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Original Article

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

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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

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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 *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 *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 *in vitro* study was approve 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 *Candida* were identified on ChromAgar by colony morphology and pigmentation according to the manufacturer's instructions, *C. albicans* produces light or dark green, *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 chlamydospores, but *C. stellatoidea* on this media produces teardrop-shaped chlamydoconidia.

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 1×10^6–5×10^6 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 10^3 yeast cells/ml. Finally, the fungal suspension with 1×10^6 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].

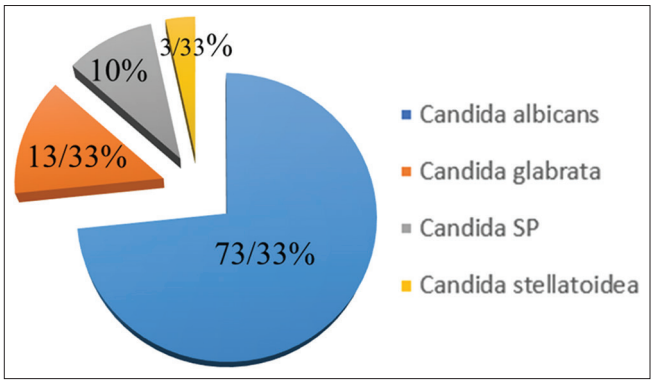

Figure 1: The frequency of fungal species found in patients in the study.

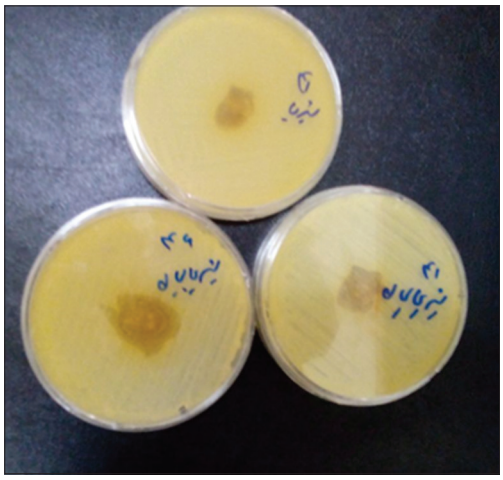

Figure 2: No inhibition zone in licorice disks.

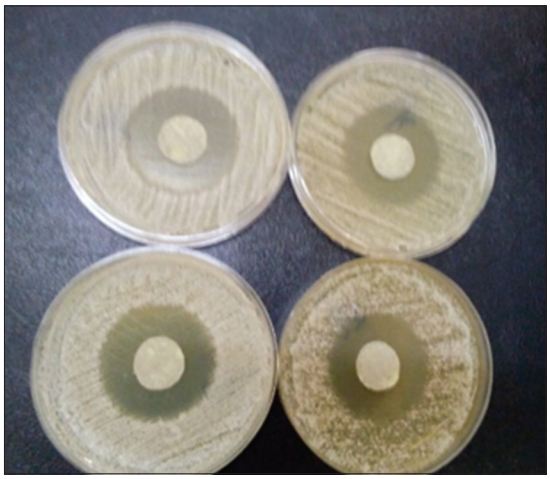

Figure 3: Inhibition zone in nystatin disks.

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]

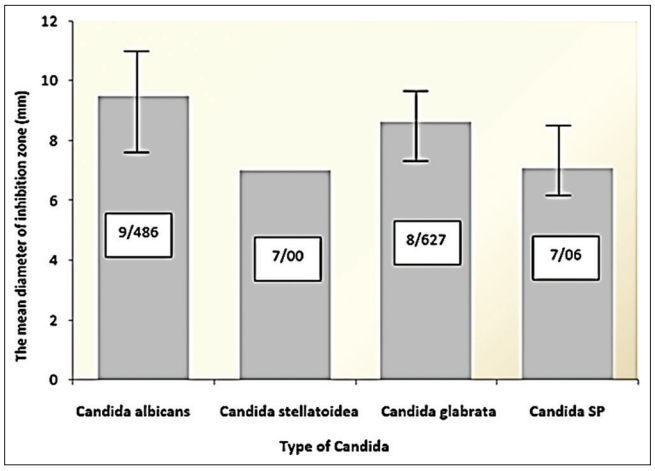

Figure 4: The average diameter of inhibition zone of fungal species using nystatin disks.

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.[25] 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.[26,27]

In Lee *et al*. 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.[7]

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.[28]

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.[11]

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.[29]

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.[30] 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*.

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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.

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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 non-financial in this article.

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