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Fabrication of ketoconazole nanoparticles and their activity against *Malassezia furfur*

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Abstract. *Paralikar P. 2015. Fabrication of ketoconazole nanoparticles and their activity against* Malassezia furfur. *Nusantara Bioscience 7: 43-47.* In the present study, ketoconazole nanoparticles were synthesized from commercially available ketoconazole powder. Sonication is the physical method used to fabricate ketoconazole nanoparticles. UV-Visible spectroscopy, FTIR spectroscopy, NTA analysis, and TEM analysis reveals the formation of polydispersed ketoconazole nanoparticles with 51nm particle size. The antifungal study demonstrates that synthesized ketoconazole nanoparticles exhibit significant activity against *Malassezia furfur* as compared with commercially available ketoconazole powder. Further, nanogel was prepared using ketoconazole nanoparticles which showed significant antimalassezial activity. After systematic trial, the ketoconazole nanoparticles containing gel can be used as antidandruff gel.

Keywords: Antimalassezial activity, ketoconazole nanoparticles, nanogel

INTRODUCTION

Nanotechnology is a rapidly growing field with its wide range of applications in the various fields like medicine, pharmacy, engineering and biotechnology for manufacturing of new materials at the nanometer scale level (Albrecht et al. 2006, Dhuldhaj et al. 2012). Nanoparticles are particles of cluster of the atoms with a size of at least 100 nm. 'Nano' is a Greek word synonymous to 'dwarf' meaning extremely small. Nanoparticles are of intense scientific interest in research, as they form an effective bridge between bulk materials and atomic or molecular structures. Because of these nanoparticles has a wide variety of potential applications in biomedical, optical, agricultural and electronic fields. The development of experimental procedures for the synthesis of nanoparticles of different chemical compositions, sizes, shapes, and controlled polydispersity is vital for its advancement (Sable et al. 2012). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution, and morphology (Savithramma et al. 2011). The interesting properties of nanoparticles are largely due to large surface area to volume ratio of the material, which governs the contribution made by the small material to bulk materials (Jena et al. 2013). Synthesis of nanoparticles can be carried out by using various chemical, biological and physical methods.

The synthesis of nanoparticles by different methods and different sources is area of research in nanotechnology. Different methods like physical, chemical and biological have been employed for synthesis of nanoparticles. The major aim of designing nanoparticles is to control size of particle, their surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug (Mohanraj et al. 2006).

Ultra-sonication is a physical method, which substantially reduces the particle size of materials in suspensions (Motlagh et al. 2010). An increase in breakdown strength was observed for sonicated samples correlating with lower sample viscosity compared to those non-sonicated and has shown to ease the fabrication process in materials. Sonication of suspension provides better dispersion if used with nanomilling (Radzuan et al. 2009).

Drug nanoparticles can be produced in stirred media mills, where size reduction, as well as mechano-chemical modification of drugs, can occur (Connors et al. 2004). Stirred media mills have been applied, in the past only to alumina and aluminum hydroxide compounds. The use of nanomill has been known to offer certain advantages. One of the major advantages along with size reduction to micron or submicron is without the use of organic solvents.

Ketoconazole (chemical name: CIS- 1-ethanoyl-4-[4-[[2-(2,4-dichlorophenyl)-2- (1H-imidazol-1-ylmethyl)-1,3-di-oxolan-4-yl]methoxy]phenyl]piperazine), is an azol-based broad-spectrum antifungal drug, which operates through inhibiting the biosynthesis of ergosterol in fungal cell and cell wall, as well as the absorption of the DNA and RNA precursors to increase the cell permeability of mycetes, and further inhibit and damage mycetes (Ying et al. 2013). Ketoconazole is classified in the Bio-pharmaceutics Classification System (BCS) as a class II drug. Although the compound has good permeability (108} 18 nm/s, Caco-2), the solubility in GI fluids is insufficient to dissolve the administered dose under normal conditions (Galia et al. 1998; Balimane et al. 2006). It is an imidazole antifungal agent, which also possesses some antibacterial activity

(Shuster 1984; McGrawth and Murphy 1991) and used to prevent and treat fungal skin infections. It is useful for dandruff treatment of skull and seborrhoeic dermatitis (Das et al. 2014).

Malassezia furfur, a lipophilic yeast which is considered as medically important yeasts because of their involvement in the etiology of some important skin disorders including *Pityriasis versicolor*, folliculitis, seborrhoeic dermatitis and dandruff. Dandruff is a condition, which causes small white flakes of skin that separate and falls from the scalp (Joshi et al. 2013). It has been investigated and reported that there is no complete cure for this disease (Vijayakumar et al. 2006). Sometimes it seems that antibiotics develop resistance due to continuous pressure exerted by drug, results of sequential alteration. Some scientist said that ketoconazole has some side effects like itching of scalp, red rashes on skin, etc. So the present work represents the nanofabrication of ketoconazole and its antimalassezial activity.

MATERIALS AND METHODS

Microorganisms

The fungal species *Malassezia furfur* (MTCC 1374), a causal organism of dandruff, was procured from microbial type culture collection, Chandigarh. The fungal strains were maintained on Sabouraud dextrose agar for further process.

Antifungal drug

The commercially available ketoconazole powder was purchased from Hi-Media® Pvt Ltd Mumbai, Maharashtra.

Synthesis of Ketoconazole nanoparticles

The ketoconazole was crushed in mortar and pestle to prepare a fine powder. Then ketoconazole suspension was prepared by dissolving in methanol. An aliquot of commercially available ketoconazole was subjected to sonication for 15 minutes at 40 pulse rate in order to reduce the bulk ketoconazole into the nanosized ketoconazole.

Characterization of synthesized nanoparticles by UV-Visible spectrophotometer

The ketoconazole nanoparticles were detected by UV-Visible spectrophotometer. Synthesized ketoconazole nanoparticles exhibit strong absorption in the visible range due to the surface plasmon resonance. The UV-Visible spectrum of control (commercially available ketoconazole suspension) and the solution of ketoconazole nanoparticles was scanned in the range of 200-800 nm.

Characterization of ketoconazole nanoparticles by FTIR

The nanoparticles were characterized by FTIR analysis (Perkin-Elmer FTIR-1600, USA), 90% of KBr powder (Hi-Media® Pvt Ltd Mumbai, Maharashtra) was mixed with 10% of nanoparticles powder. Then the powder was crushed by mortar and pestle. The mixture was filled in the cavity and it was kept in FTIR cavity holder and spectrum was recorded.

Characterization of ketoconazole nanoparticles by nanoparticles tracking analysis (NTA) (Nanosight-LM 20)

The synthesized nanoparticles were characterized by nanoparticles tracking and analysis (NTA) using LM-20 (NanoSight Ltd. UK). LM-20 is laser-based light scattering system, in which particles suspended in the liquid medium (Gaikwad et al.2013). LM-20 utilizes the properties of both light scattering and Brownian motion to obtain size distribution of the particles suspended in the liquid medium at the concentration range of 10^7-10^9 per mL (Kuralkar et al. 2014). Brownian motion of nanoparticles within path of laser beam was detected by charge coupled device (CCD) camera. The generated videos and images were analyzed for size distribution of nanoparticles.

Evaluation of antifungal activity of ketoconazole nanoparticles

Antifungal activity of ketoconazole nanoparticles was performed against *M. furfur* by Kirby-Bauer disc diffusion method (Bauer et al. 1966; Gupta et al. 2013). Sabouraud dextrose agar (Dextrose 40 g/L, Peptone 10 g/L, Agar 15 g/L) plates were inoculated with respective test fungal spore suspension and discs were placed on to the medium at four corners of the plates. Sterile paper discs impregnated with ketoconazole powder and ketoconazole nanoparticles (20 µg/mL) along *with* ketoconazole antibiotic disc impregnated with ketoconazole nanoparticles were kept in the plate. Moreover, positive control disc of methanol was used in the plates. Plates were incubated at 26±1ºC for 48-72 hours. The zone of inhibition was measured with zone measurement scale of Hi-Media®. The study was assessed in triplicate.

Preparation of antidandruff gel by ketoconazole nanoparticles

The nanogel was prepared using ketoconazole nanoparticles as a soul component of gel. Warm distilled water was taken in a flask and 2% carbapol (gelling agent) was added in it. The stock solution of ketoconazole nanoparticles was added accordingly and mixed properly. The mixing was carried with the help of magnetic stirrer for the homogenize mixing of ketoconazole nanoparticles and proper gelling. This gel was tested for its antifungal activity against *M. furfur*.

RESULTS AND DISCUSSION

Commercially available ketoconazole powder was crushed finely in mortar and pestle and then suspended in methanol. Then the suspension was sonicated in such a way that it should form nano-suspension of ketoconazole.

Further confirmation of synthesized ketoconazole nanoparticles was made by UV-spectrophotometer. The UV-Visible spectrum of ketoconazole suspension was recorded at 293 nm (Figure1). The present study showed similarity with results reported by Kedor-Hackmann et al.

(2006). In their study, authors observed UV-visible spectrum of ketoconazole nanoparticles in the range of 211 to 295 nm.

The FT-IR spectrum showed the presence of different peaks at 1628, 1477, 1232, 532 cm^{-1} in control spectrum and peaks at 1627, 1457, 1192, 527 cm^{-1} in experimental spectrum (Figure 2). The FTIR spectrums revealed the presence of different functional groups like N-H, C-N, C-C, alkyl halide linkage which are present in both controls as well as experimental spectra. The peaks at 1628 cm^{-1}in control and 1627 cm^{-1} in experimental were associated with bend vibration of -N-H group (Barman et al. 2013). The peaks at 1477cm^{-1} and 1457 cm^{-1} were due to stretching vibration of aromatic C-C linkage (Sanghi and Verma 2009). The peak at 1232 cm^{-1} and 1192 cm^{-1} was associated with stretch vibration of -C-N group (Huang et al. 2007; Kong et al. 2007). While the peaks at 532 and 527 cm^{-1} associated with alkyl halide linkage. The spectral study showed that there was no chemical interaction of the drug with solvent (methanol) in which drug dissolved by sonication method. If the drug and solvent would interact with the functional group in FTIR spectra would show band shifting of the peaks as compared with standard ketoconazole powder. The FTIR spectra obtained from the ketoconazole nanoparticles showed peaks which were similar to the characteristic peaks obtained from crude ketoconazole powder. This showed that there was no chemical interaction of the drug with solvent (methanol). FTIR spectra indicate reduction in sharpness of peaks in case of ketoconazole nanoparticles as compared to crude ketoconazole powder.

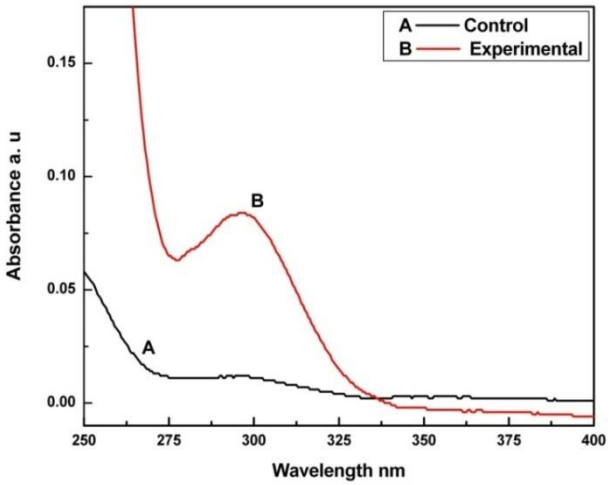

Figure 1. UV-Visible spectra of ketoconazole nanoparticles **(**293 nm).

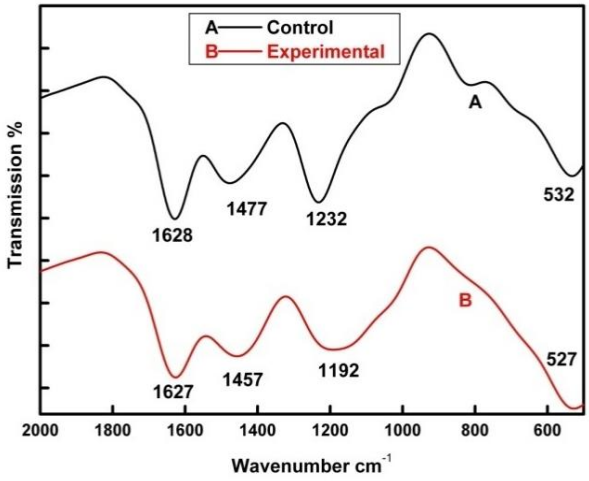

Figure 2. FTIR analysis of A=crude ketoconazole, B=synthesized ketoconazole nanoparticles.

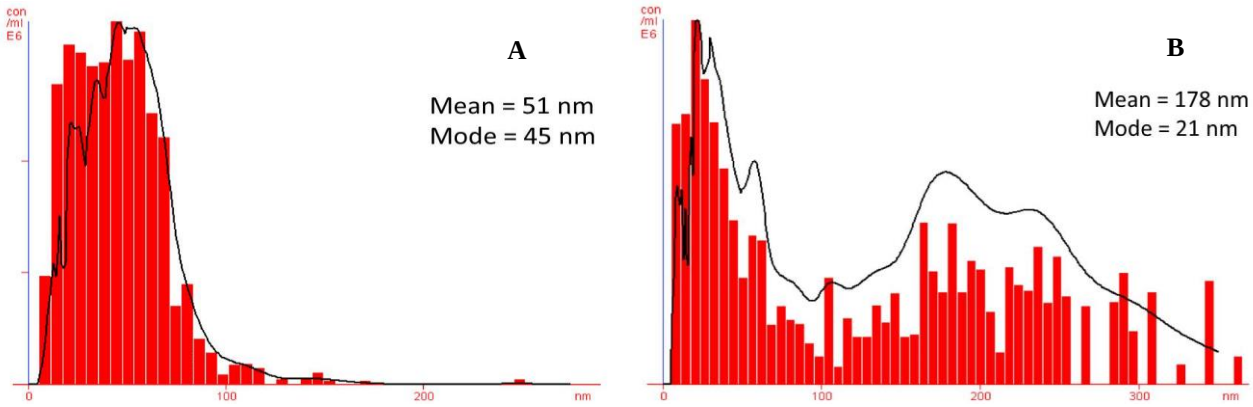

Figure 3. NTA analysis of: A. Ketoconazole nanoparticles (51 nm), and B. Crude ketoconazole powder (178 nm).

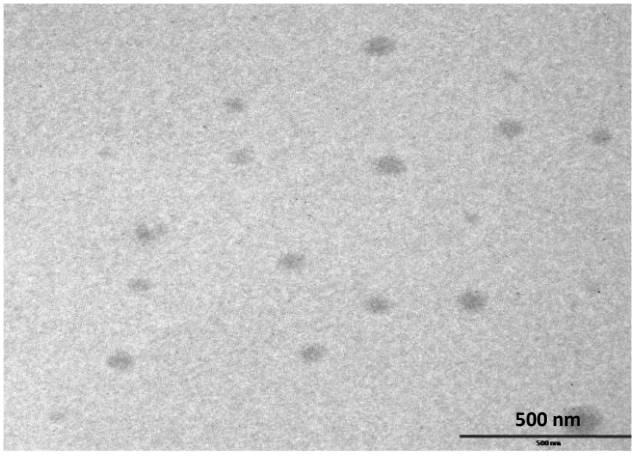

Figure 4. Transmission Electron Microscopy of ketoconazole nanoparticles particle size in range 50 to 100 nm.

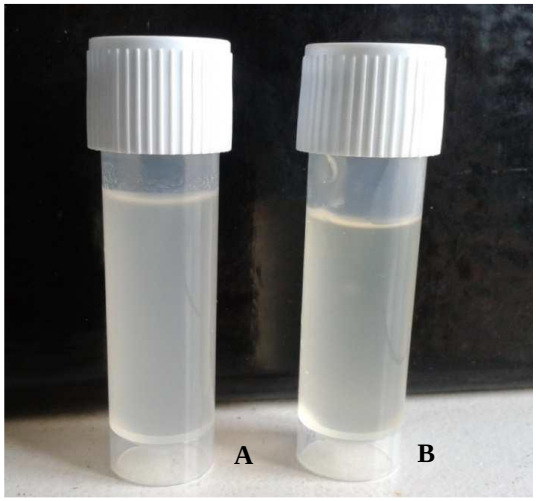

Figure 5. Formulated nanogel using ketoconazole nanoparticles

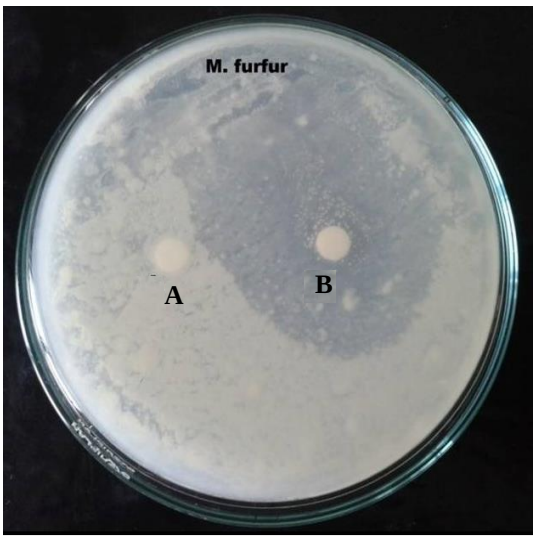

Figure 6. Antifungal activity of nanogel against *M. furfur* where, A. Control gel, and B. Nanogel

Table 1. Zone of inhibition of ketoconazole nanoparticles against *M. furfur*.

Name of Species	Zone of inhibition of ketoconazole nanoparticles	Zone of inhibition of crude ketoconazole	Zone of inhibition of control (methanol)
M. furfur	26 mm	18 mm	0 mm

The size of synthesized ketoconazole nanoparticles was analyzed by nanoparticles tracking analysis (NTA). The nanoparticles tracking analysis techniques look for individual particles and size them on the basis of particle by particle. NTA produced average size of the particles and it can also go to much smaller particle sizes (down to 10 nm) than light obscuration techniques, which is an added advantage of this technique. The particle size distribution of ketoconazole nanoparticles and crude ketoconazole powder were shown in figure 3.A and 3.B respectively. The average size diameter of ketoconazole nanoparticles was found to be 51 nm. The average size diameter of crude suspension of ketoconazole was showed particle with 178 nm which were calculated on the basis of Brownian motion of particles. This result resembles with the study carried out by Dar et al. (2013) for particle size distribution of silver nanoparticles by NTA analysis.

The direct electron microscopic visualization allows measuring the size and shape of the ketoconazole nanoparticles formed. Typical bright-field TEM image of the synthesized ketoconazole nanoparticles was showed in Figure 4. TEM micrograph showed the presence of polydispersed spherical nanoparticles having the size range of 50-100 nm.

Antifungal activity of ketoconazole Nanoparticles

The in vitro antifungal activity of ketoconazole nanoparticles was evaluated against *M. furfur*. The obtained results clearly indicate that synthesized ketoconazole nanoparticles exhibit good antimalassezial activity and it was calculated from the obtained significant zone of inhibition. Synthesized nanoparticles showed significant activity against *M. furfur* (26 mm) and while the control did not show activity against *M. furfur*.

Antifungal activity of nanogel against *M. furfur*

The in vitro antifungal activity of ketoconazole nanoparticles was evaluated against *M. furfur*, as the causative agent of dandruff. The obtained results clearly indicate that synthesized ketoconazole nanoparticles exhibit good antimalassezial activity and it was calculated from the obtained significant zone of inhibition. Synthesized nanoparticles showed significant activity against *M. furfur* (26 mm) and while the control did not show activity against *M. furfur*.

The fungus *M. furfur* releases free fatty acids due to its lipase activity that causes dermal inflammation and tissue damage which is serious problem. As antifungal drug ketoconazole interfere with fungal cell membrane and their

components by blocking synthesis pathways of certain enzymes and sterols. This results in weakening the structure and function of fungal cell membrane lead to cell damage. In this in vitro activity study, ketoconazole nanoparticles showed potent and significant antifungal activity against *M. furfur* as compare with crude ketoconazole. Similarly in vitro activity of formulated nanogel showed significant results evaluated against *M. furfur* by blocking synthesis pathways of certain enzymes and sterols.

The ketoconazole nanoparticles were synthesized using commercially available ketoconazole powder. These nanoparticles were found to be the potent antifungal agent against *M. furfur*. After further clinical trials, the ketoconazole nanoparticles can be used as potential antifungal agent against *M. furfur*. The nanogel was formulated by ketoconazole nanoparticles, which was found effective against *M. furfur*. The nanogel can be used to control the problem of dandruff after further extensive trials.

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