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An In Vitro Study of the Effect of Cinnamaldehyde on the Growth of *Candida albicans* Compared to Nystatin and Fluconazole

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Abstract

Objective: Cinnamaldehyde is the main active component of cinnamon essential oil with antibacterial and antifungal effects. This in vitro study was done to investigate the effect of cinnamaldehyde on the growth of standard strain of *Candida albicans* (*C. albicans*) in comparison to nystatin suspension and fluconazole.

Materials and Methods: The tests were designed for the three groups of this study namely cinnamaldehyde, fluconazole and nystatin. Standard strain of *C. albicans* (PTCC 5027) was used. Three methods namely disc diffusion, well diffusion and microdilution were used. Based on elevation of cinnamaldehyde concentrations, its antifungal effect was increased.

Results: The minimum inhibitory concentration (MIC) of antifungal effect of cinnamaldehyde was 0.312 μl/ml. This effect is similar to the effect of 100000 IU/ml nystatin in concentration of 20 μl/ml and 1% fluconazole in concentration of 40 μl/ml. The mean (±SD) of inhibition zone diameter in concentration of 80 μl/ml for disc and well diffusion were 60.4 (±2.2) and 70 (±3.5) mm, respectively.

Conclusion: These inhibition zones were larger than the zones for fluconazole and nystatin groups. According to the results, cinnamaldehyde has dose-dependent anti-*C. albicans* effect. In some concentrations, the cinnamaldehyde effect is similar to fluconazole and nystatin.

Keywords: *Candida albicans*, Cinnamaldehyde, Cinnamon, DMSO

Introduction

Oral candidiasis is the most prevalent opportunistic fungal infection that affects oral mucosa. It is mainly produced by *Candida albicans* (*C. albicans*) which is a saprophyte dimorphic fungus. The infection is considered by an adhesive membrane which contains fungal organisms and cellular debris. An inflamed or sometimes irritating surface will appear when this pseudo membrane has been removed. In most cases of candidiasis, only mucosal lining is affected but systemic manifestations may rarely lead to fatal conditions (1).

With increase of fungal infections, many efforts have been done towards the development of effective strategies for prevention and treatment of candidiasis and other fungal infections. The majority of clinically used antifungal drugs have disadvantages such as toxic effects, high costs and appearance of drug resistance (2).

In recent years, resistance to antifungal agents has been reported from all over the world. Fluconazole resistance has become a major concern in the treatment of fungal infections. Its efficacy may be reduced in HIV-positive patients with recurrent oral candidiasis and patients infected with fungi other than *C. albicans* (3,4).

Antifungal components of medicinal plants have been considered. To date different plants have been used for treatment of fungal diseases. The main advantage of natural antifungal agents is that they do not cause "antibiotic resistance" which is a common phenomenon during the long-term use of synthetic antibiotics. Antimicrobial properties of some herbal medicines such as cinnamon, oregano and clove are referred to their aromatic components such as cinnamaldehyde, carvacrol and eugenol (5).

The genus of *Cinnamomum* (Lauraceae) are found in southeast of Asia, Australia and South America as shrubs and short to medium trees (2,6,7). Cinnamon oil consists of different components such as cinnamaldehyde, cinnamyl acetate, eugenol, caryophyllene, terpineol, linalol and some other secondary components (8).

Cinnamaldehyde, which produces the special taste and smell of cinnamon, is naturally found in the oil of bark, leaves and roots of *Cinnamomum* (9). Cinnamaldehyde has antifungal and antibacterial (10), anti-inflammatory (11), antiviral (12), antioxidant (13) and even antitumor effects.

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According to the antifungal effects of cinnamon oil (2,6,14-17) or cinnamaldehyde as its major component (18), the present in vitro study was conducted to compare the effect of cinnamaldehyde on the growth of *C. albicans* with nystatin and fluconazole by disc diffusion, well diffusion and microdilution methods.

Materials and Methods

The current study was done on standard strain of *C. albicans* (PTCC 5027) purchased from Industrial Fungi and Bacterial Preparation Center, Tehran, Iran. Pure cinnamaldehyde (Merck, Germany), nystatin powder (Midlothian, USA), fluconazole powder (Arasto Pharmaceutical Chemicals Inc., Iran), DMSO (Merck, Germany), BHI and Sabouraud dextrose agar (Merck, Germany) were prepared. Pure DMSO (Dimethyl Sulfoxide) was used as solvent of nystatin, fluconazole and cinnamaldehyde. Then 100000 IU/ml nystatin, 1% fluconazole and different concentrations of cinnamaldehyde (2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml, 40 µl/ml and 80 µl/ml) were prepared. To evaluate the antifungal effect of cinnamaldehyde, 3 in vitro methods were used.

Disc Diffusion Method

In the first step, fresh suspension of *C. albicans* (0.5 McFarland) was cultured on Sabouraud dextrose agar plates. The solution of 80 µl/ml cinnamaldehyde in DMSO was prepared as stock solution and then diluted into 5 concentrations (2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml, and 40 µl/ml). Sterilized blank discs were soaked in 30 µl of a certain concentration of cinnamaldehyde, DMSO (negative control), 100 000 IU/ml nystatin and 1% fluconazole (as positive control). Initially, 2 discs were placed on each plate, one of them was immersed in a certain concentration of cinnamaldehyde and the other one was immersed in DMSO, but because of large inhibition zone of higher concentrations of cinnamaldehyde which prevented fungal growth around the disc of DMSO, only one disc was placed on each plate and the disc of negative control (DMSO) was placed on a separate plate. After replacing the discs on medium plates, the plates were incubated at 37°C for 48 hours, and then were studied for evaluation of inhibition zones. The basic margin of the discs was determined at 6 mm. Also, to control the growth of fungi, a cultured plate without any disc was incubated in the same condition. This procedure was repeated 5 times for each concentration (4).

Well Diffusion Method

Like previous method, fresh suspension of *C. albicans* (0.5 McFarland) was cultured on Sabouraud dextrose agar plates. Then in each plate a 6 mm diameter well was made by sterilized pipet. Thirty microliter of each concentration of cinnamaldehyde (2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml, 40 µl/ml and 80 µl/ml), DMSO (as negative control), 100 000 U/ml nystatin and 1% fluconazole (as positive control) were added in the wells and a plate was considered as a control of fungal normal growth. Then the plates were incubated at 37°C for 48 hours and the results were observed. The basic margin of the wells was determined at 6 mm. Based on previous investigations, this procedure was repeated 5 times for each concentration (14,19).

Microdilution Method

The minimum inhibitory concentration (MIC) was measured using microdilution method. To prepare stoke solution, 10 µl of cinnamaldehyde was dissolved in 1 ml of DMSO and the concentration of 10 µl/ml was obtained. This solution was diluted by 10% DMSO and concentration of 2.5 µl/ml cinnamaldehyde in 10% DMSO was obtained. Then the solution was diluted by 10% DMSO in 5 microwells with a ratio of ½ to obtain final concentration of cinnamaldehyde (0.156 µl/ml). One hundred microliter of 0.5 McFarland suspension of *C. albicans* was added to each microwell containing 100 µl cinnamaldehyde with mentioned concentration. The remaining microwells were divided into 3 groups: negative control (100 µl of 10% DMSO + 100 µl of *C. albicans* suspension), positive control (100 µl 1% fluconazole + 100 µl of *C. albicans* suspension) and no treatment (100 µl of *C. albicans* suspension). In this investigation nystatin was not used in microdilution method as positive control due to its precipitation in 10% DMSO. The microwells were incubated with the same conditions at 37°C. After 24 hours, the absorbance of the samples was measured using spectrophotometry at 450 nm. Then, the fungi cells were counted. This test was repeated 5 times for each concentration (16,18).

Statistical Analysis

One-way analysis of variance (ANOVA) post-hoc Tukey test was used to evaluate the effect of cinnamaldehyde concentrations on *C. albicans* using disc diffusion and well diffusion methods. Unpaired *t* test was used to evaluate the results of the microdilution method. Any difference between data was considered statistically significant at $P < .05$.

Results

In the present study, the antifungal effect of cinnamaldehyde on standard strain of *C. albicans* was evaluated. The results were summarized in two parts; disc diffusion and/or well diffusion methods and microdilution method. Results showed that inhibition zone diameter was increased in cinnamaldehyde used plates (Figure 1). Among the available concentrations, 2.5 µl/ml cinnamaldehyde and DMSO (negative control) made no zone of the inhibition. Hence DMSO which was used as solvent in disc and well diffusion methods had no effect on the strain tested in the present study. Both, in disc and well diffusion methods, the diameter of inhibition zones for 20 µl/ml, 40 µl/ml and 80 µl/ml cinnamaldehyde and also for 100000 U/ml nystatin and 1% fluconazole (positive controls) were significant compared to DMSO ($P < .05$). The diameter of inhibition zone at 80 µl/ml cinnamaldehyde was significant compared to other groups ($P < .05$, Figure 2). The diameter of inhibition zone of 100 000 U/ml nystatin was

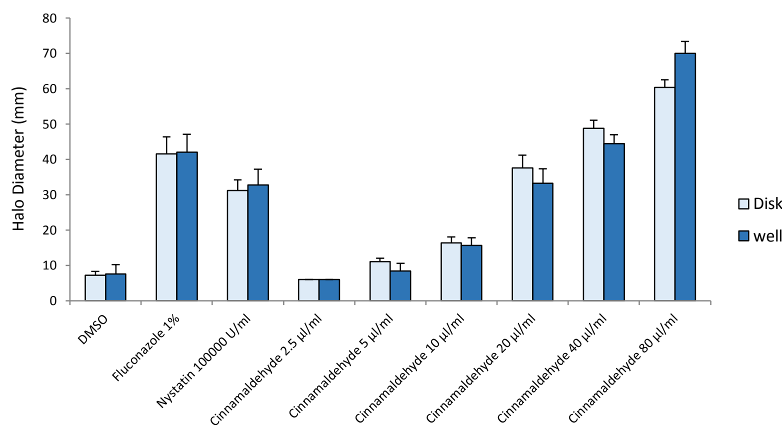

Figure 1. Mean and SD of inhibitory zone diameter (mm) in different concentrations of cinnamaldehyde, 100 000 IU/ml nystatin and 1% fluconazole (positive controls) and DMSO (negative control) 48 hours after treatment in disc and well diffusion methods.

significant compared with other groups, except 20 µl/ml cinnamaldehyde (Figure 3). The diameter of inhibition zone of 1% fluconazole was significant compared with other groups, except 40 µl/ml cinnamaldehyde (Figure 4). The results of cell counting method (Table 1) and spectrophotometry (Table 2) showed that by increasing the concentration of cinnamaldehyde, the number of fungi cells and the turbidity decreased in a way that cinnamaldehyde in 0.312 µl/ml and higher concentrations showed a significant antifungal effect ($P < .05$). Hence, the concentration of 0.312 µl/ml was considered as MIC. Also, 10% DMSO, which was considered as negative control, did not show significant difference with 0.5 McFarland suspension of *C. albicans* in cell counting method ($P = .235$) and spectrophotometry ($P = .260$).

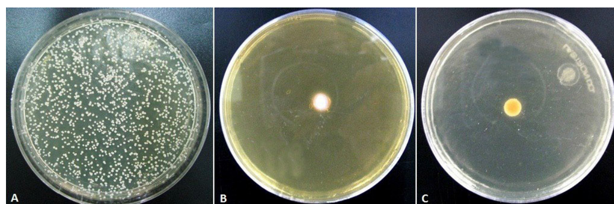

Figure 2. A) fungal cell growth (suspension 0.5 McFarland) in plates of Sabouraud dextrose agar medium. B) Inhibition of fungal cell growth in plates of Sabouraud dextrose agar medium in the presence of 80 µl/ml cinnamaldehyde in disc diffusion method. C) Inhibition of fungal cell growth in plates of Sabouraud dextrose agar medium in the presence of 80 µl/ml cinnamaldehyde in well diffusion method.

Discussion

In the present study, we examined the anti *C. albicans* effect of cinnamaldehyde in comparison to nystatin (100 000 U/ml) and fluconazole (1%). Present study showed that cinnamaldehyde can dose-dependently inhibit growth and proliferation of *C. albicans* compared to nystatin and fluconazole. Cinnamaldehyde even in concentration of 80 µl/ml inhibits *C. albicans* more than nystatin and fluconazole. Some investigators reported that the antifungal effect of cinnamon oil is mainly due to its cinnamaldehyde component (14,15,17).

The inhibitory effect of cinnamaldehyde on *C. albicans* presented in this investigation is in consistence with previously mentioned studies (15-17). Sanla-Ead et al (20) showed cinnamaldehyde and eugenol for their antimicrobial activity against 10 pathogenic bacteria and 3 strains of yeast. They reported that cinnamaldehyde and eugenol created 8.7-30.1 mm inhibition zones and with MICs of 0.78-50 µl/ml inhibited the growth of all test micro organisms. In the above mentioned study no clear inhibition zone was found in the disc diffusion method, but in the

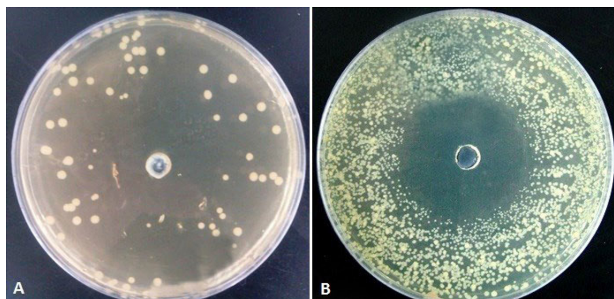

Figure 3. A) Inhibition of fungal cell growth in plates of Sabouraud dextrose agar medium in the presence of 100 000 U/ml nystatin in well diffusion method. B) Inhibition of fungal cell growth in plates of Sabouraud dextrose agar medium in the presence of 20 µl/ml cinnamaldehyde in well diffusion methods.

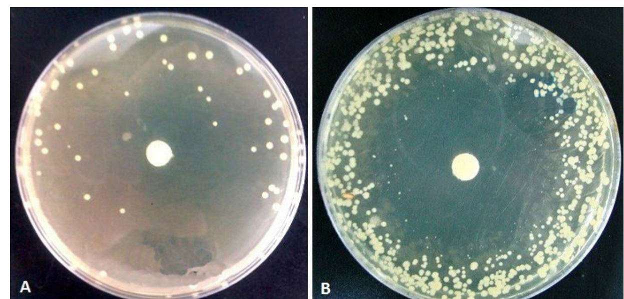

Figure 4. A) Inhibition of fungal cell growth in plates of Sabouraud dextrose agar medium in the presence of 1% fluconazole in disc diffusion method. B) Inhibition of fungal cell growth in plates of Sabouraud dextrose agar medium in the presence of 40 µl/ml cinnamaldehyde in disc diffusion method.

Table 1. Mean (±SD) of *C. albicans* Cell Count (cfu/ml) in Different Treatment Groups (Suspension 0.5 McFarland, Negative Control, Positive Control and Cinnamaldehyde in Different Doses) 24 Hours After Treatment

Groups	Mean (± SD)
suspension 0.5 McFarland	4600 (± 2561.49)
10% DMSO (negative control)	2980 (± 1188.27)
1% fluconazole (positive control)	1020 (± 3047.71)
Cinnamaldehyde2.5 µl/ml	1446 (± 575.39)
Cinnamaldehyde1.25 µl/ml	1670 (± 446.65)
Cinnamaldehyde0.625 µl/ml	1780 (± 450.83)
Cinnamaldehyde0.312 µl/ml	1800 (± 772.17)
Cinnamaldehyde0.156 µl/ml	2016 (± 689.80)

Table 2. Mean (±SD) of Optical Density at 450 nm in Different Treatment Groups (Suspension 0.5 McFarland, Negative Control, Positive Control and Cinnamaldehyde in Different Doses) 24 Hours After Treatment

Groups	Mean (± SD)
Suspension 0.5 McFarland	1.041 (± 0.34)
10% DMSO (negative control)	0.845 (± 0.10)
1% Fluconazole (positive control)	0.618 (± 0.02)
Cinnamaldehyde 2.5 µl/ml	0.589 (± 0.04)
1.25 µl/ml Cinnamaldehyde	0.597 (± 0.02)
Cinnamaldehyde 0.625 µl/ml	0.617 (± 0.12)
Cinnamaldehyde 0.312 µl/ml	0.650 (± 0.11)
Cinnamaldehyde 0.156 µl/ml	0.714 (± 0.23)

vapour diffusion method, decrease in the size and number of the bacterial and fungal colonies were considerable. Similarly in our study, cinnamaldehyde (in concentration 2.5 µl/ml) did not show inhibition zone using disc and well diffusion methods but in microdilution method, antifungal activity in 2.5 µl/ml and lower concentrations was observed and the MIC of cinnamaldehyde was achieved at concentration 0.312 µl/ml.
Taguchi et al (18) evaluated the effect of cinnamaldehyde on the growth and morphology of *C. albicans* and reported that cinnamaldehyde has both fungistatic and fungicidal activities against the fungi and can alter the cellular structure. It has been shown that cinnamaldehyde may exert its fungicidal effect in concentration higher than 40 µg/ml (19). Since cinnamaldehyde concentrations higher than 10 µl/ml in DMSO is precipitated in microdilution method, and may result in formation non-uniform medium, the antifungal effect of cinnamaldehyde will disappear. Therefore to avoid the precipitation, lower concentrations of cinnamaldehyde were used. According to the present study, the MIC was set at 0.312 µl/ml. The reason of difference between the results of the current study and other surveys may be due to homogeneity of the solution in different concentrations and better spreading of cinnamaldehyde in the fungal suspension which led to antifungal effect in lower concentrations.

Antibacterial and antifungal effect of cinnamaldehyde have been previously reported (19). It has been shown that the effect of higher concentrations of cinnamaldehyde is comparable with 2% chlorhexidine on *C. albicans* biofilms (21). The exact mechanism of antifungal action of cinnamaldehyde remains to be elucidated. Yen and Chang (22) demonstrated that cinnamaldehyde by inhibition of fungal cell wall synthesizing enzymes, destroys their cell wall which leads to osmotic cell destruction and makes yeasts susceptive to the fungicides. Another mechanism of action which has been reported for cinnamaldehyde is destruction of fungal cell plasma membrane which has 2 site of action: plasma membrane ATPase activity and ergosterol biosynthesis. Plasma membrane-ATPase is an important fungal pump which transfers substances in fungal plasma membrane. This pump is activated in the presence of glucose to extrude more H^+. Cinnamaldehyde can significantly inhibit the activity of this pump in the absence of glucose (23). Another mechanism of action may be inhibition of ergosterol biosynthesis. Since ergosterol causes the integrity and fluidity of plasma membrane and also provides proper function of membrane enzymes, by inhibitory effect of cinnamaldehyde on ergosterol biosynthesis, destruction in homogenous structure of fungal cell plasma membrane can occur (23).
Rajput and Karuppayil (24) compared the anticandida effect of 25 molecules of plant origin, and evaluated their effects on sterol by "sterol quantitation method." The results showed that cinnamaldehyde, piperine, furfuraldehyde and indole can show good anticandida effects due to inhibition of ergosterol biosynthesis. Nystatin is a polyene antifungal medicine, which acts by adhering to ergosterol and inducing destructive effect (25,26) and fluconazole is an azole which acts by decreasing ergosterol biosynthesis through inhibition of cytochrome P450 enzyme (27). It seems that antifungal mechanism of action of cinnamaldehyde is similar to nystatin and fluconazole.

Conclusion

According to the results, it can be concluded that cinnamaldehyde with MIC of 0.312 µl/ml has a dose dependent antifungal effect on *Candida albicans*. Also, more investigations on clinical application of cinnamaldehyde are recommended.

Ethical issues

Ethical of this research work was approved by Babol University of Medical Sciences.

Conflict of interests

The authors declare that they have no competing interest.

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