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ANTIFUNGAL ACTIVITY OF SILVER FUNGAL NANOPARTICLES - A NOVEL THERAPEUTIC APPROACH

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To synthesize silver fungal nanoparticles (fungal nano-Ag) and investigating antifungal activity on fungal pathogens. The isolated nanoparticles showed potent activity against clinical isolates (*Trichophyton mentagrophytes, T. rubrum, Microsporum canis, M. persicolor and Candida albicans* (IC_{80}, 1-6µg/ml). The activity of the nanoparticles was compared to Amphotericin B (1-5µg/ml) and Fluconazole (10-30µg/ml).The results showed fungal nanoparticles exerted activity on the pathogenic fungal mycelia.Thus the present study highlights the fungal nanoparticles may have considerable antifungal activity deserving further invasion for clinical applications.

Key words: Antifungal effect, Fungal clinical isolates, Fungal nanoparticles.

INTRODUCTION

Fungal infections remain an important cause of morbidity and mortality in hospitalized patients and others who in contact. The primary factor driving the emergence of antifungal resistance appears to be selective pressure. Resulting from increased research on alternative system of medicine including nanotechnology given the seriousness of fungal infections and difficulties linked with their diagnosis, restricting empiric antifungal therapy may not be the best solution for combating resistance. Furthermore, when resistance does occur it is generally after prolonged exposure to relatively low concentrations of drug such is the case when antifungal prophylaxis is given to immune compromised patients [1,2,3].

Dermatophytosis is one of the most common infectious diseases in humans which are mainly caused by invasion of stratum corneum dermatophytic fungi [4]. The various forms of a disease include tinea corporis, tinea paedis, oncomycosis, candidiasis etc. For which many antifungal drugs including terbenafine, triazoles, butenafine have been used clinically for the topical treatment of dermatophytosis [5]. The current antifungal armamentarium recommends as first line therapy showed high incidence of toxicity wide adverse events and observable resistancy. This upward trend is considering limited number of antifungal drug available because prophylaxis with antifungal may lead to emergence of resistant strains [5,6]. Therefore there is an inevitable and urgent medical need for novel antifungal formulations. In recent advances in research on metal nanoparticles, silver based fungal nanoparticles have received special attention as a possible antimicrobial agent [7,8,9,10]. Since ancient times it has been known that silver and its compounds are effective antimicrobial agents [6,11]. There, the preparation of nanosized silver particles with specific requirements in terms of size, shape and chemical properties is of great interest in the formulation of new pharmaceutical products. Many literatures have shown that the nanoparticles are having antimicrobial properties whereas the effects against fungal pathogens are mostly rare. In this investigation fungal based silver nanoparticles were synthesized and its antifungal effects on clinical pathogens were investigated.

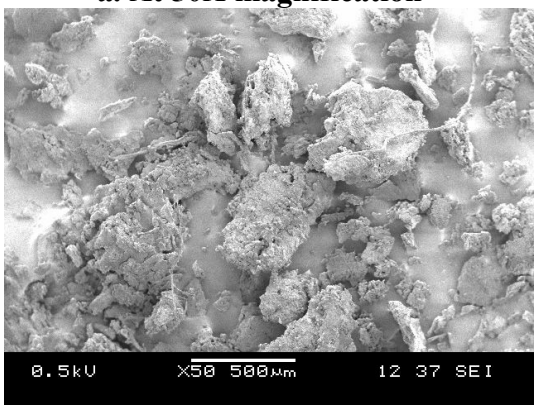
MATERIALS AND METHODS

Preparation of fungal nanoparticles

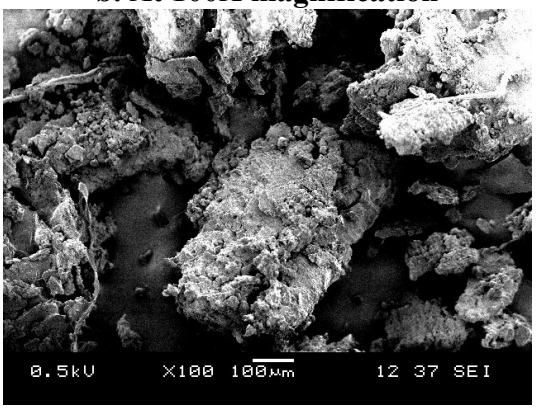
The test fungus included in this study is *Aspergillus fumigatus* where it was grown in Sabouraud dextrose broth for the production of fungal based nanoparticles which can be used for antibacterial assay and their physico chemical characterization.

Figure I: Scanning Electron Imaging of *Aspergillus fumigatus* mediated silver nanoparticles in various magnifications

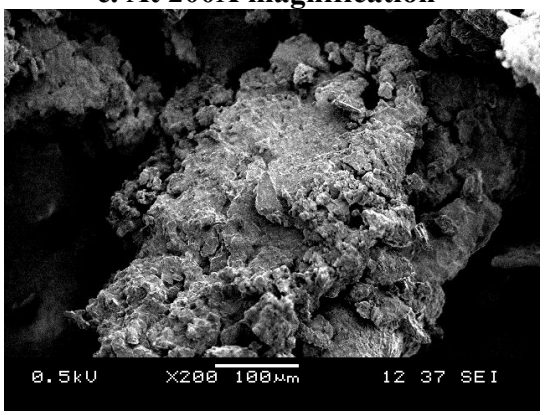
a. At 50X magnification



b. At 100X magnification



c. At 200X magnification



d. At 500X magnification

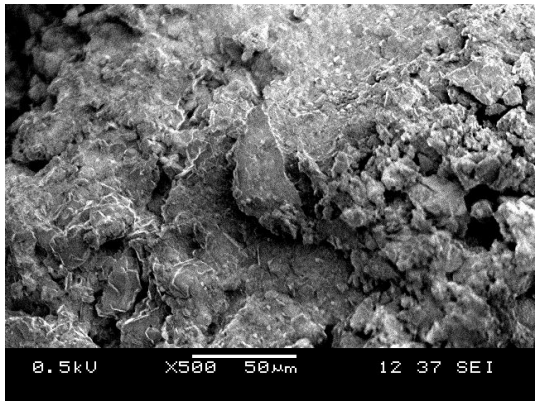


Table I: Periodical color change from yellow to brown of *Aspergillus fumigatus*

S. No.	Time interval	Observation of nanoparticle formation by *Aspergillus fumigatus*
1	10 mins	-
2	30 mins	-
3	1 hr	+
4	2 hr	++
5	4 hr	+++
6	8 hr	++++
7	16 hr	+++++
8	28 hr	+++++++

- No colour change; + Dark yellow; ++ Reddish yellow; ++ Reddish yellow; +++ Red; ++++ Reddish brown; +++++ Tinge brown; ++++++ Brown threads

Table II: Antifungal activity of *Aspergillus fumigatus* mediated silver nanoparticles

Fungal strains (No. of strains)	IC_{80} (µg/ml)		
	Fungal nano Ag	Amphotericin B	Fluconazole
T. mentagrophytes (13)	1-5	1-2	20-32
T. rubrum (7)	1-6	1-2	20-25
M. canis (11)	2-6	2-4	17
M. persicolor (8)	-	2-4	20-28
C. albicans (7)	2-5	4-6	12-18

In this study, the isolated nanoparticles were subjected to antileptospiral activity by which a novel drug to be formulated to overcome drug resistance. After appropriate time of incubation and the water bubbles on the fungal mat is the indication of the fungal broth to be subjected for filtration [12,13]. With the usage of glass rod, the fungal mat was crushed thoroughly and then filtered through Whatmann No.1 filter paper in aseptic condition and then the filtrate was stored for further use under aseptic condition at 4°C. The extra cellular production of metal nanoparticles was found that aqueous silver ions when exposed to test fungal strains are reduced in solution, there by leading to the formation of silver hydrosol.

The reduction of the metal ions occurs by a nitrate – dependent reductase and a shuttle quinine extra cellular process [13,14]. From the collected fungal filtrate, 5ml was taken in sterile BOD bottles and 100ml of 10^{-3}m $AgNo_3$ solution was added under aseptic condition. This mixture was kept at room temperature for 28 hours. Periodical checking from pale yellow to brown colouration was noted and recorded. The sizes and morphology of nanoparticles were examined by using a scanning electron microscope (JOEL 2010-F SEM with oxford EDS unit at voltage of 200 KV operated at Scanning mode using an HAADF detector).

Determination of antifungal susceptibility

A total of 46 strains of 5 fungal isolates were used in this study. *Trichophyton mentagrophytes*, *T. rubrum, Microsporum canis, M. persicolor, Candida albicans* were obtained from the Department of Laboratory Medicine, Kovai Medical Center and Hospital, Coimbatore. These fungal members were cultured in Sabouraud dextrose agar at 35°C. The isolates were confirmed by standard microscopy and other special tests according to standard clinical mycology manual.

The Minimum Inhibition Concentrations for *Trichophyton, Microsporum* and *Candida* species were determined by broth micro dilution technique based on the national commity for clinical standard (NCCLS: now renamed as clinical and laboratory standards institute CLSI; 2000) method out lined in documents M-27A [15] and M-38P [16] respectively.

The Sabouraud dextrose medium buffered to pH 6 with 2% olive oil and 1% Tween 80 was used as the culture medium and the inoculums size of *Candida* was 0.5×10^3 to 2.5×10^3cells/ml, *Trichophyton* was 0.4×10^4 to 5×10^4 cells/ml and *Microsporum* was 0.6×10^4 to 4×10^4 cells/ml. The micro dilution plates inoculated with fungi were incubated at 35°C and the turbidity of the growth control wells was observed every 24 hours. The 80% inhibition concentration. (IC_{80}) was defined as the lowest concentration that inhibited 80% of the growth by a comparison with growth in control plates. The growth was assayed with a micro plate reader [Bio Rad 2010 (model 680)

with 100-240 voltage at 50-60 hertz] by monitoring absorption at 405nm. In this investigation, Amphotericin B and Fluconazole were used as positive control towards fungi.

Effect of nanoparticles on dimorphic transition

Candida albicans cells were maintained by periodic sub culturing in liquid yeast extract peptone dextrose (YPD) medium. Cultures of yeast cells were maintained in liquid YPD medium at 37^{o}C were mycelia formation was induced by supplemented with 20% Bovine serum albumin (BSA). The dimorphic transition in *C. albicans* was investigated from cultures containing 2mg/ml of nanoparticles (at the IC_{80}) which is incubated for 48 hours at 37^{o}C [17,18].

RESULTS

The aqueous silver ions when exposed to *Aspergillus fumigatus* are reduced in solution, thereby leading the formation silver hydrosol [12,14]. The formations of silver fungal nanoparticles were confirmed by change in colour to brown from pale yellow. The periodical analysis of colour change of *A. fumigatus* was observed and interpreted (Table I). The size dependent phenomenon was well studied using Scanning electron microscope and the results showed fungal nanoparticles were spherical form and its average size 3nm (Figure I – a,b,c,d).

The biosynthesis of silver nanoparticles was clearly observed and noted within 10minutes of silver ions coming in contact with filtrate and were quite stable even upto 4 months at 25^{o}C[14]. Fungal nano Ag in an IC_{80} range of 1-6µg/ml showed significant antifungal activity against *Trichophyton mentagrophytes , T. rubrum, Microsporum canis* and *Candida albicans*. In this study, the inhibition rate over *M. persicolor* is very less exhibited as less potent activity. Toward all fungal strains fungal nano Ag exhibited activity with amphotericin B showing IC_{80} values of 10-30µg/ml. The antifungal activity of fungal nano Ag was incorporated in the Table II. The dimorphic transition in *candida albicans* was investigated from cultures containing 2mg/ml of fungal nano Ag (IC_{80}), were incubated for 24-48 hours at 37^{o}C. The dimorphic transition to mycelia forms was detected by phase contrast light microscopy (Nikon, Japan).

The dimorphic transition of *C. albicans* from yeast to mycelia form is responsible for pathogenicity with mycelia shapes being found during the entry of host tissue. The serum induced mycelia were significantly inhibited from extending in the presence of fungal nano Ag, at the same time the formatting of mycelia is normal in the absence of fungal nano Ag. These results highlighted and suggested that fungal based silver nanoparticles are a potential compound in the treatment of fungal infections. Many studies showed the antimicrobial effects of fungal nanoparticles but the effects against fungal pathogens of clinical isolates are mostly unknown. Various studies highlighted the importance of fungal based nanoparticle are effective against various pathogens including drug resistant strains [11,14,19,20].

DISCUSSION

The chemical method of preparing nanoparticle and its significance over dermatophytes to produce effective antifungal agent was standardized [6]. The primary significance of current investigation is the observation of fungal based nanoparticles could inhibit the growth of dermatophytes and opportunistic fungal pathogen. To our knowledge this is the first study to apply fungal based nanoparticles successfully to dermatophytes. The major significance of this work is the fact that the nanoparticle formulation methodology is cost effective and time consuming. Therefore it could be identified and expected that the nanoparticles produced from *Aspergillus fumigatus* may have potential as an antifungal agent overcome the problems of drug resistancy and reducing the drug mediated adverse events.

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