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Investigation of the antifungal effects of curcumin against nystatin-resistant *Candida albicans*

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ABSTRACT

Background: Emergence of nystatin-resistant *Candida albicans* (*C. albicans*) strains has raised some concerns in the recent years. Recent scientific evidence proves that turmeric, especially curcumin, has anti-inflammatory and anti-fungal activity. The aim of this study was the investigation of the antifungal effects of curcumin against nystatin-resistant *C. albicans*.

Materials and Methods: This *in vitro*, experimental study evaluated standard-strain (ATCC 16201) and 10 nystatin-resistant *C. albicans* strains. The antifungal activity and minimum inhibitory concentration (MIC) of curcumin were evaluated using the CLSI-M27-A3, and the MIC of curcumin was compared with that of nystatin. The results were analyzed using the one-way ANOVA.

Results: The MIC of curcumin was 15.6, 32.25, 15.6, 7.8, 32.25, 15.6, 15.6, 15.6, 32.25, and 15.6 μg/mL for the 10 resistant strains and 62.5 μg/mL for the standard strain of *C. albicans*. Curcumin in the above-mentioned concentrations significantly inhibited the proliferation of nystatin-resistant *C. albicans* strains ($P < 0.001$).

Conclusion: According to this research, it was shown that curcumin with MIC value of 7.8–32.25 μg/mL has inhibitory effects on nystatin-resistant *C. albicans* strains.

Key Words: *Candida albicans*, curcumin, nystatin, nystatin-resistant *Candida albicans*

INTRODUCTION

Oral candidiasis is the most common opportunistic infection of the oral mucosa caused by *Candida albicans* (*C. albicans*). The presence of *C. albicans* as part of the normal oral flora has a mean prevalence of 35%.[1] A significant correlation exists between oral candidiasis and the effect of local predisposing factors such as wearing denture, smoking, and use of topical and inhaled steroids, and systemic factors such as the immune system status and endocrine status, since these factors can lead to transformation of *C. albicans* from a commensal microorganism to a pathogenic microorganism.[1] Antifungal medications have many side effects and are associated with high recurrence rate. The commonly used antifungal agents belong to the family of azoles or polyenes. Polyenes such as nystatin are among the first-line medications for the treatment of primary oral candidiasis.[2] However, nystatin has drawbacks such as bitter taste, poor acceptance by patients, mucosal irritation, and nausea.[3] Furthermore, empirical and extensive use

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of antifungal agents has led to the development of resistant species, especially in immunocompromised patients.[4,5] Infections caused by the resistant species do not often respond to the conventional treatments and have a prolonged course.[6] Resistant *C. albicans* strains are a growing dilemma worldwide.[7,8] Nowadays, a growing interest in alternate materials[9-11] and medicinal herbs has developed worldwide due to their easy availability, fewer side effects, and significant therapeutic effects even on resistant strains.[12,13] The anti-inflammatory and antifungal effects of turmeric, and particularly curcumin, which is one of the most effective constituents of turmeric, have been well documented.[14] Curcumin has been used for the treatment of many diseases.[15] Several studies demonstrated antibacterial, antiviral, antifungal, and antimalarial activities of curcumin[16] and it can inhibited the proliferation of *C. albicans*.[17-19] Curcumin can reduce the metabolic activity of *C. albicans* biofilms.[20] Antifungal drugs-resistant *Candida* strains are a critical problem in therapeutic strategies. It has been shown that Curcumin is a potent fungicidal compound against susceptible and resistant *Candida* species[21] while some other studies refuted its optimal antifungal efficacy.[22] Considering the limited number of studies on the antifungal efficacy of curcumin on nystatin-resistant *C. albicans* strains, this study aimed to determine the antifungal effects of curcumin against nystatin-resistant *C. albicans.*

MATERIALS AND METHODS

This *in vitro*, experimental study was conducted on standard-strain *C. albicans* (ATCC 10261) and 10 nystatin-resistant *C. albicans* strains isolated from the samples taken from patients with denture stomatitis by a sterile swab. Three repetitions (three rows of a microplate for each strain) were considered for the exposure of each strain to curcumin (per each dilution). Nystatin was used as the internal control and one row (12 wells) of a microplate was assigned to this group. Nystatin (Cas no: 1400-61-9) was purchased from Hakim Pharmaceuticals (Tehran, Iran).

Sampling

Sterile swabs were used to collect the samples from the denture surface of patients with denture stomatitis. Three samples were collected from each patient after obtaining written informed consent. The *Candida* isolates were subcultured on CHROMagar *Candida* (Paris, France) and green colonies identified as *C. albicans* on the basis of the manufacturer's instructions. Their resistance to nystatin was confirmed according to CLSI M27-A3.[23]

Preparation of curcumin

The curcumin powder was purchased from Sigma-Aldrich (catalogue number: c1386). The curcumin solution was prepared with a concentration of 0.5 mg curcumin in 1 cc dimethyl sulfoxide and dispersed in autoclave-sterilized distilled water for 15 min at 121°C.

Preparation of fungal suspension

Roswell Park Memorial Institute (RPMI; Invitrogen, Gibco) 1640 medium was prepared as instructed in the brochure. It was then filtered and refrigerated. Next, a 24-h culture of *C. albicans* (ATCC 10261) on Sabouraud Dextrose Agar (Merck, Germany) in the logarithmic phase was used to prepare a fungal suspension. Colonies were collected and added to distilled water in a test tube. The optical density of the suspension was read by a spectrophotometer at 536 nm wavelength with distilled water blank. The transmittance value had to be 75% to 77% (for yeasts) indicating the presence of 5×10^3 CFU/mL. According to previous study,[15] the suspension was diluted 1:10 by using sterile distilled water (9 mL of distilled water plus 1 mL of the primary suspension). The suspension was then diluted 1:100 by addition of RPMI (9.9 mL of RPMI plus the secondary suspension). After the addition of RPMI and curcumin plus the fungal suspension, the number of yeast cells in each well reached 2.5×10^3 cells/mL.

Antifungal activity

The broth microdilution technique was performed. For this purpose, 100 µL of RPMI culture medium was added to all wells of the microplate. Next, 100 µL of curcumin with 500 µg/mL concentration was added to the first well of row A. After pipetting up and down, 100 µL of the first well (containing curcumin and culture medium) was transferred to the second well by a sampler. After pipetting up and down, 100 µL of the second well was transferred to the third well and so on. Finally, 100 µL of the last well was discarded. The same process was repeated for the second and third rows. Accordingly, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.9, 0.95, and 0.47 mg/mL concentrations of curcumin were obtained as such. The 11th well was the positive control which included

the culture medium and fungal suspension. The 12th well was the negative control which only included the culture medium. Afterward, 100 µL of the fungal suspension (separately from each isolate) was added to all wells until the 11th well. The microplate was then capped and placed in a shaker for 3 min for homogenization. It was then incubated for 24, 48, and 72 h, and the minimum inhibitory concentration (MIC) was determined at each time point. After 24 h, the positive and negative controls were evaluated. Fungal growth was noted in the positive control well while the negative control well remained clear. The test was repeated in triplicate.[23] According to CLSI M27-A3, the MIC values for nystatin were the lowest concentration that caused complete inhibition of growth (sensitive: MIC ≤1 µg/mL; resistant: MIC >1 µg/mL), as recommended for other polyenes, for example amphotericin B.

Statistical analysis

Data were analyzed using the SPSS version 22 (Armonk, NY, IBM Corp) by the one-way ANOVA.

RESULTS

Table 1 presents the mean of MICs of curcumin in 24, 48, and 72 h for the 11 groups of standard-strain and resistant isolates of *C. albicans* determined according to CLSI M27-A3. ANOVA revealed a significant difference in MIC of different study groups ($P < 0.001$). Nystatin in the range of 1–2 µg/mL had inhibitory effects on *C. albicans*. The MIC value ranges for nystatin resistant and ATCC strains were 7.8–32.25 and 62.5 µg/mL, respectively. The results indicated the inhibitory effects of curcumin in higher concentrations than nystatin on nystatin-resistant *C. albicans* strains.

DISCUSSION

This study assessed the effect of curcumin in different concentrations on 10 nystatin-resistant *C. albicans* clinical isolates and the standard-strain *C. albicans* (ATCC 10261) by determining the MIC. The results indicated the optimal antifungal effect of curcumin on the 10 resistant isolates and the standard strain *C. albicans*. Curcumin in higher concentrations than nystatin had inhibitory effects on nystatin-resistant strains.

Upnow, no study performed on the antifungal effect of curcumin against nystatin-resistant *C. albicans* clinical isolates. However, our results regarding the optimal antifungal effect of curcumin on *C. albicans* were in agreement with those of Mustafa *et al*.[17] Babaii and Zamaninejad,[24] Garcia-Gomes *et al*.[19] Golpour *et al*.[22] and Alalwan *et al*.[20] evaluated the efficacy of an alcohol-free chitosan-curcuminoid mouthwash, compared with chlorhexidine, for treatment of denture stomatitis. Of all, 80% of patients using the chitosan-curcuminoid mouthwash were treated while this rate was 30% in those using the chlorhexidine mouthwash. Babaii and Zamaninejad evaluated the inhibitory effect of curcumin, compared with nystatin, on *C. albicans in vitro*.[24] They used colony counting, cup bioassay and assessment of growth inhibition zone around dried curcumin discs in methyl sulfate to assess its antifungal efficacy. The results of all three techniques confirmed the antifungal effects of curcumin, which was dose-dependent. Their results were in agreement with the present findings, although nystatin-resistant strains were evaluated in the present study and the CLSI protocol was applied. Garcia-Gomes *et al*. evaluated the synergistic effect of curcumin plus fluconazole on *C. albicans* clinical isolates.[19] Similar to the present study, they used the broth microdilution method and reported the results in accordance with the present findings, although nystatin-resistant strains were evaluated in the present study. Golpour *et al*. assessed the antifungal effects of curcumin entrapped in nanomicelle particles on expression of *CDR1* gene by fluconazole-resistant *C. albicans in vitro*.[22] They showed that curcumin inhibited the fungal growth and had a synergistic effect with fluconazole on fluconazole-resistant *C. albicans*. Their results were in line with the present findings despite using different isolates.

In the study of Tsao and Yin on 200 clinical isolates of *Candid*a species showed fungicidal activity for

Table 1: The mean of minimum inhibitory concentrations of curcumin for the standard-strain and resistant isolates of *Candida albicans* determined by the broth microdilution technique

Isolate number	1	2	3	4	5	6	7	8	9	10	ATCC 10261	*P*
Nystatin (range 1-2), MIC (µg/mL)	2	2	2	2	2	2	2	2	2	2	1	<0.001
Curcumin MIC (µg/mL)	15.6	32.25	15.6	7.8	32.25	15.6	15.6	15.6	32.25	15.6	62.5	

MIC: Minimum inhibitory concentration

curcumin with MIC value of 32–128 μg/mL.[25] In the present study, MIC values for nystatin resistant and ATCC strains were 7.8–62.5 μg/mL. The difference in MIC values can be due to the evaluation of different *Candida* species in Tsao and Yin study and the examination of nystatin-resistant *C. albicans* in the present study.

However, the present results were different from those of Nosratzehi *et al*.,[26] Neelofar *et al*.,[21] and Khan *et al*.[27] Nosratzehi *et al*.[26] reported inferior inhibitory effect of curcumin compared with nystatin, which may be due to their different methodology, since they used the paper disc diffusion technique while we used the broth microdilution technique. Neelofar *et al*. evaluated the effect of curcumin on standard-strain *C. albicans* (ATCC 10261) and *Candida glanrata* (ATCC 90030) regarding their proliferation, the amount of sterol, and release of proteinase, compared with fluconazole.[21] Its MIC was found to be 250 μg/mL while this value was 62.5 μg/mL in the present study, which may be due to the use of different culture media because we used RPMI while they used YEPD culture medium. Furthermore, nystatin-resistant strains were evaluated in the present study. Khan *et al*. evaluated the antifungal effects of curcumin and methylcinnamaldehyde on 38 *C. albicans* strains including three standard, 24 fluconazole-sensitive and 11 fluconazole-resistant strains.[27] The MIC for the sensitive groups ranged from 350 to 650 μg/mL while it ranged from 250 to 500 μg/mL for the resistant strains, while Its values in present study were 62.5 μg/mL for ATCC stain and 7.8–32.25 μg/mL for resistant strains. They used, YPD culture medium which was different from the RPMI medium used in the present study, also different geographical regions for the *Curcuma longa* could be important fact that can explain these differences. Curcumin is the effective constituent of the rhizome of turmeric with the generic name *C. longa* and the chemical name diferuloylmethane.[28] It accounts for 2%–8% of the turmeric composition and is responsible for the yellow-gold color of turmeric and the majority of its therapeutic properties.[29] Curcumin is a polyphenol with strong biological properties. It is known as a contemporary bio-regulator. It reportedly has anti-cancer, cholesterol-reducing, and immune-boosting properties. It can prevent cardiovascular diseases, peroxidation of cell membrane, and inflammation.[26] The anti-inflammatory properties of curcumin are attributed to the reduction in level of lysosomal enzymes in macrophages.[14] Evidence shows that curcumin can inhibit the adhesion of *C. albicans*[20] which may be due to high amounts of chitin in the cell wall.[24] Curcumin can also decrease the production of lipopolysaccharides by the prostaglandin E2.[15] Another effective factor in the antifungal activity of curcumin can be due to the reduction of proteinase secretion and membrane changes related to ATPase activity.[21]

The present results confirmed the antifungal effects of curcumin on both standard and nystatin-resistant *C. albicans* strains. Thus, it may be suitable for use in the formulation of mouthwashes for the treatment of resistant cases of candidiasis. Future clinical trials are required on the efficacy of curcumin for the treatment of resistant cases of candidiasis.

CONCLUSION

According to this research, it was shown that curcumin with MIC value of 7.8–32.25 μg/mL has inhibitory effects on nystatin-resistant *C. albicans* strains.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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