SHORT COMMUNICATION

# In Vitro Assessment of Anti-*Streptococcus Mutans* Potential of Honey

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## Abstract

**Background:** Honey is one of the traditional medicines used for treatment and prevention of various illnesses. This study aims to assess the *in vitro* antimicrobial effect of honey on *Streptococcus mutans*.

**Methods:** This experimental study was conducted in Shiraz University of Medical Sciences. The Minimal Inhibitory Concentration/MIC effect of a type of commercial honey on *S. mutans* isolates was investigated by Agar well diffusion and broth microdilution assays. For comparison a carbohydrate solution with sugar content similar to that of natural honey was used as a control.

**Results:** The MIC values of honey for 88.3% of tested strains were 75% v/v that were significantly different of the MIC values of the carbohydrate solution ($p$<0.001) and the average inhibition zone of undiluted honey on the *S. mutans* isolates was 13 mm. No inhibition zone was observed for undiluted carbohydrate solution in any isolates ($p$<0.001).

**Conclusion:** Honey (*Apis mellifera*) has bacteriostatic activity when tested in vitro. However, pharmacological standardization and clinical evaluation of this effect are essential before using honey as a preventive measure for dental caries.

**Keywords:** *In vitro*; *Streptococcus mutans;* Honey

## Introduction

Honey is mentioned in the Holy Quran. The use of honey as a remedy has been reported only in folk medicine but now it is reborn in modern medicine. It has been demonstrated that honey possesses important biological activities and therapeutic properties, its use in modern medicine being evaluated more and more. It has been used for treatment of respiratory diseases, ulcers, wounds, eczema, psoriasis, and dandruff.[1-4] Reportedly, honey has an inhibitory effect on aerobic and anaerobic bacteria, yeast, fungi and viruses.[5-10] Moreover, it can enhance antibody production against thymus-dependent and thymus-independent antigens.[11] Honey increases antioxidant agents, serum iron and blood indices, and trace elements. It can decrease immunoglobulin E, liver and muscle enzymes, and fasting blood sugar in healthy subjects.[12] It can also lower the concentration of prostaglandins in the plasma of normal individuals,[13] lower C-reactive protein, homocysteine, blood lipids in healthy and hyperlipidemic subjects, and cause lower elevation of plasma glucose level in comparison with dextrose and sucrose in diabetic patients.[14]

*S. mutans* and other cariogenic bacteria are the major etiological agents in dental caries. Factors associated with cariogenicity include adhesion, acidogenicity and acid tolerance.[15] Acidogenic bacteria such as *S. mutans* and *Lactobacillus* species produce metabolic products such as lactic acid and acetic acid with PKa values of 3.86% and 4.75%. Such acids can reduce the plaque pH below 5.5 and the critical pH for enamel demineralization, leading to the initiation and development of caries.[16] Little information is available regarding the inhibitory effect of honey on

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oral bacteria. The aim of the present study was to determine the antimicrobial *in vitro* effect of honey on *S. mutans* isolates.

## Materials and Methods

A commercial type of honey (Khomein Honey; Iran), commonly used locally, was purchased and used in the study. It was dark yellow in color and of multi-floral origin. To obtain different honey concentrations v/v (75%, 50% and 25%), nutrient broth was added to the tubes. Honey and broth were mixed thoroughly by a vortex.

*Streptococcus mutans* strains were isolated from the saliva. Seventy volunteers' unstimulated saliva samples were collected by spitting the whole saliva into sterile containers in an educational dental clinic and transported to laboratory immediately. Ten microlitres of undiluted saliva samples were cultured on mitis salivarius bacitracin (MSB) agar (Himedia, Mumbai, India). The plates were incubated at 37°C for 48 hours in an atmosphere of 80% $N_2$, 10% $H_2$, and 10% $CO_2$.

Agar well diffusion and broth microdilution assays were performed on 60 *S. mutans* isolates.

A sizable colony of each *S. mutans* isolates was emulsified in 4 ml of distilled water, yielding approximately $1.0 \times 10^6$ CFU/ml, and used to swab sensitivity test agar plates. Wells of 7 mm diameter were made and 50 µl of each undiluted honey was placed into each well with a pipette. For comparison of a carbohydrate solution with sugar content similar to that of natural honey (39% D-fructose, 31% D-glucose, 8% maltose, 3% sucrose and 19% water) was used as a control. The plates were incubated in an appropriate atmosphere ($H_2$:$CO_2$:$N_2$ 10:10:80) at 37 ºC for 48 hours. The diameter of inhibition zones was measured (mm) and the average was recorded. Each test was done in triplicate.

SPSS software (version 13, Chicago, IL, USA) was used for statistical analysis. Fisher's exact test was used to compare the groups. A *p*-value of less than 0.05 was considered as statistically significant.

A bacterial suspension equal to McFarland standard of 0.5 was prepared in saline for each *S. mutans* isolate and further diluted in nutrient broth to a concentration of approximately $1 \times 10^6$ CFU/ml. Fifty µl of this suspension was inoculated into the wells of the panels, which also contained 50 µl of different honey concentrations v/v (100%, 75%, 50%, 25%). This resulted in the final concentration of approximately $5 \times 10^5$ CFU/ml. After 48 hours of incubation at 37°C in an appropriate atmosphere, ($H_2$:$CO_2$:$N_2$ 10:10:80), the MIC was determined as the lowest concentration of honey that inhibited the visible growth of *S. mutans* isolates. For comparison, the MIC of the carbohydrate solution (mentioned before) was determined similarly. Each test was done in triplicate.

## Results

Of the 70 saliva samples, 60 strains were isolated and confirmed as *S. mutans* (85.7%), using standard biochemical tests. The average inhibition zone of undiluted honey (100% v/v) on the 60 *S. mutans* isolates was 13 mm (lowest 10 mm, highest 15 mm). No inhibition zone was observed for undiluted carbohydrate solution in any isolates ($p<0.001$). Fifty three out of 60 (88.3 %) *S. mutans* isolates had MIC values of 75% v/v for testing the honey sample. The other 7 isolates were inhibited only in undiluted honey. The growth of 9 (15%) isolates was inhibited in undiluted carbohydrate solution. Other concentrations of carbohydrate solution (75%, 50% and 25% v/v) did not have any inhibitory effect on the isolates ($p<0.001$).

## Discussion

Natural products have recently been demonstrated as an alternative to synthetic substances for prevention of tooth decay.[17-19] Honey and Propolis (honey product) have been considered as a candidate for this reason.[20-23] However, the chemical composition of honey varies depending on several factors, including the collection site and the species of the plant employed as a source, as well as the climatic conditions in which the plants grow.[24] The antibacterial properties of honey against medically important bacteria have been well documented but this information is not completely available for the oral bacteria and specifically for oral *Streptococci*. Natural honey has antibacterial activity against certain bacteria, viruses and fungi.[2-10] This study also showed that honey had a similar inhibitory effect on *S. mutans*. These bacteria are important acidogenic agents in the oral cavity and use dietary sugars as a fermentable substrate. Honey has a high sugar content (80%),[25] with a low pH.[26] Analysis of the major components of honey obtained from the United States and South Africa shows that

they are surprisingly similar,[27] and environmental factors, therefore, play a minor role in the final composition of the major components (fructose and glucose). Since honey is an important sweetening agent and supply of fermentable sugar to the oral bacteria, it can be regarded as a potential cariogenic food. However, the antibacterial activity of honey might influence this potentially harmful effect. For instance, it has been shown that other bee products such as propolis control dental caries.[21-23] The mechanism of the antibacterial effect of honey remains speculative at present. Possible explanations are presence of hydrogen peroxide,[28] flavonoids,[29] and hypertonic sugar concentration.[30] Hydrogen peroxide is formed in honey by the action of the enzyme glucose oxide which produces gluconic acid and hydrogen peroxide from glucose. It was found that on dilution of honey the enzyme became activated.[28] The hydrogen peroxide that was formed accumulated in the growth medium, thereby inhibiting the growth of bacteria.[28] Flavonoids are flower pigments that occur naturally in plant products and in honey. This product has been shown to have antibacterial properties.[29] High concentration of sugar is sometimes used as a preserving agent in the making of fruit preserves, candies and condensed milk. At high sugar concentrations, hypertonic condition exists, causing plasmolysis of the microbial cell which results in growth inhibition and death.[30] A combination of these factors probably causes antibacterial properties of honey. It seems that the hypertonic sugar concentration plays a major role in this activity .Cavanagh,[31] showed that yeasts were more resistant to honey than bacteria. Some species even grow in pure honey, whereas bacteria grow at a concentration of 20%. This is consistent with the finding that bacteria are more sensitive to hypertonic sugar than yeasts.[29] Sela,[30] showed that total salivary bacterial count was not significantly different between the normal subjects and head and neck irradiated patients after consumption of honey, whereas the *S. mutans* count decreased significantly.[32] We used a carbohydrate solution with sugar content similar to that of natural honey as a control. No inhibition zone was observed in any isolates in agar well diffusion assay and only 15% of the isolates were inhibited by undiluted carbohydrate solution in broth of microdilution assay. It seems that the carbohydrate content of honey has not a significant inhibitory effect on *S. mutans*. Further studies on anti *S. mutans* effect of honey, especially in clinical trials, are required to determine whether honey can be used as a preventive measure for dental caries.

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**Conflict of interest:** None declared.

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