DOI: https://doi.org/10.53350/pjmhs211551408

ORIGINAL ARTICLE

The effect of antifungal and Ago and Zno nanoparticles on Trichophyton mentagrophytes

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ABSTRACT

This study aimed to determine the main species of dermatophytes which caused skin infection and effect of antifungal and Ago and Zno nanoparticles on them. The result of this study showed that out of 80 sample, 54 sample were positive to fungal isolation with ratio 67.5%. and according to culture and PCR results %38.8 of isolated type belong to Trichophyton mentagrophytes. Trichophyton mentagrophytes resistant to Nystatin and Fluconazole while sensitive to Griesofulvin, Clotrimazole and Flucytosin. MIC of Ago and Zno nanoparticle against Trichophyton mentagrophytes were 250 and 275 µg /ml while MFC were 275 and 300 µg /ml respectively. Results of RAPD PCR showed that both Ago and Zno nanoparticle effect in genetic material of Trichophyton mentagrophytes

Key words: Trichophyton mentagrophytes, nanoparticle**,** RAPD PCR

INTRODUCTION

Skin is first line define against infection, it protects the body from external physical, chemical and biological effects, as well as it has physiological functions such as balancing the body temperature, its complex system formed about 15% from total body weight (1).

Dermatophytes belong to deuteromycota fungi, it consist from three genus which are: Microsporum, Trichophyton and Epidermophyton. Its classified according to sources of infection in to: Anthropophilic species, Zoophilic species and Geophilic species (2). Dermatophytosis is most common types of skin infection, these group of fungi called keratophilic, it have Keratinase which help them in use of keratin as source of protein (3).

Clinical of Dermatophytes infection can be classified according anatomical location of infection in: Tinea capitis, Tinea ungium, Tinea corporis, Tinea manum, Tinea barbae, Tinea pedis, Tinea faieci and Tinea cruris (4).

Similarities between fungi cell and host cell and development of fungi resistant against antifungal drugs lead to difficulty in treatment of mycotic infection (5).

Nanoparticles is one alternatives treatment to antifungal, which is a particles in size 10-100 nanometer, Manufactured by Down-Up Fabbrication or Up- Down Fabbrication, have different physical and chemical properties in compare with its origin, act as antifungal and a microbial agents (6).

MATERIAL AND METHODS

Sample collection and fungal diagnosis: (80) pathological skin samples were collected from outpatient clinics Under the supervision of dermatologists. The samples direct cultivation in Sabouraud dextrose agar and incubated at 25-30 ° C for 7days. After colony development, one colony selected for phenotypic examination and Microscopic examination applied according to (7) and a group of biochemical tests were applied (Hair penetration test, Urease enzyme test and Protease production test) according to (8).

PCR test for confirmation diagnosis of Trichophyton mentagrophytes

A- DNA extraction : DNA was extracted according to (7).
B- DNA amplification mixture: as in Table (1)
C- Thermocycler program: as in table (2) according to (9)

Table (1) Compounds used in preparation of Reaction Mixture

Compounds used in preparation of Reaction Mixture	Reference	Amount
Taq PCR Master Mix KIT (Qiagen, Germany) Which contain Taq DNA Polymerase (2.5 Unit), PCR Buffer with 3mM MgCL2, 200µMdNTP	3	25
Panderm_F(5'GAAGAAGATTGTCGTTTGCATCGTCTC3	7	0.3 from 100pM Solution.
Panderm_R(5'CTCGAGGTCAAAAGCACGCCAGAG3'	Out product size 336bp	0.3 from 100pM Solution.
DNA Template	Samples	3
DNA free water (Qiagen, Germany)	8	21.4
Total		50

- Antifungal sensitivity test: applied by disc methods against Nystatin , Griesofulvin , Fluconazole, Clotrimazole , Flucytosin and according to (10).
- Minimum inhibitory concentration (MIC)and Minimum fungicidal concentration (MFC) of nanoparticles (Ago and Zno) : applied by tube methods according to (11). with final nanoparticles concentration (100,200, 225, 250, 275, 300, 325, 350 µg /ml).
- Study effect of nanoparticles on genetic materials of Trichophyton mentagrophytes by RAPD PCR:

A- DNA was extracted according to (12).
B- Preparation of RAPD-PCR reactions according to (11), were performed using the (GoTaq® G2 Green master mix.
C- Thermocycler program: as in Table 3.

Table (2): showed thermocycler program

Stage	Temperature	Time	No. of cycles
First Denaturation step	95c°	3 mints	1cycle
Denaturation step	94c°	45sec.	35 cycles
Primer-annealing step	56 c°	45sec.	
DNA extension step	72 c°	1mint	
Final DNA extension	72 c°	10 mints	--------
End Temperature	4 c°	---------	----------

Table (3):Thermocycler program

Step	Temperature (C˚)	Time	No. of cycles
Initial denaturation	95	4min	1
Denaturation	92	30sec.	40
Annealing	36	45sec.	
Extension	72	45sec.	
Final extension	72	5min	1

D- Primers used in RAPD PCR as in table (4)

Table (4): Primers used in RAPD PCR

No	Primer code	Nucleotide sequence 5 to 3
1	OP V-20	CAGCATGGTC
2	OP Q-02	TCTGTCGGTC
3	OP G-05	CTGAGACGGA
4	OP P-04	GTGTCTCAGG
5	OP U-12	TCACCAGCCA

RESULTS

A- Result of fungal isolation :
Out of 80 sample , 54 sample were positive to fungal isolation with ratio 67.5%. Table 5.

Table (5): Number and ratio of fungal isolation

Gander of patient	Number of sample	Number of positive case	Ratio
Male	43	31	72.0%
Female	37	23	62.1%
Total	80	54	67.5%

B- from table (6) show that's highest infection case was Tinea capitis and lowest was Tinea crusis

Table (6): type of tinea and ratio of fungal isolation

Type of tinea	Clinical case		Isolation	
	No	%	No	%
Tinea capitis	21	26.2%	16	76.1%
Tinea corpis	19	23.7%	13	68.4%
Tinea manum	14	17.5%	8	57.1%
Tinea pedis	11	13.7%	7	63.6%
Tinea faciei	8	10%	5	62.5%
Tinea ungium	5	6.25%	4	80.0%
Tinea crusis	2	2.5%	1	50.0%
Total	80	100%	54	67.5%

C- Genus and Species of isolated fungi: from table (7) showed that highest isolated species was Trichophyton mentagrophytes (Figure 1 and Figure 2) and lowest isolated species was Epidermophyton floccosum

Table (7):Genus and Species of isolated fungi

Dermatophyte species	Number	Ratio
Trichophyton mentagrophytes	21	%38.8
Trichophyton tonsurans	15	%27.7
Trichophyton verrucosum	3	%5.5
Trichophyton rubrum	3	5.5%
Microsporum canis	6	%11.1
Microsporum ferrugineum	4	%7.4
Epidermophyton floccosum	2	%3.7
TOTAL	54	%100

Figure (1): SDA showed Trichophyton mentagrophytes colony

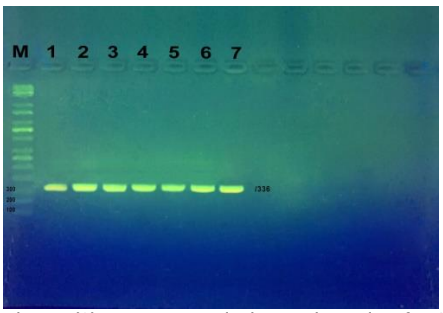

Figure (2): Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-7) positive result at 336bp for Trichophyton mentagrophytes

D- Result of antifungal sensitivity test: as in table (8).

Table (8): antifungal sensitivity test

Inhibition diameter Zone (mm) / Isolates	Antifungal agents				
	Nystatin	Griesofulvin	Fluconazole	Clotrimazole	Flucytosin
Trichophyton mentagrophytes	ـــ	16	ـــ	14	16
Trichophyton rubrum	ـــ	12	4	12	8
Trichophyton.tonsurans	ـــ	12	ـــ	10	ـــ
Trichophyton verrucosum	ـــ	14	ـــ	12	ـــ
Microsporum canis	ـــ	18	8	16	16
Microsporum ferrugineum	ـــ	20	6	18	14
Epidermophyton floccosum	ـــ	16	ـــ	12	8

Table (9): MIC and MFC of of Ago and Zno nanoparticle against Trichophyton mentagrophytes

Concentration of nanoparticles (µg /ml)	Type of nanoparticles	
	Ago	Zno
100	Turbidity/ growth	Turbidity/ growth
200	Turbidity/ growth	Turbidity/ growth
225	Turbidity/ growth	Turbidity/ growth
250	Clear / growth	Turbidity / growth
257	Clear / sterile	Clear / growth
300	Clear / sterile	Clear / sterile
325	Clear / sterile	Clear / sterile
350	Clear / sterile	Clear / sterile

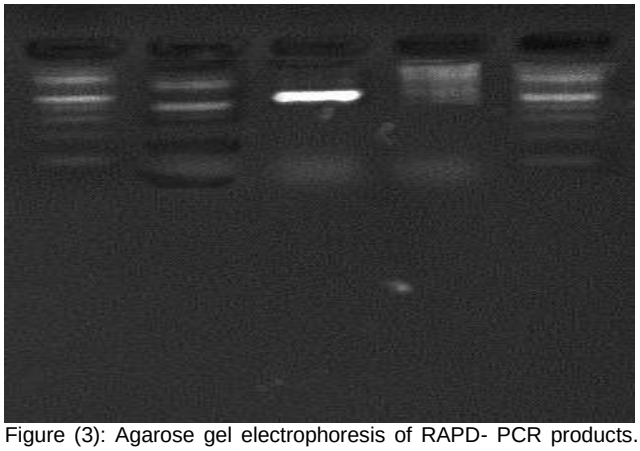

Figure (3): Agarose gel electrophoresis of RAPD- PCR products. lines (1-5) positive result of Trichophyton mentagrophytes with5different primers, before treatment with Nanoparticle

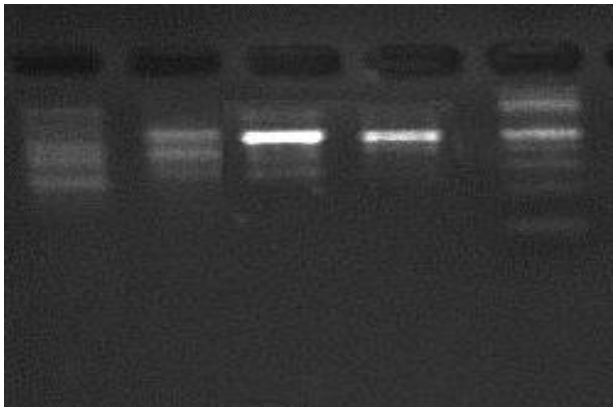

Figure (4): Agarose gel electrophoresis of RAPD- PCR products. lines (1-5) positive result of Trichophyton mentagrophytes with5different primers, after treatment with Ago Nanoparticle

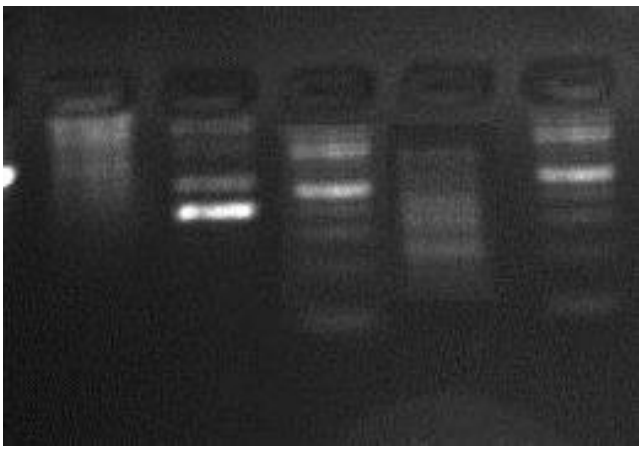

Figure (5): Agarose gel electrophoresis of RAPD- PCR products. lines (1-5) positive result of Trichophyton mentagrophytes with5different primers, after treatment with Zno Nanoparticle

E- Result of antifungal activity of nanoparticle(Ago and Zno) against Trichophyton mentagrophytes: from table (9) showed that MIC of Ago and Zno nanoparticle against Trichophyton mentagrophytes were 250 and 275 µg /ml while MFC of Ago and Zno nanoparticle against Trichophyton mentagrophytes were 275 and 300 µg /ml respectively.

F- Result of RAPD PCR test: from figure 3,4,5 showed that both Ago and Zno nanoparticles were effected in genetic materials which showed as appear or disappear and increase or decrease in thickness of bands

DISCUSSION

The common type of skin diseases were Superficial fungal infections which affecting millions of healthy and immunocompromised people worldwide (13).

n the current study showed presence of 32.5% of clinical infected cases were negative to culture that's may be due to take of patient to antifungal drugs or error in transport of sample or culture technique, or due to contamination of sample with other organism or maybe the infected not fungal origin (14). In present study showed that main type of infection were Tinea capitis that's perhaps due to factors related with the community culture, such as Clean the barbers, Free barber tools from the causes of disease.

In current study showed that the dominants fungal isolated type was Trichophyton mentagrophytes that's

agreement with (15) and disagreement with (16). The dominance of fungal type in compare with other type depend on geographic location, type of samples, season and Community habits.

In this study show high effect of Ago nanoparticles as antifungal and according to RAPD PCR showed effect in genetic materials, that's agreement with (17). Ago nanoparticles have high specific surface area and high fraction of surface atoms in compare silver metal, that's may be gives him the antifungal characteristic (18). The antifungal activity of AgNPs due to their ability to accumulation on fungal membrane of microorganisms, formation of pores which lead to change in permeability of cell wall. Also AgNPs can cause inhibit cellular respiration, DNA replication, and cell division, which result in the loss of cell viability, and lead to cell death (19).

In the current showed obvious effect of Zno nanoparticles on Trichophyton mentagrophytes and according to RAPD PCR showed effect in genetic materials. This agreement with (20, 21, 22). These activity may be due to electrostatic interaction between negative charge of cell membrane and positive charge of nanoparticles (23). Zn o have ability to cause damage to the cell membrane directly leads to the leakage of minerals, al may be act as antifungal due to toxic properties of metal oxide NPs, also have ability to bind with thethiol(-SH)groups of protein present in the cell wall. This interaction decreases the cell permeability which leads to cell lyses (24, 25,26).

Conflict of Interests: The authors of this paper declare that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

Source of Funding: The authors have no sources of funding, so it is self-funding research.

Ethical Approve: We declare that the study does not need ethical approval.

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