Research Article

Evaluation of Antifungal Effect of Silver Nanoparticles Against *Microsporum canis*, *Trichophyton mentagrophytes* and *Microsporum gypseum*

Seyyed Amin Ayatollahi Mousavi [1, 2], Samira Salari [1, 2*], Sanaz Hadizadeh [1]

[1]Department of Medical Mycology and Parasitology, School of Medicine, Medical University of Kerman, Kerman, Iran
[2]Research Center for Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran

[*]*Corresponding author*: Samira Salari, Department of Medical Mycology and Parasitology Faculty of Medicine, Kerman Medical University, Kerman, Iran. Tel: +98-3433224616, Fax: +98-3433239843, E-mail: sa_salari@kmu.ac.ir

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Background: Dermatophytosis is the common cutaneous infections in humans and animals, which is caused by the keratinophylic fungus called dermatophytes. In recent years, drugs resistance in pathogenic fungi, including dermatophyte strains to the current antifungals have been increased.
Objectives: The aim of this study was to evaluate the antifungal efficacy of AgNPs against *Microsporum canis*, *Trichophyton mentagrophytes*, and *Microsporum gypseum*.
Materials and Methods: The antifungal susceptibility of nanosilver particles compared with griseofulvin (GR). Its efficacy was investigated against three strains of dermatophytes by both agar dilution and broth microdilution test (BMD).
Results: The average minimum inhibitory concentration (MIC) AgNPs on *M. canis*, *T. mentagrophytes* and *M. gypseum* were 200, 180 and 170 $\mu g.mL^{-1}$, respectively. Whereas these strains showed MIC of 25, 100 and 50 $\mu g.mL^{-1}$ for GR.
Conclusions: Our finding indicated that the AgNPs was less active than GR but it had anti-dermatophytic effect.

Keywords: AgNPs; Antifungal efficacy; *Microsporum canis*; *Microsporum gypseum*; *Trichophyton mentagrophytes*

1. Background

Silver nanoparticles (AgNPs) are the particles with size of 2-100 nm, which contain 20-15,000 silver atoms (1). These particles are used in medicine, dental cements, treatment of wounds and burns, water purification, and textile engineering (2-4). Several studies have been carried out concerning the antimicrobial properties of AgNPs against various pathogens such as viruses, fungi, and some bacterial species. Most of which have confirmed the antimicrobial properties of AgNPs (5, 4, 6). The mechanisms of action of AgNPs referred to their accumulation on the membrane of microorganisms, formation of pores, change in permeability of cell wall, and inhibition of respiration process. In addition, it has been shown that AgNPs can greatly inhibit cellular respiration, DNA replication, and cell division, which result in the loss of cell viability, and lead to cell death (7, 8).

Dermatophytosis is the most common cutaneous fungal infections with worldwide distribution. Dermatophytes can grow in keratinized tissues such as hair, nails, and the outer skin layer (9, 10). This infection occurs in humans skin, pets, and farm animals. Dermatophyte species divided into three genera: Epidermophyton, Microsporum, and Trichophyton, and consist of 40 accepted species (11, 12). Clinical features of dermatophytosis are observed as tinea capitis, tinea corporis, tinea barbae, tinea faciei, tinea cruris, tinea pedis, tinea manuum, tinea unguium (onychomycosis), and allergy to dermatophyte antigens (13).

Depending on different types and severity of infection, various therapeutic agents such as griseofulvin and oral and/or topical formulations of azoles or allylamines, particularly itraconazole and terbinafine are used in the treatment of dermatophytosis (14, 15).

2. Objectives

According to increase in number of antifungal-resistance reports in some strains including *M. gypseum* and *T. mentagrophytes* (16-18), antifungal efficacy of AgNPs against *M. canis*, *T. mentagrophytes*, and *M. gypseum* was evaluated in this study.

3. Materials and Methods

3.1. Reagents and Fungal Strains

Nanosilver (Nanocid®) was purchased from Nano

Nasb Pars Co, Tehran, Iran. The silver nanoparticles with average particle size of 4 nm were synthesized by a novel process that involved the photo-assisted reduction of Ag^+ to metallic nanoparticles and theirbio-stabilization based on undisclosed US-patent (United State Patent Application under No. US/2009/0013825) (17). Dermatophyte strains including *M. canis* PTCC 5069, *M. gypseum* PTCC5070, and *T. mentagrophaytes* PTCC 5054 were purchased from Iranian Research Organization for Science and Technology (IROST) in Tehran, Iran.

3.2. Susceptibility Testing

3.2.1. Broth Microdilution Method

Antifungal susceptibility testing was performed by microdilution assay and agar dilution method, according to guideline of Clinical and Laboratory Standards Institute (CLSI) in M38-A document for filamentous fungi (19). For broth microdilution test, dermatophyte strains were subcultured on Potato Dextrose Agar (PDA) (Merck Co., Darmstadt, Germany) and incubated at 30°C for 5-7 days. Conidia were moved to sterile saline and allowed to rest for 15 min. Conidia was counted by a hemocytometer, and the suspension was adjusted to 1×10^4 CFU.mL^{-1} in RPMI 1640 medium (with L-glutamine, without sodium bicarbonate; GIBCO-BRL, Grand Island, NY) buffered with MOPS (3-(N-morpholino) propanesulfonic acid; Serva, Feinbochemica GmbH, Germany). Serial dilutions of drugs (200-0 µg.mL^{-1} for AgNPs and griseofulvin) and inoculum were combined in 96-well microtiter plates and incubated at 32°C for 5 days (20). Inhibited growth by 90% of dermatophyte strains compared with the positive control determined as minimum inhibitory concentration (MIC). Griseofulvin was used as positive control for the evaluation of antifungal activity. A plate for each fungal strain with no AgNPs was used as negative control. The experiments were performed for each fungi sample in triplicate.

3.2.2. Agar Dilution Method

The inhibitory effects of various concentrations of AgNPs (0, 40, 80, 120, 160, 170 and 200 µg.mL^{-1}) were assayed on three dermatophyte strains. An *in vitro* assay was carried out on a PDA (Merck Co., Darmstadt, Germany) treated with different concentrations of AgNPs as above and GR (0, 3.125, 6.25, 12.5, 25, 50, 100, 200 µg.mL^{-1}). Various concentrations of AgNPs and GR were poured to PDA medium prior to plating in petri dish. Inoculum containing 1×10^4 CFU.mL^{-1} of dermatophyte strains was added to the hole in center of the plates. The plates were incubated for 14 days in 28°C. When the control plate was covered completely with fungal growth, the MIC was read. The MIC was determined as the lowest AgNPs and GR concentration that inhibited visible growth (21, 22). The experiments were replicated three times.

3.3. Data Analysis

Data were expressed as mean±SD of at least three independent experiments. One-way ANOVA was used to calculate statistical significance between positive control and culture medium treated with AgNPs at p-value < 0.05.

4. Results

The inhibitory effects of AgNPs at various concentrations were tested on the growth of *M. canis* PTCC 5069, *M. gypseum* PTCC 5070, and *T. mentagrophytes* PTCC 5054. Comparison between MICs of AgNPs and GR indicated that the antifungal efficacy of GR on dermatophayte strains was significantly higher than AgNPs ($p<0.001$). Susceptibility results of dermatophayte strains to AgNPs and GR are illustrated in (Figure 1). *M. Canis* had the highest resistance (200 µg.mL^{-1}), following *T. mentagrophytes* (180 µg.mL^{-1}) and *M. gypseum* (170 µg.mL^{-1}). Mean MIC for GR were 25, 100 and 50 µg.mL^{-1}, respectively. The colony diameter dermatophyte strains (mm) in various concentrations of griseofulvin and AgNPs are shown in (Tables 1 and 2) respectively.

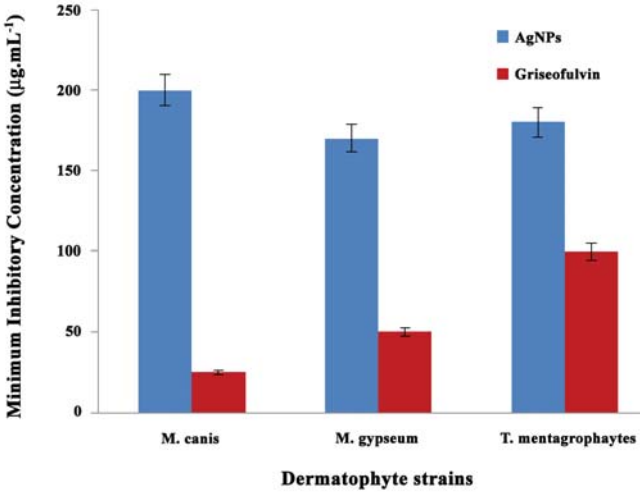

Figure 1. The minimum inhibitory concentration (MIC) of dermatophyte strains against AgNPs compared with griseofulvin (µg.mL^{-1})

Table 1. Colony diameter dermatophyte strains (mm) in various concentrations of griseofulvin

	Griseofulvin concentrations (µg.mL^{-1})									
Strains	**0**	**0.78**	**1.56**	**3.125**	**6.25**	**12.5**	**25**	**50**	**100**	**200**
M. canis	54(±3.06)	32(±3.34)	22(±2.78)	20.21(±1.98)	16(±1.38)	3(±0.9)	-	-	-	-
M. gypseum	63(±2.01)	35(±1.53)	28(±2.06)	21(±1.71)	15.1(±3.06)	8(±1.82)	2(±1.01)	-	-	-
T. mentagrophaytes	68(±2.5)	32(±3.7)	27(±2.9)	22.5(±2.07)	22(±2.1)	19(±1.39)	12(±1.87)	5(±1.5)	-	-

Table 2. Colony diameter dermatophyte strains (mm) in various concentrations of AgNPs

	AgNPs concentrations (µg.mL^{-1})							
Strains	**0**	**40**	**80**	**120**	**160**	**170**	**180**	**200**
M. canis	48(±2.6)	41(±2.7)	36(±2.02)	29(±3.04)	25(±2.8)	23(±2.33)	17(±1.00)	-
M. gypseum	58(±3.00)	49(±1.53)	34(±2.4)	22(±1.33)	12(±1.7)	-	-	-
T. mentagrophytes	55(±4)	51(±1.8)	47(±2.56)	35(±2.00)	21(±2.31)	15(±1.29)	-	-

5. Discussion

Dermatophytosis is caused by the keratinophylic fungus called dermatophytes (23). Transmissibility from infected humans or animals to human is one important public health problem caused by dermatophyte species (24). In some cases, treatment of the disease with the current therapeutic agents can result in the damage of host tissues due to the similarity between eukaryotic cells in human and fungi structure, emergence of drugs resistance to fungal strains, and treatment failures (25, 26). Different research groups have investigated the efficacy of AgNPs on yeasts, molds, bacteria, and viruses (5, 27). But, information about anti-dermatophyte activities of nano-silver particles is few (28, 29).

This study was performed to investigate a new antifungal drug for the treatment of dermatophyte infection caused by *M. Canis*, *T. Mentagrophytes*, and *M. gypseum*. Our findings revealed that GR had higher anti-dermatophyte activity than AgNPs. Comparison of the three tested dermatophyte strains showed that *M. canis* was more resistant to AgNPs. Dermatophyte strains demonstrated an antifungal activity to AgNPs with the following order of resistance: *M. canis*> *T. mentagrophytes*> *M. gypseum*. Ability of AgNPs in destroying of fungi, pore in cell wall and plasma membrane are the potential mechanisms of its inhibitory effect on different organisms (7, 30). Here, the most GR-susceptible strains were *M. canis* followed by *M. gypseum* and *T. mentagrophytes*.

Previous data indicated that the AgNPs had good antifungal and antimicrobial effects (31-33). Atef *et al*. (33) reported the growth inhibition of the AgNPs on *T. mentagrophytes* and *C. albicans*. In their study, MIC100 AgNPs against *C. albicans* and *T. mentagrophytes* were 4±2.0 µg.mL^{-1} and 5±0.10 µg.mL^{-1}, respectively. Kim *et al*. (29) showed that AgNPs had inhibitory effects on the growth of *T. mentagrophytes*, *C. albicans*, *C. tropicalis*, and *C. glabrata*. AgNPs (IC80, 1-7 µg.mL^{-1}) exhibited greater efficacy than fluconazole (IC80: 10-30 µg.mL^{-1}), but less active than Amphotericin B (IC80: 15 g.mL^{-1}).

Petica *et al*. (32) indicated that the colloidal solutions containing up to 35 ppm AgNPs could inhibit the growth of Aspergillus, Penicillium, and Trichoderma species. Moreover, the inhibition effects of low concentrations of AgNPs on yeasts and *E. coli* were noted by Sondi *et al*. (8). Khaydarov *et al*. (34) reported that the AgNPs MIC for *E. coli* and *S. aureus* were 3 and 2 mg.L^{-1}, respectively. Azimi *et al*. (35) demonstrated that the greater antifungal effect of AgNPs on *S. mutans* and *S. sanguis* than *Nigella sativa* oil. The inhibitory effects of AgNPs on the growth of Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* and *S. mutans* were confirmed (36, 31). All of which appeared to be in agreement with our findings and other results reported about the antimicrobial activity of AgNPs.

Rathod *et al*. (37) demonstrated that synthesized AgNPs by *Rhizopus stolonifer* has a considerable antifungal activity on *T. mentagrophytes* and Candida species compared with Amphotericin B and flucona-

zole. Similarly, the antifungal effect of AgNPs alone and combined with griseofulvin against *T. rubrum* was studied. The results showed that AgNPs had superior efficiency than fluconazole (40 $\mu g.mL^{-1}$), but less antifungal efficiency than griseofulvin (0.8 $\mu g.mL^{-1}$). They confirmed that the antifungal activities of fluconazole and griseofulvin were increased in the presence of AgNPs (28). Gajbhiye *et al*. (38) showed that the increasing inhibitory effect of fluconazole was occurred in combination with AgNPs against *C. albicans*, Phoma, Glomerata and Trichoderma species. In conclusion, our data showed that (1) AgNPs had antidermatophytic effect and (2) the AgNPs was less active against dermatophyte strains.

Conflict of interest

The authors report no conflicts of interest in this work.

Authors' Contribution

SS performed the experiments, analyzed data and wrote the manuscript. SAAM designed, provided consultation, supervised the study and analyzed data. SH performed the experiments.

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