mL of RPMI (Negative control) and ninth well, as a positive control, had 1 mL of RPMI and 100 µL of fungi suspension.

3. Preparation of yeast suspension: After 24 hours incubation in 35°C, five *C. albicans* colonies in PDA medium (diameter, 1 mm) were mixed in 5 mL of physiology serum. After 15 seconds of vortex, they were diluted by RPMI medium to 1/100 and after that 1/20; therefore, this suspension had 0.5 × 10³ to 2.5 × 10³ cells.

4. Preparation of serial dilution of fluconazole plus nanosilver: Chosen plate had 12 wells. We added 1 mL of RPMI to each one and then added 1 mL of nanosilver stock solution (4 µg/mL) to first well; After turning up and down, we infused 1 mL of this well to second well and went on to sixth well. Therefore, sixth well had 0.0625 µg/mL of Nanosilver stock. Seventh well had just 1 mL of RPMI (Negative control) and eighth well, as a positive control, had 1 mL of RPMI and 100 µL of fungi suspension. Then we added 1 mL of fluconazole (16 µg/mL) to each one except seventh and eighth wells.

5. Drug sensitivity tests: We added 100 µL of yeast suspension to each well except negative control, and incubated them at 35°C for 48 hours.

4. Results

Result of fungi static and fungicidal activities of fluconazole against *C. albicans* showed: 1) The MIC of fluconazole concentration for standard sample was 16 µg/mL (Table 1).
2) The growth of 20 fluconazole-resistant *C. albicans* was inhibited at MICs > 512 µg/mL (Table 1).

Result of fungi static and fungicidal activities of nanosilver against *C. albicans* showed: 1) The MIC of nanosilver for standard sample was 4 µg/mL (Table 2). 2) The MICs of nanosilver for 20 resistant *C. albicans* were 2 µg/mL (58%) and 4 µg/mL (42%).

Result of fungi static and fungicidal activities of nanosilver plus fluconazole (8 µg/mL) on *C. albicans* showed: 1) The combination had better inhibitory effect on the growth of standard *C. albicans* when MIC of fluconazole (8 µg/mL) was combined with MIC of Nanosilver (0.0625 µg/mL) (Table 3). 2) Results on 20 resistant *C. albicans* showed there are several MIC of nanosilver: 40% of resistant *C. albicans* samples grew on 0.25 µg/mL, 11% on 0.0625 µg/mL, 22% on 0.03125 µg/mL, and 27% had no growth on 0.03125 (Table 3).

### 5. Discussion

Between azoles, Fluconazole has excellent in vitro activity against *C. albicans* at a wide range of body sites and tissues (24, 25). In addition, fluconazole is effective against some other *Candida* species, including *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* (25, 26). While the most important problem in treatment by the chemical antimicrobial agents is multidrug resistance, silver, as an antimicrobial agent in various fields, has brought some hope (17). It is well known that inorganic drug such as silver ions and its compounds have strong antimicrobial effects (27). In our study, the MICs of fluconazole and nanosilver against Standard *C. albicans* were 8 µg/mL and 2 µg/mL, respectively. Investigation of our finding shows the followings:

1) The study of results in first stage showed that MIC for standard and drug resistant *C. albicans* were 256 to 512 µg/mL and ≥ 64 µg/mL, respectively. Moreover, these results were similar to another research such as a study by Pfaller et al. that reported MIC ≥ 64 µg/mL (28), or study by Enwuru et al. on HIV-positive patients that showed fluconazole’s MIC of 64 µg/mL against *C. albicans* (29).

2) In second stage, comparison between MIC of nanosilver and fluconazole showed that nanosilver inhibited *C. albicans* growth seven-fold to nine-fold more than fluconazole did.

3) In third stage, the MIC analysis showed that nanosilver combined with fluconazole had the most effective activity against *C. albicans*. Another study by Kim et al. in 2008 showed that amphotericin plus nanosilver and fluconazole plus nanosilver were the most effective combinations against trichophyton/mentagrophytes and *C. albicans*, respectively (30). According to our study, fluconazole with insignificant amount of nanosilver exhibit higher antifungal activity against pathogenic *C. albicans* compare with fluconazole and nanosilver alone. The nanosilver inhibits growth of these fungi at very low concentrations that are

### Table 1. The Growth of Standard and Fluconazole-Resistant Candida albicans on Difference Fluconazole Concentrations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fluconazole Concentrations, µg/mL</th>
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<tr>
<td></td>
<td>0.5</td>
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<tr>
<td>Standard</td>
<td>+</td>
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<td>sample</td>
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<table>
<thead>
<tr>
<th>Variables</th>
<th>Nanosilver Concentrations, µg/mL</th>
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<tbody>
<tr>
<td></td>
<td>0.5</td>
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<tr>
<td>Standard</td>
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### Table 3. The Growth of Standard and Fluconazole-resistant Candida albicans on Difference Nanosilver Concentrations Combined with 8 µg/mL Fluconazole

<table>
<thead>
<tr>
<th>Variables</th>
<th>Nanosilver Concentrations (0.5-0.0625 µg/mL) Plus 8 µg/mL of Fluconazole</th>
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<tr>
<td>22% of resistant <em>Candida albicans</em> samples</td>
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<tr>
<td>11% of resistant <em>Candida albicans</em> samples</td>
<td>+</td>
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<tr>
<td>40% of resistant <em>Candida albicans</em> samples</td>
<td>+</td>
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<tr>
<td>27% of resistant <em>Candida albicans</em> samples</td>
<td>+</td>
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<td>Standard sample</td>
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comparable to those of current antifungal drugs. Although the nanosilver exhibit no significant cytotoxic effects on human fibroblasts in these concentrations (31), clinically prescription of these particles needs more clinical trial studies.

**Authors’ Contributions**

Shadi Alimehr: study concept and design, acquisition of data, and drafting the manuscript. Sassan Rezaie: study concept and design, acquisition of data, and drafting the manuscript. Hamide Shekari Ebrahim Abad: critical revision of the manuscript for important intellectual content.

**References**