Nanoantimicrobials: A critique on Susceptibility,Mechanism of action and Toxicity on Opportunistic fungal pathogens

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BACKGROUND: Burden of serious mycotic infections in high risk patients and development of multi-drug resistance warrants the inevitable need for development of novel antifungal drugs with better efficacy. Recently, Nanoscale materials have emerged as a promising alternate to conventional antimicrobial agents and often regarded as next generation antibiotics. The knowledge on antifungal behavior and their precise mechanism of action on fungal pathogen is not yet elucidated and were reported in this study.

MATERIALS AND METHODS: The silver nanospheres were synthesized via simple green chemistry approach using Silver nitrate as precursor and aqueous extract of *Allium cepa* as reducing agent. The nanodimension of the Ag nanospheres were characterized by UV- visible spectroscopy, HRTEM, XRD, FTIR Spectroscopy, EDX spectroscopy, Zeta potential analysis and ICP-OES spectroscopy. The antifungal potential of nanospheres were tested on 30 different yeast and mould pathogens including the causative agents of Candidiasis, Cryptococcosis, Trichosporonosis, Geotrichosis, Rhodotorulosis, Aspergillosis, Mucormycosis, Penicilliosis, Fusariosis, Dermatophytosis and Phaeohyphomycosis by micro broth dilution assay following CLSI guidelines. The antifungal action mechanism was investigated using Scanning Electron Microscopic analysis, Ergosterol and Reactive Oxygen Species (ROS) profiling, Lactate dehydrogenase leakage, Propidium Iodide (PI) influx and Lipid peroxidation assays. The cytotoxicity of the nanospheres was assessed on mammalian system using MTT and hemolytic assays.

RESULTS: The synthesized silver nanospheres exhibited strong SPR at 417nm with average size of 7.5nm, negatively charged, crystalline, polydispersed and were highly stable. The nanospheres showed broad spectrum activity against all the tested fungal species with MIC ranging between 0.8 - 0.1µg/ml. The results of mechanistic investigation suggested that the antifungal mechanism of the nanospheres is due to the increased generation of ROS which subsequently cause number of deleterious events including disruption of the cellular components and fungal cell membrane. In addition, the lethal dose of synthesized nanospheres in mammalian cells was several orders of magnitude than that of MIC values.

CONCLUSION: The silver nanospheres exhibited potential antifungal behavior on both yeast and mould pathogens with lesser MIC values compared to conventional antifungal drug and showed lesser cytotoxic effects on mammalian cells. Increased ROS generation is the key antifungal mechanism exhibited by silver nanospheres as evidenced from this study. Further analysis of the antifungal mechanism on selective fungal cell targets would help us to elucidate the therapeutic potential of silver nanospheres.

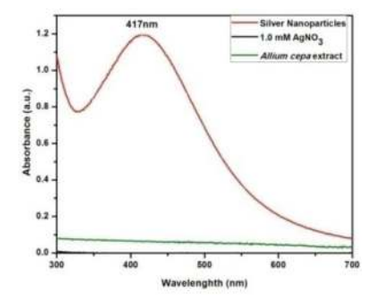

Fig 1: UV-vis Spectrum of Ag nanospheres

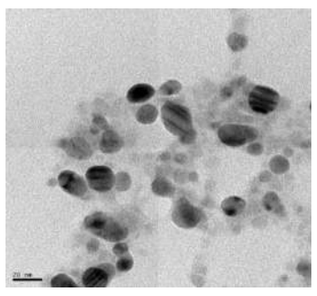

Fig 2: HRTEM micrograph of Ag nanospheres

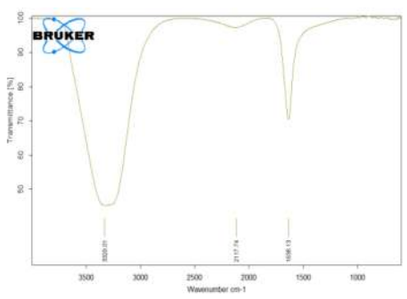

Fig 3: FTIR spectrum of Ag nanospheres

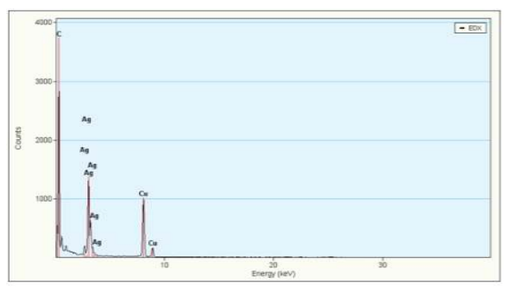

Fig 4: EDX spectrum of Ag nanospheres

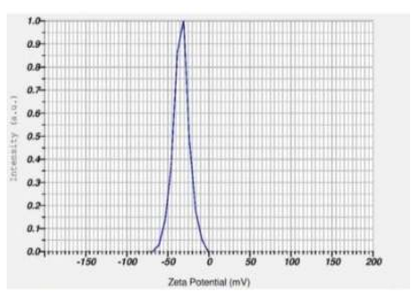

Fig 5: Zeta potential of Ag nanospheres

Species Tested	MIC (µg/ ml)
T. asahii	0.5
C. parapsilosis, G. candidum C. neoformans, R.mucilaginosa M. gypseum	0.1
C. albicans, C. glabrata, A. fumigatus, A. versicolor, P. purpurogenum, P. oxalicum, S. racemosum, C. berthollotiae, F. solani, T.mentagrophyte, T. rubrum, A. alternata, C. verruculosa	0.2
A. nidulans, A. ochraceus, C. pallescens, Mucor spp, Rhizomucor spp. Paecilomyces variotii,	0.4
A. niger , A. terrus, R. microsporus, Paecilomyces lilacinus	0.8
A. flavus	>0.8

Table 1: MIC of Ag nanospheres against Fungal species.

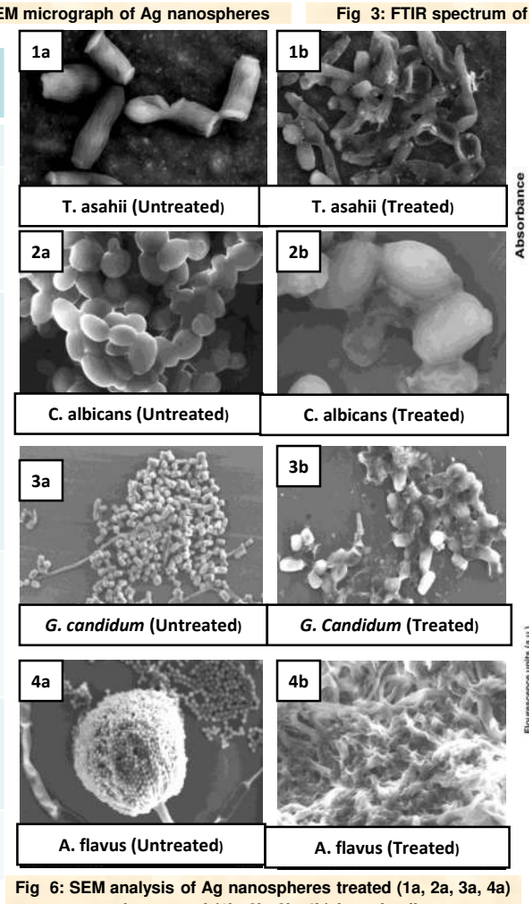

Fig 6: SEM analysis of Ag nanospheres treated (1a, 2a, 3a, 4a) and untreated (1b, 2b, 3b, 4b) fungal cells

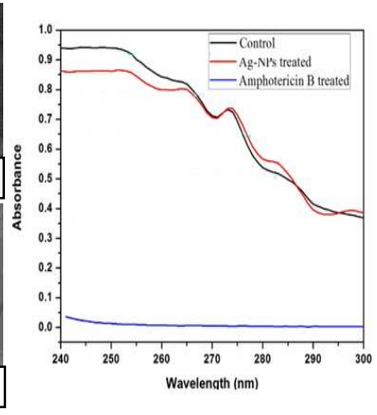

Fig 7: Ergosterol profile of Ag nanospheres treated and untreated fungal cells

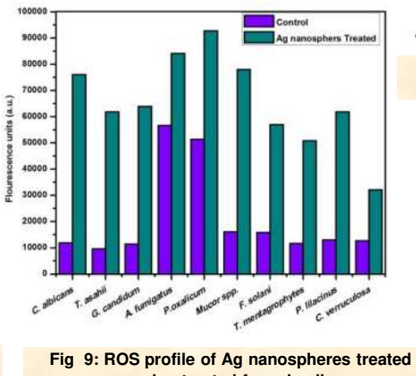

Fig 9: ROS profile of Ag nanospheres treated and untreated fungal cells

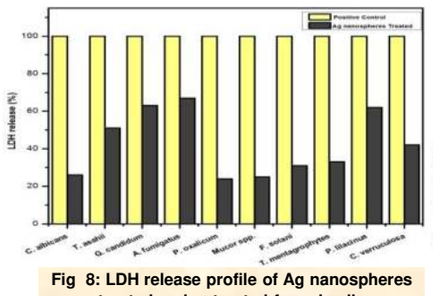

Fig 8: LDH release profile of Ag nanospheres treated and untreated fungal cells

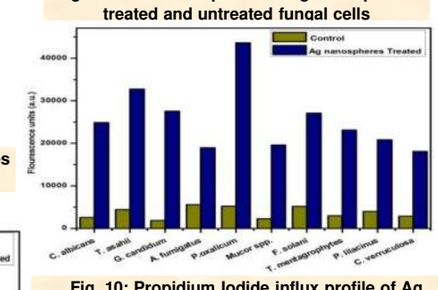

Fig 10: Propidium Iodide influx profile of Ag nanospheres treated and untreated fungal cells

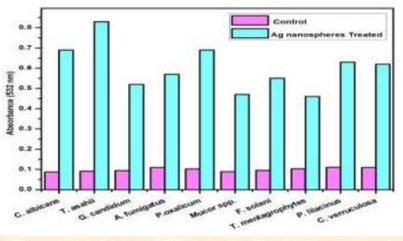

Fig 10: Lipid peroxidation profile of Ag nanospheres treated and untreated fungal cells

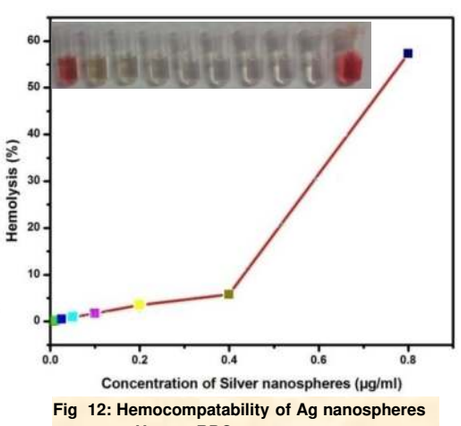

Fig 12: Hemocompatability of Ag nanospheres on Human RBC

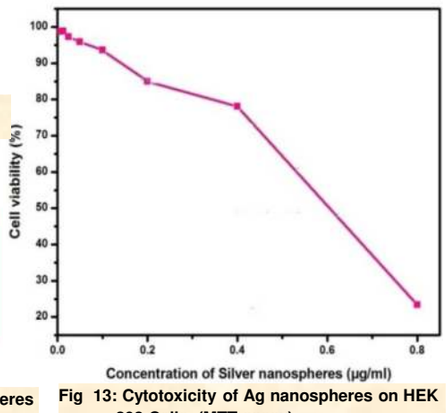

Fig 13: Cytotoxicity of Ag nanospheres on HEK 293 Cells (MTT assay)

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