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Evaluation of Nickel nanoparticles as a potential antifungal agent against *T. tonsurans*

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Abstract

The present study main goal is to assess the in vitro antifungal activity of nickel nanoparticles (NiNPs) against some species of *Trichophyton* causing dermatophytosis infections. Specimens were taken and the identification of the fungi was determined by their macroscopic and microscopic features. The predominant species was *T. tonsurans*.

The nickel nanoparticles at various levels of concentrations (200–300 µg/ml) were used for the evaluation through OD600. A concentration of 225 µg/ml was determined as the minimum inhibitory concentration (MIC), while 250–300 µg/ml was fungicidal (MFC).

Statistical analysis (ANOVA) revealed highly significant differences between concentrations tested ($p < 0.05$). These results reveal the potential of using nanoparticles as an alternative or complementary therapeutic in antifungal resistance.

Keywords: Antifungal; NINPS; Nanoparticles; Dermatophytes; ROS

1. Introduction

The skin is the human body's largest organ and acts as a primary barrier to external agents, including viruses, chemicals, and mechanical insults. It is also important in maintaining body temperature and preventing loss of fluids (1). It is however, susceptible to all kinds of infections, especially fungal infections because its lining is very conducive to growth of various forms of microorganisms.

They are highly relevant due to their capacity to hydrolyze keratin in human and animal's skin, hair and nail, as one of the most important etiological agents of the common superficial mycoses (2). *Trichophyton* is one of the most common pathogenic genera in dermatophytes, which is composed of various species that cause different types of infections. The most common species encountered worldwide is *Trichophyton rubrum,* which is the leading cause of chronic skin and nail infections (3). It is closely followed by *T. mentagrophytes* which is responsible for skin and hair infections and is usually transmitted from animals (zoophilic strains) (4). *T tonsurans* is also considered as a major factor in scalp infections especially in children (`5). *T. verrucous* is recognized as an occupational pathogen in farmers because of its transfer from cattle and sheep (6). Other significant species are *T. Schoenlein*, the causative agent of favus of the scalp, and *T, violaceus*, which is common in some parts of Africa and Asia (7).

Due to the difficulties of application of antifungal agents, including inhibitory effects and side effects, recent research has focused on new and/or safer therapeutic options (8). In this contrast, a new promising approach is the use of

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nanomaterials in medicine, due to its own characteristics-such as the narrow size, the large surface area and reactivity-which can facilitate the inhibition of the wall of the fungal cells and their basic structure (9).

Nickel nanoparticles (NINPS) are one of these nanomaterials which have increasingly received attention, highlighting their proved antibacterial and antifungal actions. It has recently been reported that these EOs were efficient for some of the pathogenic yeasts of *Candida albicans* as well as against the filiform spores of *Aspergillus Niger*, through ROS production, disturbance of membrane permeability, and prevention of the germination of spore (10,11).

Therefore, in the present study, an attempt has been made to determine the antifungal activity of nickel nanoparticles against the selected *Trichophyton spp*., in vitro, as a preliminary model system in order to search for other alternative or additional therapeutic options against dermatophytid infections.

2. Materials and Methods

2.1. Samples and Fungal Identification

Specimens from lesions for dermatophytic infection were obtained clinically and were plated on Sabouraud´s dextrose agar. Identification was made on the basis of macroscopic (color and texture of colonies) and microscopic (microconidia and macroconidia) morphology. (12).

2.2. Preparation of Nickel Nanoparticle Solutions

The NiNP solutions were prepared with the dissolving of the nanoparticles in the distilled water, and the concentrated solutions were diluted to get the following concentrations:

- **200 µg/ml**: 0.4 ml NiNP solution + 0.6 ml distilled water
- **225 µg/ml**: 0.45 ml NiNP solution + 0.55 ml distilled water
- **250 µg/ml**: 0.5 ml NiNP solution + 0.5 ml distilled water
- **275 µg/ml**: 0.55 ml NiNP solution + 0.45 ml distilled water
- **300 µg/ml**: 0.6 ml NiNP solution + 0.4 ml distilled water

The fungal inoculum density was standardized using a haemocytometer to adjust the spore count (13).

2.3. Antifungal Activity Assay

Fungal spore/conidia were collected from the surface of fresh fungal culture (7–10 days old).

The turbidity was set to be comparable with 0.5 of McFarland scales and then it was diluted to obtain the inoculum at the final density.

Antifungal assay the broth microdilution method was performed as described in references CLSI M38 or EUCAST E.Def 11.0 for antifungal activity determination.

The growth of fungi was quantified on the basis of turbidity at OD600 values after the indicated incubation.

The minimum growth inhibitory concentration (MIC) was considered as the lowest concentration of EUMI that did not allow the visible growth of the fungal cells and the minimum fungicidal concentration (MFC) was the minimum concentration that inhibited the sub culture of the fungal cells on fresh fungal medium. (14,21).

2.4. Statistical Analysis

All experiments were performed in triplicate for each concentration. One-way ANOVA was employed to evaluate the significance of differences, and a p-value of < 0.05 was considered statistically significant.

3. Results and Discussion

Diagnosis established that the clinical samples were of different *Trichophyton* species, such as *T. rubrum, T. mentagrophytes, T. verrucosum, and T. terrestris*. Among these *T. tonsurans* was the dominant species (40–50% of the

isolates) followed by *T. mentagrophytes* with a lower frequency. In contrast, *T. verrucosum* and *T. terrestre* mutants were rare. These results are in accordance with other studies (15).

3.1. Effect of NiNP Solution on Dermatophytes

Turbidity readings showed that the concentration of 200 µg/ml allowed considerable growth for the fungi (OD = 0.900 ± 0.050). The minimum inhibitory concentration (MIC) was 225 µg/ml (OD = 0.377 ± 0.025). Concentrations between 250–300 µg/ml represented the MFC, and the optical density values were 0.010 ± 0.010, 0.003 ± 0.006 and 0.000 ± 0.000, respectively.

ANOVA statistics for the tested concentrations was found to be $F = 710.78$ ($p < 0.0001$), illustrating a significant difference among the tested concentrations.

These findings are also in agreement with previously reported studies (16,17) that showed the penetration of the fungal cell wall by nanoparticles and changes in the composition of the cell membrane, followed by the leakage of intracellular content, and consequently, cell death. The interval MIC and MFC indicates that the action of nanoparticles on fungi is a multi-step process, meaning at lower concentration they will just prevent the division of fungi but at higher concentration, will be responsible for the cell death.

In a previous investigation, green method synthesized Ni/NiO NPs demonstrated strong activity against *Candida albicans* and *Aspergillus niger*, and produced large zones of inhibition on agar diffusion assays (18). Furthermore, nickel nanoparticles were reported to suppress the growth of *F. oxysporum* at low concentrations via membrane disruption and metabolic disorders (19). These results are consistent with our observation in our study in which the nickel nanoparticles clearly inhibited the growth of Trichophyton spp.

The main benefit of the nickel nanoparticles is the ability to use them as either an alternative or combination treatment with general antifungal agents, specifically in an era when prevalence of drug resistance is growing for azoles and terbinafines (20). However, practical use of nanoparticles directly in clinic needs to be evaluated extensively such as cytotoxicity, biocompatibility, and side effects, before nanoparticles can be truly utilized in treatment.

This report sets the stage for future applications of nickel nanoparticles against Trichophyton dermatophytes. Additional studies are required to establish the most efficient doses, administration modality, and safety of NAM for translating their use to human patients.

Table 1 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of NiNPs against *T. tonsurans*

compound	**Concentration µg/ml**				
	200	225	250	275	300
MIC	Turbid	Clear	Clear	Clear	Clear
MFC	Growth	Growth	Sterile	Sterile	Sterile

Table 2 Mean turbidity measurements (OD ± SD) at different concentrations of NiNPs

Observation	**SD**	**Mean OD**	**Concentration(µg/ml)**
Clear growth	0.050	0.900	200
MIC	0.025	0.377	225
MFC	0.010	0.10	250
MFC	0.003	0.003	275
MFC	0.000	0.000	300

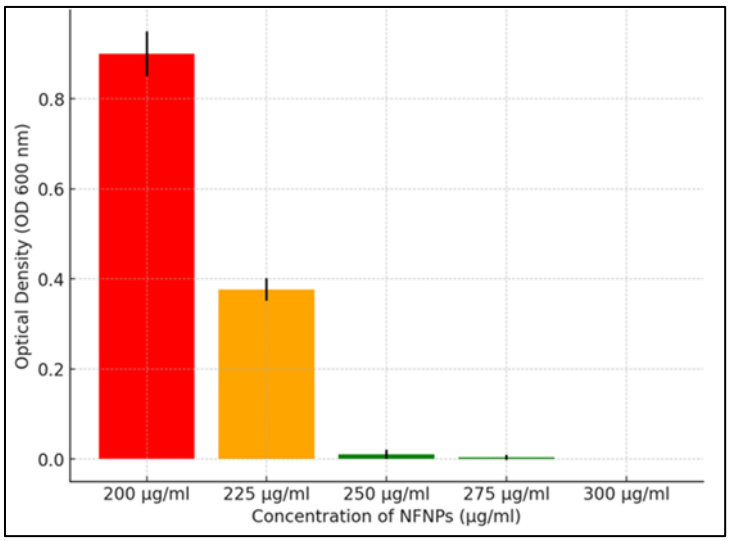

Figure 1 Effect of nickel nanoparticles (NiNPs) on the growth of T. tonsurans at different concentrations

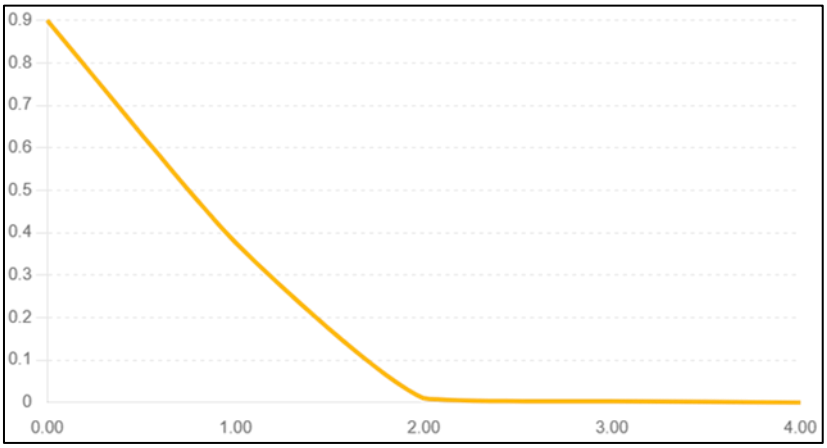

Figure 2 Curve illustrating the reduction in turbidity with increasing concentrations of NiNPs

The antifungal activity of nickel nanoparticles against Trichophyton was evident from the results, changing from growth inhibitory activity (MIC) at 225 µg/ml to complete fungicidal activity (MFC) at concentrations ≥250 µg/ml.

The discrepancy between the MIC and MFC illustrates the need for higher concentrations for total elimination than for simply growth suppression. These results further support the potential use of nanoparticles as a new therapeutic modality, especially because of the growing number of resistant dermatophytes to conventional antifungals.

4. Conclusion

- *T. tonsurans* was the most common species in the isolated samples.
- The antifungal activity of nickel nanoparticles (NiNPs) was evident.
- The MIC was CC1 (225 µg/ml), and the MFC was ≥250 µg/ml.
- The obtained results showed very significant differences ($p < 0.05$) between the tested concentrations.

- Additional investigation is required to examine cytotoxicity and possible clinical application

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was conducted in compliance with ethical principles outlined in the declaration of Helsinki and approved by the relevant institutional authorities

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