

Inhibitory Effect of Curcumin on *Candida-albicans* compared with Nystatin: an *in-vitro* Study

Babaii Neda¹, Zamaninejad Shiva²

¹Associate Professor of Oral and Maxillofacial Medicine, Faculty of Dentistry, Babol University of Medical Science, Babol, Iran

²Assistant Professor of Pediatric Dentistry, Faculty of Dentistry, Golestan University of Medical Science, Gorgan, Iran

Received 9 July 2016 and Accepted 13 September 2016

Abstract

Introduction: Curcumin is the active ingredient in the traditional herbal remedy and dietary spice turmeric (*Curcuma longa*). Curcumin has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activity. on basis of recent studies; it has antifungal and antibacterial effects. The aim of this study was *in-vitro* evaluation of antifungal effect of curcumin on candida albicans and comparing it with nystatin. **Methods:** after preparing curcumin powder, 3 laboratory methods were used to evaluate antifungal effect. The first method was cell count technique, used to evaluate the amount of candida albicans after time, in different concentrations of curcumin in Dimethyl sulfoxide (DMSO). The second was cup bioassay, in which inhibitory a zone of curcumin in DMSO was evaluated in sabouraud culture plates; and in third method, inhibitory zones of dried disks; which contained curcumin in DMSO were evaluated. **Results:** the result of all three methods showed that curcumin has antifungal effect and this effect increases in more concentrations. **Conclusion:** curcumin has apparent and dose dependent antifungal effect on candida albicans.

Key word: curcumin, antifungal effect, candida albicans, dimethyl sulfoxide

Introduction

Candidiasis is a very important fungal infection of oral cavity. The most important risk factors are endocrine alterations such as diabetes mellitus, pregnancy and renal failure, immune depression, poor oral hygiene, smoking, alcoholism and long term administration of drugs¹⁻³. Oral candidiasis occurs because of increase in *Candida* species, chiefly *Candida Albicans*. Some pathogenicity factors in *C. albicans* cause better adherence to cell membrane than other species (4).

Management of oral candidiasis is typically drug based but the extensive use of azoles has resulted in resistance in *Candida* species and subsequent treatment failures. Problems associate to common antifungal drugs have led to new therapeutic methods to be thought (5).

Curcumin is the active substance of the herbal remedy and the spice turmeric (*curcuma longa*)⁶. It is the element of yellow colour in spices turmeric and curry that is isolated from the rhizomes of the plant *Curcuma longa* so that this material is natural, healthy and safe (7). Spice turmeric has a lot of benefits in traditional medicine such as treatment of Jaundice, rheumatoid arthritis, liver disease, eye infections and dental pain (8, 9). Also the remedial effect of turmeric in new medicine has been approved such as anti-inflammatory, hypoglycemic, antioxidant, wound-healing, and antimicrobial activities (10, 11). The mechanism of its antifungal activity is not known but it has been suggested that its fungicidal mechanism may involve chitin accumulation on the cell wall outer layer¹². However, curcumin has been suggested as an irresistible antiviral, antibacterial and antifungal agent, but it is still obscure that is curcumin as effective as

Babaii N, Zamaninejad S. Inhibitory effect of curcumin on candida albicans compared with Nystatin: an invitro study. J Dent Mater Tech 2016; 5(4): 196-201.

common antifungal drugs especially against *C. Albicans* or not 13.

This study wants to evaluate the effect of curcumin against *C. Albicans* species in vitro. Also, compare the fungal effect of curcumin with the famous antifungal drug, Nystatin.

Methods and Materials

This is a prospective lab trial study with no ethical concerns. Pure standard specimen of *C.albicans* (PTCC5027, Merck, Germany) prepared in a way that is described below:

1. Surface of vial containing fungi, was disinfected by alcohol and broken within cotton dipped in alcohol near the heat.
2. 1ml of sterilized BHI culture (assembled as manufacturer orders and sterilized in autoclave) added to vial. After mixing, a few of solution pulled into a siring and then added to Sabouraud Dextrose agar (assembled as manufacturer orders and sterilized in autoclave) and BHI cultures.
3. Concentration of *C. Albicans* in the BHI culture was made approximately 105 CFU/ml. microplates dipped about three hours in Sodium hypochlorite (NaOCl) 2.5% for disinfection. Then rinsed completely and dipped in distinct water for 12 hours. After that the samples were dried with sterile gauze.

Three laboratory methods were used to evaluate antifungal effect that in all methods the plates that have been infected by bacteria excluded from the study and the process repeated for those plates.

- A) Cell count technique, was used to evaluate the amount of *C. Albicans* after time, in different concentrations of Dimethyl sulfoxide (DMSO) :

The main solution was prepared in the form of curcumin inside DMSO solvent, with a concentration of 5gram in 100cc. Therefore, a solution consists of 10g curcumin in 200qlit DMSO was obtained. Then the solution diluted by DMSO solvent in a ratio of 1/2 in 5 microplates, as in the last plate, curcumin concentration in solvent got to 0.156g in 100cc. The content of solution in each microplate was 200μlit.

In the other side, Nystatin was prepared into the manufacturer program and 50μlit of that was mixed with 50μlit of liquid BHI culture. Then 100μlit of fungal suspension that was made in BHI culture, added to each microplate. To ensure that the solvent has no effect on antifungal activity of curcumin, the second and third testimonial prepared (table.1).

Previously mentioned, microplates were placed in 37°c autoclave in a same condition for 24 and 48 hours

to study the growth. This experiment was repeated 19 times.

- B) Cup bioassay technique, in which inhibitory zones of curcumin in DMSO was evaluated in sabouraud culture plates:

Three cups with a diameter of 6 mm were created by the beak of sterile pipet in the sabouraud culture plates. The main solution was prepared in the form of curcumin inside DMSO solvent with a concentration of 5%, then the main solution diluted in a ratio of 1/2 in 2 experimental tubes (concentrations:5%, 2.5%, 1.25%), and poured from prepared concentrations in the plate as following bellow:

Plate1: 3 cups (30qlit of 5%curcumin, 30qlit DMSO, 30qlit Nystatin)

Plate2: 3 cups (30qlit of 2.5%curcumin, 30qlit DMSO, 30qlit Nystatin)

Plate3: 3 cups (30qlit of 1.25%curcumin, 30qlit DMSO, 30qlit Nystatin)

Plate4: 3cups (30qlit of 5%curcumin, 30qlit of 2.5%curcumin, 30qlit of 1.25%curcumin)

Plate5: 3 cups (30qlit of 5%curcumin, 30qlit of 2.5%curcumin, 30qlit Nystatin), for elimination of probable effect of DMSO on curcumin.

Plate6: 3 cups (10qlit of 5%curcumin, 10qlit of 2.5%curcumin, 10qlit Nystatin), for observation of probable changes with changing in using volumes.

Then the plates were placed in 37°c autoclave for 48 hours to evaluate the results. This procedure was repeated 3times for each plate.

- C) Disk diffusion, that inhibitory zones of dried disks which contained curcumin in DMSO was evaluated:

The main solution was prepared as foresaid and diluted in a ratio of 1/2 in 5 microplates (5%, 2.5%, 1.25%, 0.625%, 0.313%, 0.156%). 20μlit of each solution was poured on one of the disks by a sampler. In lower concentrations in the pilot examination was not seen any effect. 20μlit Nystatin was poured on each of disks that constituted Nystatin disks as well. The disks were placed in autoclaved to dry. In addition, they placed under UV ray for 24 hours because of infection control. Some disks of DMSO were prepared the same and autoclaved, too. Three disks were placed in each plate including one of curcumin concentrations, one Nystatin disk and one DMSO disk. The disks were placed on the culture and were autoclaved in 37°c for 48 hours. This procedure performed for 18 plates (3 times for each concentration).

Statistical analysis:

Shapiro-wilk test was used to determine the parameter setting for the data. As the data was non-parametric, Wilcoxon and Kruskal-wallis tests were used to evaluating the results.

Table1. Component of experimental microplates

Tube No.	Tube volume number1-6	Testimonia I1 volume	Testimonia I2 volume	Testimonia I3 volume
suspension	0.1	0.1	0.1	0.1
curcumin-DMSO solution	0.1	-	-	-
Nystatine	-	-	-	0.05
DMSO	-	-	-	-
BHI culture	-	0.1	0.1	0.05
Final volume	0.2	0.2	0.2	0.2

*Curcumin concentration in tube number1 was 0.05gr/cc and in tube number6 was 156×10^{-5} gr/cc

*All the volumes are in millilitre (cc) measuring unit.

Results

A) Evaluation of microplates showed that in testimonial1 microplate there was apparent growth of fungi but fungal growth was significantly lower in testimonial2 microplate. In other microplates, fungal growth was lower, especially in higher concentrations of curcumin (chart.1).

As the curcumin concentration increases, the fungal growth decreases (chart1). Also from testimonial2 (DMSO) to 5% curcumin there is a decreasing process, that is a sign of curcumin antifungal effect alone without the effect of DMSO.

Wilcoxon nonparametric test was used to compare fungal computation based on 24 and 48 hours groups. The difference was statistically significant, so that after 48 hours more antifungal effect was seen in comparison with 24 hours in each group.

Also By the results of Kruskal-wallis test that used to compare different groups of 24hours with each other, as well as 48 hours groups, the difference was significant. That means as curcumin concentration increases, antifungal activity increases too (table.2).

B) Evaluation of plates showed that around DMSO cup, there was an inhibition halo. There was a halo around curcumin cup too. In higher concentrations, it was significantly more than DMSO cup. The halo around nystatin cup was the biggest one (table3).

C) There was inhibition halo around the disks approximately similar to those around the cups. In this experiment, bigger halos in more concentrations were seen as well.

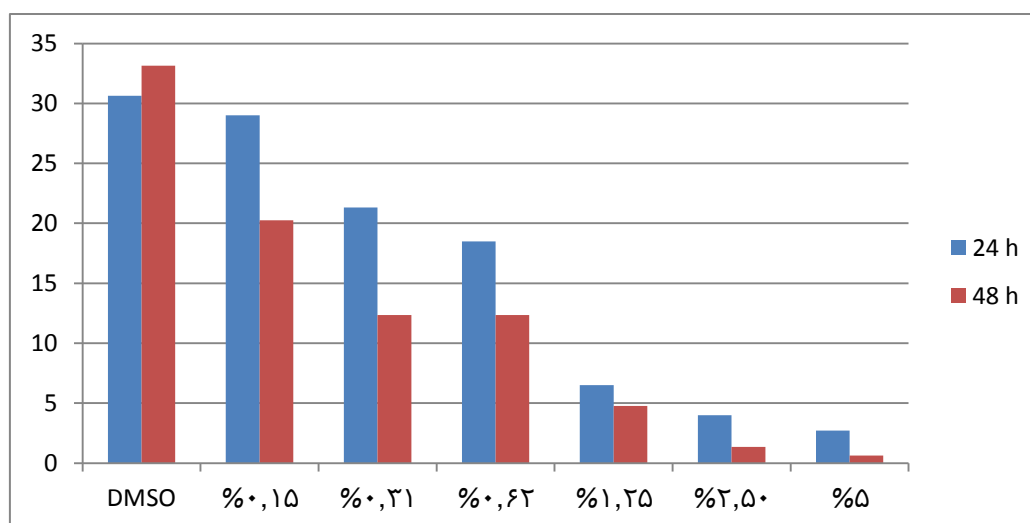


Chart.1 Inhibitory effect of different concentrations of curcumin on C. Albicans growth, 24 and 48 hours after receiving curcumin

Table 2. Average \pm standard deviation of *Candida Albicans* cell count in different treatment groups (control and curcumin in various concentrations), 24 and 48 hours after beginning the treatment

Treatment groups Time Of effect	control	DMSO	5% curcumin	2.5% curcumin	1.25% curcumin	0.625% curcumin	0.313% curcumin	0.156% curcumin
After 24 hours	1160.79 46.95 \pm	30.63 \pm 16.73	27.74 \pm 18.17	20.21 \pm 16.59	17.53 \pm 15.69	26.21 \pm 5.23	34.21 \pm 4.89	26.58 \pm 2.69
After 48 hours	1587.63 \pm 37.59	33.15 \pm 15.77	0.63 \pm 1.16	1.36 \pm 1.25	4.78 \pm 6.94	12.36 \pm 14.90	12.36 \pm 10.44	20.26 \pm 17.81
Comparison of P values	0.071	0.037	0.003	0.007	0.007	0.008	0.008	0.034

Table3. Diameters of inhibitory halo of *C. Albicans* growth in different treatment groups (control and various curcumin concentrations), 48 hours after placing different concentrations of curcumin by the cup bioassay technique

Cup	5% curcumin	2.5% curcumin	1.25% curcumin	0.156% curcumin	DMSO	Nystatin
Diameter of Inhibitory halo	~6 mm	~6 mm	~3 mm	~2 mm	~2 mm	20-22mm

Discussion

Curcumin is the effective substance of *Curcuma longa* that has anti-fungal activity based on Shyh Ming Tsoa, CVB Martins, and M Sharma studies (14, 15, 16). And its antifungal activity against *C. Albicans* has been confirmed by Livia N. Dovigo study.

In 2000 Shyh Ming Tsoa showed that confection of curcumin with Amphotericin B or fluconazole provides more antifungal effect for systemic fungal infections 14. M sharma study confirmed the synergic effect of curcumin confecting Poliens or Azoles as well 16. In our study, as a secondary finding, due to antifungal activity of DMSO, confection of curcumin and DMSO, was increased the solution's antifungal activity.

In a study by Garia Gomes AS et al in 2012 concluded that curcumin have a great capability to inhibit Fluconazole resistance of the isolate of *C. Albicans* (17).

In another study in 2008 by CVB Martins et al, it was concluded that curcumin was much more efficient than Fluconazole for restraining *C. Albicans* adherence to the epithelium of buccal mucosa 15. In the current

study, *C. Albicans* growth restrained by nystatin drug much more than curcumin. However, curcumin showed a significant effect too. This difference may be attributed to the different types of drug used in the studies. Moreover, they studied *C. Albicans*' adherence inhibition *in-vivo*, but in our study, growth inhibition of *C. Albicans* was evaluated, *in vitro*.

In the current study, first we tried cell count method, and curcumin diluted initially by distinct water, that redounded many huge crystallizations and prevented cell counting by microscope. To solve the problem we used another solvent named DMSO, as was used in Livia N. Dovigo et al, as well as Rana Pratap Singh et all studies (18,19). However, during the experiments it was revealed that DMSO has antifungal activity, itself. Whereas it have not been noted in other studies. To solve the problem, we decreased solvent concentration to 50% (in less concentrations curcumin sedimented). But the results didn't significantly change.

Next step, we decided to use Alcohol as solvent. The steps of experiment performed with methanol but it was revealed that Methanol has antifungal activity

too. So we decided to use 50% DMSO that provided more elucidate solution.

Due to the noted results, we decided to use cup bioassay method to eliminate antifungal effect of solvent with another method.

In our study, inhibition halo observed for curcumin extract, and halo diameter was 6mm for 5% curcumin and it was bigger than DMSO halo.

These results are in contradiction to Ranna Pratap Singh study. In their study, the diameter of inhibition halo of curcumin was 18mm and for DMSO there was no halo (19).

This may be because in their study the curcumin concentration was 20mg/ml in DMSO solvent, but in our study maximum concentration was 0.05mg/ml and as noted before, the amount of antifungal activity is proportionate to concentration of curcumin.

In the other method, we used disks on the plates. In this experiment a little inhibition halo was created around DMSO disks, and the halo around curcumin disks was bigger than DMSO disks, especially in more concentrations. Whereas in Monika Sharma et al study, the use of curcumin alone or an antifungal drug (Nystatin or Amphotericin B) alone, did not create any halo after 48 hours. However, when curcumin was used simultaneously with each of two antifungal drugs, there was a 1-1.5mm halo because of synergic effect of drugs (16).

This may be because they used 23µg/ml concentration of curcumin and also 0.078µg/ml Amphotericin B, so that because of very low concentrations may each one alone was not effective. In another study, curcumin was shown to enhance the activity of azole and polyene antifungals (20).

In the current study due to more concentrations of curcumin, the halo created around curcumin disks, alone. Additionally, P-value for different concentrations of curcumin was significant, as it was in Livia N. study (18).

Conclusion

According to this study, we can conclude that curcumin has antifungal activity against *C. Albicans*, and this effect generally increases by increasing the dosage.

References

1. Bensadoun RJ, Patton LL, Lalla RV, Epstein JB. Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: update 2011. *Support Care Cancer*. 2011; 19:737-744.
2. Dorko E, Baranová Z, Jenca A, Kizek P, Pilipcinec E, Tkáčiková L. Diabetes mellitus and candidiasis. *Folia Microbiol (Praha)*. 2002; 50: 255-261.
3. Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. *Oral Microbiol Immunol*. 2008; 23:377-383.
4. Castellote L, Soriano Y. Clinical and microbiological diagnosis of oral candidiasis. *J Clin Exp Dent*. 2013; 5(5):279-286.
5. Dovigo LN, Carmello JC, Souza Costa CA, Vergani CE, Brunetti LI, Vanderlei Salvador Bagnato, et al. Curcumin-mediated photodynamic inactivation of *Candida albicans* in a murine model of oral candidiasis. *J Med Mycol* (2013) 51 (3): 243-251.
6. Hatcher H, Planalp R, Cho K, Torti FM, Torti SV. Curcumin: From ancient medicine to current clinical trials. *J Cell Mol Life Sci*. 2008. 65(11), 1631-1652
7. Dias Ribeiro AP, Pavarina AC, Dovigo LN, Brunetti IL et al. Phototoxic effect of curcumin on methicillin-resistant *Staphylococcus aureus* and L929 fibroblasts. *J Lasers Med Sci*. 2013; 28:391-398.
8. Fallah Hoseini H, Zahmatkesh M, Haghghi M. Aplocations of *Curcuma Longa L.* in traditional and modern science: a review article. *J verbal plants*. 2010; 33(9):1-10.
9. Mukhopadyay A, Basu N, Ghatak N, Gujral PK. Anti-inflammatory and irritant activities of curcumin analogues in rats. *J Agents Actions*. 1982; 12:508-512.
10. Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern

- targets. *Trends Pharmacol Sci.* 2009; 30(2):85–94.
11. Gupta, S.C., Patchva, S. & Aggarwal, B.B. Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials. *AAPS J.* 2013; 15: 195-218.
 12. Huang L, Zhang J, Song T, Yuan L, Zhou J, Yin H, et al. Antifungal curcumin promotes chitin accumulation associated with decreased virulence of *Sporothrix schenckii*. *Int Immunopharmacol.* 2016; 34:263-270.
 13. Moghadamtousi S, AbdulKadir H, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. *J BioMed Research International.* 2014.
 14. Tsao SM, YIN MC. Enhanced Inhibitory Effect from Interaction of Curcumin with Amphotericin B or Fluconazole against *Candida* Species. *J Food and Drug Analysis.* 2000; 8(3):208-212.
 15. Martins CVB, Silva DL, Neres ATM, Magalhaes TFF, Watanabe GA, Modolo LV, et al. *J Antimicrobial Chemotherapy.* 2009; 63:337–339.
 16. Sharma M, Manoharlal R, Singh Negi A, Prasad R. Synergistic anticandidal activity of pure polyphenol curcumin in combination with azoles and polyenes generates reactive oxygen species leading to apoptosis. *J. FEMS.* 2010; 10: 570–578
 17. Garcia-Gomes AS, Curvelo JA, Soares RM, Ferreira-Pereira A. Curcumin acts synergistically with fluconazole to sensitize a clinical isolate of *Candida albicans* showing a MDR phenotype. *J Med Mycol.* 2012.
 18. Dovigo LN, Pavarina AC, Ribeiro APD, Brunetti IL, Costa CAS, Jacomassi DP, et al. Investigation of the Photodynamic Effects of Curcumin Against *Candida albicans*. *J Photochemistry and Photobiology.* 2011; 87:895–903.
 19. Singh RP, Jain DA. Evaluation of Antimicrobial activity of Volatile Oil and total Curcuminoids extracted from Turmeric. *International Journal of ChemTech Research.* 2011; 3(3):1172-1178.
 20. Gupta SC, Patchva S, Koh W, Aggarwal BB. Discovery of curcumin, a component of golden spice, and its miraculous biological activities. *Clinical and Experimental Pharmacology and Physiology.* 2012; 39(3): 283-299.

Corresponding Author:

Shiva Zamaninejad

Pediatric dentistry faculty of dentistry, Golestan University of medical science, Gorgan, Iran

Phone numbers: 00989113718638

E-mail address: sh.zamani66@gmail.com