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Antifungal Activity of Biogenic Selenium Nanoparticles

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Abstract: Fungal infections can occur in various parts of the body and can sometimes be difficult to treat. Although selenium sulfide (SeS_2) has long been used as an effective inorganic antifungal agent, the antifungal activity of selenium nanoparticles has not yet been investigated. In the present study, biogenic selenium nanoparticles produced by *Klebsiella pneumoniae* were examined for their antifungal effectiveness against test strains of two important clinical fungal genera, *Malassezia* and *Aspergillus*. The inhibitory effect of prepared selenium nanoparticles on spore germination was also evaluated in *Aspergillus* species. The minimal inhibitory concentrations (complete visual growth inhibition) of these biogenic selenium nanoparticles ranged from 10-260 μg/ml for all fungal test strains. The highest antifungal activity of the nanoparticles was observed against *Malassezia sympodialis* (Pityrosporum ovale), *Malassezia furfur* and *Aspergillus terreus*. The nanoparticles inhibited germination of spores of all *Asperigillus* species tested. The greatest inhibition was observed for *Aspergillus terreus* and *Aspergillus fumigatus*, with complete inhibition seen in the presence of 40 and 80 μg/ml of biogenic selenium nanoparticles, respectively.

Key words: Selenium nanoparticles · *Aspergillus* species · *Malassezia* species · Antifungal activity

INTRODUCTION

Nanotechnology allows the fabrication of different nanoparticles that can exhibit novel antimicrobial properties [1-4]. In the case of selenium nanoparticles, a number of biological effects (e.g., antioxidant activity) have been recently investigated and reported [5-13]. Selenium sulfide (SeS_2) has long been known as an effective antifungal agent and is frequently incorporated into antidandruff formulations for treatment of scalp fungal infections [14-18]. However, fungal infections can also affect other areas of the body and can sometimes be difficult to treat [19].

Although several different selenium preparations have been reported to show antifungal activity, our literature survey failed to uncover any information on the antifungal activity of elemental selenium nanoparticles (Se^0), leaving this area as an important one to address [14-18]. In the present paper, we investigate the antifungal properties of biogenic selenium nanoparticles produced by *Klebsiella pneumoniae* against several fungal test strains, including *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Malassezia sympodialis* (Pityrosporum ovale) and *Malassezia furfur*.

MATERIALS AND METHODS

Synthesis of Selenium Nanoparticles: Biogenic selenium nanoparticles were prepared using a recently described method [20]. Briefly, a uniform inoculum was prepared by aseptically transferring a loopful of *Klebsiella pneumoniae* from a Tryptic Soy Agar (TSA) plate to 100 ml of sterile Tryptic Soy Broth (TSB) and growing the culture to an OD600 of 1.0. This solution constituted

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the inoculum. Fresh TSB at pH 7.2 was then prepared, sterilized and supplemented with 200 mg/l Se^{+4} (equal to 559.19 mg of selenium chloride) and 1% (v/v) of inoculum was added. The culture flask was incubated at 37 °C for 24 hours. *K. pneumoniae* cells containing red selenium particles were disrupted using a wet heat sterilization process in a laboratory autoclave at 121°C, 1.2 kg/cm^2 for 20 minutes. The released selenium nanoparticles were centrifuged at 25000 g for 15 minutes and washed three times with distilled water. The washed sample was sonicated for 10 minutes (Tecna6, Techno-Gaz, Italy) and its physical and chemical properties were confirmed by Transmission Electron Microscopy (TEM) and Energy Dispersive Spectroscopy (EDS), respectively.

Antifungal Activity: The antifungal activity of the biogenic selenium nanoparticles was tested against the following isolated clinical test strains: *A. niger* (TUMS R124), *A. terreus* (TUMS R126), *A. flavus* (TUMS R142), *A. fumigatus* (TUMS R138), *M. sympodialis* ((TUMS R 150) and *M. furfur* (TUMS R151). All clinical test strains were obtained from the Department of Medical Mycology and Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran (Iran). The identity of the isolates was confirmed by Dr. Sassan Rezaie. A conventional serial agar dilution method in Sabouraud Dextrose Agar (SDA) was used to test susceptibility of *Aspergillus* spp. and a Leeming and Notman Agar (LNA) method was used for *M. sympodialis* and *M. furfur* [21] with an inoculum of approximately 10^4 colony-forming units (CFU)/ml. The SDA and LNA culture media were supplemented with serial concentrations of biogenic selenium nanoparticles ranging from 10 to 300 μg/ml. The data are reported as MICs; i.e., the lowest concentration of selenium nanoparticles capable of inhibiting visible growth after 48 h of incubation at 25°C. Voriconazole (Pfizer, Ballerup, Denmark) was used as a control antifungal drug. Spore germination of *Aspergillus* spp. was further monitored in the SDA culture plates and the lowest concentration of selenium nanoparticles inhibiting spore germination was designated as the MIC*. All antifungal experiments were repeated three times.

RESULTS AND DISCUSSION

The prevalence of fungal opportunistic pathogens exhibiting multiple resistances to antibiotics constitutes a serious problem, especially in compromised hosts [19]. In the present study, biogenic selenium nanoparticles were prepared and their antifungal activity against important clinical test strains of *Malassezia* and *Aspergillus* fungal species was investigated (Figure 1 and Table 1). The upper-right illustration in Fig. 1 (B) shows the representative TEM images of the biogenic selenium nanoparticles released after the sterilization process and three washes with distilled water. The selenium particle size histogram (Fig.1A) shows a size range from 100 to 600 nm, with an average size between 200 to 300 nm. The EDS analysis of the biogenic selenium nanoparticles confirmed the presence of elemental selenium signals (Fig. 1C). The selenium nanocrystallites displayed optical absorption bands with peaks at 1.5, 11.2 and 12.5 keV, which is typical of the absorption of metallic selenium nanocrystallites [22].

Table 1: Antifungal activity of biogenic selenium nanoparticles against different species of *Aspergillus* and *Malassezia*

	Antifungal activity of biogenic selenium(μg/ml)		Antifungal activity of antifungal control drug(μg/ml)
Test strains	MIC	MIC*[a]	MIC
Aspergillus niger	260	240	0.5
Aspergillus terreus	60	40	1
Aspergillus flavus	220	220	1
Aspergillus fumigatus	100	80	1
Malassezia sympodialis	10	-[b]	0.06
Malassezia furfur	50	-[b]	16

(a) MIC for spore germination

(b) Not applicable for these non-sporulating test strains

(c) Standard deviations in all antifungal assays were negligible.

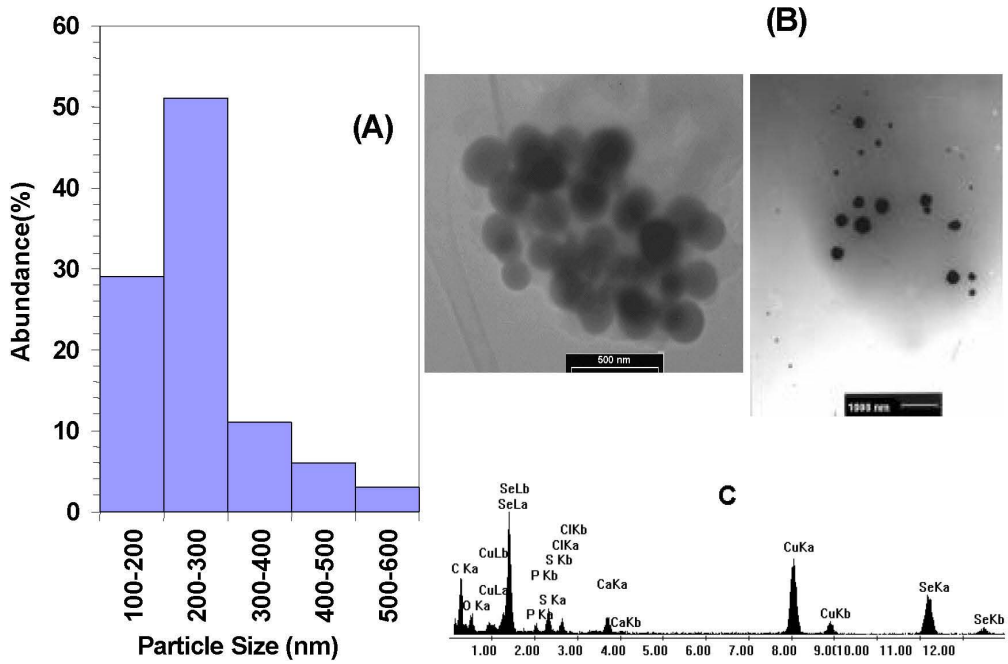

Fig. 1: The characterization of biogenic selenium nanoparticles prepared by *Klebsiella pneumoniae*. (A) the particle size distribution histogram (B) Transmission electron micrographs and (C) EDS spectrum of separated selenium nanoparticles

Biogenic selenium nanoparticles showed a good antifungal activity against all test strains (Table 1), with MICs ranging from 10-260 µg/ml for all isolates. *M. sympodialis, M. furfur* and *A. terreus* were the most sensitive to selenium nanoparticles, with MICs of 10, 50 and 60 µg/ml, respectively. In contrast, selenium nanoparticles were less effective against *A. niger* and *A. flavus* (Table 1). Voriconazole, a potent antifungal drug used as a control, showed strong antifungal activity against all test strains. The MICs for inhibition of spore germination (indicated as MIC*s) for biogenic selenium nanoparticles varied from 40-240 µg/ml for spores of all *Aspergillus* species. The highest inhibition was observed for spore germination of *A. terreus* and *A. fumigatus,* which was completely inhibited by treatment with 40 and 80 µg/ml of biogenic selenium nanoparticles, respectively. For other *Aspergillus* species (*A. niger* and *A. flavus*), spore germination was inhibited at higher concentrations of selenium nanoparticles (Table 1).

Although a number of different soluble metal ions or metal complexes show significant antimicrobial activity against a wide range of microorganisms [23-27], use of these materials as topical or systematic applications have been limited for various reasons, such as toxicity to biological systems [23-27]. In the case of selenium, its antioxidant and pro-oxidant effects, or its bioavailability and toxicity, depend on its chemical form [28]. Elemental selenium powder with a redox state of 0 is not soluble in water and is generally considered to be biologically inert. Thus, the toxicity of elemental selenium (Se^0) is less than that of selenate or selenite ions [28]. However, elemental selenium, when supplied in the form of nanoparticles, clearly can serve as a potent ingredient for the preparation of new antifungal formulations. This study is the first to examine the antifungal activity of selenium nanoparticles and confirms the efficacy of elemental selenium supplied at the nanoscale level against different fungal test strains.

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