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Antifungal Effects of Silver Nanoparticle alone and with Combination of Antifungal Drug on Dermatophyte Pathogen Trichophyton Rubrum

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[*]***Abstract*—Nano science has been emerged as a powerful tool to develop new approaches in the field of designing new antimicrobial drugs. Antimicrobial effect of silver Nano particles (Ag-NPs) were investigated on many bacteria and yeast, but there is no information about their role on dermatophyte fungi. *Trichophyton rubrum* is the most common agent of superficial mycoses world wide, and major causative agent for onychomycosis and tinea pedis. The aim of this study is to investigate the effect of Ag-NPs alone and in combination with fluconazole and geriseofulvin on dermatophyte pathogen *T.rubrum.***

***T.rubrum* has been isolated from the patient who referred to medical Mycology Department in Tehran University of medical Sciences. Antifungal susceptibility against Ag-NPs, drug alone and in combination was performed by the broth microdilution method described in NCCLS document M38-A[22]. Results demonstrated that Ag-NPs can inhibited the mentioned fungus at 10microgeram per mili liter(µg/ml). Regards to this concentration, Ag-Nps shows less inhibitory efficiency than geriseofulvin (0.8 µg/ml), but more efficiency than flocunazole (40µg/ml). However, the antifungal activity of flocunazole and geriseofulvin were increased in presence of Ag-NPs in combination test.**

Keywords-Silver nanoparticle, Dermatophyte, Trichophyton rubrum, Antifungal activity

I. INTRODUCTION

Dermatophytosis is a superficial fungal infection in keratinized substrates and caused by a group of filamentous fungi called dermatophytes. Among these fungi, *Trichophyton rubrum (T. rubrum)* is known to account for as many as 69.5% of all dermatophyte infections [5, 6,13].
Silver or silver ions have long been known to have strong inhibitory and bactericidal effect as well as a broad spectrum of antimicrobial activities. It is expected that the high specific surface area and high fraction of surface atoms of silver nano particles will lead to high antimicrobial activity compared to bulk silver metal[18]. Silver nanoparticles (NPs), exhibiting very strong bactericidal activity against both gram-positive and gram-negative bacteria including multi resistant strains [20,24]. In addition, silver NPs kill bacteria at low concentrations (units of mg/L)[24,27], which do not reveal acute toxic effects on human cells[3,4]. Besides, silver NPs have not been shown to cause bacterial resistance currently complicating antibiotic therapy of bacterial infections[1]. Regards to mycoses, NPs can be considered as potential antifungal agent[1]. However, the antifungal effect of silver NPs has received only marginal attention and just a few studies on this topic have been published[9,11,25,29]. Recent studies revealed the effects of silver NPs on some species of fungi particularly candida genus. However, only few studies have been performed for the mention effects on dermatophyte fungi such as *T.mentagrophytes*[1,15,21]. To the best of our knowledge, there is no study carried out for other dermatophyte pathogenes such as *T. rubrum*.

In this study, we investigate the effects of silver NPs alone and in combination with fungal antibiotics as fluconazole and griseofulvin on *T. rubrum*.

II. MATERIALS AND METHOD

A. Antifungal agent dilution

The mentioned antibiotics were obtained from their respective manufacturers: fluconazole (pars darou.Tehran, Iran) geriseofulvin (Darou pakhsh, Tehran, Iran). Silver nanoparticle has been obtained from klebsiela pnomonia by a method as described before [26]. Fluconazole and silver-NP was dissolved in distilled water, while geriseofulvin was dissolved in 100% dimethyl sulfoxide (Sigma-Aldrich). Antifungal susceptibility testing of the study isolates was performed by the broth microdilution method described in NCCLS document M38-A. The final concentrations ranged from 1.25 to 64 µg/ml for fluconazole, 0.05 to 16 µg/ml for griseofulvin and 5 to 100 µg/ml for silver nanoparticle.

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B. *Broth microdilution method*

The broth microdilution assay for antifungal susceptibility testing on *T.rubrum* was performed in accordance with NCCLS protocol[22]. The inoculum suspensions were prepared from seven-day old *T.rubrum* cultures which was grown on potato dextrose agar at 28°C. The fungal colonies were covered with approximately 10 ml of distilled water, and the suspensions were made by scraping the surface with the tip of a sterile loop. The obtained mixture containing fungal conidia and hyphal fragments was removed and transferred to sterile tube. The final suspension with 0.5 mac farland concenteration of conidia and hyphal fragment was then prepared. The amount of 100 µ1 of suspension was inoculated to microtiter plate containing RPMI 1640 culture medium with 3-N-morpholino propanesulfonic acid (MOPS) and specific antifungal drug as well as silver NP. The micro dilution plates were incubated at 28°C for one week. The plates have been investigated every 24h. Each assay was carried out in duplicate. For all the antifungal agents tested, the MIC was read as the lowest concentrations that prevented any discernible growth.

III. RESULT

The antifungal activities of fluconazole and griseofulvin, that are widely used against many fungal infections, were used as positive control for comparison with antifungal activity of Ag-NPs. The results of MIC were determined by means of the broth microdilution method after six days of incubation of *T. rubrum* in culture media containing the mentioned medicines and Ag-NPS (table I).

Different serial Dilution from silver NPs (Stock concentration 10 µg/ml) as well as antifungal drugs; fluconazole (Stock concentration 40 µg/ml) and griseofulvin (Stock concentration of 0.8 µg/ml) were used in broth dilution method.(table II).

The results of MIC indicate the inhibitory activities of Ag-Nps in both; alone and in combination with anti fungal medicines .

TABLE I. THE MINIMUM INHIBITORY CONCENTRATIONS OF FLUCONAZOLE,GRISEOFULVIN, AG-NPS ON TRICHOPHYTON RUBRUM.

T. rubrum	Fluconazole(µg/ml)	Griseofulvin(µg/ml)	Ag-NPs (µg/ml)
1	40	0.4	10
2	10	0.8	5-10
3	40	0.8	10
4	20	0.8	10
5	40	0.4	10

TABLE II. COMBINATION TEST SILVER NANOPARTICLE WITH FLUCONAZOLE AND GRISEOFULVIN

T.rubrum	Ag-NPs +Fluconazole	Ag-NPs +Griseofulvin
1	2.5+10	2.5+0.2
2	1.25+5	2.5+0.2
3	2.5+10	2.5+0.2
4	2.5+10	2.5+0.2
5	2.5+10	2.5+0.2

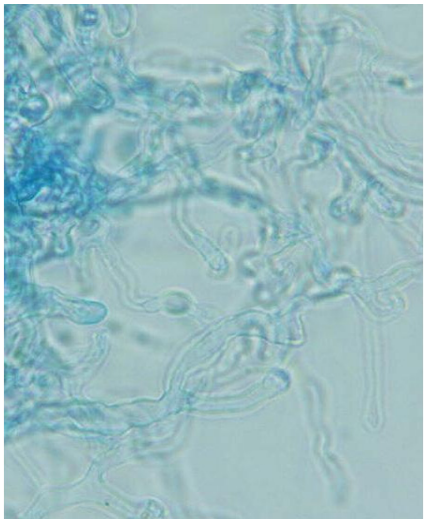

Figure 1. *T.rubrum* incubated in mixture of Ag-Nps (2.5 µg/ml) and fluconazole(10 µg/ml). deformed mycelial bodies as been observed.

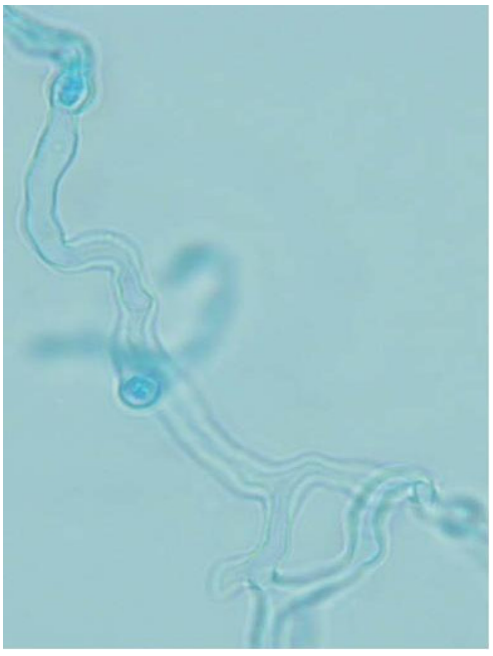

Figure 2. T.rubrum incubated in mixture of Ag-Nps (2.5 µg/ml) and griseofulvin(0.2 µg/ml). deformed mycelial bodies as been observed.

IV. DISSCUSION

It is well known that Ag ions and Ag-based compounds have strong antimicrobial effects [10].These inorganic NPs have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is multi drug resistance. Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed in medical devices desperately. Ag ions and Ag salts have been used for decades as antimicrobial agents in various fields because of their growth-inhibitory capacity against microorganisms [14].

In the present study, obtained results of antifungal activity reveal that the growth of *T. rubrum* was inhibited at concentration of 10 µg/ml Ag-Nps alone. In combination tests, flocunazole (20 µg/ml) together with Ag-NPS (2.5 µg/ml) and geriseofulvin (0.4 µg/ml) with Ag-NPS(2.5 µg/ml) revealed inhibitory effects on *T.rubrum*. These results indicate that the antifungal effects of drugs increased in presence of Ag-NPs.

Previous studies demonstrate significant antifungal activity of Nano-Ag, in an IC80 range of 1-7 µg/ml against *T. mentagrophytes* and *Candida species* [15]. Minimum inhibitory concentration for *C. albicans* I,II and *C.tropicalis* were 0.21,0.42,0,84 mg/l respectively[1]. In other investigations, Keuk-Jun Kim shows the inhibition of *C.albicans* at 2 µg/ml concentration of Ag-NPs[16]. The study of Jun Sung Kim et al, revealed the MICs of Ag-NPs for yeast, *Echerichia coli*, and *Staphylococc aureus* respectively: 6.6, 3.3, and 33 nM[14,15].

In addition, the MIC studies of silver NPs against bacteria indicated the values of 1.69 mg/L against *Escherichia coli* and 6.75 mg/L against *Enterococcus faecalis*[17].The inhibition of bacterial growth by spherical nanoparticles was observed at less silver content than truncated triangular nanoparticles[23].

STEM images show that the smaller sized nanoparticles (~5 nm) efficient antibacterial activity[20].

The silver nanoparticles show efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. These particles release silver ions in the cells, which enhance the cellular activity[19]. Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles [7,8,12].

Sondi and Salopek-Sondi[28] reported that Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. Amro et al[1] suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins

Nano-Ag breaks down the membrane permeability barrier of *C.albicans*, it is possible that nano-Ag perturbs the membrane lipid bilayers, causing the leakage of ions and other materials as well as forming pores and dissipating the electrical potential of the membrane[16].

As mentioned above, Ag-Nps have inhibitory effect on several micoorganismes, but the effect of nano-Ag against dermatophyte fungi is mostly unknown. To the best of our knowledge, the present study is the first study about the effect of Ag-Nps on *T.rubrum*. Our observation revealed that Ag-Nps could inhibit the growth of *T.rubrum*(~10 µg/ml). However, this effect has revealed to be weaker compare to that from Griseofulvin (0.4-0.8 µg/ml), but this inhibitory role of Ag-Nps were shown more in combination with antifungal same medicine (2.5+0.2 µg/ml respectively).

The Comparison of the mentioned results with those obtained from other investigations demonstrate that antimicrobial concentrations are different, and dependent on size, shape and mode of action [23].Further researches about the role of Ag-Nps on cellular functions of dermatophyte pathogens may help us to gain more information concerning the using methods of these Nps against cutaneous fungal infections.

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