

# Antifungal Effect of Magnesium Oxide, Zinc Oxide, Silicon Oxide and Copper Oxide Nanoparticles Against *Candida albicans*

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## Absrtact

**Background:** Candidiasis is the most common fungal infection in human and warm-blooded animals. *Candida albicans*, is an opportunistic pathogen in immune suppressed hosts, like HIV infected and under chemotherapy patients. Since, antifungal drugs are limited and challenged by resistance. Thus discovering agents with antifungal properties and minimum side effects and toxicity is essential. Nano-agents such as metal oxide nano-particles have unique properties such as high surface to volume ratio that introduce them as appropriate antimicrobial agents.

**Materials and Methods:** In this experimental study, antifungal effects of 4 nano-metal oxides; magnesium oxide, zinc oxide, silicon oxide and copper oxide (MgO, SiO<sub>2</sub>, ZnO and CuO) were investigated in vitro against *Candida albicans* and compared with amphotericin B. Solution acetic acid was used for preparing nanoparticles suspensions. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of these nano-particles were evaluated.

**Results:** The results showed that MIC of nano-MgO and nano SiO<sub>2</sub> was greater than 3200 µg/mL, but MIC and MFC of nano-ZnO was recorded 200 µg/mL and 400 µg/mL, respectively. The MIC and MFC of nano-CuO was 400 µg/mL. The MIC and MFC of amphotericin B was 0.5 µg/mL and 2 µg/mL, respectively.

**Conclusions:** It is concluded that, ZnO and CuO nanoparticles have anti *C. albicans* properties and may be used in treatment of infections caused by this fungus that should be investigated in vivo.

**Keywords:** Nanoparticles, In Vitro, *Candida albicans*

## 1. Background

Developing resistance microbial strains against antibiotics is one of the important challenges in treatment of diseases. The limited choice of antifungal agents is also one of the most challengeable problems about fungal diseases [1, 2]. *Candida albicans* is a polymorphous fungus that lives as natural floor of human and warm-blooded animals. But the conversions of host body in immunosuppressive diseases like HIV can let this yeast to cause different diseases. Some diseases from *Candida* (called candidiasis), are vulvovaginal candidiasis, oral lesions (thrush), mucocutaneous candidiasis in human and thrush and vaginal candidiasis in animals [3, 4].

Conventional drugs have several disadvantages including adverse effects, non-specificity of their action, and a lesser effectiveness due to ineffective in cancer chemotherapy and anti-diabetic therapy [5].

Among the new agents used as antimicrobials, nanoparticles are under especial attention. Nanoparticles have large surface to volume ratio that introduce

them to better penetration in tissues and cells [6]. Some metal oxide nanoparticles including ZnO, CuO and silver have anti-microbial effects [7, 8]. For example, Panacek et al. reported silver nanoparticle had better antifungal effect against *Candida* by lesser concentration [9]. The antibacterial and antifungal activity of conventional (bulk) ZnO has been reported by Yamamoto [10] and Sawai and Yoshikawa [11]. Also it has been demonstrated ZnO nanoparticles possess significant antifungal properties against *Botrytis cinerea* and *Penicillium expansum* and the inhibitory effects increase by its concentrations [12]. ZnO nanoparticles increased intensity of lipid and protein bands in *E. coli* [7].

## 2. Objectives

Because the information about the anti-candidiasis effect of SiO<sub>2</sub>, MgO, CuO, and ZnO nanoparticles has not been assessed, this effect was investigated in vitro at present study.

### 3. Materials and Methods

In this experimental study, the nanoparticles were provided from Iolitec Company, Germany. The particle size of SiO<sub>2</sub>, MgO, CuO and ZnO respectively was 10, 40, 60 and 20 nm. The standard strain of the fungus, *C. albicans*, with reference number of PTTC10231 was obtained from the Iranian Center of industrial bacteria and fungi collections, Tehran.

#### 3.1. Preparing Culture Media

Sabouraud dextrose agar medium was used for the first subcultures to harvest single colonies of the yeast. Sabouraud dextrose agar plates were used to determine MFC ranges. The RPMI 1640 culture medium was used for microdilution test.

#### 3.2. Fungal Suspension Preparation

The standard strain of *C. albicans* was inoculated on Sabouraud dextrose agar medium and incubated for 48 hours in 37°C, then, some colonies of the fungus were dissolved in distilled water, until the stiffness of suspension changed equal to 0.5 MC farland. The 0.5 MC farland was prepared according to references [1, 13]. The yeast concentration in this stiffness is about  $1 \times 10^6 - 5 \times 10^6$  CFU/mL.

#### 3.3. Minimum Inhibitory Concentration Determination

MIC was determined visually after 48 hours incubation at 37°C and was defined as a concentration of drug which completely inhibited growth. Since the nanoparticles were insoluble in water, according to the producer factory guidelines, 5% acetic acid in distilled water was used for solvent. Sixteen milligram of each nanoparticle was solved in 1 mL 5% acetic acid and the resulted solutions were diluted 5 folds by the culture medium (RPMI 1640). After preparing the primary concentration, the final concentrations were selected 3200-1600-800-400-200-100-50-25 µg/mL.

One hundred micro liter RPMI 1640 culture medium and 100 micro liter yeast suspension in 0.5 MC Farland dilution was added to these wells containing 100 µL nanoparticles suspension. As control, only 200 µL culture medium and 100 µL yeast suspension were added to all the wells of one column. Also, in one column, serial dilutions of acetic acid were prepared as negative control (because the acid has inhibitory effect on yeast growth). In one column also, serial dilutions of amphotericin B (as positive control) (64 µg/mL to 0.5 µg/mL) was added to culture medium and yeast suspension. The trails were done in two separate microplates double repeatedly for each nanoparticle. The plates were incubated in 37°C for 48 hours.

#### 3.4. Minimum Fungicidal Concentration Determination

The content of each clear well (no growth seen) was homogenized with a micropipette and the entire volume (100 µL) of each of these wells was subcultured, onto two 90 × 15 mm Sabouraud Dextroseagar (Difco) plates. To avoid anti-fungal carryover, aliquots were deposited as a spot onto the agar plate and allowed to soak. After the plate was dry, streaking was performed as described by Moore et al. to separate cells and remove them from the drug source [14]. All plates were incubated at 37°C for 48 hours. If *C. albicans* had growth on a plate, the equivalent concentration in microplates was recorded as MFC.

### 4. Results

MIC for nano SiO<sub>2</sub> and nano MgO was greater than 3200 µg/mL. The MICs for nano ZnO and nano CuO were 200 µg/mL and 400 µg/mL, respectively. The MIC of amphotericin B was 0.5 µg/mL.

The amounts of MFC for nano ZnO and nano CuO were both 400 µg/mL. MFC for amphotericin B was 2 µg/mL. The result of MICs and MFCs for the nanoparticles and amphotericin B was illustrated in Table 1. MFC for nano SiO<sub>2</sub> and nano MgO was not observed.

**Table 1.** Mean of MIC and MFC of Nanoparticles and Amphotricin B Against *Candida albicans*

Substance	Minimum Inhibitory Concentration (MIC)	Minimum Fungicidal Concentration (MFC)
Amphotericin B, µg/mL	0.5	2
Nano ZnO, µg/mL	200	400
Nano CuO, µg/mL	400	400
Nano SiO <sub>2</sub> , µg/mL	> 3200	Not observed
Nano MgO, µg/mL	> 3200	Not observed

## 5. Discussion

Our study showed the amphotericin B had better antifungal effect than nanometals, but this property for nano-ZnO was greater than nano-CuO; while nano-MgO and nano SiO<sub>2</sub> did not have antifungal effect against *Candida albicans* at in vitro condition.

According to our knowledge, this is the only study investigating the effect of these nanoparticles on *C. albicans*. Some studies investigated the effect of other nanoparticles on this yeast. Different studies are available about the effect of nano ZnO on non *C. albicans*. The antifungal effects of nano ZnO were evaluated against pathogenic fungi (*Penicillium expansum* and *Botrytis cinerea*) [12, 15]. It was showed that nano-ZnO can considerably inhibit the growth of these two fungi in concentrations more than 3 mmol/L (138 µg/mL) and completely stop their growth in concentrations more than 6 mmol/L (276 µg/mL) [12, 15]. According to this study, nano-ZnO has the potential of antifungal activity. Our study did not show any antifungal effects of SiO<sub>2</sub> and MgO nanoparticles in concentrations ≤ 3200 µg/mL. The information about antifungal effects of these nanoparticles is limited. Some nanoparticles as silver induce apoptotic cell death in *C. albicans* by increasing of hydroxyl radicals [16]. However, shape of nanoparticle can influence its antimicrobial effects [17]. Garcia-Saucedo et al. investigated the toxicity of SiO<sub>2</sub> nanoparticles on yeast *Saccharomyces cerevisiae*, and showed that this nanoparticle is low effective or no effective on this yeast [18]. Similar studies on amphotericin B effect on *C. albicans* are available [19-21] and the results are close to the recent study.

Nanotechnology offers the possibility of designing new drugs with greater cell specificity and drug-release systems that act selectively on specific targets. This allows the administration of smaller but more effective doses, minimizing adverse effects. Nanotechnology can also be used to optimize drug formulations, increasing drug solubility and altering the pharmacokinetics to sustain the release of the drug, thereby prolonging its bioavailability [5].

Results of our study show by different size nanoparticles, although may be change their effects in similar size particles. Unfortunately we do not access to more data for comparison.

It can be totally concluded that regardless to their mechanism of action, nano ZnO and nano CuO, can be effective against *C. albicans*, and this subject can be examined more on different strains of this yeast and other fungi, and also can be investigated in vivo in next studies.

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## Footnotes

**Authors' Contribution:** All authors had equal role in design, work, statistical analysis, and manuscript writing.

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