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Antifungal activity of gold nanoparticles against dermatophytes isolated from infected patients at Nasiriyah educational hospital

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Abstract

Objective

Fungal skin infections, particularly those created by dermatophytes, represent a meaningful global public health concern, especially in regions with warm climates and restricted access to antifungal therapies. Dermatophytes are a group of keratinophilic fungi that infect the skin, nails, and hair. Traditional antifungal treatments are often prolonged and may be compromised by resistance or side effects. Recent advancements in nanotechnology have introduced novel antimicrobial agents, containing gold nanoparticles (AuNPs). This research investigates the antifungal efficacy of AuNPs against clinical dermatophyte isolates from patients in Nasiriyah, Iraq, aiming to evaluate their potential as alternative therapeutic agents. Gold nanoparticles are attended promising for addressing a range of medical challenges, containing the treatment of dermatophytic infections.

Materials and methods

This investigation was conducted in the laboratories of Mazaya College, a private university, applying clinical samples gathered from patients attending the Dermatology Department at Nasiriyah Teaching Hospital, in accordance with official authorization from the Dhi Qar Health Department. Sampling took place between March and September 2023. A total of 100 samples were gathered from male and female patients of numerous age groups suffering from skin fungal infections. Commercially prepared gold nanoparticles were tested at different concentrations to evaluate their antifungal efficacy. The fungal isolates were treated with varying concentrations of AuNPs, and inhibition of growth was measured.

Results

The results demonstrated that gold nanoparticles inhibited dermatophyte growth in a concentration-dependent manner. At concentrations of 25, 50, 75, and 100 µg/mL, the observed inhibition rates were 36.25%, 59.3%, 78.1%, and 96.35%, respectively. The data indicate that higher concentrations of AuNPs result in remarkably greater antifungal activity. Statistical analysis affirmed that the differences between all tested concentrations were meaningful ($p < 0.05$).

Conclusions

This investigation affirms the strong inhibitory effect of gold nanoparticles on dermatophyte growth, with efficacy increasing at higher concentrations. These results support the potential apply of AuNPs as an alternative or adjunct treatment for dermatophytic infections. Further in vivo investigations and clinical trials are recommended to establish their safety, effectiveness, and optimal dosing protocols for clinical application.

Keywords: Dermatophytes, gold nanoparticles, antifungal activity, nanotechnology, skin infection

Paper Type: Research Paper.

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Introduction

Dermatophytes are a group of phenotypically and functionally related fungi capable of colonizing keratinized tissues like the skin, hair, and nails of humans and animals, leading to a

situation known as dermatophytosis. These fungi are traditionally classified into three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton* (Janardhan & Vani, 2016). Fungal skin infections are among the most widespread contagious diseases globally. Although their distribution is extensive, the overall infection rate remains below 20% of the global population (Kadhim et al., 2015).

In the United States alone, the annual cost of treating fungal infections is estimated at approximately $206 billion, with a mortality rate as high as 40% (Marin-Felix et al., 2017). Dermatophytosis remains one of the most common situations encountered by dermatologists and has emerged as a growing public health concern due to its rising prevalence and persistence worldwide (Gnat et al., 2020). Notably, an increasing number of dermatophyte infections have become resistant to conventional treatments, often presenting as multifocal, recurrent, and chronic lesions (Narasimhalu et al., 2016). Nanoscience, a rapidly advancing interdisciplinary field, centralizes on the synthesis and application of nanoparticles—materials typically measuring between 1 and 100 nanometers in size. Numerous methods, containing chemical, physical, and biological approaches, are employed to generate nanoparticles of metals like gold, silver, zinc, and platinum (Mohammadabadi et al., 2009; Heidarpour et al., 2011; Mohammadabadi & Mozafari, 2018). Among these, chemical and physical methods often involve high costs and need specialized laboratory environments that may pose risks to human health and the ecosystem (Chaturvedi et al., 2012). Due to their high surface area-to-volume ratio, metal nanoparticles exhibit distinct physicochemical and biological characteristics, making them useful across a wide range of applications (Sharma et al., 2009). Gold nanoparticles (AuNPs), in particular, have demonstrated meaningful antimicrobial activity. Their biocidal properties are attributed to their capability to improve cellular permeability, induce oxidative stress, and promote cell death (Al-Kawmani et al., 2020). Furthermore, AuNPs are attended biocompatible at low concentrations and have minimal toxicity to human cells. Key parameters influencing the efficacy of AuNPs include their shape, size, and the biosynthetic situations under which they are generated, like pH, temperature, and the biological agents involved. These factors play a pivotal role in determining the functional properties of AuNPs intended for medical apply, containing the activity of biomolecules like proteins, enzymes, and organic acids through synthesis (Heidarpour et al., 2011; Mohammadabadi & Mozafari, 2019). Importantly, biosynthesized AuNPs are designed to selectively target pathogenic cells without harming healthy tissues (Mortazavi et al., 2005; Zarrabi et al., 2020). Recent investigations have highlighted the therapeutic potential of biologically synthesized gold nanoparticles against pathogenic microorganisms and cancer cells. For instance, Dehghani et al. (2023) successfully synthesized AuNPs applying *Verbena officinalis* extract, which demonstrated potent antifungal activity against *Candida albicans*, *Aspergillus flavus*, and

Aspergillus niger, underscoring their promise as eco-friendly antifungal agents. Similarly, Shabani et al. (2023) announced the green synthesis of ruthenium-templated AuNPs applying rutin extract, which exhibited strong biocompatibility and selective cytotoxic effects on MCF-7 breast cancer cells when exposed to laser irradiation.

These results support the growing interest in green-synthesized AuNPs for a wide array of biomedical applications, containing antifungal therapy. Despite these advances, investigations on the antifungal properties of gold nanoparticles, particularly in relation to dermatophytic infections, remain restricted. The persistent spread of fungal skin diseases, especially those created by drug-resistant strains, poses a meaningful challenge in clinical dermatology. This investigation, therefore, aimed to address this gap by investigating the antifungal potential of gold nanoparticles against dermatophytes, thereby contributing to the development of novel, effective treatments for fungal skin infections.

Materials and methods

Sample collection: Approximately 100 clinical samples, containing hair, skin, and nail specimens, were gathered from patients attending the Dermatology Consultation Department at Al-Nasiriyah Teaching Hospital, located in Dhi Qar Governorate, Iraq. Prior to sample collection, relevant patient information was recorded, containing age, gender, place of residence, occupation, and anatomical site of infection. Informed consent was achieved from all participants, and ethical approval for the investigation was secured from the institutional review board.

Sample examination: Gathered samples were transported under sterile situations to the laboratory at Mazaya University College for direct microscopic analysis. Samples were achieved from visibly infected areas of the skin, scalp, and nails. For each specimen, a drop of 10% potassium hydroxide (KOH) solution was applied to aid in the digestion of keratinized tissue, followed by placement on a clean glass slide. After permitting the preparation to stand for 5 minutes, the slides were examined under a light microscope at 40× magnification to detect fungal elements like hyphae, spores, or arthroconidia.

Fungal cultivation: Remaining portions of the clinical samples were inoculated onto Sabouraud Dextrose Agar (SDA), a standard mycological growth medium supplemented with 0.05 g/mL chloramphenicol and 5.0 g/mL cycloheximide to inhibit bacterial and non-dermatophytic fungal contamination, respectively (Figure 1). The inoculated plates were incubated at 25°C for a period of two weeks to promote fungal growth. Plates that exhibited no visible growth after this incubation period were classified as negative. For positive cultures, colony morphology—containing surface texture, pigmentation, and growth rate—was

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documented. For further microscopic identification, a portion of the colony was stained with lactophenol cotton blue and examined microscopically, as described by Putriningsih and Arjentinia (2017).

Fungal identification: Preliminary evaluations were conducted after five days of incubation. All cultures were maintained for up to three weeks at 28°C to permit slow-growing dermatophytes to develop fully. Positive isolates were subcultured on fresh SDA slants for purification. Once isolated, fungal colonies were maintained at 25°C for short-term growth and later preserved at 4–6°C for storage. Identification of dermatophyte species was based on macroscopic and microscopic characteristics following the taxonomic keys described by De Hoog et al. (2000) and Elewski and Charif (1997).

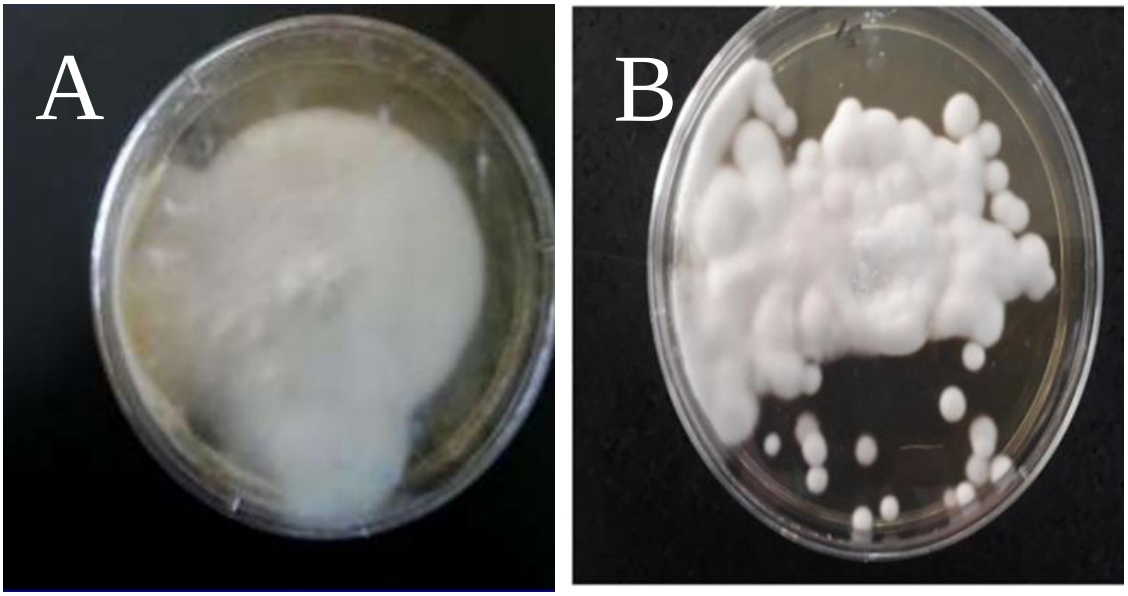

Figure 1. Morphological features of *Trichophyton rubrum* cultured on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and cycloheximide. (A) Surface view of fungal colony morphology; (B) Microscopic aggregation of fungal hyphae.

Preparation of fungal vaccine: The fungal vaccine was prepared following the methodology described by McGinnis (1980), with modifications. Actively growing colonies of the identified fungus were harvested from SDA plates applying sterile inoculation needles. A portion of each colony was suspended in sterile vials containing 5 mL of normal saline solution. The suspensions were vigorously mixed applying a vortex mixer to obtain homogenous fungal suspensions suitable for apply in antifungal susceptibility testing.

Antifungal testing and gold nanoparticle preparation: The antifungal activity of numerous concentrations of gold nanoparticles (AuNPs) was evaluated against *T. rubrum*. Gold

nanoparticles were prepared in concentrations of 0 (control), 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL, as outlined by Zhou et al. (1999). For each concentration, 5 µg of the nanomaterial was accurately weighed, diluted with distilled water, and thoroughly mixed applying a vortex mixer to ensure even dispersion.

The antifungal assay was conducted as follows:

1. SDA plates were prepared and pre-incubated for 24 hours at 28°C to ensure sterility.
2. *T. rubrum* suspensions were diluted in physiological saline to a standardized inoculum density.
3. The fungal suspension was evenly spread on the surface of SDA plates applying a sterile loop.
4. Gold nanoparticle solutions at the specified concentrations were applied to the plates immediately after inoculation.
5. Plates were incubated at 28°C for 12 days.
6. The diameter of the fungal growth inhibition zone was measured in millimeters applying a calibrated ruler.

All tests were carried out in triplicate to ensure reproducibility, and results were analyzed based on established antifungal susceptibility testing guidelines (Namidi et al., 2021).

Results and discussion

Treatment of *T. rubrum* with gold nanoparticles: The effect of numerous concentrations of gold nanoparticles (AuNPs) on the growth inhibition of *Trichophyton rubrum* is illustrated in Figure 2. The results demonstrated a clear and meaningful inhibitory effect of gold nanoparticles, with the degree of fungal suppression increasing proportionally with nanoparticle concentration. This aligns with the growing body of evidence suggesting that inorganic nanomaterials, particularly metal-based nanoparticles, possess considerable potential for biomedical applications due to their unique physicochemical properties and bioactivity. In recent years, there has been increasing scientific interest in utilizing inorganic natural resources, containing metal nanoparticles, due to their structural diversity and bio-functional potential. Metals like gold (Au), silver (Ag), zinc (Zn), copper (Cu), carbon (C), iron (Fe), titanium (Ti), and palladium (Pd) are frequently employed in nanoparticle synthesis. Among them, gold nanoparticles have emerged as a promising candidate for medical and biological applications due to their biocompatibility and ease of surface functionalization. These nanoparticles can be synthesized through numerous physical, chemical, and biological methods. However, biological synthesis has gained prominence due to its lower cost, reduced toxicity, and environmental sustainability (Reeda et al., 2021; Vadlapudi and Kaladhar, 2014). The overuse and often indiscriminate application of conventional antibiotics and antifungals has led to an alarming rise in resistance among

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pathogenic organisms. This trend has remarkably reduced the efficacy of classical treatments for infectious diseases, which continue to spread and evolve. As a result, new medical interventions are urgently needed to manage these infections effectively. As noted by Yassin and Mohammed (2020), antibiotics alone are increasingly insufficient in controlling certain pathogenic fungi, prompting the exploration of novel antimicrobial systems. Nanoparticles, especially gold nanoparticles, have been identified as key components in this new frontier of medical research (Yassin and Mohammed, 2021). In the current investigation, antifungal assays showed that AuNPs generated a strong inhibitory effect on the growth of *T. rubrum*, and this inhibition was dependent on the concentration applied. The inhibition percentages observed for each concentration were as follows:

36.25% ± 2.01 at 25 µg/mL, 59.30% ± 1.98 at 50 µg/mL, 78.10% ± 1.78 at 75 µg/mL, and 96.35% ± 0.635 at 100 µg/mL.

These results are existed in Table 1, and the corresponding inhibition zones are illustrated in Figure 2. Statistical analysis applying the Least Significant Difference (LSD) method revealed meaningful differences among all tested concentrations ($p < 0.05$), with an LSD value of 9.562. These data affirm the dose-dependent antifungal activity of gold nanoparticles against *T. rubrum*. The antifungal mechanism of gold nanoparticles is believed to involve multiple pathways. AuNPs interact directly with the fungal cell wall, causing structural disintegration, and can penetrate into cells, where they interfere with essential processes like protein synthesis, mitochondrial function, and DNA replication. This multifaceted action ultimately disrupts fungal metabolism and leads to cell death.

Furthermore, gold nanoparticles may enhance the efficacy of conventional antifungal drugs when applied in combination, potentially reducing the needed dose and minimizing associated side effects. Although the short-term results are highly encouraging, it is important to attend the potential long-term implications of nanoparticle apply. Extended or repeated exposure to gold nanoparticles may pose risks to human tissues and the environment. While current research indicates that AuNPs are generally biocompatible, comprehensive clinical investigations are still needed to fully understand their safety, especially for dermatological applications involving chronic or repeated exposure (Yassin and Mohammed, 2021). In this investigation, the antifungal activity of gold nanoparticles was evaluated applying both the agar diffusion method and the microdilution technique. The best antifungal activity was observed at the highest tested concentration (100 µg/mL), which generated an inhibition rate of 96.35%. This was associated with a 20 µL sample containing *T. rubrum* fungal inoculum. The minimum inhibitory concentration (MIC) of AuNPs against *T. rubrum* was determined to be 25 µg/mL, corresponding to an inhibition rate of 36.25%.

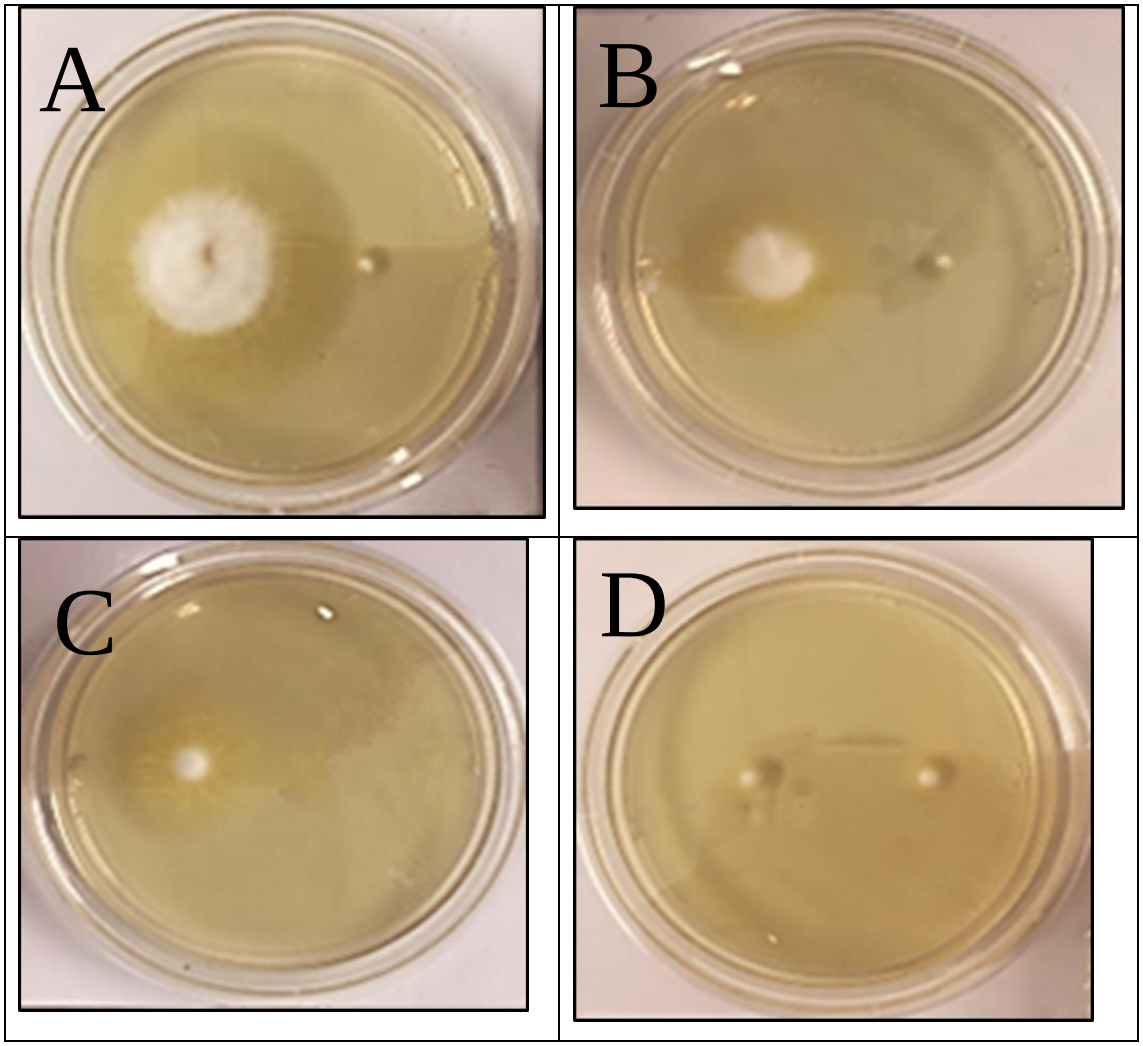

Figure 2. Effect of numerous concentrations of gold nanoparticles on the inhibition of *T. rubrum* growth. A: 25 µg/mL, B: 50 µg/mL, C: 75 µg/mL, D: 100 µg/mL.

Table 1. Effect of different concentrations of gold nanoparticles on inhibiting the growth of *T. rubrum*

Nano Gold Concentration (µg/mL)	Inhibition (%) ± SE
25	36.25 ± 2.01
50	59.30 ± 1.98
75	78.10 ± 1.78
100	96.35 ± 0.635
LSD ($p < 0.05$)	9.562

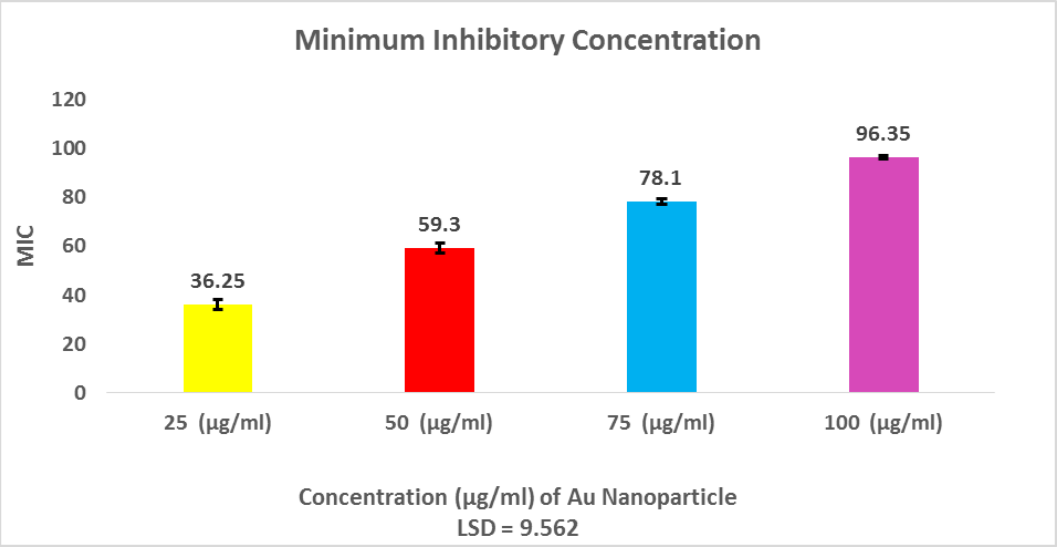

Figure 3. Concentration-dependent inhibition of *T. rubrum* by gold nanoparticles at 25, 50, 75, and 100 µg/mL

The inhibitory activity improved steadily with higher concentrations: 59.3% at 50 µg/mL, 78.1% at 75 µg/mL, and a maximum of 96.35% at 100 µg/mL, as shown in Figure 3. Additionally, it was observed that *T. rubrum* is capable of growing efficiently on keratin agar medium, affirming its keratinophilic nature, a hallmark characteristic of dermatophytes. In the absence of AuNPs, the fungus exhibited robust growth.

Interestingly, the inhibition zone for conventional antifungal antibiotics was consistently smaller than that observed with nanogold treatments, denoting superior antifungal performance by gold nanoparticles. The results of this investigation support the hypothesis that biologically synthesized AuNPs have meaningful potential in the treatment of dermatophytic infections. Their capacity to disrupt fungal growth at multiple concentrations, combined with their environmentally friendly synthesis positions them as a compelling alternative or supplement to conventional antifungal therapies.

Conclusions: The results of this investigation clearly demonstrate the potent antifungal activity of gold nanoparticles (AuNPs) against *Trichophyton rubrum*, a common dermatophyte responsible for skin, hair, and nail infections. A concentration-dependent inhibitory effect was observed, with the highest inhibition (96.35%) achieved at 100 µg/mL of AuNPs. Statistical analysis affirmed meaningful differences between all tested concentrations ($p < 0.05$), emphasizing the efficacy of AuNPs as a promising antifungal agent. Gold nanoparticles appear to exert their antifungal effects through direct interaction with the fungal cell wall, disruption of essential cellular functions, and inhibition of fungal growth.

These results highlight the advantages of applying biologically synthesized nanomaterials, which offer reduced toxicity, cost-effectiveness, and environmental safety compared to conventional chemical methods. Given the limitations and resistance associated with traditional antifungal therapies, the integration of nanotechnology, particularly gold nanoparticles offers a promising alternative strategy for managing dermatophytic infections. However, further in vivo investigations and clinical trials are essential to fully evaluate the safety, efficacy, and potential cytotoxicity of AuNPs, especially for long-term therapeutic apply in humans.

Overall, this investigation contributes to the growing body of evidence supporting the application of nanotechnology in medical mycology and suggests that gold nanoparticles could serve as an effective adjunct or substitute to conventional antifungal treatments in the near future.

Author contributions

N.S. contributed to the conceptualization, investigation, visualization, provision of resources, and participated in the review and editing of the manuscript. S.M. was responsible for formal analysis, software application, investigation, funding acquisition, and also participated in the review and editing of the manuscript. Both authors have read and approved the final version of the manuscript for publication.

Data availability statement

The data supporting the results of this investigation are available from the corresponding author upon reasonable request.

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Ethical considerations

This investigation was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board and Research Ethics Committee of University of Al-Qadisiyah, Iraq. Informed consent was achieved from all participants, ensuring their voluntary participation and confidentiality. Participants were informed of the investigation's purpose, procedures, and their rights to withdraw at any time without consequences.

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Conflict of interest

The authors declare that there are no conflicts of interest related to the publication of this investigation.

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فعالیت ضد قارچی نانوذرات طلا در برابر درماتوفیت‌های جدا شده از بیماران مبتلا در بیمارستان آموزشی ناصریه

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چکیده

هدف: عفونت‌های قارچی پوستی، به ویژه آن‌هایی که توسط درماتوفیت‌ها ایجاد می‌شوند، نگرانی قابل توجهی در سلامت عمومی جهانی، به ویژه در مناطقی با آب و هوای گرم و دسترسی محدود به درمان‌های ضدقارچ هستند. درماتوفیت‌ها گروهی از قارچ‌های کراتین‌دوست هستند که پوست، ناخن‌ها و مو را آلوده می‌کنند. درمان‌های ضد قارچی سنتی معمولاً طولانی مدت بوده و ممکن است با مقاومت یا عوارض جانبی همراه باشند. پیشرفت‌های اخیر در نانوتکنولوژی عوامل ضد میکروبی نوینی مانند نانوذرات طلا (AuNPs)را معرفی کرده است. این مطالعه به بررسی اثربخشی ضد قارچی نانوذرات طلا در برابر جدایه‌های بالینی درماتوفیت‌ها از بیماران در ناصریه عراق، با هدف ارزیابی پتانسیل آن‌ها به عنوان عوامل درمانی جایگزین پرداخته است. نانوذرات طلا به عنوان یک گزینه امیدوارکننده برای مقابله با چالش‌های مختلف پزشکی از جمله درمان عفونت‌های درماتوفیتی در نظر گرفته می‌شوند.

مواد و روش‌ها: این مطالعه در آزمایشگاه‌های دانشکده Mazaya که یک دانشگاه خصوصی است انجام شد و از نمونه‌های بالینی جمع‌آوری‌شده از بیماران مراجعه‌کننده به بخش پوست بیمارستان آموزشی ناصریه، طبق مجوز رسمی از اداره بهداشت ذی‌قار استفاده شد. نمونه‌برداری بین ماه‌های مارس و سپتامبر ۲۰۲۳ انجام شد. در مجموع ۱۰۰ نمونه از بیماران مرد و زن در گروه‌های سنی مختلف که از عفونت‌های قارچی پوستی رنج می‌بردند، جمع‌آوری شد. نانوذرات طلا آماده تجاری در غلظت‌های مختلف آزمایش شدند تا اثر ضدقارچی آن‌ها ارزیابی شود. جدایه‌های قارچی با غلظت‌های مختلف نانوذرات طلا درمان شدند و میزان مهار رشد اندازه‌گیری شد.

نتایج: نتایج نشان داد که نانوذرات طلا رشد درماتوفیت‌ها را به طور وابسته به غلظت مهار می‌کنند. در غلظت‌های ۲۵، ۵۰، ۷۵ و ۱۰۰ میکروگرم در میلی‌لیتر، نرخ‌های مهار به ترتیب ۳۶/۲۵٪، ۵۹/۳۰٪، ۷۸/۱۰٪ و ۹۶/۳۵٪ بودند. داده‌ها نشان می‌دهند که غلظت‌های بالاتر نانوذرات طلا منجر به فعالیت ضدقارچی به طور معنی‌داری بالاتر می‌شوند. تحلیل آماری تأیید کرد که تفاوت‌های بین تمام غلظت‌های آزمایش‌شده معنادار است ($p < 0.05$).

نتیجه‌گیری: این مطالعه اثر مهاری قوی نانوذرات طلا بر رشد درماتوفیت‌ها را، با اثربخشی بیشتر در غلظت‌های بالاتر تأیید می‌کند. این یافته‌ها از پتانسیل استفاده از نانوذرات طلا به عنوان درمان جایگزین یا مکمل برای عفونت‌های درماتوفیتی پشتیبانی می‌کنند. پیشنهاد می‌شود مطالعات بیشتر در شرایط In vivo و کارآزمایی‌های بالینی برای تعیین ایمنی، اثربخشی و پروتکل‌های دوز مناسب برای استفاده بالینی انجام شود.

واژه‌های کلیدی: درماتوفیت‌ها، عفونت پوستی، فعالیت ضدقارچی، نانوتکنولوژی، نانوذرات طلا

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