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METAL NANOPARTICLES ACTIVITIES ON HUMAN PATHOGENIC FUNGI

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ABSTRACT
The application of nanotechnology in the synthesis of effective medicines that are used for the treatment of different infections is progressively taken attention by many studies in the present days. Replacement of the conventional forms of antifungals with a nanoparticle formula is the most successful application of nanotechnology. Metal nanoparticles that have been prepared from different metals such as silver, gold, zinc oxide, and iron in size of 10-100 nm are widely used either as antifungals or to enhance other conventional antifungals. Silver nanoparticles (AgNPs) are the most effective type of metallic nanoparticles. Many other forms of nanoparticles, such as polymers and lipid-associated nanoparticles are also proven to have antifungal activities. Activities of metal nanoparticles show significant action on several pathogenic yeasts and filamentous fungi. Some of these fungi have resistance to many conventional antifungals. *Candida albicans* and *Aspergillus* spp., which cause invasive fungal infection, revealed more susceptibility to metal nanoparticles, particularly to AgNP. Other positive properties of prepared nanoparticles are their low adverse effects with little toxicity to human tissues. Several mechanisms of action of AgNP have been proposed. Oxidation state with the releasing of reactive oxygen species (ROS) as free radicals is the most acceptable mechanism. Further studies are always required to determine the specific mechanism and adverse effects of AgNP. Applications of AgNP with many conventional antifungals and their *in vivo* activities are also needed further studies.

KEYWORDS: Aspergillus; Candida; Nanoparticle; Silver.

INTRODUCTION
Nanotechnology is a new field of technology that developed in fast steps nowadays. Its applications can be sensed in different parts of modern life. Antifungal limitations and adverse of many of their conventional groups have been decreased by the application of nanoparticles.[1] Other characteristics associated with the success of nanoparticles are their high efficacy in killing resistant species of pathogenic fungi and their few toxicity or adverse effects in human tissue.[1-3] Metal nanoparticles in size of 10-100 nm are the most effective type of nanoparticles to be used for the treatment of different invasive fungal infections.[4-6] Drug carriers and diagnosis of infection are important applications of metal nanoparticles in medicine.[2,6]

Several metals are entering in preparation of antifungal nanoformulations such as silver, zinc oxide, gold, iron, cobalt, copper, and magnesium oxide.[7-8] Silver nanoparticles (AgNPs) are the most common formula used for preparing antifungal nanomaterials.[8-9] It has strong inhibitory activity against pathogenic yeast, such as *Candida albicans*, and some filamentous fungi, such as *Aspergillus* spp. with low MIC compared to conventional antifungals.[2, 8-10] Many mechanisms of action of AgNP are proposed. The oxidation state of AgNP and releasing of reactive oxygen species (ROS) as free radicals is the most satisfactory mechanism.[5,11] Other mechanisms with the most physiochemical characteristics of AgNP and types of affected fungi are discussed in the present review.

Nanoparticle General Characters
Nanoparticle is a new term referring to the particle of matter in a nanoscale within the size of 1-1000 nm.[12-13] The synthetic nature of these particles may take the form of colloidal dispersions or solid states.[4,13] Nanoparticles with sizes of 10-100 nm have unique physical and chemical characteristics to be used in different medical and industry applications in modern life.[4-6] Several metals, such as silver, gold, iron, zinc, titanium, palladium, and copper oxide have been used for preparing metal nanoparticles.[5] Silver is the most common metal used due to its many suitable properties, including antimicrobial activities, chemical stability, strong conductivity, and effective catalytic action.[14]

Important of nanoparticles as therapeutic drugs

A new era of therapeutic and diagnostic applications has been opened by nanotechnology, with a focus on drug delivery and the detection of biological and chemical agents.[2,6] These functions of nanoparticles as therapeutic agents or as carriers have become real due to their special physiochemical properties.[5] The continuous debate about using nanoparticle formulations as an alternative to conventional antifungal agents is an important decision in today's world. Although the available antifungal agents achieved satisfactory results in the treatment of different fungal infections, they also have many unsuitable characteristics. Such characteristics may include the poor solubility as with many azoles in water and the high toxicity of polyenes, which limited the therapeutic benefits of these groups.[12]

Increasing the rates of resistance to the most common antifungals is another serious problem in reducing the therapeutic activities of antifungals.[11] Preparation of known antifungals in nanoparticle formula can reduce many of these inappropriate characteristics. Studies proved that nanoformulations usually have less toxicity to human tissue, enhance the ability of drug solubility, increase the efficacy of action, and overcome many biological barriers.[2, 12-13] Slowing the release of active antifungal ingredients in the human body when they are incorporated with nanoparticles is another benefit of nanoformulations for the treatment of refractory fungal infections.[2] The use of nanoparticles as a carrier to hold and release antifungals is usually facilitated by the small size of nanoparticles, simple design, and biocompatibility with other materials.[13] However, the number of nanoparticle formulations is still few in the market.[12]

Nanoparticles are designed in many formulas when they are used as delivery drugs for other antifungals.[2, 8, 12, 15] The first formula is metallic nanoparticles by compatibility of metal, such as silver or gold with antifungal. The second formula is polymeric structure, which can also be synthesized in the form of polymeric micelles. The third formula incorporates of nanoparticles with lipids to form a coat around antifungal agents. This lipid may be synthesized as phospholipids vesicles that can be taken many forms, e.g. liposomes, ethosomes, transferosomes, and transethosomes. The fourth structure is non-phospholipid vesicles such as spanlastics and niosomes. Solid lipid nanoparticles are the fifth formula. Other formulas are also available such as nanoemulsions and dendrimers. Nanoparticle complexes with different green molecules are another form of nanoformulations proposed to be used for the treatment of fungal infections.[2]

Antifungal action of AgNP

Treatment of fungal infections is an important challenge nowadays due to the limited number of available antifungals.[2,11] The toxicity of antifungals to mammalian cells and emerge of resistance species are the other factors associated with the difficulty of treatment or control of invasive fungal infections.[2,5,11] Pathogenic fungi developed many mechanisms of resistance including, alteration in cellular pathways or drug targets, overexpression of efflux pump or specific targets, reduce the amount of target enzyme, and biofilm production.[11] From another aspect, treatment of infections caused by resistant species needs a high dose of antifungals, which results in toxicity to the human cells.[11] For all of these reasons, new antifungal agents are always needed.

Nanotechnology is a promising technique to create new antifungals or enhance conventional groups.[1] Several nanoformulations are currently prepared to control fungal infections with high therapeutic efficacy, fewer adverse effects, and more effectiveness in killing resistance species.[1-3] The physicochemical properties of such nanomaterial are important to take into consideration when starting the design of nanoparticles, such as the size, shape, and influence of its surface chemistry.[11] Several metallic nanoparticles such as silver, zinc oxide, gold, iron, cobalt, copper, lead, and magnesium oxide are successfully entering for preparing effective antifungal nanomaterials.[7-8] An example of such activities includes the strong antifungal action of AgNP on several pathogenic fungi for plants (*Chaetomium globosum* and *Mortierella alpina*) and humans (*A. fumigatus*, *Trichophyton rubrum, C. albicans*, and *C. tropicalis*); and AuNP on *C. albicans*; iron oxide nanoparticles on *A. niger* and *Penicillium chrysogenum.*[8-9] The antifungal effects of AgNP are mainly dependent on its high efficacy on antifungal resistance species and its ability to reduce virulence factors.[5] Aghamoosa et al. (2014) found that the size of AgNP may affect the antifungal activities of these nanoparticles.[16] Three sizes of AgNP, including 10, 20, and 40 nm were examined on *C. albicans* and two of filamentous fungi (*Trichophyton rubrum* and *A. fumigatus*). The MIC of all sizes on all fungi ranged from 4 to 16 µg/ml. AgNP with 40 nm was the strong effective size, followed by nanoparticles with 20 nm. Meantime, a size of 10 nm showed more effectiveness on *C. albicans* and *A. fumigatus* and little on *T. rubrum*.

Antifungal activity of AgNP on yeasts

Candida albicans is the common type of yeast with a high rate of invasive fungal infections worldwide due to its possessing many virulence factors.[4] Treatment of *Candida* infections by conventional antifungals is usually associated with several limitations such as high toxicity, expensive, recurrent infection, and emerging antifungal resistance.[4] Applications of metal nanoparticles either alone or in combination with antifungals to form nanoformulations have shown a significant reduction in most of these limitations.[3] Growth and virulence factors of *C. albicans* are inhibited by synthetic silver, gold, or iron nanoparticles.[4,6]

Silver nanoparticles (AgNPs) revealed more *in vitro* inhibitory effects on *C. albicans* with low MIC compared to conventional antifungals.[10] The MIC of AgNP on *C. albicans* was from 0.125 µg/ml to 0.5 µg/ml in compared to fluconazole (1–64 µg/ml) or caspofungin (0.062–1 µg/ml). Two clinical isolates of *C. albicans* were more affected by AgNP (MIC 0.27–0.97 µg/ml) than AmB (MIC 0.5–2 µg/ml).[17] Many *C. albicans* and non-albicans *Candida* were killed by synthetic caspofungin with gold nanoparticles (CAS-AuNPs) by forming pores in fungal cell walls.[3] Additionally, the antifungal action of caspofungin on *Candida auris* has been enhanced by loading caspofungin on zinc oxide nanoparticles (CAS-ZnO NPs) by reducing the MIC50 value of caspofungin from 15.47 ng/ml to 10.34 ng/ml.[18] The activities of AgNP are also demonstrated on several strains of resistance *C. albicans*.[5,10] Metal nanoparticles also showed *in vivo* therapeutic activities for the treatment of different types of candidiasis such as oral and cutaneous candidiasis in rodent models.[4] The opportunistic pathogenic yeast, *Trichosporon asahii*, which is a non-*Candida* opportunistic pathogen shows resistance to many antifungals.[19] It was susceptible to the AgNP with low MIC (0.5 mg/ml) compared to six conventional antifungals (Amphotericin B, 5-flucytosine, caspofungin, terbinafine, fluconazole, and itraconazole).[20] Moreover, biofilm produced by *C. albicans* is significantly reduced under the effect of AgNP.[4] The combination of propolis extract with AgNPs revealed fungicidal action on mature biofilms of *C. albicans* at lower cytotoxicity concentrations.[1]

Antifungal activity of AgNP on filamentous fungi

The activities of AgNP are not only studied on human pathogenic yeasts, it is also evaluated on important medical filamentous fungi. The effect of nanomaterials, including AgNP, on many species of *Aspergillus* and for treatment of their infections, aspergillosis, has been investigated by many studies.[2, 8-9] The AgNP associated with the extract of growing *Bacillus thuringiensis* MAE6 that was synthesized by green methods showed antifungal action on four species of *Aspergillus*, including *A. niger*, *A. terreus*, *A. flavus*, and *A. fumigatus* at a concentration of 500 µg/ml.[21] The MIC of AgNP on these species were 125, 62.5, 15.62, and 62.5 µg/ml, respectively. Clinical isolates of *A. niger*, *A. fumigatus*, and *A. flavus* from nasal debridement by swabs and biopsy were significantly affected by AgNP in MIC of 1–64 µg/ml and MFC of 16–512 µg/ml.[22]

Many other filamentous fungi are affected by AgNPs. Twelve clinical isolates of *Rhizopus arrhizus*, as one cause of mucormycosis, were inhibited by AgNP in MIC of 1–64 µg/ml and MFC of 16–512 µg/ml.[22] Clinical isolate of *Fusarium oxysporum* from nasal swabs and biopsy was affected by 1µg/ml (MIC) of AgNP.[22] *Trichophyton rubrum*, as one of the causative agents of dermatophytosis, was also inhibited by AgNP.[8] The AgNP at different sizes showed effective action on *T. rubrum* in MIC of 4–16 µg/ml.[16] However, therapeutic applications of AgNP for the treatment of invasive fungal infections, such as mucormycosis and coccidioidomycosis still need further *in vivo* studies.[2]

Mechanism of Action

Antifungal activities of AgNPs have been explained by many mechanisms of action. The oxidation state of AgNP plays an important role in binding with microorganisms and shows toxicity in their growth.[5] Such toxicity depends on many factors that relate to the chemical structure of AgNP, such as the nanoparticle size, shape, and capping nature.[5] Although the mechanism of AgNP action in fungal cells is not well understood, the main proposed target of AgNP is the cell membranes (plasma membrane and cell wall) and the cellular components.[5-6, 20] The release of positive silver ions from AgNP is the first step of antifungal action.[11] These ions can attach to the negatively charged surfaces of fungal cell membranes causing structural changes and loss of membrane functions.[6] Examination of the cellular structure of *Aspergillus* spp. that was treated with synthesized AgNP and extract of *Bacillus thuringiensis* by the green process revealed a reduction in the integrity of the cell wall with separation of the cell membrane from the cell wall toward the cytoplasm.[21] Morphological changes resulting from harm to cell walls were observed after treating cells of *Candida* spp. with AgNP.[17] Additionally, the growth of *T. asahii* was inhibited by AgNP through the destructive activity of AgNP in the cell walls and cellular components.[20]

Many other mechanisms of antifungal action of AgNPs have been proposed. One suggested mechanism is the inactivation of the cellular enzymes by binding silver ions with the disulfide bonds of these enzymes.[6] Generally, oxidation stress by releasing reactive oxygen species (ROS) as free radicals is the strong proposal mechanism.[6] These ROS have many cellular destructive actions, such as denaturation of proteins, damage to the integrity of the cell membranes by inactivation of enzymes and proteins, DNA damage, decreasing the levels of ATP and protein pump, and blocking mitochondrial functions.[4, 6, 8]

The AgNP can play a synergistic action with fluconazole to prevent the production of biofilm by *C. albicans* through the role of nanoparticles to facilitate entering of fluconazole across the cell membrane and the action of silver (Ag) in disturbance efflux transporter activity.[1] In comparison between the effect of caspofungin and caspofungin-ZnO NP on *C. auris*, caspofungin enhances the resistant properties of *C. auris* by increasing contents of chitin in the cell wall and overexpression of the genes targeted by caspofungin, while caspofungin-ZnO NP has an opposite role to all of the previous activities.[18]

CONCLUSIONS

Controlling infections with resistant species of *Candida* and systemic filamentous fungi is considered a challenge in a world filled with high rates of immunocompromised

patients. The appropriate medical characteristics of AgNP and other metal nanoparticles have opened a promising path to provide alternative drugs for the treatment of refractory fungal infections. Generally, almost all of the successful results regarding the antifungal activities of metal nanoparticles are obtained in *in vitro* studies. Clinical trials for the *in vivo* efficacy of these new synthetic compounds on invasive fungal infections are recommended.

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