In Vitro comparison of the Effects of Ginger Extract, Fluconazole and Nystatin on Candida glabrata and Candida kruzei

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INTRODUCTION

Candidiasis is one of the most prevalent and important opportunistic fungal infections in human beings which caused by candida yeast species like albicans, glabrata, and kruzei. Different forms of candidiasis, in acute and chronic forms, affect various parts of body such as skin, oral and genital mucosa, bronchus, GI tract and lungs. These infections diffuse usually in immunosuppression status like HIV and other contributing factors and strike internal organs such as kidney and liver [1]. Antifungal drugs, in various formulations, are being used topically (like nystatine and clotrimazol) and systemically (azoles and amphotericin B) [2]. In recent years, numerous studies have been reported the failure of treatment in patients with different clinical types of candidiasis. Long-term consumption of antifungal agents has been caused adverse effects and also drug-resistance against these against led to the limitations in antifungal therapies [3, 4]. Drug resistance is reported more in individuals with compromised immune system and long-term use of antifungal agents [5]. The number of infections caused by candidal species other than albicans (like kruzei and glabrata) is increasing and these microorganisms respond poorly toazole treatments such as fluconazole which is the most effective azole in management of fungal infections in immunosuppress and HIV positive individuals). This drug resistance leads to the increase of infection prevalence [6]. Side effects of the common antifungal agents include nausea, vomiting, hepatic dysfunction, arrhythmias, neuropathies, and etc. Therefore, recent researches are directed in finding effective antifungal agents with natural origin and less side effects [5]. Among herbal extracts, inhibitory
effect of ginger extract on microorganisms has been evaluated by researchers. Antimicrobial effect of ginger extract has been investigated against staphylococcus aureus, pseudomonas aeruginosa, and escherichia coli and revealed that this extract has certain inhibitory effect on these species [7]. In another study antifungal activity of ginger extract was assessed against fluconazole-resistant candida albicans species isolated from patients with genital candidiasis. It revealed that ginger extract has a inhibitory impact on all candida albicans isolates [8].

In traditional medicine ginger is administrated for cure of movement inabilities, nausea and vomiting during pregnancy, and etc. numerous studies did not report any side effects for ginger except for sedation and drowsiness [6-8]. Antimicrobial and antifungal effects of ginger extract has been proved against some of bacterial and fungal species as candida albicans in vitro, and since no study has been performed on other candidal species like glabrata and kruzei which are increasing with raising immunosuppress and HIV affected patients, therefore this study was aimed to evaluate the antifungal property of ginger extract on candida glabrata and kruzei and compare its effect with common antifungal agents.

**MATERIALS AND METHODS**

In this in-vitro experimental study, disc diffusion method was employed for comparison of the effect of ginger extract with two commonly used antifungal agent, nystatin and fluconazole. Standard species of candida glabrata (BSM 11226) and candida kruzei (BSM 70079) were tested in this study. 0.5 McFarland suspension was prepared by 24-hour cultured microorganisms in sterile physiologic serum. For each fungal species, antibiogram test was carried out on 15 sabouraud dextrose agar. Plates were inoculated using sterile swab soaking in prepared fungal suspension and dispersed on agar medium. After that quaternary discs containing ginger extract, nystatin, fluconazole, and a blank one were placed on each plate with a same distance from each other. After completion of these processes, all plates were incubated at 35°C for 18-24 hours and then diameter of growth inhibition zone around discs were measured using a millimeter ruler. There should not be any growth inhibition around blank disc to confirm test validity.

For preparation of ginger extract, 500 grams of arid herb was grinded and wetted in 100 milliliters of 99% ethanol for 24 hours. Prepared solution was distillated and eventually 45 grams of arid extract were obtained. In a 96-well cell culture microplate, 100 microliters of sabouraud dextrose medium were added to each well, subsequently, 100 microliters of ginger extract were added to first well and later dilutions were prepared in next wells. Then, 100 microliters of fungal specimen were added to each well and after 18-24 hours incubation at 35°C, wells were evaluated in respect of turbidity. The well before the first turbid well was considered as MIC. This was performed for both of fungal species. Obtained MIC for both fungal species was 25 µg/ml. Antibiogram discs of fluconazole and nystatin were prepared from Padtan Teb, Tehran, Iran. Fluconazole discs each contained 25 µg fluconazole in a 6.4 mm disc and nystatin discs contained 100 units or 20 µg pure nystatin. Ginger extract discs were prepared by solving 25 mg of extract in 1ml of total ethylic alcohol (99.6%). Sterile disks were placed on a sterile glass and 10 µl of prepared 25 mg/ml of extract were poured on them to absorb completely. Subsequently, discs were transferred into 37 °C incubator for 30-60 minutes in order to evaporation of alcohol and obtaining arid discs.

Collected data first was reported by descriptive statistics. Kolmogorov-Smirnov analysis was used for evaluation of data normality. Comparison of mean growth inhibition zone of tested fungi and antifungal agents was done by Kruskal-Wallis test [6] in both fungal species. U-Mann Whitney analysis was used for pair-wise comparisons. P<0.05 was considered significant in this study. This study was done in Tabriz medical university grant number 2013472975 between 2012 till 2013.

**RESULTS AND DISCUSSION**

**Results:**

The greatest growth inhibition diameter in candida kruzei and glabrata was recorded around ginger extract discs (table 1).

<table>
<thead>
<tr>
<th>Candida</th>
<th>Experimental antifungal agent</th>
<th>Mean (std. deviation)</th>
<th>minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glabrata</td>
<td>Ginger extract</td>
<td>31.06 (0.7)</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Nystatin</td>
<td>17 (0.5)</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>18.3 (0.5)</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Kruzei</td>
<td>Ginger extract</td>
<td>27.5 (0.6)</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Nystatin</td>
<td>17.5 (0.5)</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>19.5 (0.5)</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>

Kolmogorov-Smirnov analysis revealed non-parametric data distribution (P>0.05). Kruskal-Wallis test indicated that mean of growth inhibition zone of tested materials are statistically significant in case of both
fungal species (P=0.000). U-Mann-Whitey analysis depicted that all pair-wise comparisons are statistically significant (P=0.000). In other words, mean growth inhibition zone diameter of ginger extract was more than nystatin and fluconazole (Fig. 1 and 2).

**Fig. 1:** Error bar of growth inhibition zone diameter of experimental antifungal agents against Candida glabrata

**Fig. 2:** Error bar of growth inhibition zone diameter of experimental antifungal agents against Candida Kruzei
Discussion:

In this study antifungal effects of ginger extract were investigated against candida kruzei and glabrata. For comparison of antifungal effect of ginger extract, effects of two commonly used antifungal agents (nystatin and fluconazole) were assessed, too. For this, disc diffusion agar method was used since it a simple and reliable method and it was employed in similar studies [7, 8]. In our study antifungal effect of ginger extract was greater than nystatin and fluconazole in respect of both candida species.

In previous studies antifungal effects of protein in ginger rhizome was evaluated and revealed that this protein had inhibitory effect on some of fungi such as fusarium oxysporum [9]. Taechowisan et al isolated a material called CMUAC 130 from ginger which had inhibitory effect on ftyopathogen fungi growth as fusarium [10]. Nguefack et al indicated that ginger extract could prevent the proliferation fusarium moniliform, aspergillus flavus, and aspergillus fumigatus and can inhibit these fungi growth in-vitro [11]. Ficker et al evaluated antifungal properties of 36 herbal extracts on 13 human fungal pathogens and reported that among these extracts, ginger and jimpijapa extract had inhibitory effect on various fungal species. Also it was revealed that ginger extract is one of the extracts which precluded the growth of fungi which were resistant to amphotericin B and ketoconazole [12]. Agarwal et al depicted this extract’s inhibitory effect on Spilosoma insect species [13]. Other researches have evaluated the antifungal effects of its rhizome and assigned it as an effective extract on aspergillus and ftyopathogens [14]. Mohammadi et al assessed its antifungal properties.
against clinical isolates of fluconazole-resistant candida albicans. Their findings indicated that ginger extract had inhibitory effect on all tested species and they declared ginger as an effective agent on candida albicans in laboratory setting [8]. There have not been performed any studies on antifungal effect of finger extract on non-albicans candida species. Therefore, our study aimed this matter. Similar to previous studies, our findings also depicted that its antifungal properties is stronger than nystatin and fluconazole.

Although, antimicrobial effects of common antimicrobial agents on different microorganisms are compared to the standards deducted by CLSI (Clinical and Laboratory Standard Institute), there is not any reference standards for other materials such as herbal extracts. Former studies just have reported antifungal properties of these extracts descriptively and have not compared with any references. In this study, we compared the antifungal effects on ginger extract with two commonly used antifungal agents (nystatin and fluconazole), for the first time and indicated that antifungal effect of this extract is much more than nystatin and fluconazole against candida kruzei and glabrata species. According to CLSI standards in 2011, growth inhibition zone diameter of nystatin against candida kruzei and glabrata is 25 mm and this is 22 mm for fluconazole [15, 16]. The mean inhibition diameter obtained for nystatine against candida kruzei and glabrata was 17 mm and this was 19 mm for fluconazole in our study which illustrates these antifungal agents’ resistance to nystatin and fluconazole. But these diameters were 31 and 27.5mm for ginger extract against candida kruzei and glabrata, respectively. These diameters shows that these microorganisms were sensitive to ginger extract in MIC concentration.

Ginger is administrated for cure of movement inabilities, nausea and vomiting during pregnancy, and etc. Numerous studies did not report any side effects for ginger except for sedation and drowsiness [6-8]. Antimicrobial and antifungal effects of ginger extract has been proved against some of bacterial and fungal species as candida albicans in vitro, and in our study its antifungal effect were proved on candida glabrata and kruzei which are increasing with raising immunosupress and HIV affected patients, therefore because of ginger’s inexpensiveness and less side effects, it’s administration is suggested for fungal infections. However, further animal and human studies are needed to confirm this.

Conclusions:

Antifungal inhibitory effect of ginger extract against candida kruzei and glabrata was greater than nystatin and fluconazole. These fungi were resistant to nystatin and fluconazole but sensitive to ginger extract. Suggestions: Regarding greater antifungal effect of ginger extract in comparison with two commonly used antifungal agents, and also because of ginger’s inexpensiveness and less side effects, its administration is suggested for fungal infections. However, further animal and human studies are needed to confirm this.

REFERENCES