Effect of nystatin and licorice on yeasts isolated from the oral lesions of patients with cancer under chemotherapy (in vitro study)

Faezeh Khozeimeh1, Parvin Dehghan1, Negin Yaghoobi2, Mehrnoush Maheronnaghsh3, Mona Bazazzadeh4, Seyede Negin Noorbakhsh2

1Dental Research Center, Department of Oral Medicine, Dental Research Institute, Isfahan University of Medical Sciences, Isfahan, Departments of 2Parasitology and Mycology and 3Mycology and Parasitology, School of Medicine, Isfahan University of Medical Sciences, 4Department of Prosthodontics, Dental Research Center, Dentistry Research Institute, Tehran University of Medical Science, Tehran, Iran, 5Department of Advanced Education in General Dentistry, Columbia University College of Dental Medicine, New York, USA

ABSTRACT

Background: Oral candidiasis is one of the most common manifestations of patients with cancer under chemotherapy. Due to many side effects of chemical antifungal products and various advantages of herbal extracts like licorice, this study was performed to compare the antifungal effects of nystatin and licorice on yeasts isolated from oral mucosa of patients with cancer receiving chemotherapy.

Materials and Methods: In this in vitro study, a total number of 30 patients with oral candidiasis who received chemotherapy were examined. The samples were prepared by using swabs taken from the lesions, and after 48 h, they were transferred and cultured on Sabouraud dextrose agar. The antifungal effect of licorice was compared with nystatin using agar disk diffusion method. These data were entered in SPSS statistical software and were analyzed with Kruskal–Wallis and Mann–Whitney tests (α = 5%).

Results: Four types of candida were identified among all 30 oral lesions (Candida albicans, Candida glabrata, Candida stellatoidea, and Candida sp). The mean inhibition zone diameter around nystatin showed a significant difference (P < 0.001) between C. albicans (9.486), C. glabrata (8.627), C. stellatoidea (7.00), and C. sp (7.06) but the inhibition zone diameter around licorice was almost zero in all groups.

Conclusion: Licorice extracts did not show any antifungal effects whereas nystatin showed the most antifungal effect against C. albicans.

Key Words: Chemotherapy, glycyrrhiza, nystatin, oral candidiasis

INTRODUCTION

Chemotherapy and radiotherapy are used as treatment plans for leukemic patients. Unfortunately, they are associated with short-and long-term side effects. Interference with function and integrity of oral mucosa, dysphagia, ulceration, and fungal infection are among their deteriorating side effects.[1,2] There are invasive fungal infections in 14% of leukemic patients who undergo chemotherapy which are fatal and difficult to diagnose.[3] Candida albicans is the most common opportunistic infection that can be isolated from human

How to cite this article: Khozeimeh F, Dehghan P, Yaghoobi N, Maheronnaghsh M, Bazazzadeh M, Noorbakhsh SN. Effect of nystatin and licorice on yeasts isolated from the oral lesions of patients with cancer under chemotherapy (in vitro study). Dent Res J 2022;19:61.
body and can cause local and systemic infections, which are mostly seen after long-term antibiotic use and in patients with immune deficiencies.\[4,5\]

Three categories of drugs are used for the treatment of fungal infections:
1. Drugs with complete absorption in gastrointestinal tract (ketoconazole, etc.)
2. Drugs with partial absorption in gastrointestinal tract (clotrimazole, etc.)
3. Drugs without any absorption in gastrointestinal tract (nystatin and amphotericin B).\[6\]

Main disadvantages in using chemical antifungal drugs such as nystatin are drug toxicity, poor taste, drug resistance, and gastrointestinal adverse reaction.\[7,9\] Medical plants are important sources of bioactive compounds with a long history of infection treatment.\[10\] Using herbal products can cause an obvious reduction in opportunistic pathogens such as \textit{C. albicans}.\[11\] Licorice root is one of the native plants of Mediterranean countries, central parts of south Russia and Asia. The word licorice is derived from two Greek words (glycos + rhiza) which means sweet root.\[12\] Clinical and experimental studies suggest that licorice has several useful pharmacological properties including anti-inflammatory, antiviral, antibacterial, antioxidant, anticancer properties and also can regulate the immune system.\[13,14\] Other advantages of using licorice are fewer side effects, toxicity, and resistance compared to chemical drugs.\[7,11\] Although few studies have been done on antifungal activity of licorice,\[11\] there are some controversies about its antifungal effects \textit{in vitro}.\[7,13\] Therefore, the aim of this study was to compare the antifungal effects of licorice and nystatin on species isolated from oral candida lesions of patients with cancer under chemotherapy.

**MATERIALS AND METHODS**

**Patients**

This \textit{in vitro} study was approved in research and ethics committee of Isfahan (NO:393719). Carried out among 30 patients with myeloid leukemia (18 patients had acute myeloid leukemia and 12 patients had chronic myeloid leukemia) at chemotherapy section, Seyed al-shahada University Hospital, Isfahan University of Medical Sciences, Isfahan, Iran. They were within the age range of 45–65 years who were receiving chemotherapy.

**Identification of oral candidiasis**

Patients had pseudomembranous or erythematous candidiasis\[15\] which were identified by oral and maxillofacial disease specialist.

**Chemotherapy regimen**

Chemotherapy regimen consisted of (cytarabine 100 mg/m\(^2\)/day for 7 days and daunorubicin 45 mg/m\(^2\) for 3 days).

**Inclusion criteria**

1. Patients were over 18 years of age
2. Leukemic patients who had received chemotherapy for a maximum of 10 days
3. Individuals who did not use any local or systemic drugs for the treatment of fungal lesions
4. Patients without systemic disease
5. Their oral candidiasis was confirmed by oral specialist.

**Exclusion criteria**

1. Pregnant women
2. Inability to provide informed consent
3. Patients without any yeast in their direct smear samples.

In the present study, 48 patients entered in first step, but after we excluded patients without any yeasts in their direct smear samples, 30 patients were evaluated.

In the next step, a consent form was filled by all patients participated in the study. (Research project number: 393719).

**Sampling**

To provide samples, the patients were required to rinse their mouth with normal saline and then two swabs were taken by pulling on lesion. We used one of the swabs to provide direct smear sample and Giemsa staining. The second swab was transferred to the Sabouraud dextrose agar (SDA) medium (SDA, Biolife Italian, Milan, Italy) to cultivate species in the laboratory of mycology.

**Laboratory process**

After 48 h of incubation at 35°C, cultivated fungi in SDA medium were transferred to chrome agar (ChromAgar, Darvash, Tehran, Iran) and cornmeal agar media (Corn meal agar, Biomark, Mumbai, India) to separate different species. The candida species were recognized in chrome agar medium by changing the colony color, and in cornmeal agar medium, they formed clamidoconidios. Some species of \textit{Candida} were identified on ChromAgar by colony morphology and pigmentation according to the manufacturer’s instructions, \textit{C. albicans} produces light or dark green, \textit{C. glabrata} produces...
light pink, and other species produces white, dark blue, purple blue, and purple. *C. albicans* on CMA media produces pseudohyphae and large, spherical chlamydocarps, but *C. stellatoidea* on this media produces teardrop-shaped chlamydocarps.

All isolates of Candida species were subcultured at 30°C for 24 h on SDA plates. At least five colonies of yeasts were suspended in 5 ml of sterile saline (0.85%). The resulting suspension was vortexed for 15 s, and the turbidity of each suspension was adjusted at 0.5 McFarland standard (corresponding to $1 \times 10^5 - 5 \times 10^6$ cells/ml) with the use of Neubauer slide by the method of the National Committee for Clinical Laboratory Standards (NCCLS). A working suspension was made by dilution of the stock suspension with sterile saline which resulted in $10^4$ yeast cells/ml. Finally, the fungal suspension with $1 \times 10^5$ yeasts was prepared with the use of Neubauer slide for assessment of the antifungal effects of two mentioned drugs. Then, we transferred $10^3$ antifungal cells to SDA medium and put nystatin and licorice disks on the medium.

The mean diameter of zone of inhibition around each disk was measured with caliper and this test was repeated 3 times for each recognized species.

To prepare licorice disks, the amount of 20 g pectin and 10 g cellulose derivative was combined with 2000 g water and was stirred vigorously to obtain a homogenous mixture, then 40 g licorice extract was completely dissolved in 100 g water and was added to the mentioned mixture by a dropper in steel trays and was dried at 50°C. These disks had to be kept away from light and mixture. The same method was used for preparation of nystatin disks except for instead of 40 g licorice extract, 40 g nystatin was used (nystatin powder 1% prepared by Jaber Ebn Haian, Tehran, Iran laboratory).

**Statistical analysis**

Finally, the data were entered in SPSS Ver. 22 (SPSSInc., Chicago, IL, USA) statistical software and were analyzed with Kruskal–Wallis and Mann–Whitney tests ($\alpha = 5\%$).

**RESULTS**

In this study, four different fungal species were obtained from 30 patients including 22 cases of *C. albicans*, 1 case of *C. stellatoidea*, 4 cases of *C. glabrata*, and 3 cases of Candida that did not fit in previous groups and could be identified by molecular methods, which were named *Candida SP* (species). Each patient showed one particular type of Candida and the frequency of each group is demonstrated in Figure 1.

Given that no inhibition zone was observed around licorice disks, it can be concluded that licorice has no *in vitro* antifungal activity [Figure 2].

![Figure 1: The frequency of fungal species found in patients in the study.](image1)

![Figure 2: No inhibition zone in licorice disks.](image2)

![Figure 3: Inhibition zone in nystatin disks.](image3)
About the antifungal activity around nystatin disks, the following results were obtained [Figure 3].

The Kruskal–Wallis test indicated that there was a significant difference between zone’s diameters in 3 groups. \((P < 0.001)\). Mann–Whitney test showed that there is a significant difference between \(C. albicans\) and \(C. glabrata\) \((P < 0.026)\), \(C. albicans\) and Candida spp. \((P < 0.001)\), and also \(C. glabrata\) and Candida spp. \((P < 0.001)\) [Figure 4].

The greatest average diameter of inhibition zone with using nystatin disks was for \(C. Albicans\) (9.48 mm) and then \(C. Glabrata\) (8.62 mm), Candida sp (7.06 mm), and \(C. stellatoidea\) (7.00 mm), respectively.

**DISCUSSION**

Candidiasis is a common infection, particularly in leukemic patients. In these patients, not only usual forms of candida but also unusual types can cause several problems that can be fatal.\[3,16\] Chemotherapy and bone marrow transplantation are usual treatments for malignant tumors that can affect the immune response and increase systemic fungal infection risk and eventually lead to death.\[17\] Articles mentioned higher rates of nonalbicans species (such as \(C. glabrata, Candida tropicalis, Candida krusei\)) in immunocompromised patients. Some problems such as higher mortality, serious infection, and increasing resistance to antifungal drugs are reported as their consequences.\[18-20\]

Our data confirm that \(C. albicans\) was responsible for the majority of the oral candidiasis. Similar to our finding, Hamzehee et al. reported that the most common candida species in patients with leukemias and lymphomas undergoing chemotherapy was \(C. albicans\) (57.14%). Other species identified in their culture were \(C. glabrata\) (14.28%), \(C. parapsilosis\) (14.28%), \(C. krusei\) (7.14%), and \(C. kefyr\) (7.14%).\[21\]

On the other hand, Jain M et al. reported \(C. tropicalis\) as the most common species observed in oral cancer patients undergoing chemotherapy. Their result showed that \(C. albicans, C. stellatoidea, and C. krusei\) had equal prevalence (20% of patients). The reason of this difference may be due to age, climatic conditions, type of cancer, and type of chemotherapy regimen patients received. Moreover, they evaluated the relationship between prevalence of Candida and treatment modality in patients. They reported 50% of patients undergoing radiotherapy, 75% of patients undergoing chemotherapy, and 81.25% of patients undergoing combined radiotherapy and chemotherapy showed Candida-positive culture. They concluded that in patients undergoing radiotherapy or chemotherapy, more bizarre species are observed.\[22\]

Although there are many chemical drugs for the treatment of candidiasis, they are not constantly effective against fungal infections and drug resistance has been reported.\[9,10\] Among synthetic drugs used to treat candidiasis, nystatin due to the least side effects (regardless of its bitter taste) is administered topically for treatment of oropharyngeal candidiasis.\[9,23\]

In this study, samples were taken directly from the mouth of leukemic patients under chemotherapy. The advantage of this method was that we could determine the type and abundance of fungal species in these patients. According to the result of this study, nystatin has antifungal effects against all separated species of Candida and the mean dimension of inhibition zone around nystatin disks in \(C. albicans\) was significantly highest (9.48), then \(C. glabrata\) (8.62), Candida sp (7.06), and \(C. stellatoidea\) (7.00) had less antifungal effects, respectively.

Carrillo-Muñoz et al. compared the effect of nystatin and other antifungal agents on several species of candida including \(C. albicans\) and \(C. glabrata\) using minimum inhibitory concentration (MIC) method and concluded that the effect of nystatin in \(C. albicans\) is more than \(C. glabrata\) that was consistent with the results of this study.\[24\]
Medical plants have many advantages compared to chemical drugs such as their safety for both human and environment and their compatibility with human body, and also, they are biodegradable with less toxicity and fewer side effects. It is noted that licorice can be used as a therapeutic agent in different fields of dentistry such as managing recurrent aphthous ulcer (reducing pain and alleviating healing), anticaries activity, preventing factor in gingivitis, and periodontitis and can be used as a chemotherapeutic agent for treatment of oral cancers.

In Lee et al.'s study, licorice products were tested both in vitro and in mice body. They concluded that none of the products derived from licorice had antifungal effect against C. albicans species in vitro, but one of the derivatives of licorice root (liquiritigenin) showed antifungal effect against C. albicans strains in mice body. Their research showed that the improving effect of this product on T helper type 1 mediated immunity can be helpful confronting with diffused candidiasis in the body.

Fatima et al. prepared disks of ethanol, ethyl acetate, and glabridin that were extracted from licorice and evaluated their antifungal effect on drug-resistant strains of C. albicans using MIC method. In their in vitro study, the products extracted from licorice had appropriate antifungal effect against the fungal strains and their result is in contrast with the results of our research. The reason of this inconsistency can be different fungal species used in their study and type of products extracted from licorice that was based on alcohol.

In another study conducted by de Oliveira et al., effect of several herbs on C. albicans species including licorice was compared with nystatin in vitro. They placed disks containing 10^6 C. albicans cells per ml in SDA media and measured colony-forming unit/ml (number of C. albicans colonies per milliliter) after 48 h. They concluded that antifungal effect of licorice is the same as nystatin, which was contrary to our results. Inconsistency with our results may be due to differences in products derived from licorice.

In another study, Giancarlo Statti et al. evaluated antifungal effect of licorice extract containing methanol and found out that from nine licorice samples collected from different areas, seven samples showed antifungal effect and concluded that the licorice effect is different under the influence of sunlight exposure and other environmental factors on different fungal species.

The present study had limitations. First, despite the advantages of disk diffusion method that was used in this study, there were other suggested methods such as MIC. The reason for not using MIC method was the large number of samples and its high cost and also, since we have found the results to be quite resistant in licorice group, MIC method would probably not add further information in this regard. Second, the antifungal effect of licorice on human body can be different from its in vitro effect due to positive and enhancing effects of it on immune system including lymphocytes and macrophages.

Finally, it can be pointed out that licorice plant alone had no distinctive effect on fungi species, but when it is used with an alcohol base or with alcoholic products, its antifungal effects can be observed. Furthermore, licorice effects on fungal species can be affected by sun exposure and environmental factors in which this herb is grown. Therefore, it is suggested to use different types of licorice planted in different areas with distinct geographical characteristics and that antifungal activities of these plants should be compared with each other in future studies. Moreover, it is suggested that the pure licorice plant should be compared with alcohol and oil base licorice for the antifungal activity.

**CONCLUSION**

Based on the present study’s results, licorice did not show any antifungal effect but nystatin illustrated the most antifungal effect on C. albicans.

**Acknowledgment**

This manuscript has been read and approved by all the authors, requirements for authorship have been met, and each author believes that the manuscript represents honest work.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

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

**REFERENCES**

1. Shu Z, Li P, Yu B, Huang S, Chen Y. The effectiveness of probiotics in prevention and treatment of cancer therapy-induced oral mucositis: A systematic review and meta-analysis. Oral Oncol 2020;102:104559.
2. de Almeida EM, Ferreira HJ, Alves DR, da Silva WM. Therapeutic potential of medicinal plants indicated by the Brazilian public health system in treating the collateral effects induced by chemotherapy, radiotherapy, and chemoradiotherapy: A systematic review. Complement Ther Med 2020;49:102293.

3. Glimmer V Jr., Prentice AG. Evidence-based review of antifungal prophylaxis in neutropenic patients with haematological malignancies. J Antimicrob Chemother 2005;56 Suppl 1:i23-32.

4. Sharma A. Oral candidiasis: An opportunistic infection: A review. Int J Appl Dent Sci 2019;5:23-7.

5. Krcmery V Jr., Oravcova E, Spanik S, Mrazova-Studena M, Trupl J, Kunova A, et al. Nosocomial breakthrough fungaemia during antifungal prophylaxis or empirical antifungal therapy in 41 cancer patients receiving antineoplastic chemotherapy: Analysis of aetiology risk factors and outcome. J Antimicrob Chemother 1998;41:373-80.

6. Worthington HV, Clarkson JE. Prevention of oral mucositis and oral candidiasis for patients with cancer treated with chemotherapy: Cochrane systematic review. J Dent Educ 2002;66:903-11.

7. Lee JY, Lee JH, Park JH, Kim SY, Choi JY, Lee SH, et al. Liquiritigenin, a licorice flavonoid, helps mice resist disseminated candidiasis due to Candida albicans by Th1 immune response, whereas liquiritin, its glycoside form, does not. Int Immunopharmacol 2009;9:632-8.

8. Revie NM, Iyer KR, Robbins N, Cowen LE. Antifungal drug resistance: Evolution, mechanisms and impact. Curr Opin Microbiol 2018;45:70-6.

9. Lyu X, Zhao C, Yan ZM, Hua H. Efficacy of nystatin for the treatment of oral candidiasis: A systematic review and meta-analysis. Drug Des Devel Ther 2016;10:1161-71.

10. Darah I, Jain K, Suraya S, Lim S, Hazarina N, Adnalizawati AS. Screening for antyeast activities from selected medicinal plants. J Trop For Sci 2006;18:231-5.

11. de Oliveira JR, Vilela PG, de Oliveira FE, Belato KK, Carvalho CA, Jorge AO, et al. Antifungal effect of plant extracts on Candida albicans biofilm on acrylic resin. Braz Dent Sci 2013;16:77-83.

12. Messier C, Epifano F, Genovese S, Grenier D. Licorice and its potential beneficial effects in common oro-dental diseases. Oral Dis 2012;18:32-9.

13. Ali EM. Phytochemical composition, antifungal, antiaflatoxigenic, antioxidant, and anticancer activities of Glycyrrhiza glabra L. and Matricaria chamomilla L. essential oils. J Med Plant Res 2013;7:2197-207.

14. Jiang M, Zhao S, Yang S, Lin X, He H, Wei X, et al. An “essential herbal medicine” – Licorice: A review of phytochemicals and its effects in combination preparations. J Ethnopharmacol 2020;249:112439.

15. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rzik MA. Oral candidiasis: A disease of opportunity. J Fungi (Basel) 2020;6:15.

16. Nair V, Shetti A. Diversity and clinical presentation of Candida species in human immunodeficiency virus patients with oral candidiasis. Saudi J Oral Sci 2019;6:96-100.

17. Costa EM, Fernandes MZ, Quinderé LB, Souza LB, Pinto LP. Evaluation of an oral preventive protocol in children with acute lymphoblastic leukemia. Braz Oral Res 2003;17:147-50.

18. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Adherence and biofilm formation of non-Candida albicans Candida species. Trends Microbiol 2011;19:241-7.

19. Sadeghi E, Ebrahimi-Rad M, Mousavi SF, Shams-Ghafrarokhi M, Razzaghi-Abyaneh M. Emergence of non-Candida albicans species: Epidemiology, phylogeny and fluconazole susceptibility profile. J Mycol Med 2018;28:51-8.

20. Kolaczewska A, Kolaczkowski M. Drug resistance mechanisms and their regulation in non-albicans Candida species. J Antimicrob Chemother 2016;71:1438-50.

21. Hamzehsee H, Kalantar-Neyestanaki D, Mohammad MA, Nasibi S, Mousavi SA. Identification of Candida spp. isolated from oral mucosa in patients with leukemias and lymphomas in Iran. Iran J Microbiol 2019;11:114-9.

22. Jain M, Shah R, Chandolia B, Mathur A, Chauhan Y, Chawda J, et al. The oral carriage of Candida in oral cancer patients of Indian origin undergoing radiotherapy and/or chemotherapy. J Clin Diagn Res 2016;10:17-20.

23. Mehta RT, Hopfer RL, Gunner LA, Juliano RL, Lopez-Berestein G. Formulation, toxicity, and antifungal activity in vitro of liposome-encapsulated nystatin as therapeutic agent for systemic candidiasis. Antimicrob Agents Chemother 1987;31:1897-900.

24. Carrillo-Muñoz AJ, Quindós G, Tur C, Ruesga MT, Miranda Y, del Valle O, et al. In-vitro antifungal activity of liposomal nystatin in comparison with nystatin, amphotericin B cholesteryl sulphate, liposomal amphotericin B, amphotericin B lipid complex, amphotericin B desoxycholate, fluconazole and itraconazole. J Antimicrob Chemother 1999;44:397-401.

25. Haghiriolasadat F, Vahidi A, Sabour M, Azimzadeh M, Kalantar M, Sharafadini M. The indigenous Cuminum cuminum L. of Yazd province: Chemical assessment and evaluation of its antioxidant effects. J Shahid Sadoughi Univ Med Sci 2011;19:472-81.

26. Sidhu P, Shankargouda S, Rath A, Hesarghatta Ramamurthy P, Fernandes B, Kumar Singh A. Therapeutic benefits of liquorice in dentistry. J Ayurveda Integr Med 2020;11:82-8.

27. Al-Snai A. Iraqi medicinal plants with antifungal effect – A review. IOSR J Pharm 2019;9:16-56.