Original Article

Comparison of the effect of honey and miconazole against *Candida albicans in vitro*

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Abstract

Background: One of the most common causes of vaginitis is candidiasis. The aim of this study is to compare the effect of honey and miconazole against *Candida albicans, in vitro*.

Materials and Methods: The different W/V concentrations of honey were prepared at 20, 40, 60, 80, and 95% and different dilutions of miconazole were prepared in 0.05, 5, and 50 µg/ml. A microdilution of 100/000 cells per ml of a two-day old culture of *Candida albicans* was prepared in normal saline, after culturing the strain of PTCC 5027 in RPMI 1640 medium. Ten microliters of this dilution was added to 1 ml of the RPMI 1640 medium containing different concentrations of honey and to 1 ml of the RPMI 1640 medium containing different dilutions of miconazole. The cultures were incubated at 35°C for 12, 24, and 48 hours.

Results: The growth rate of *Candida albicans* was determined in the cultures. The results indicated that the honey prevented the growth of *C. albicans* greatly only at an 80% concentration, whereas, miconazole inhibited it completely.

Conclusions: As *Candida albicans* is a normal vaginal flora, the inhibitory effect of honey without the fungicide effect is a very good trend in the treatment of vaginal candidiasis.

Key Words: *Candida albicans*, honey, *in vitro*, miconazole

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INTRODUCTION

The *Candida* species are part of the normal flora of approximately 25% of the women, being a commensal saprophytic organism on the mucosal surface of the vagina. When the ecosystem of the vagina is disturbed, *C. albicans* becomes an opportunistic pathogen. *Lactobacillus*, an aerobic gram-positive rod, is found in 62 to 88% of asymptomatic women, and is the regulator of the normal vaginal flora. *Lactobacilli* make lactic acid, which maintains the normal vaginal pH of 3.8 to 4.5 and inhibit the adherence of bacteria to the vaginal epithelial cells. Approximately 60% of the vaginal *Lactobacilli* strains make hydrogen peroxide, which inhibits the growth of bacteria and destroys the human immunodeficiency virus (HIV) *in vitro*. *Lactobacilli* inhibit the growth of fungi in the vagina. When the

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relative concentration of the *Lactobacilli* declines, a rapid overgrowth of the *Candida* species occurs. Following the traditional regimen of 10 to 14 days of oral broad-spectrum antibiotics the percentage of women who have vaginal colonization of *Candida* increases threefold.[1]

It is estimated that 75% of the women experience at least one episode of vulvovaginitis candidiasis (VVC) during their child-bearing years, and approximately 40-50% experience a second attack.[2]

Candida albicans is responsible for 85 to 95% of vaginal yeast infections. Factors that cause an increased susceptibility to VVC include antibiotic therapy, pregnancy, uncontrolled diabetes mellitus, using oral contraceptives (especially high-dose formulations), immunosuppressants, and occlusive synthetic clothing.

Symptoms of VVC include pruritus and vaginal discharge. Other symptoms may include vulvar burning, dyspareunia, vulvar dysuria, and vaginal irritation. Discharge is not always present, and there may only be a small amount.[3] Treatment agents include butoconazole, clotrimazole, miconazole, and terconazole.[2] Some of side effects of azoles are pruritus, vulvovaginal burning, stinging, erythema, urticaria, irritation, headache, and skin rash.[4]

Honey has been used since ancient times as part of traditional medicine. It also functions as an antibacterial, antioxidant, antitumor, anti-inflammatory, and antiviral.[5] The belief that honey is a nutrient, a drug, and an ointment has been carried into our days. In long human tradition honey has been used not only as a nutrient, but also as a medicine. Honey contains numerous compounds such as organic acids, proteins, amino acids, minerals, polyphenols, vitamins, and aroma compounds. It has been found to contain significant antioxidant activity including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, amino acids, and proteins.[6-8]

Honey inhibits the growth of microorganisms and fungi. The antibacterial effect of honey, mostly against gram-positive bacteria, is well-documented. Both bacteriostatic and bactericidal effects have been reported for many strains, some of them are pathogenic. The antimicrobial effect of honey is due to different substances and depends on the botanical origin of honey. The low water activity of honey inhibits bacterial growth. Honey glucose oxidase produces the antibacterial agent hydrogen peroxide, but the capacity for peroxide production also depends on the honey catalase activity.[8]

Varidi A. *et al*. in their study on nine infants with large, open, infected wounds reported that all infants showed marked clinical improvement after five days of treatment with topical application of 5-10 ml of fresh unprocessed honey, twice daily. They concluded that honey is useful in the treatment of post-surgical wounds that are infected and do not respond to conventional systemic or local antibiotic treatments.[9]

Honey maintains a moist wound environment that promotes healing, and its high viscosity helps to provide a protective barrier to prevent infection. In addition, the mild acidity and low-level hydrogen peroxide release assists both tissue repair and contributes to the antibacterial activity of honey.[4]

Al-Waili NS and Saloom KY have concluded that topical application of crude undiluted honey could eradicate bacterial infections fast, reduce the period of antibiotic use and hospital stay, accelerate wound healing, prevent wound dehiscence and need for re-suturing, and result in minimal scar formation in women with postoperative wound infections due to gram-positive and gram-negative bacteria following Cesarean sections and hysterectomies.[10]

The incidence of *Candida* infections is escalating worldwide. The serious nature of these infections is compounded by increasing levels of drug resistance. Certain honeys have significant antifungal activity against clinical isolates of the *Candida* species. Most importantly, the minimum inhibitory concentration of these honeys would be achievable in a clinical setting.[11]

In a study by Mercan *et al*., honey exhibited high anticandidal activity on *C. albicans, P. aeruginosa*, *E. coli*, and *S. aureus*. The honey samples that were obtained from Izmir proved more effective as inhibitors against *P. aeruginosa*, *E. coli*, and *S. aureus*. The honey that was obtained from Muğla exhibited high anticandidal activity on *C. albicans*.[12]

In a study by Al-Wailli, a mixture of honey, beeswax, and olive oil was effective in reducing the symptoms of dermatitis, and eradicated *C. albicans* from 50% of the culture-positive patients, during the seven-day trial.[9]

As there were differences between the reported studies and not much was known of the effect of Iranian honeys, we managed to study the *in vitro* effect of honey from Central Iran, and compared it with the effect of miconazole on *Candida albicans*. As *Lactobacillus* was a normal vaginal flora that protected the vaginal

ecosystem, and the disturbance of this ecosystem was important in vaginal infections, including candidiasis, the effect of honey on *Lactobacillus* was also studied.

MATERIALS AND METHODS

Preparation of different viscosities of honey: Standard and substandard honey was obtained from the Chahar Mahal and Bakhtiari province in Iran.

The degree of purity was detected in the food laboratory at the Shahrekord University of Medical Sciences, using the Brix method. The percentage of saccharose was 0.5 and 5.6%, respectively, in these honeys. Considering the maximum standard level of saccharose, which was 5%, the purity was 99.5% (standard level) and 94.6% (substandard level), respectively, but the ratio of fructose to glucose was at a standard level in both of them.

The RPMI 1640 medium (Sigma, Germany) was used for the preparation of different V/V concentrations. The concentrations of both the honeys were prepared at 20, 40, 60, 80, and 95%. One milliliter of each dilution and also undiluted honey were added to the tubes.

Preparation of different dilutions of miconazole: Different dilutions of miconazole were prepared at 0.05, 0.5, 5, and 50 µg/ml in distilled water.

Preparation of microorganisms: A human *Candida albicans* strain PTCC5027 and *Lactobacillus* sp1332 were obtained from the Iranian Science and Industrial Research Institute and was then cultured in Sabouraud's dextrose agar (Merck, Germany) with Chloramphenicol. This strain was cultured in corn meal agar (QLAB, UK) to produce Chlamydoconidia, and in human serum for the production of germ tubes, and they were confirmed.

Laboratory tests

A microdilution of 100/000 cells per ml of a two-day *Candida albicans* culture was prepared in normal saline.

Ten microliters of the above-mentioned dilution was added to 1 ml of RPMI 1640 medium containing different concentrations of honey, so the final dilution was 1000 cells per culture. A culture containing 1 ml RPMI 1640 with 1000 cells was considered to be the control. The cultures were incubated at 35°C for 12, 24, and 48 hours. One hundred microliters in each tube were then cultured in the solid Sabouraud dextrose agar containing Chloramphenicol. The colonies were counted after 48 hours incubation, at 35°C. The method was the same for both standard and substandard honey.

Different concentrations of miconazole powder were prepared at 0.1, 1, 10, and 100 µg/ml in distilled water, and 1 ml of each was added to 1 ml RPMI 1640 medium, so the final concentration was reduced to half. Therefore, the final concentrations of miconazole were 0.05, 0.5, 5, and 50 µg/ml. Twenty microliters of *Candida albicans* suspension were added to each culture to get 1000 cells/ml. A culture containing only 1 ml RPMI 1640 and 1000 cells was considered to be the positive control. The cultures were incubated at 35°C for 12, 24, and 48 hours. One hundred microliters of each tube was then cultured in Sabouraud dextrose agar containing Chloramphenicol. The colonies were counted after 48 hours incubation at 35°C.

Lactobacillus was cultured in Trypticase Soy Broth (Merck, Germany). Different concentrations of honey were added to the suspension after incubation, getting a 0.5% McFarland concentration. *Lactobacilli* re-cultured on Mueller Hinton Agar (Merck, Germany) after 24 hours incubation, at 37°C, and the growth of bacteria was monitored at different time points.

RESULTS

None of the different concentrations of standard honey inhibited the growth of *C. albicans* completely. *C. albicans* grew slightly throughout the control time in the media with 80% honey. It showed the inhibitory effect of honey on *Candida*. It grew exceedingly in the culture containing 20-60% honey, and at an intermediate level in a culture with 95% concentration. For more accuracy, five different concentrations close to 80% (70, 75, 80, 85, and 90%) were prepared and their effect on the growth of *Candida* was detected using the same method. A concentration of 80% had the greatest inhibitory effect, whereas, higher and lower concentrations prevented the growth of *Candida* at the intermediate level [Table 1]. The results were similar for the substandard honey [Table 2]. Although the honey could not prevent the growth of *Candida albicans* completely, it had a high inhibitory effect at 80% concentration.

C. albicans growth was inhibited completely in the media containing miconazole. The *Candida* grew well in all conditions in the control culture. As shown in the tables, honey prevented the growth of *C. albicans* greatly only at 80% concentration, whereas, miconazole inhibited it completely.

Neither of the two types of honey inhibited the growth of *Lactobacillus* and complete growth could be seen at different concentrations.

Table 1: The growth degree of *Candida albicans* in cultures containing different concentrations of standard honey, miconazole, and control in different time points

Time (hour)	Concentration													Control positive media
	Different concentrations of Miconazole (μg/ml)				Different concentrations of honey (V/V)									
	0.05	0.5	5	50	20	40	60	70	75	80	85	90	95	
12	-	-	-	-	+ + +	+++	+++	+++	++	+	++	++	++	++++
24	-	-	-	-	+ + +	+++	+++	+++	++	+	++	++	++	++++
48	-	-	-	-	+ + +	+++	+++	+++	++	+	++	++	++	++++

Degree of growth: -: none, +: mild, ++: moderate, +++: severe, ++++: complete

Table 2: The growth degree of *Candida albicans* in cultures containing substandard honey, miconazole, and control in different time points

Time (hour)	Concentration													Control positive media
	Different concentrations of Miconazole (μg/ml)				Different concentrations of honey (V/V)									
	0.05	0.5	5	50	20	40	60	70	75	80	85	90	95	
12	-	-	-	-	+ + +	+++	+++	+++	++	+	++	++	++	++++
24	-	-	-	-	+ + +	+++	+++	+++	++	+	++	++	++	++++
48	-	-	-	-	+ + +	+++	+++	+++	++	+	++	++	++	++++

Degree of growth: -: none, +: mild, ++: moderate, +++: severe, ++++: complete

DISCUSSION

The antimicrobial properties of honey have been confirmed in different studies, which could be the effect of the following factors:

- Osmotic effect: Eighty-two percent of honey contains a high concentration of carbohydrates, but a low volume of water. It could inhibit the growth of bacteria by cell dehydration. Fungi are more resistant to high osmotic pressure than bacteria
- Acidity: The honey pH, 3.2-4.5, is due to the organic acids particularly glutamic acid, pyruvic acid, malic, and citric acid, whereas, the minimum pH for bacteria growth is 7.2-7.4
- Hydrogen peroxide (H2O2) is often produced in diluted honey as a result of glucose oxidation.
- Stimulation of the immune system by B and T-cell cytogenesis and neutrophil activation.[5,13-15]

In this study, 80% of the honey could prevent the growth of the *Candida* greatly, but not inhibit it completely. This effect was similar with 75, 85, and 90% honey. Koc AN *et al*. demonstrated that all honeys had antifungal activity at a high concentration of 80% (v/v), on fluconazole-resistant strains.[16] In the study by Al-Waili NS *et al*., honey inhibited the growth of *Candida* at 30-100% concentration in Nutrient agar media.[17] The inhibition of growth, but not killing has been reported in other studies.[3,8]

Our data showed the effect of the concentration on the degree of inhibition. The inhibition was low up to 70% concentration, intermediate at 75, 85, and 90%, and high at 80%. The degree of inhibition was also low at 100%. It was not clear why the honey concentrations above and below 80% had less effect. Such phenomenon could be seen in alcohol, as 70% ethanol had the highest inhibitory effect on the growth of bacteria.

Similar results have been reported in the study by Al-Waili, as they found complete inhibition of *Candida* growth in Sabouraud's agar culture with 66% honey, but in Sabouraud's glucose agar media containing wax, olive oil, and honey at 33 and 50% concentrations, the degree of growth of *Candida* was intermediate and high.[18]

Lusby PE and colleagues have studied the effect of honey on different pathogenic bacteria and found that the 20% concentration had low or no effect on the inhibition of the growth of *Candida*, but the effect augmented with increasing the concentration.[13] In the Theunissen F. *et al*. study, 2.5-15% honey had a low effect on the growth and the highest concentration (25%) had the highest inhibition, 29.4%.[19] The authors concluded that the honey hydrocarbons, up to the specific levels, help growth, but the inhibitory effect appeared at the higher hypertonic concentration. The influence of honey and starch on *Candida albicans* and *Aspergillus* was investigated in the study by Boukraâ L. *et al*., where the minimum inhibitory effect of honey on *Candida* was found at 42 and 46%, which was reduced to 28 and 38%, by adding starch to honey. The investigators assumed that the starch amylase increased the anti-*Candida* effect by increasing the osmotic pressure.[15] This finding augments the role of carbohydrates and their oxidation on the antimicrobial effects.

Al-Waili *et al*. studied the antimicrobial effects of honey on human isolates in the United Arab Emirates and found the effective concentration for prevention of the growth of *Candida* at more than 70%. The growth was completely inhibited when 80% honey was added to the culture two to six hours after the initiative time. However, the *Candida* had grown in a subculture. Thus, the honey stopped or slowed down the growth, but did not kill the *Candida*.[16] The study also mentioned that the time of adding honey influenced the inhibitory effect. It may explain the difference between this study and our findings.

A concentration of more than 80% reduced the inhibitory effect in our study, which was compatible with the Al-Wailli *et al*. study. Theunissen F explained that the fungi were more resistant than bacteria against high osmolarity honey. This effect was similar to the anti-bacterial effect of alcohol, which was the highest at 70%.[19]

In the Mercan *et al*. study the antimicrobial effect of Izmir honey was found to be more on *E. coli* and *Staphylococcus aureus* than on *Candida*, whereas, Mugla honey had the greatest effect on *Candida*.[12] In the Frans *et al*. study, the inhibitory effect of the three types of honey on *Candida* was different, which was supposed to be due to the diversity of the plants.[19] This could explain the differences between the various studies. Moreover, the *Candida* species and the laboratory tests might not be the same in different studies.

Using two types of honey in our study, we had similar results. It could be concluded that the total concentration of the carbohydrates and the osmolarity, but not their ratio, play a role in the anti-*Candida* effects. It is notable that honey does not interfere with the *Lactobacillus,* which is the normal flora of the vagina.

CONCLUSION

Miconazole can inhibit the growth of *Candida* completely and kill them, whereas, honey only has inhibitory effects. However, honey does not remove the normal vaginal flora, *Lactobacillus*.

A comparative study on the differences between the honeys from different areas, with different properties, could be helpful, and the inhibitory effect of honey on the human vaginal isolates would help to get more comprehensive data.

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