Original Article

Antibacterial Activity of Honey on Cariogenic Bacteria

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Abstract

Objective: Honey has antibacterial activity. The aim of this study was to evaluate the antibacterial activity of honey on Streptococcus mutans and Lactobacillus.

Materials and Methods: In this in vitro study, solutions containing 0%, 5%, 10%, 20%, 50% and100%(w/v) of natural Hamadan honey were prepared. Each blood (nutrient) agar plate was then filled with dilutions of the honey. The strains of bacteria were inoculated in blood agar for 24 hours at 37^{o}C and were adjusted according to the McFarland scale (10×10 cfumcl $^{-1}$). All assays were repeated 10 times for each of the honey concentrations. Data were analyzed by non parametric Chi-Square test. Statistical significance was set at α=0.05.

Results: Significant antibacterial activity was detected for honey on Streptococcus mutans in concentrations more than 20% and on Lactobacillus in 100% concentration (P<0.05).

Conclusion: It seems that antibacterial activity of honey could be used for prevention and reduction of dental caries.

Key Words: Honey; Antibacterial Agents; Streptococcus Mutans Lactobacillus; Dental Caries

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INTRODUCTION

Honey is super saturated nectar collected by bees from a wide variety of plants. Although it is very well known as a food, it is not well-recognized as a medicine [1]. The medicinal and antimicrobial activities of honey for wound healing have been introduced for approximately 4500 years, almost as long as bacteria have been known. Honey has been used as a medicine since ancient times [2-4]. In Islamic countries, it is also based on the belief in the words regarding honey in the Holly Quran that 1900 years ago and that the Lord taught the bee to build its cells on trees and in men's habitations, then to eat all the products of earth and find with skill the spacious paths of its

Lord, there issues from within their bodies a drink of varying colors, wherein is healing for men,verily in this is a sign for those who give thought [5,6]. The composition of the honey sample depends on the composition of the nectar, from which it originates. The natural antioxidants and flavonoids exhibit a wide range of biological effects including antibacterial, antiinflammatory, antiallergic, anti-thrombotic and vasodilatory action [7,8]. In addition, its physical properties provide ideal moist conditions for healing and a stimulating effect on the growth of wound repair tissues [9]. Unlike other antiseptics, honey has no harmful effects on tissues because slow enzymatic production of hydrogen peroxide is one thousandth hydrogen peroxide 3% [9]. Honey contains about 181 substances. There is scientific evidence about honey in several experimental and clinical conditions; for example, it has been reported effective in the treatment of gastrointestinal disease through gastric protection against gastric lesions, healing of wounds and burns, and as an antimicrobial agent [10-13]. Honey can be kept for long periods of time without spoiling, because of its high osmotic pressure and antibacterial activity [7]. It has been noted that honey clears infection, removes malodors, reduces inflammation and pain, subsides edema and exudation, and it has healing properties by stimulating angiogenesis. Honey has the potential to be useful for periodontal therapy, prevention of infection in wounds following extraction or oral surgery, other oral infections, erosion of mucosa, radiotherapy-induced stomatitis and oral ulcers with no adverse reaction [14-18].

It has been revealed that honey has antimicrobial activity against aerobic, anaerobic, gram positive and gram negative bacteria, moulds and yeasts with unique properties because of its bacteriostatic and bactericidal effect [19]. Honey differs from other sweets because of enzymes such as invertase, diastase and glucose oxidase. Moreover, honey is effective on infections which cannot be treated with conventional drugs [18,20]. Studies have demonstrated that many bacterial and fungal pathogens that are sensitive to honey include Bacillus anthracis, Corynebacterium diphtheriae, Escherichia coli, Haemophilus influenza, Klebsiella pneumoniae, Listeria monocytogenes, Mycobacterium tuberculosis, Pasteurella multocida, Proteus species, Pseudomonas aeruginosa, Salmonella choleraesuis, Salmonella typhimurium, Serratia marcescens, Shigella species, Staphylococcus aureus, Streptococcus mutans, Streptoccus pneumoniae, Streptococcus pyogenes, Vibrio cholerae, Alcaligenes faecalis, Citrobacter freundii, Enterobacter aerogenes, Klebsiella pneumoniae, Mycobacterium phki, Staphylococcus epidermis, E coli and Helicobacter pylori [21-23]. Recently, studies have focused on new aspects of honey application for various therapeutic purposes, especially on its antibacterial properties. Since Streptococcus mutans and Lactobacillus, as two main cariogenic bacteria of the oral cavity, play an important role in dental caries, understanding the antibacterial effect of honey on these cariogenic bacteria is necessary.

Only limited studies have been performed about the antibacterial activity of honey against cariogenic bacteria [1]. The aim of this study was to evaluate the antibacterial activity and the ability of honey to prevent growth of these bacteria (Streptococcus mutans and Lactobacillus).

MATERIALS AND METHODS

Preparation of honey samples

This study was carried out with natural, untreated and unpasteurized honey samples obtained directly from beekeepers in Hamadan, West of Iran. The samples were originated from blossoms of wild flowers and did not contain artificial preservatives or diluents.

Assay of antibacterial activity

The honey samples were screened for their antibacterial activity, according to the agar well-diffusion method proposed by the CLSI

against the two reference strains: (1) Streptococcus mutans PTCC 1683 and (2) Lactobacillus spp. PTCC 1643. The above bacteria were grown (100 mL) in Trypticase Soy Broth (TSB) at 37◦C for 18 hours. The following honey concentrations (w/v) were prepared in sterile saline solution; 5%, 10%, 20%, 50%, and 100% and their antibacterial activity was evaluated against the bacterial strains.

Accordingly, a 100 mL aliquot of each honey dilution was added to each well on blood agar inoculated with bacterial concentration similar to 0.5 Mac Farland tube, and were incubated at 37◦C for 18 hours. Antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding the wells. Control plates were prepared with no honey added. All assays were repeated 10 times for each honey concentration.

Statistical analysis

Linear regression lines were estimated for determination of the minimum active dilution of each honey against the reference strains. Data were analyzed by non parametrical Chi-Square test and SPSS 12 for Windows (SPSS Inc., Chicago, Ill, USA). The level of confidence was set at 95% (α= 0.05).

RESULT

The results are shown in Table 1. There was no significant similarity between the two isolates in their sensitivity to honey (P>0.05). Honey in concentration more than 20% had significant antibacterial activity on Streptococcus mutans and in 100% on Lactobacillus (P<0.05).

DISCUSSION

Honey inhibits the growth of a wide range of bacterial species in vitro [24]. However, there have been few studies about the efficacy of honey against the two main cryogenic bacteria streptococcus mutans and Lactobacillus [25]. Honey is a delicious, natural sweet food and sweeteners are one of the most common causes in developing dental caries.

Thus, the use of a low harmful sweetener in the diet is very important, especially, if it is confirmed that honey has antibacterial activity against cariogenic bacteria in vitro and in vivo. The effective factors in the antimicrobial activity of honey include enzymatic glucose oxidation reaction, physical properties, high osmotic pressure, low water activity, low PH, acidic environment, low protein content, high carbon

Table 1. Antibacterial Effect of Honey on Bacterial Growth

Honey Concentrations	S.M Sample Number	S.M Growth Number (Percent)	L.B Sample Number	L.B growth Number (Percent)
0%(Control)	10	10(100) [a]	10	10(100) [a]
5%	10	10(100) [a]	10	10(100) [a]
10%	10	10(100) [a]	10	10(100) [a]
20%	10	0(0) [b]	10	10(100) [a]
50%	10	0(0) [b]	10	10(100) [a]
100%	10	0(0) [b]	10	0(0) [b]

Superscripts indicate statistically significant difference between values a and b (P=0.000).

to nitrogen ratio, low redox potential due to the high content of reducing sugars, a viscosity that limits dissolved oxygen and other chemical agents, phytochemicals, antioxidants, volatiles, flavonoid, beeswax, nectar pollen and propolis [7,25-27]. This study tested honey, something that is used by people in this region and that is available for them. Because the antibacterial activity of honey varies with the source and processing, results of this study showed that natural honey of this region had an antibacterial effect on cariogenic bacteria (Streptococcus mutans and Lactobacillus) in experimental studies. Other studies reported that the antibacterial activity of honey increased when the honey was diluted [24]. In the present study, results were not in agreement with this finding and 100% honey concentration had an antibacterial effect on two cariogenic bacteria. Honey in dilutions greater than 20% was effective against Streptococcus mutans.

These differences may be due to the type of honey that was used, water content of this honey and honey sources. Researchers evaluated honey from different regions in Egypt for antimicrobial effects. While all samples had antibacterial activity on negative and positive gram bacteria, they were ineffective against Candida albicans and Aspergillus niger [28]. Research showed that Helicobacter pylori is sensitive to honey [29], but honey enhanced the growth of bifidobacteria [30]. Staphylococcus aureus, the principal contaminant agent in many clinical infections, has developed resistance against several antibiotics and honey is a costly treatment option in this case. Manuka honey has been reported to be highly effective against various pathogens, including methicillin-resistant staphylococcus aureus (mRSA) and vancomycin-resistant Enterococci (VRE) [25].

Findings of a study showed that coagulase-negative Staphylococci, Staphylococcus aureus, Pseudomonas aeruginosa and Entrococcus species are susceptible to honey [2]. One study indicated growth inhibition and acid production of Streptococcus mitis, Streptococcus sorbinus and Lactobacillus casei with 7%, 7.5-8.5% and 8-12% of honey [1].

It was reported that the antibacterial activity of wild flower honey significantly decreased streptococcus mutans in the head and neck irradiated cancer patients that had xerostomia due to radiation [31]. Results of this study were in agreement with the findings of previous studies and demonstrated that honey had antibacterial activity (Streptococcus mutans and Lactobacillus). Another result which was obtained in this study, was the antibacterial effect of honey tested in concentrations higher than 20% for Streptococcus mutans. However, the greatest inhibition of two the bacteria was seen at 100% concentration. A study exhibited that other honeys such as manuka had good antibacterial activity at very low concentrations [21].

An explanation for the difference of results may be due to methodological difference between the studies because the agar dilution method used by others were slightly different from that used in this study. However, it can be due to variation in the composition of the honey being used [21].

In addition, the other honeys have no similar effect on all the bacteria. Although the data obtained from this study could not fully represent the profile of the antibacterial activity of honey in cariogenic bacteria and confirm the use of honey clinically, the present study established that honey had in vitro antibacterial activity and honey might be useful for the prevention of dental caries. Further studies are recommended regarding the antibacterial activity of honey in clinical situations.

CONCLUSION

Under limitations of this study, results demonstrated that natural honey had an antibacterial activity on Streptococcus mutans and Lactobacillus bacteria. This effect dependent on to the concentration of honey used.

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