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Inhibition of Candida infections by synthesized Zinc oxide nanoparticles and mechanism thereof

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ABSTRACT

Candida infections are very serious cause of health issues and consequently, there is critical requirement for novel drug lead compounds with potent antifungal activity and least side effects. Minimum inhibitory concentration (MIC) was established by microdilution technique. Drug resistance in microorganisms has imitated significant susceptibility to present medicines; hence alternatives to fight with resistant fungal pathogens are desirable. Our focus was to prepare as well as categorize zinc oxide nanoparticle with Candida sp. and to explore them in terms of their antifungal potential. ZnO NPs have been successfully amalgamated through hydrothermal process utilizing zinc nitrate precursor and calcination at 150 °C for 12 h. The synthesized ZnO NPs determined antifungal activity against fungal isolates. The ZnO NPs were characterized using standard physicochemical characterization viz. XRD, SEM, FTIR and EDX. The characteristic antifungal property of ZnO NPs against *C. albicans* (ATCC 10231) was estimated. The ZnO NPs depicted antifungal potential which advocate their use as material for development of antifungal drugs to be used in food industry, cosmetics, water purification, textile industry etc.

Keywords: *Candida albicans*; Candidemia; Antifungal drugs; ZnO NPs; Infections

1. INTRODUCTION

During last few decades fungal infections in human beings have frighteningly amplified especially in immunocompromised persons [1]. However, development of novel techniques has resulted in discovery of new medicines. Nevertheless, this has given rise to longevity and survival of numerous patients. The existing statistics point out that comparative percentage of microbes responsible for nosocomial bloodstream infectivity has altered during current times and Candida species has been recognized among the most frequent pathogens. Candidemia has not only been found responsible for soaring death rate but also has lengthened the treatment duration; ultimately giving rise to costly hospital expenses. Additionally; the outcome of all this is more incapacitated patients having very weak immunity susceptible to infectivity, primarily originated by fungi. Candida species are universally dispersed, inhabit plants as well as alimentary tracts of mammals; lives commensally on human mucocutaneous surfaces [2]. It has been reported that approximately 50-70% of total yeast isolates in gastrointestinal tract of human beings are identified

as *Candida albicans*. Frequently utilized antifungal molecules are Amphotericin B, Itraconazole and Fluconazole. Although the aforementioned drugs are being utilized to treat various fungal infections, however, these drugs face various limitations such as side effects as well as appearance of resistance due to prolong exposure [3]. The available medicines are mainly restricted to Amphotericin B and to some lipid preparations [4] azole pharmaceuticals, for instance; Fluconazole, Itraconazole, and Flucytosine (5-Fluorocytosine). However, these drugs are found to have side effects and complications; therefore, their use is being limited. These complications include dose-limiting nephrotoxicity due to Amphotericin B, swift resistance with Flucytosine, drug-drug complications, fungistatic activity as well as azoles resistance.

Considering the limitations of present available antifungals; there is an imperative requisite for novel antifungal drugs with broad spectrum fungicidal activity. It is also important to develop drugs with least or none after effects [5, 6]. Traditionally, medicinal plants and plant products have been found as an outstanding source of pharmaceuticals with admirable antifungal potential. However, Candida species infectivity and their high rise antifungal resistance is major dilemma, therefore, innovative approaches are required to control virulent aspects which result in severe pathogenicity [7]. Therefore, effective substitutes to encounter against multiple drug resistant pathogenic fungi are required [8]. Nanostructured materials deserve increasing attention owing to outstanding antimicrobial potential even at very small quantity. The objective of present work was to synthesize ZnO NPs using zinc nitrate precursors.

ZnO NPs are non-toxic and possesses various attractive functionalities. The ZnO NPs are synthesized by various techniques [9-12]. Its (ZnO) noteworthy functions enable its use in light emitting diode, electronic material, capacitors or gas sensors etc. [13]. Furthermore, ZnO is incorporated into cosmetics and medical gels in order to formulate them sunlight- shielding and antiseptic [10, 12-13]. In this study we have characterized ZnO NPs and estimated for anti-fungal effect using standard serial dilution approach.

2. MATERIALS AND METHODS

2.1. ZnO nanoparticles synthesis

ZnO NPs were synthesized by direct hydrothermal process. About 2 g of Zn(NO3)2.6H2O (Alfa Aesar Company) was mixed in 70 ml of distilled water. The zinc solution was kept continuously stirring for 10 minute. After it 2 M NaOH was added drop by drop till the solution attained the pH 10. The final solution was then shifted to autoclave reactor and heated at 150 °C for 12 hrs. Subsequently, the solution was kept outside until it reached room temperature. The solution was filtered and precipitate was washed several times with distilled water and one time with ethanol. It was kept in the oven for drying at 80 °C for 12 hours.

2.2. Characterization

The zinc oxide powder sample was checked for its crystallinity by using X-ray diffraction (XRD, Rigaku). In order to evaluate the particle sizes and morphologies of sample powder, SEM (Scanning electron microscope) images are taken. The image of the sample was taken from SEM. The chemical constituents and functional group of the sample were analyzed by EDS (Energy dispersive spectrum) and FT-IR (Fourier transform infrared, Thermo scientific instrument) spectroscopy. The wavelength spectra of the samples were recorded by UV-Visible spectroscopy (UV-Vis, Shimadzu).

2.3. Antifungal activity

The clinical isolate of *C. albicans* ATCC 10231 was used in this study and maintained and cultured as previously described [14]. MICs was assessed on Sabroud agar (SDA) dishes as mentioned by Jalal, M. et al., 2016 [15] with important modifications. Fluconazole (25 mg, Hi-Media, India) disks were used for sensitivity test of *C. albicans* [16]. The *Candida albicans* was cultured and maintained in SDA. Briefly, after spreading the plates with representative fungus, the inoculated plates were kept in an incubator for 30 minutes for incubation. The fungal culture growth was monitored after 72 h incubation period. The experiments were executed in triplicate.

3. RESULTS AND DISCUSSION

The XRD spectrum of ZnO NPs calcined at 160 °C is shown in fig. 1. The position of the peaks with 2θ angle of 32.05°, 34.6°, 36.4°, 47.8°, 57.02, 63.15, 66.78, 68.26, 69.28, 72.68 and 77.11° correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) planes [18, 19]. The peaks agree well with those of standard ZnO which confirms the synthesis of hexagonal wurtzite ZnO. All reflection peaks in XRD are also noticeably broad showing the fine nature of ZnO NPs.

The particle sizes and morphologies was evaluated SEM images (figure 2) and it shows the sample consisting of homogeneously distributed spherical particles in nano size. The diameter of the ZnO NPs is found to be approx. 100–200 nm size.

The energy dispersive spectrum (EDS) of the sample (figure 3) was shown with spectrum that it contains mainly Zn and O peaks. The elemental ratio of Zn and O was approximately 1:1. The signal from carbon is due to the carbon substrate tape and signals from platinum are due to Pt coating on sample.

The functional groups of the ZnO NPs are determined by FT-IR (Fig. 4). A prominent peak at 3431 cm−1 signifies the O-H stretching and bending vibrations of water molecules adsorbed on ZnO. In the wave number range of 1632 cm−1 and 2355 cm−1 the peaks are ascribed to the adsorption of water and carbon dioxide molecules on the metal surface [25]. The peak at 1383.9 cm−1 and 1634.6 cm−1 corresponds to C=O asymmetric and symmetric vibration. The band present at 875 cm-1 is ascribing to the formation of tetrahedral coordination of Zn. The peak at 443 cm−1 and 546 cm-1 signifies the stretching mode of Zn-O vibration.

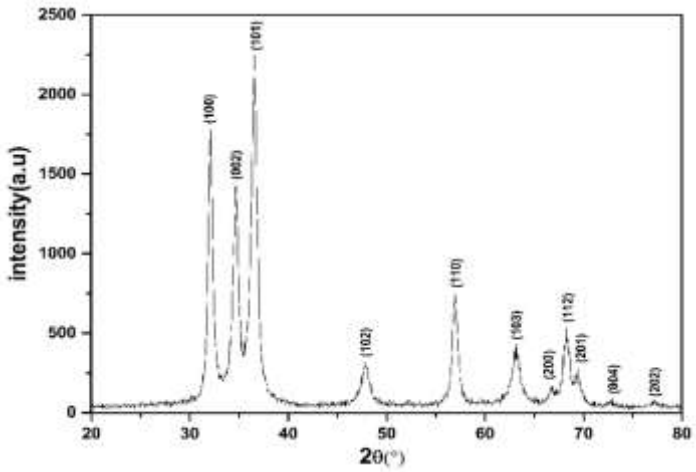

Fig 1. XRD spectra of synthesized ZnO nanoparticles calcined at 150 ºC

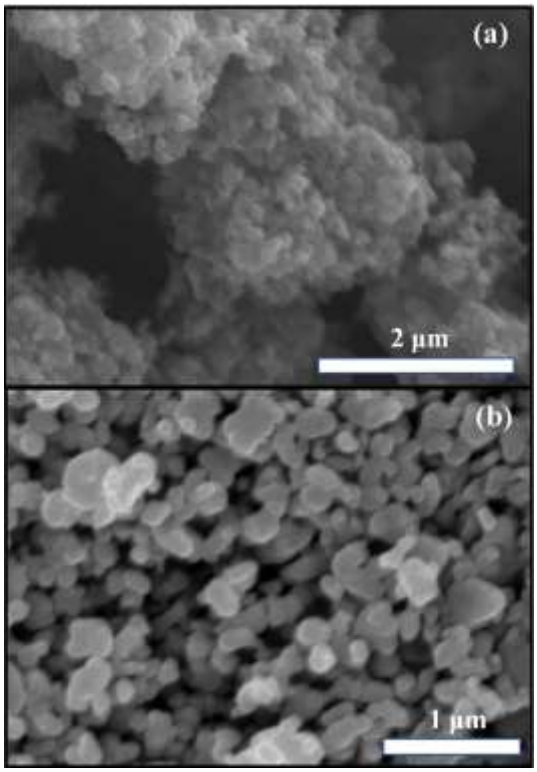

Fig 2. SEM images of synthesized pure ZnO nanoparticles

The anticandidal action of ZnO NPs is determined employing broth dilution method to conclude MIC. The obtained MIC for *C. albicans* is established to be 75 µg/ ml. The representative fungal isolate utilized is *C. albicans*. Herein, it is found that high concentration (600 µg) of ZnO NPs possesses commendable antifungal potential (Fig. 5).

Numerous reports have shown that species of Candida are etiological means of human infectivity. Almost 90% of contagions are due to *C. albicans* and its related species [17]. Approximately, fifty percent of candidiasis is due to the *C. albicans*. This pathogen is also accountable for critical nosocomial candidemias which is responsible for increased deaths [18]. Increased infection rate due to *C. albicans* as well as elevated confrontation to current antifungals; projected the demand to develop innovative antifungal medicines. Nevertheless; previously, there is no study which account for probable antifungal mechanism of ZnO NPs. Our research investigation for the first time reports antifungal potential of ZnO NPs. Antifungal activity of ZnO NPs unwrap a suitable substitute to accessible antimicrobial drugs. The most common method of toxicity of ZnO NPs could be role of ROS as well as Zn2+ ion on N-acetylglucosamine (N-acetyl-D-glucose-2-amine) or and β-1 3-D-glucan synthase. ROS are produced by the nanoparticles [19], whereas Zn2+ ions are created in aqueous medium by nanoparticles [20, 21]. The other report

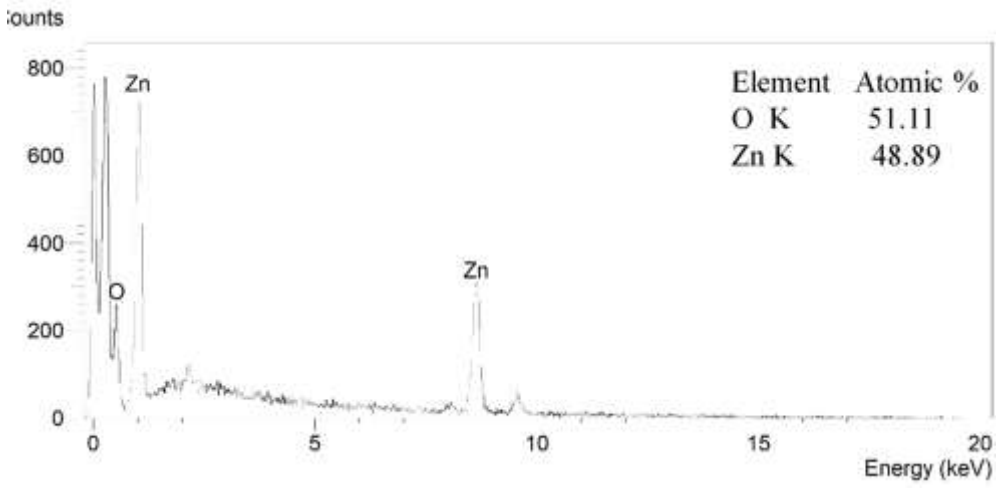

Fig 3. EDX spectra of synthesized pure ZnO nanoparticles

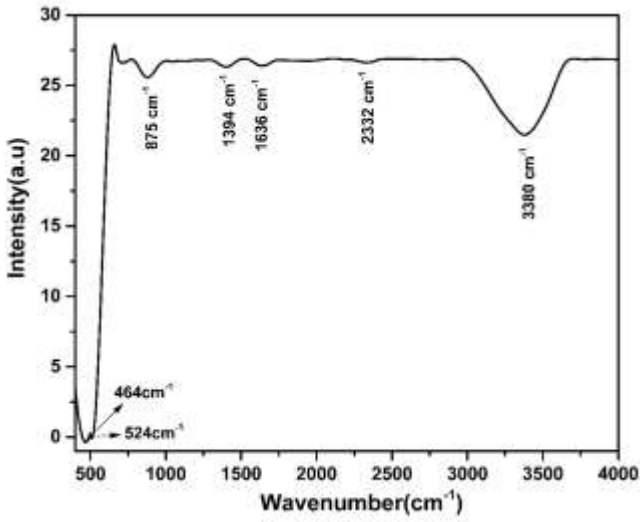

Fig 4. FT-IR spectra of the pure ZnO nanoparticles

had signified that nanoparticles develop oxidative stress [21] and destruction is either caused in direct way or by production of inflammatory mediators. Similarly, previous studies evaluated toxicity of Zinc encrusted planes for multiple resistant fungal isolates [22]. Herein, the ZnO NPs have been tested for very prevalent human pathogen and was found evidently effective against *C. albicans* fungus. Zinc is a non-toxic element therefore turning Zinc into a very promising biocidal material in order to trim down microbial contamination will be very helpful.

4. CONCLUSION

Conclusively, therapeutic collapse as well as progress in drug resistant fungal isolates has directed necessity to discover innovative anticandidal drugs. This investigation was about synthesis and characterization of ZnO NPs and to explore them in terms of their anti-candiasis potential. The ZnO NPs depicted excellent antimicrobial potential which

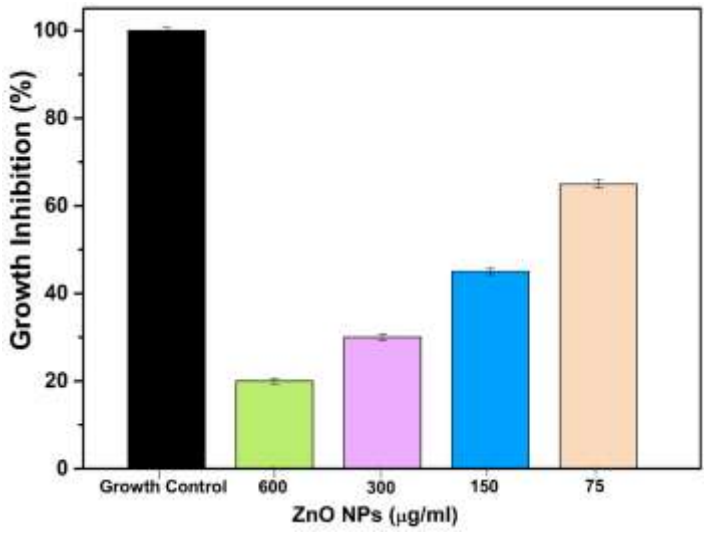

Fig 5. In vitro antifungal action of ZnO NPs against *C. albicans*. Absorbance for control in absence of ZnO NPs as well as with different concentrations of ZnO NPs was determined. *C. albicans* growth was articulated as percent growth of control culture.

strongly recommends their use as material of choice for future antimicrobial agents. Herein, we report that dose-dependent anticandidal potential of ZnO NPs suggest that ZnO NPs possibly can be utilized for preparation of novel anticandidal drugs to restrain candidal adherence to healthcare instruments. However, before their utilization, their compatibility with human cells should be ensured. Besides anticandidal activity, ZnO NPs have depicted potential uses such as anticancer, antibacterial, antidiabetic, anti-inflammatory etc. Therefore in recent future the ZnO NPs can be utilized as novel applicant for bioimaging, to amplify bioavailability of beneficial drugs. Conclusively, considering the aforementioned properties of ZnO NPs can be a novel lead molecule for biomedical diagnostic and therapeutic fields.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

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