International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-1, 2017, 228-233
http://www.bipublication.com

Research Article

Comparison the Effect of Aqua-Alcoholic Root Extract of Glycyrrhiza Glabra and Chlorhexidine on Candida Albicans (Invitro)

Nahid Askarizadeh[1], Peyvand Moeini[2], Melika Arabinezhad[3*], Amin Nowruzi[4] and Soudabeh Taheri[5]

[1]Associated Professor, Department of Pediatric Dentistry. Dental Branch, Tehran, Iran.
[2]Assistant Professor, Department of Pediatric Dentistry. Dental Branch, Tehran, Iran.
[3]Doctor of Dental Surgery, Dental Branch, Tehran, Iran.
[4]Resident of oral and maxillofacial surgery. Dental Branch. Tehran. Iran.
[5]Lecturer, Head of microbiology Lab Center. Shahid Beheshti Medical University, Tehran, Iran.
*Corresponding Author: Melika Arabinezhad Email: Melika_arnzhd@yahoo.com

ABSTRACT

Introduction: Candida Albicans as the most common opportunistic microorganism is responsible for sever oral cavity infection in human beings. To control this infection, usually mouthwashes and medications such as Chlorhexidine and Nystatin have been used. Recently with regard to the side effects of chemical mouthwashes, herbal products in form of mouth rinses have been increasingly used and studied. Glycyrrhiza Glabra is an effective herbal mouth rinse to decrease and control tooth caries. The aim of this study was to compare the antifungal effect of aqua-alcoholic root extract of Glycyrrhiza Glabra(G.G) and Chlorhexidine on Candida Albicans (C.A).

Materials and methods: In this experimental study antifungal effect of glycyrrhiza glabra aqua-alcoholic root extract was evaluated using disk diffusion test (DIT) by measuring growth inhibition hallow diameter in both case and control groups. Minimal Inhibitory Concentration (MIC) was assessed by Broth Dilution Test (BDT) and then Minimum Fungicidal Concentration (MFC) was evaluated by Agar Dilution Method (ADM) in specific fungal culture media.Each test was performed three times in both groups and results were statistically analyzed by ANOVA test primarily and complementary Scheffe test if needed ($p<0.01$).

Findings: Chlorhexidine, showed significant antifungal effects on C.A but Glycyrrhiza Glabra root extract had no significant effect on C.A. The antifungal effects of chlorhexidine 0.2% as compared to chlorhexidine 0.12% and also to aqua-alcoholic extract of Glycyrrhiza Glabra was significantly higher ($p <0.01$).

Conclusion: It seems that aqua-alcoholic root extract of Glycyrrhiza Glabra has no significant antifungal effect on candida albicans, and in comparison with chlorhexidine is obviously less effective.

Keywords: candida albicans; Glycyrrhiza Glabra; chlorhexidine; Disk Diffusion Test, drug sensitivity tests

[I] INTRODUCTION:

Candida Albicans (C.A) is a fungal microorganism that causes localized or diffuse infections in skin and mucosa specially in patients with systemic disease and immune system deficiency (1). In healthy people using dentures C.A can lead to diseases such as denture-induced stomatitis. C.A related oral infection and its treatment is one of the most challenging issues in dentistry. Diffuse C.A oral infection specially in immunocompromised patients have been traditionally treated by using systemic antifungal drugs such as nystatin. The most common disadvantages of these products are drug resistance and drug toxicity and bad taste (2).

For localized infection of oral cavity CHX and Nystatin mouthwashes have been used widely in recent years. Although chemical side effects of these synthetic products are concerning and

recently great attention have been paid to introduce herbal products with minimal side effects. Glycirrhiza Glabra root extract (licorice) is one of the most known herbal drugs in traditional medicine. There are more than 100 phenolic component in this product and some of these metabolites such as flavonoids have inhibitory effects on bacterial growth and colonization (3). Many investigations have assessed the effect of this herbal extract on oral microorganisms both in vitro (4,5,6,7) and in vivo(8). Almost all of these studies have focused on bacterial species and specially streptococcus mutans (6), and unfortunately there are a few studies about this herbal product antifungal potency (4,5,7). Loe et al (1960) showed that CHX can prevent plaque accumulation on teeth and mucosa of oral cavity (9). There are many studies and investigation on CHX effectiveness on decreasing bacterial load in oral cavity (10,11,12,13). Martins et al (2015) evaluated the antifungal effects of GGRE on C.A(7). Candida Tropicalis was the most sensitive to GGRE followed buy Candida Glabrata. C.A was reported to be the least sensitive to GGRE. C.A had an inhibitory growth hallow about 10-12 mm and MFC, MIC was lesser than 1.5 mg/ml. GGRE has some inhibitory effect on C.A but increase of GGRE concentration does not promote this effect necessarily. In this experimental in vitro study the authors assessed the GGRE inhibitory effects on C.A growth and colonization.

[II] METHODS AND MATERIALS:

G. Glabra previously dried and reduced in smaller fragments three years after its harvesting from Neyshabour farms in Iran. To obtain phenolic extract , a hydro methanolic extraction was carried out. The sample (1g) was extracted with 50 cc of distilled water at $25^{\circ}C$ and 200 rpm, during 24 hour and then filtered through Whatman Paper. The obtained residue was then extracted again three times in three days. Then the extracts were evaporated and then lyophilized. Finally about 29 gram of purified extract was dissolved in water to evaluate anti C.A activity in different concentrations.

Disk Diffusion Assay:

Condida Albicans PTCC5027 strain was obtained from Iranian Research Organization for Science and Technology and yeast specific culture medium (Sabouraud Dextrose Agar - SDA) and general yeast culture medium (Blood Agar) were obtained also from Yeast Information Bank. All samples were grown in Sabouraud Dextrose Agar (SDA) at $37^{\circ}C$ for 24 hour. After this period , one loop impregnated with C.A was transferred to sabouraud Dextrose Broth and incubated for 24 hour. An aliquot containing approximately 1.5×10^{8} yeast (cells/ml) was spread in SDA petri dishes. Then an aliquot of GGRE with a known Concentration (100mg/ml) was placed on a sterile blank disk, previously placed on the inoculated petri dishes. The same disk diffusion assay was performed with CHX in %0/2 and %0/12 Concentrations. All three plates were incubated at $37^{\circ}C$, for 24 hour in anaerobic environment. After 24 hour , the evaluation of inhibitory properties was performed measuring the corresponding zones of inhibition (mm). This assay was repeated three times in similar situation and average inhibitory hallow diameter was recorded for each plate.

Minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations:

To determine extract MIC we used both microdilution Broth and macrodilution Broth techniques in Brain Heart Infusion (BHI) media. To achieve this goal, we transferred yeast from SDA media to tubes containing physiologic serum to reach the value of 0.5 in Mc Farland Scale. The turbidity and haziness of physiologic serum in this scale confirmed that The number of yeast Strains in physiologic serum is about $1/5\times10^{8}\ \frac{CFu}{Ml}$. Then to evaluate MIC and MFC we prepared $\frac{1}{20}$ concentration of our extract in 0.5 Mc Farland

Scale. To evaluate MIC with microdilution broth technique we used 10 microtiter plates. 100cc of BHI Solution was injected into each microtiter plate using multichannel sampler. Then 100cc of GGRE aqua-alcoholic solution was injected into first microtiter plate and this solution was mixed with BHI Solution in a shaker machine. Then 100cc of first microtiter plate solution was transferred to second plate and this sequence was done for each tube to reach the minimal concentration of GGRE in 10^{th} microtiter plate. Finally 100cc of the solution in 10^{th} microtiter plate was discharged from this plate and therefore we had ten microtiter plates containing 100 cc of GGRE aqua-alcoholic solution which their concentration were continuously decreasing .First to 10^{th} microtiter plate solution concentration were 50,25,12.5, 6.25, 3.12, 1.56, 0.78, 0.39 , 0.19 and 0.09% respectively. Then 100cc of physiologic serum containing C.A yeast in concentration of $\frac{1}{20}$ of 0.5 Mc Farland scale was transferred to each microtiter plate. Then each microtiter plates containing same concentration of C.A and different GGRE concentration were incubated for 24 hour and this process was repeated three times for each GGRE concentration. To measure MIC all of these sequences were repeated for CHX solution in both 0/2 % and 0/12 % concentration as control group in same fashion we described for GGRE aqua-alcoholic solution. Simultaneously we assessed MIC by macrodilution Broth method. This technique is performed in same manner we described for microdilution Broth technique but in higher volume (1cc of solutions in minitubes). For each experiment we defined positive and negative control groups in both macrodilution and microdilution broth methods:

Positive control*:* the tube contents were turbid (hazy) because of C.A growth.

Negative control: The tube contents were transparent of C.A growth inhibition.

After incubating each tube in $37^{\circ}C$ for 24 hour the tube contents were assessed visually to determine MIC. If the tube contents were transparent we concluded that the GGRE or CHX solution have inhibited C.A growth and if the tube contents were turbid (hazy) we concluded that the GGRE or CHX solutions had no inhibitory effect on C.A growth. The concentration of first tube which was obviously transparent was considered as MIC. To determine exact MIC of GGRE solution it was necessary to define MFC first. To achieve this goal all microtube contents (including hazy and transparent macro tubes) were cultured on SDA media. The incubation time was 24 hour in 37^{c} temperature. The first petri dish with no C.A growth was considered as minimal fungicidal concentration (MFC) and one degree thinner concentration than MFC was considered as minimal inhibitory concentration (MIC).

Each test was repeated three times for each group in three different days by the same technician. Finally all data was collected and analyzed statistically by ANOVA test and the p-value ≤ 0.01 was considered significant statistically.

[III] RESULT:

Table 1 shows the anti-candida potential of GGRE solution evaluated by disk diffusion assay. Inhibitory hallow diameter of all three groups are presented in table1 and shows that the most inhibitory hallow diameter was observed in CHX 0.2% group (21 ± 1.73) followed by CHX 0.12% (18.66 ± 1.52).

The least diameter was related to GGRE aqua alcoholic solution (7.66 ± 2.58).

The disk diameter was 6mm and inhibitory hallow diameter of GGRE (7.66 ± 2.58) shows that its inhibitory effect on C.A growth is visually and significantly much less in comparison with CHX inhibitory hallows in both concentrations. ANOVA test showed that the difference between GGRE aqua- alcoholic solution inhibitory hallow and CHX is statistically significant (p-value $\leq$ 0.01)

Table1: Disk Diffusion Assay (Disk diameter=6mm)

Coefficient Variation	Diameter (in mm)	Inhibitory hallow diameter groups
8	18.66 ± 1.52	Chlorhexidine 0.12%
8	21 ± 1.73	Chlorhexidine 0.2%
34	7.66 ± 2.58	GGRE Aqua-alcoholic extract

Then the MIC and MFC of all three study groups were evaluated and the results are presented in table2. These results shows that the most MIC and MFC are related to GGRE aqua- alcoholic solution followed by CHX 0.12%. the lowest minimal inhibitory concentration and minimal fungicidal concentration are related to 0.02% CHX.

Table 2. MIC and MFC index in all three groups

MFC $\frac{mg}{ml}$	MIC $\frac{mg}{ml}$	Results / Groups
0.125	0.0625	CHX 0.12%
0.0625	0.0312	CHX 0.2%
100 > MFC > 50	100 > MIC > 50	GGRE aqua-alcoholic solution

[IV] DISCUSSION:

Table 1 shows the anti Candida Albicans potential effect of GGRE in comparison with CHX in both 0.2 and 0.12 percent concentration by disk diffusion assay. Considering the inhibition hallow, among all the tested groups, 0.2% CHX had the most powerful antifungal effect and GGRE solution had the least antifungal potential. Karomi et al.(2012) evaluated the antifungal effect of GGRE against C.A and obtained similar inhibition zones when compared with the present experiment (15). They also concluded that this effect was dose dependent to the licorice concentration. Irani et al.(2012) evaluated the antibacterial effect of licorice aqueous and ethanolic extracts and also achieved similar results(16). All of these results were in part confirmed in the present study and by the subsequent determination of MFC and MIC. Kalati et al. (2013) evaluated the antifungal potential of GGRE solution in comparison with an antifungal drug; nystatin (1). In their in vitro study they used disk diffusion assay for GGRE and nystatin as study groups and methanol and blank disk as control groups. No growth inhibitory hallow was seen around blank and methanol disk. The largest inhibitory hallow was seen around nystatin disk with an average of 32 mm. there was also no inhibitory hallow around GGRE disk. Finally they concluded that GGRE has almost no in vitro antifungal potential which is similar to our conclusion. Fatima et al (2009) evaluated the antifungal potential of Glabridin and Glycyrrhiza Glabra(4). Their goal was to assess which of this two extracts have more antifungal potential on clotrimazole, nystatin and amphotericin B resistant Candida Albicans species. They concluded that Glabridin has fungicidal potential on all species resistant to these drugs but it was significantly most effective on amphotericin B resistant Candida Albicans. This study support the antifungal effect of GG which is not similar to our results.This study used drug sensitivity tests and measured MIC and MFC like our study but disk diffusion assay has not been used in this study.

Martins et al.(2015) evaluated Glycyrrhiza Glabra extract antifungal potential on Candida Albicans species(7). They used disk diffusion assay and concluded that GGRE is most effective on

Candida Tropicalis and least effective on Candida Albicans. Finally they concluded that GGRE has anti C.A potential although this potential is not dose dependent.

[V] CONCLUSION:

In this present in vitro study we concluded that based on disk diffusion assay and MIC and MFC measuring Glycyrrhiza Glabra aqua-alcoholic root extract solution as a herbal mouthwash does not have significant anti-Candida Albicans potential in comparison with chlorohexidine. GGRE growth inhibition hallow was significantly smaller than CHX in both 0.2 and 0.12 percent concentrations. MIC and MFC measuring showed that GGRE concentrations in this study have no significant fungicidal effects.

ACKNOWLEDGEMENTS:

The authors are grateful to Iranian Research Organization for Science and Technology. The authors are also so thankful to Shahid Beheshti Medical University Microbiology Laboratory.

Conflict of Interest:

The authors have no conflict of interest.

REFERENCE:

1. Arbabi-Kalati F, Porzamani M. Comparison the antifungal effect of licorice and nystatin, in vitro study. Journal of Dental Medicine. 2013 Apr 15;26(1):71-4.
2. Lee JY, Lee JH, Park JH, Kim SY, Choi JY, Lee SH, Kim YS, Kang SS, Jang EC, Han Y. Liquiritigenin, a licorice flavonoid, helps mice resist disseminated candidiasis due to Candida albicans by Th1 immune response, whereas liquiritin, its glycoside form, does not. International immunopharmacology. 2009 May 31;9(5):632-8.
3. Emami ahmad. Medical herbalist. Ir: Iranian students booking agency ;1385.P.125-32 [Persian]
4. Fatima A, Gupta VK, Luqman S, Negi AS, Kumar JK, Shanker K, Saikia D, Srivastava S, Darokar MP, Khanuja SP. Antifungal activity of Glycyrrhiza glabra extracts and its active constituent glabridin. Phytotherapy Research. 2009 Aug 1;23(8):1190-3.
5. Messier C, Grenier D. Effect of licorice compounds licochalcone A, glabridin and glycyrrhizic acid on growth and virulence properties of Candida Albicans. Mycoses. 2011 Nov 1;54(6):e801-6.t
6. Ahn SJ, Cho EJ, Kim HJ, Park SN, Lim YK, Kook JK. The antimicrobial effects of deglycyrrhizinated licorice root extract on Streptococcus mutans UA159 in both planktonic and biofilm cultures. Anaerobe. 2012 Dec 31;18(6):590-6.
7. Martins N, Ferreira IC, Henriques M, Silva S. In vitro anti-Candida activity of Glycyrrhiza glabra L. Industrial Crops and Products. 2016 May 31;83:81-5.
8. Jain E, Pandey RK, Khanna R. Liquorice root extracts as potent cariostatic agents in pediatric practice. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2013 Jul 1;31(3):146.
9. Löe H, Theilade E, Jensen SB. Experimental gingivitis in man. Journal of periodontology. 1965 May;36(3):177-87.
10. Fathilah AR, Himratul-Aznita WH, Fatheen AR, Suriani KR. The antifungal properties of chlorhexidine digluconate and cetylpyrinidinium chloride on oral Candida. Journal of dentistry. 2012 Jul 31;40(7):609-15.
11. Barkvoll P, Attramadal A. Effect of nystatin and chlorhexidine digluconate on Candida albicans. Oral surgery, oral medicine, oral pathology. 1989 Mar 31;67(3):279-81.
12. Shino B, Peedikayil FC, Jaiprakash SR, Ahmed Bijapur G, Kottayi S, Jose D. Comparison of Antimicrobial Activity of Chlorhexidine, Coconut Oil, Probiotics, and Ketoconazole on Candida albicans Isolated in Children with Early Childhood Caries: An In Vitro Study. Scientifica. 2016 Mar 14;2016.
13. Aroonrerk N, Dhanesuan N. Candida inhibitory effects of six commercial

mouthwashes. Annals of microbiology. 2007 Sep 1;57(3):449-52.

14. Hosseini A, Shafiee-Nick R, Mousavi SH. Combination of Nigella sativa with Glycyrrhiza glabra and Zingiber Officinale augments their protective effects on doxorubicin-induced toxicity in h9c2 cells. Iranian journal of basic medical sciences. 2014 Dec;17(12):993.
15. Karomi MF. Huda SA : effect of methanolic extracts of different parts of liquorice GG on growth of some pathogenic bacteria and fungus candida albicans. Iraq Acad. Sci.J.4.140-150.
16. Irani M. Sarmadi M. Bazarnov HS : Leaves antimicrobial activity of Glycyrhizza Glabra L. Iran. J. Pharm. Res. 9: 425-428. 2010.