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Preliminary evaluate of anti-dermatophytic ointment from Biosynthesis MgO nanoparticles in vivo

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ABSTRACT

Due to the presence of antibiotic resistance and toxicity that occurs as a result of long-term treatment with antifungals, the aim of this study was to propose an anti-fungal ointment formulation of MgO nanoparticles after conducting tests related to the antifungal activity that characterized by the nanoparticles of MgO in vitro, where the effect was tested these molecules on the genes responsible for the secreting of protease in isolates of *T. rubrum*, accordant of these results, the effective composition of the proposed product was determined, where the effective concentration was 20%, which showed effective results in healing.

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1-Introduction

T. Rubrum is a dermatophyte fungus that has two stages of development: sporophyte, teardrop-shaped, relatively low metabolic level, and inactive, as it degrades storage compounds because it contains a group of metabolic enzymes (Teutschbieten *et al.*, 2010) in these At this stage, in the presence of carbon sources, the enzymes required for germination and a set of mRNAs and ribosomes are already present, allowing rapid initiation of the germination process and fungal growth (Mendes *et al.*, 2018). In the innate stage, both metabolism and protein synthesis are active; Thus, enzymes associated with respiratory metabolism are regulated, as are mitochondrial proteins, and proteins that are translation-related (Lamarre *et al.*, 2008). The genome of *T. Rubrum* can survive for a period of up to 6 months during bad environmental conditions through the presence of conidia surrounding it. Infection occurs as a result of conidia adhering to the skin and forming a mycelium. Obvious damage to the skin occurs due to the penetration of the growing fungus deep into the layers of the skin. Due to its dispersal and growth characteristics, *T. rubrum* has been considered

a good model system for use in the study of human pathogenic filamentous fungi. (Xu *et al.*, 2015). Several antifungal compounds, especially azoles, have been used to treat dermatophytes, but fungal resistance to azole derivatives is common. Furthermore, azole drugs may have many adverse effects and drug interactions, as they interfere with the activity of hepatic enzymes, the central nervous system, thyroid and sex hormones, and the biosynthesis of testosterone. Therefore, there is an urgent medical need for new antifungal agents. Nanoparticles are promising antimicrobial agents because they have superior properties due to their large surface area to volume ratio that increases antimicrobial activity. Among the different nanoparticles are magnesium oxide nanoparticles, which are considered to have great biological activity (Stoimenov *et al.*, 2002). Therefore, the research dealt with a proposed formula for an ointment prepared from nanoparticles prepared by biological methods and using plant extracts.

2. Material & Methods:

2.1. Fungal Strain: *Trichophyton rubrum* was obtained from the patients which suffering from skin infection and attended to AL-Diwaniyah hospital

2.2. MgO Nanoparticles - nanoparticles were by biological methods and by using aqueous extract of the leaves of the *Sesbania* plant, and their properties were studied in the previously published paper by (Radhi and Jasim, 2021) was used in this study.

2.3. Prepare concentration of MgO nanoparticles- Preparation of MgO nanoparticle concentrate - 20 mg of MgO nanoparticles are dissolved in 100 ml of Sabouraud Dextrose broth to prepare 20% MgO nanoparticles (He, *et al.*, 2011)

2.4. Zinc oxide NP impact on the genes responsible for producing the protease enzyme - To examine how MgO NP affects the genes involved for producing of protease in isolates of *T. rubrum*. Using three replications for the treatment with 20% MgO NP and three replications for the negative control in 250 mL flasks containing SDB. This was inoculated with a disc (5 mm) from a fungus culture, cultured for two weeks, and then underwent real-time polymerization chain reaction and quantitative experience.

2.4.1. Real-time quantitative reverse transcription PCR (RT-qPCR):

To measure quantitative reporter DNA (mRNA) levels a quantitative real-time polymerase chain reaction (PCR) assay is used to denote the amount of gene expression as well as the gene (*Act1*) standing gene score is used to calculate gene expression (Nagura *et al.*, 2020). As follows:

- Extraction of Total RNA using the Trizol kit supplied by the Korean company Pioneer, and this kit was used according to the manufacturer's instructions

Table (1) Primer of DNA

Primers	Sequence 5'-3'		Amplicon
PCR detection gene ITS1/ITS4 gene	F	TCCGTAGGTGAACCTGCGG	692bp
	R	TCCTCCGCTTATTGATATGC	
PCR subtilisin-like protease gene	F	ACGGAGTTGCTAAGAAGGCC	589bp
	R	AAACATGGGGAGAGGCCATG	
qPCR subtilisin-like protease gene	F	ACGACAACACTGATGGCAAC	145bp
	R	TTGATCACGCCAGAGGTAGAAC	

qPCR Control Actin gene	F	AACGTGAAATCGTCCGTGAC	150bp
	R	ATCGCTCGTTTCCAATGGTG	

-Quantitative Real-Time PCR (qPCR):

Using the Accupower 2x Green Star qPCR kit provided by Pioneer of Korea, a qPCR assay is performed, reacting with the swollen genes in the Real-Time PCR device to contain the green dye.

-Real-time PCR data analysis:

Using the Levac method, real-time quantitative PCR data are analyzed, and this method was developed by (Livak and Schmittgen.,2001).

2.4. Study on laboratory animals

2.4.1. Ointment formulation

The pharmacological composition of nanoparticles was performed on the basis described by (Pajuet *al.*,2013). To prepare the ointment of the drug, 4 g of the nanoparticles were mixed with 16 g of white Vaseline, for 20%. Components of the medicinal prepared were mixed with stirring until homogeneous and the formation of the preparation. In the control treatment, 20 g of Vaseline only, without the nanoparticle.

Table 2. MgO nanoparticles formulation

Components	Formula(%)
	20%
MgO nanoparticle	4
White Vaseline	16g
Total amount	20g

2.4.2. Experimental infection:

Nine laboratory rats (3-4 months, 30-40 g) were used in this experiment, which were housed in cages and kept under standard conditions. The infection was infected with a wet swab that was wiped from the surface of the fungus culture and rubbed on the surface of the back, neck and head areas after cleaning.

2.4.3. Treatment.

The animals were divided into 3 groups (each group 3 mice). One group was treated with white Vaseline as a negative control. Another group was treated with 10% Terbinafine cream as a positive control. The remaining group was treated with 20% of the prepared nanoparticle ointment, the treatment was done twice a day. The effect of the ointment was determined every two days from the third to the fourteenth day by hair transplantation on Sabouraud Dextrose agar, the percentage recover was calculated using the equation given by (Wahabeet *al.*,1982).

$\% \text{ culture recovery} = \frac{\text{Total no. of sites positive for culture in each set}}{\text{Total no. of sites in each set}} \times 100$

2.5. Statistical Analysis

Using the statistical analysis method, the results of laboratory experiments were analyzed to find out the significant differences between the rates to be analyzed statistically through the chi-square (QI-SQUARE). Differences between groups by the value of the least significant difference (LSD) at the probability level ($P \leq 0.05$)

3. Results & Discussion:

The results of the study showed that there was a significant difference between the nanoparticles treatment at a concentration of 20% and the control treatment as a result of the effect of the prepared nanoparticles on the gene expression of the protease enzyme production gene (Table 3, Figure 1, 2).

Table(3)Effect on the gene expression of the protease enzyme production gene in *T.rubrum*

treatment	Gene expression
Control	8.645±1.05*
20% MgO nanoparticles	1.738±0.40*

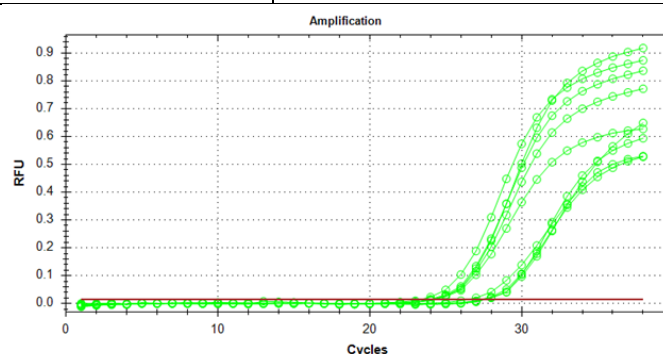


Fig.(1) Amplification curve used to assess how the protease gene in *T.rubrum* interacts with real-time PCR

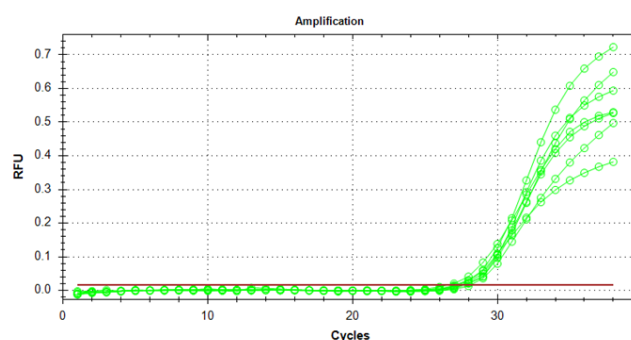
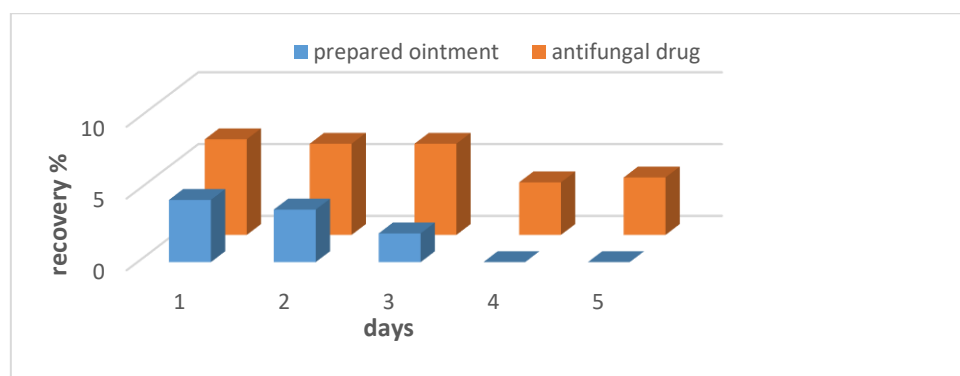


Fig.(2) Amplification curve to assess Real-Time PCR interaction of HKG gene

These results can be attributed to the unique properties of nanoparticles, the most important of which is their small size, which qualifies them to enter the cell and access the genetic material in it. Figure (3,4) shows the effectiveness of the proposed ointment that was prepared in this study in treating the infection that was experimentally induced in laboratory animals compared with the antifungal used to treat such infections. The two figures show that the prepared ointment gave a high affecting when using it. In addition, in this study, an oil composition is used for ease of application on the infected area, and Vaseline is used for therapeutic and cosmetic purposes, as it maintains skin moisture, and supports the absorption of the material (nanoparticles) from the affected tissues. (Selvan, *et.al.*, 2012).



Fir. 3.recovery percentage after treated with prepared ointment

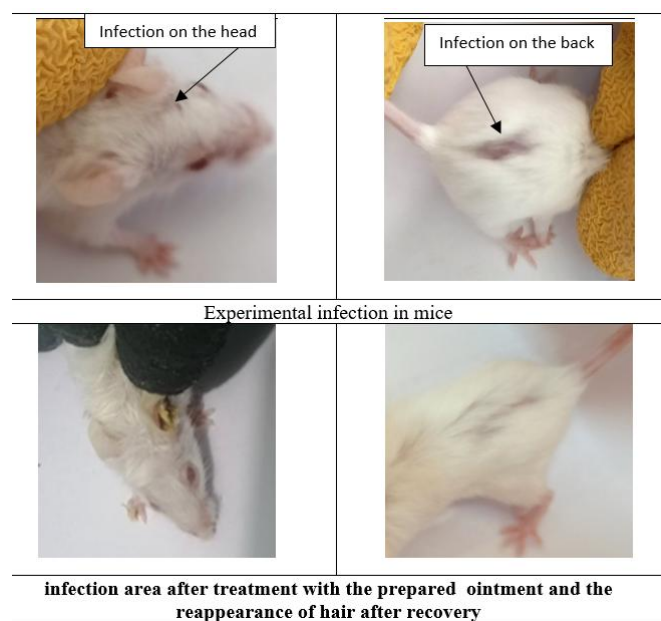


Fig.4.Efficacy of the ointment prepared from nanoparticles

4.Conclusion: our results suggest that the MgO nanoparticles may be an alternative to an antifungal agent for the healing of ringworm caused by *T. rubrum* in humans. moreover, clinical experiments with these nanoparticles formulations are wanted so as to recommend them for application as nanoparticles based antifungal factors.

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