Advanced Herbal Medicine, 2014; 1 (1): 42-47

herbmed.skums.ac.ir

In vitro comparison of the effect of honey and clotrimazole against *Candida albicans* isolated from vagina

Shayeste Banaeian- Boroujeni [1*], Simin Taghipoor[2]
[1]*Midwifery Dept., Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran;*
[2]*Parasitology Dept, Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran*
Received: 28/July/2014 Accepted: 17/Oct/2014

Original article

ABSTRACT

Background and aims: *Candida albicans* is the most common cause of Vulvovaginitis Candidiasis that is the most common vaginitis in human. It is estimated that more than 90% of these infections are caused by *Candida albicans*. Medical treatment for these infections is carried out with chemotherapeutic drugs such as azoles. The increasing resistance of *C. albicans* to the azoles as well as their probable side effects is public concerns. Therefore, research for new natural component having antifungal activity has been considered to be very important. This study was designed to compare the effect of honey and clotrimazole against *Candida albicans* isolated from vagina and standard strain.
Methods: In this clinical trial study, Samples of vaginal discharges were prepared from 100 women with vulvovaginitis candidiasis. Isolation and complete identification of Candida species were performed and the suspension of *Candida albicans* prepared for insemination. Different concentrations of honey and clotrimazol were prepared and 10 µl of yeast suspension was added and incubated. Then 10 µl of these medium was cultured. Finally, the number of yeasts was counted and MIC50 and MIC90 were determined in comparison with positive control.
Results: Different concentrations of honey and clotrimazol inhibited the growth of *Candida albicans*. In MIC 50 the mean concentrations of honey and clotrimazol were 1.15±0.49 g/ml and 60.26± 6.24 µg/ml respectively. In MIC 90 the mean concentrations of honey and clotrimazol were 3.10± 3.15 g/ml and 2.38±2.36 µg/ml respectively. This means that honey in 3.10 g/ml concentration had the same effect as clotrimazol with concentration of 2.38 µg/ml.
Conclusion: Honey markedly inhibited the growth of *Candida albicans* and its effect was comparable to cloitrimazol.

Keywords: *Candida albicans,* Antifungal activity, Honey, Clotrimazol.

INTRODUCTION

Vulvovaginitis Candidiasis (VVC) is the most common infection of the female genital tract. it has been estimated that up to 75% of women experience at least one episode of Vulvovaginitis Candidiasis during their child-bearing years, and approximately 40% to 50% of them experience chronic recurring.[1] More than 90% of this vaginitis is produced by a gram-positive fungus named Candida albicans and 5% to 10% of them produced by other species such as *Candida glabrata* or Candida tropicalis. *Candida spp* is part of the normal flora of vagina in women. When the ecosystem of the vagina is disturbed, C. albicans can become an opportunistic pathogen. Hormonal factors, depressed cell-mediated immunity, and antibiotic usage are

[*]**Corresponding author:** Midwifery Dept., Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran, Tel: 00983833334463, E-mail: shbanaeian@yahoo.com

the three most important factors that alter the vaginal ecosystem. Lactobacilli inhibit the growth of fungi in the vagina. Therefore, when the relative concentration of lactobacilli declines, rapid overgrowth of Candida spp. can cause VVC.[2] Medical treatment for these infections is carried out with chemotherapeutic drugs including butoconazole, clotrimazole, miconazole, and terconazole.[1] Some of side effects of azoles are pruritus, vulvovaginal burning, stinging, erythema, urticaria, irritation, headache and skin rash.[3] There is also evidence of an increased azole resistance among isolates of Candida Species isolated from women with VVC or from HIV-infected children. The resistance to specific azole can develop cross-resistance to other azoles concomitantly, and this resistance correlates with refractory mucosal candidiasis. Pelletier et al (2000) concluded it is not known whether resistance to clotrimazole develops as a consequence of previous exposure to clotrimazole itself or to other azole drugs as a cross-resistance phenomenon.[4] Therefore, the types of antibiotics used to treat vaginitis must be very selective in order not to kill the beneficial bacteria (Lactobacilli) that help in preservation of vaginal health and ecosystem as being one of the probiotic bacteria.[5]

Honey is a natural product that is not only used as nutrition but also used in wound healing and as an alternative treatment for clinical conditions ranging from gastrointestinal tract (GIT) problems to ophthalmic conditions. It has been used as wound barrier against tumor implantation in laparoscopic oncological surgery and no infection reported from the application of honey to open wounds. It plays a potential therapeutic role in the treatment of gingivitis and periodontal disease.[6] In recent years, several in vitro studies on the antifungal effect of honey have been done and the results have shown considerable variations. In some articles honey inhibited the growth of *Candida albicas* in different concentrations such as 42% and 46% [7], 66%[8], 70% [9] and 30-100%.[10]

Despite several studies about antifungal effect of honey, there are minimal studies compared honey and antifungal agents such as asols. In previous study we compared the effect of two types of honey and miconazole on the *Candida albicans* and Lactobacillus Strains. We concluded that in both of honeys a concentration of 80% had the greatest inhibitory effect on Candida albicans. Neither of the two types of honey inhibited the growth of Lactobacillus. Myconazol in all concentrations inhibited the growth of *Candida albicans* and Lactobacillus as well.[11] Because the vaginal Candida albicans may be resistant to azoles and also the side effect of chemical drugs on beneficial microorganisms, this study managed to compare the effect of honey and clotrimazol on Candida albicans isolated from vagina of women suffered from candidiasis.

METHODS:

Preparation of the *Candida albicans* strains: In this study, 100 women who referred to gynecologic clinic of Shahrekord Hajar hospital (in Chahar Mahal & Bakhtiary province) complaining of vulvovaginitis candidiasis and having typical symptoms of candidiasis were selected. After license, a sample of vaginal discharge was prepared with the sterile swab in test tube containing 0.5 cc sterile normal saline and sent to the laboratory immediately. In the laboratory, the samples cultured in Sabouraud dextrose agar (Merck, Germany) containing Chloramphenicol and incubated at 35 °C for 48 h. Yeasts were identified by staining. In the next step, yeast colonies were passaged on corn meal agar medium (QLAB,UK) containing tween 50 (to produce chlamydoconidia) and simultaneous for production of germ tubes into test

tubes containing 0.5 cc human serum (for 3-2 h at 35 °C). Strains that produced chlamydoconidia in corn meal agar medium and germ tubes human serum medium, were detected as Candida albicans. Of 100 samples were sent to laboratory, 23 Candida albicans were detected and arrived to study. Also one Human Candida albicans strain (PTCC 5027) was prepared from Iranian Science and Industrial Research Institute and used as standard.

Preparation of suspension: New culture of *Candida albicans* was used to prepare the suspension of yeast in sterile normal saline. The number of Candida albicans was counted under the microscope and 2.5×10^3 yeast was determined for insemination.[12]

Honey: The natural; and purified honey was obtained from Chahar Mahal & Bakhtiari province in Iran. The degree of purity was detected in food laboratory at the Shahrekord University of Medical Sciences by brix method. The percentage of saccharose was 0.5% and the honey with 99.5% purity was used.[12] concentrations (0.5-15 g/ml) (20-95%) of honey were prepared in RPMI1640 (Roswell Park Memorial Institute) (Sigma, Germany) medium. 1 ml of these concentrations was added to the sterile tubes and 10 µl of yeast suspension was added to them. One tube contained 1ml RPMI and 10 µl of yeast suspension without honey was determined as positive control. All of tubes were incubated at 37°C for 37 h. After this time, 10 µl of contents of tubes were cultured in Sabouraud dextrose agar medium and were incubated at 37°C for 37 h. At the end, the numbers of yeasts were counted and MIC50 and MIC90 were determined in comparison with positive control tube. This process was repeated for all of 23 samples and standard strain of Candida.

Drug and stock solution preparation: 2048 µg of powder of clotrimazol (Tehran Chemi, Tehran) was desolved in 1ml of DMSO (Dimethyl sulfoxide) and maintained for 30 minutes. DMSO was diluted ($\frac{1}{20}$) to destroy the attraction effect on yeast. The Broth micro-dilution with RPMI1640 medium (with glutamine without bicarbonate) was used based on National Committee for Clinical Laboratory Standards (NCCLS).[13] The test was carried out in 96-well flat-bottomed micro-titration plates. The 1-1024 µg/ml concentrations were obtained. 1µl of yeast suspension was added to the plates. One well contained medium and yeast suspension without clotrimazol was determined as positive control. All of plates were incubated at 37°C in shaker incubator for 48 h. After this time, 10 µl of contents of plates were cultured in Sabouraud dextrose agar medium and were incubated at 37°C for 48 h. At the end, the number of yeasts were counted and MIC50 and MIC90 were determined compared with positive control tube. This process repeated for all of 23 samples and standard strain of Candida.

RESULTS:

Different concentrations of honey and clotrimazol inhibited the growth of standard strain and all of vaginal isolated Candida. Minimum, maximum and mean concentrations of honey and clotrimazol are showed in tabe1. The frequency of Candida isolates in different concentrations of clotrimazol and honey in MIC 50 and 90 are showed in table 2. In MIC 90 the highest frequency of samples was seen in 5g/ml concentration of honey. The highest frequency of Candida isolates in honey was seen in 1 g/ml concentration in MIC 50 and in 1.5 g/ml in MIC 90 respectively. Honey inhibited the growth of standard Candida albicans 50% and 90% in 45 and 60 g/ml concentration respectively. Also clotrimazol inhibited the growth of standard Candida albicans 50% and 90% in 1 and 256 µg/ml concentration.

Table 1: Comparison of minimum, maximum and mean of honey and clotrimazol in MIC 50 and 90

MIC 90						MIC 50					
Clotrimazol (µg/ml)			Honey (g/ml)			Clotrimazol (µg/ml)			Honey (g/ml)		
MIN	MAX	Mean±SD	MIN	MAX	Mean±SD	MIN	MAX	Mean±SD	MIN	MAX	Mean±SD
8	1024	2.38± 2.36	1	13.5	3.10±3.15	1	256	60.26± 5.26	0.64	2.25	1.15±0.49

Table2: The frequency of *Candida* isolates in different concentrations of clotrimazol and honey

MIC 90				MIC 50			
Clotrimazol (µg/ml)		Honey (g/ml)		Clotrimazol (µg/ml)		Honey (g/ml)	
Concentration	frequency	concentration	frequency	concentration	Frequency	Concentration	Frequency
8	3	1	3	1	2	0.81	5
32	2	1.22	4	2	2	0.64	2
64	2	1/5	5	4	3	1	10
128	5	2.25	4	8	1	1.5	3
256	6	3.5	3	16	1	2.25	3
512	4	6	1	32	4		
1024	1	8.5	2	64	3		
		13.5	1	128	6		
				256	1		

DISCUSSION:

The results of this study showed that honey could markedly inhibit the growth of both vaginal Candida albicans and Standard Candida albicans. Also, clotrimazol inhibited the growth of these fungi. The criterion of clotrimazol was µg/ml but that of honey was g/ml. This means that the minimal concentration of honey which is parallel to the clotrimazol effect is many folds. Clotrimazol was the pure substance but honey is composed of different compounds that some of them are probably effective agents for inhibition of fungi and bacteria. This explains the existing difference between used concentration of clotrimazol and honey. In this study in MIC 90 the mean concentration of honey and clotrimazol were 3.10± 3.15 g/ml and 2.38±2.36 µg/ml respectively. This means that honey in 3.10 g/ml concentration had the same effect as clotrimazol in 2.38 µg/ml concentration. Also in MIC 50 the mean concentrations of honey and clotrimazol were 1.15± 0.49 g/ml and 60.26 ± 5.26 µg/ml, respectively. This means that honey in 1.15 g/ml concentration had the same effect as clotrimazol in 60.26 µg/ml concentration.

In Zarin Far et al (2007) study the minimum concentration of clotrimazol that inhibited the growth of Candida albicans in MIC90 was 1 µg/ml.[13] The study of Al-Waili et al (2005) showed that honey inhibited the growth of Candida at 30-100% concentrations in Nutrient agar media.[9] In Ahmed Moussa study the MIC of the tested honey concentrations were in range of 70.09 – 93.48) against C. albicans.[14] Estevinho et al reported that the honey concentration (%w/v) inhibited 10% of the C. albicans growth ranged from 31.0%. Osmotic effect, acidity and effect of hydrogen peroxide are the presumptive function of antifungal activity of honey.[15] Several factors such as phytogeographic regions, botanical origin and variety of components may influence the antifungal activity of honey.

In this study the frequency of samples decreased with increase of honey concentrations. In other words, honey has less impact in higher concentration. This result was also obtained in our previous study.[11] Ahmad Moussa also reported that undiluted honey was not able to inhibit the growth of C. albicans.[14]. In our previous study honey did not inhibit the growth of Lactobacillus. As Lactobacillus is the main vaginal flora and the regulator of normal vaginal ecosystem, this effect of honey is important.

CONCLUSION:

Honey could markedly inhibit the growth of Candida albicans and its effect and it is comparable to clotrimazol.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENT:

The authors are grateful to thank the employers of gynecologic clinic of Shahrekord Hajar hospital (in Chahar Mahal & Bakhtiary province).

REFERENCES

1. Soper DE. Genitourinary infection and sexually transmitted deseases. In: Break J S. Break and Novak's gynecology. Philadelphia: Williams & Wilkins; 2012.
2. Linda OE, Gretchen ML. Infections of the lower and upper genital tract. In: Lentz GM, Lobo RA, Gershenson DM, Katz VL. Comprehensive Gynecology. 6th ed, Philadelphia: Mosby; 2012.
3. Barbar B, Hodgson RN OCN, Robert J. Kizior BS RPh. Saunders Nursing Drug Handbook 2009, 1st ed. USA: Saunders Pub; 2009.
4. Pelletier R, Peter J, Antin C, Gonzalez C, Wood L, Walsh TJ. Emergence of resistance of Candida albicans to clotrimazole in human immunodeficiency virus-infected children: in vitro and clinical correlations. J Clin Microbiol. 2000; 38(4): 1563-8.
5. Razzak MS, Al-Charrakh AH, Al-Greitty BH. Relationship between lactobacilli and opportunistic bacterial pathogens associated with vaginitis. N Am J Med Sci. 2011; 3(4): 185-92.
6. Khan FR, Ul Abadin Z, Rauf N. Honey: nutritional and medicinal value. Int J Clin Pract. 2007; 61(10): 1705-7.
7. Boukraa L, Bouchegrane S. Additive action of honey and starch against Candida albicans and Aspergillus niger. Rev Iberoam Micol. 2007; 24(4): 309-11.
8. Al-Waili NS. Mixture of honey, beeswax and olive oil inhibits growth of Staphylococcus aureus and Candida albicans. Arch Med Res. 2005 Jan-Feb; 36(1): 10-3.
9. Al-Waili NS, Akmal M, Al-Waili FS, Saloom KY, Ali A. The antimicrobial potential of honey from United Arab Emirates on some microbial isolates. Med Sci Monit. 2005; 11(12): BR433-8.
10. Al-Waili NS. Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. J Med Food. 2004; 7(2): 210-22.
11. Banaeian-Borujeni S, Mobini GR, Pourgheysari B, Validi M. Comparison of the effect of honey and miconazole against Candida albicans in vitro. Adv Biomed Res. 2013; 2: 57.
12. Zarin Far H, Yadegari MH, Katiraei F, Etminan Rad S. Comparison of the ketokonazol and clortimazol against Candida albicans in women with chronic vaginitis in laboratory conditions. J Ilam Univ Med Sci. 2007; 15(1):1-7.

13. Cuenca-Estrella M, Lee-Yang W, Ciblak MA, Arthington-Skaggs BA, Mellado E, Warnock DW, et al. Comparative evaluation of NCCLS M27-A and EUCAST broth microdilution procedures for antifungal susceptibility testing of *candida* species. Antimicrob Agents Chemother. 2002; 46(11): 3644-7.
14. Moussa A, Noureddine D, Saad A, Abdelmelek M, Abdelkader B. Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: *Candida albicans* and Rhodotorula sp. Asian Pac J Trop Biomed. 2012; 2(7): 554-7.
15. Estevinho ML, Afonso SE, Feas X. Antifungal effect of lavender honey against *Candida albicans*, Candida krusei and Cryptococcus neoformans. J Food Sci Technol. 2011; 48(5): 640-3.

How to cite the article: Banaeian-Boroujeni SH, Taghipoor S. In vitro comparison of the effect of honey and clotrimazole *against Candida albicans* isolated from vagina. Adv Herb Med. 2014; 1(1): 42-47.