

Evaluation of Antimicrobial Action of Honey on Cariogenic Bacteria - An *in vitro* Study

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Abstract :

Introduction:

Dental plaque consists of various pathogenic microorganisms. Since ancient times it has been proved that honey has anti-inflammatory, antimicrobial, antiviral, anti-parasitic, antimutagenic and anti-tumour effect, this study highlights the potential antibacterial properties of honey.

Objective :

The main objective of this study is to know about the antimicrobial role of crude and two processed commercially available honey on cariogenic bacteria.

Method :

The antimicrobial activity of three different honey samples is tested on *Streptococcus mutans* by agar well diffusion technique and the results recorded by measuring the zone of inhibition in mm diameter.

Result :

The result of our present study showed that the crude honey showed more activity than the other two variety of honey compared with the control.

Keywords : Agar well diffusion, honey, *Streptococcus mutans*, inhibition zone, propolis.

INTRODUCTION :

Honey is one of the oldest traditional medicines considered as traditional remedy for microbial infections. In ancient times, honey was considered the food of gods and the symbol of wealth and happiness. Honey was used to treat the infected wounds as long ago as 2000 years before the bacteria were discovered to be the cause of infection. Honey produced by honeybees (*Apis mellifera*) is one of the traditional medicines considered to be important in the treatment of respiratory ailment, gastrointestinal infection and various other diseases. It is been used effectively as a dressing for wound, (including surgical wounds), burns and skin ulcers to reduce pain and odour quickly. The antibacterial properties of honey may be particularly useful against bacteria which have developed resistance to many antibiotics. Honey is thus used as an ideal topical wound dressing agent in surgical infections, burns and wound infections (Betts and Molan, 2002). The use of honey as a medicine has continued into the present-day medicine. 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⁽¹⁾. It can also lower the concentration of prostaglandins in the plasma of normal individuals,⁽²⁾ 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.⁽³⁾

It has been shown that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria. Dental plaque consists of various pathogenic

microorganisms like *Streptococcus mutans*. Since ancient times it has been proved that honey has anti-inflammatory, antimicrobial, antiviral, antiparasitic, antimutagenic and antitumour effect. So, this study was conducted to evaluate the antimicrobial effect of honey on *Streptococcus mutans* after application of honey.

Dental caries and periodontal diseases are chronic diseases of human beings. Both these diseases are initiated by dental plaque. Dental plaque present at localized area on tooth surface can cause dental caries. So, plaque bacteria are responsible for both dental caries and periodontal diseases. Dental plaque consists of various pathogenic microorganisms of which *Streptococcus mutans* is the most pathogenic. *S. mutans* and other cariogenic bacteria are the major etiological agents in dental caries. Factors associated with cariogenicity include adhesion, acidogenicity and acid tolerance.⁽⁴⁾ 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.⁽⁵⁾ Little information is available regarding the inhibitory effect of honey on oral bacteria. The antifungal activity of the honey, especially anti-*Candida* activity (Irish et al., 2006, Koc et al., 2008 and Ahmad et al., 2012) has also been reported. The aim of the present study was to determine the antimicrobial *in vitro* effect of honey on *S. mutans* isolates.

MATERIAL AND METHODS:

1. Honey samples

Three honey samples, one crude and two branded, processed and commercially available (S1, S2 & S3) were

purchased from super market. Samples were stored in dark place at a room temperature (25-35c).

2. Bacterial Strains

Strains of streptococcus mutans were collected from Saveetha Dental College and Hospitals, poonamalle high road, chennai-77.

3. Agar well diffusion method

Broth culture of the test organisms compared to Mac Farland's standard 0.5 were prepared. Lawn culture of the test organisms were made on the Muller-Hinton agar [MHA- M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. Well measuring 4 mm depth was made on the agar with sterile cork borer.

100µl of the three different honey samples were added to the wells. 0.2% of Chlorohexidine was used on the positive control. The plates were incubated overnight and the zone of inhibition of growth was measured in mm diameter. All the test were done in triplicate to minimize the test error.

RESULT AND DISCUSSION:

The study was conducted to assess the antimicrobial effect of honey. A total of three honey samples from different origins were evaluated for their antimicrobial activity against the streptococcus mutans. Streptococcus colony inhibition zone were measured after honey application. The average diameter of the inhibition zones produced by these samples were around 16.2-20.8mm. The growth of bacteria was also inhibited by these honey samples; although to a lesser extent. The inhibition zone for each honey sample were found to be in significant amount around the wells.



The inhibition zone for each honey sample were measured properly.

Sample 1: The inhibition zone was measured as 20.8mm

Sample 2: The inhibition zone was measured as 16.2mm

Sample 3 The inhibition zone was measured as 0.5mm

Based on these results, it was found that sample 1 had maximum inhibition of the streptococcus mutans, and sample 2 had a little lesser inhibiting than sample 1 while

sample 3 had the minimum inhibition of mutans colony.

The antimicrobial properties of honey can be attributed to several factors like high osmotic pressure, low pH. Natural products have recently been demonstrated as an alternative to synthetic substances for prevention of tooth decay.⁽⁶⁻⁸⁾ Honey and Propolis (honey product) have been considered as a candidate for this reason.⁽⁹⁻¹²⁾ 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.⁽¹³⁾ 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.^(14,15) This study also showed that honey had a similar inhibitory effect on *S. mutans*.

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.⁽⁹⁻¹²⁾ The mechanism of the antibacterial effect of honey remains speculative at present. Possible explanations are presence of hydrogen peroxide,⁽¹⁶⁾ flavonoids,⁽¹⁷⁾ and hypertonic sugar concentration.⁽¹⁸⁾ It seems that the carbohydrate content of honey has not a significant inhibitory effect on *S. mutans*.⁽¹⁹⁾

CONCLUSION :

The present study concluded that honey has antimicrobial effect on streptococcus mutans after a definite time interval. Results can be due to production of hydrogen peroxide, inhibition of glucosyltransferase activity or presence of polyphenols in honey. All these factors are responsible for antimicrobial effect of honey on streptococcus mutans. Study findings are important for public health intervention. Results suggest that honey can be used to develop an oral hygiene product such as a tooth-pastes and mouthwashes to prevent dental caries. It can also be used in chewing gum and candies which are most frequently used by children. 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. Another limitation of the present study is that commercially available honey was used for testing the effect on streptococcus mutans. Artificial honey contains various sugars and other constituent which can alter the results of the study. So, further studies are required to be conducted using natural honey.

REFERENCE :

1. Al-Waili NS. Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. *J Med Food* 2003; 6:135-40. [12935325] [doi:10.1089/109662003322233549]
2. Al-Waili NS, Boni NS. Natural honey lowers plasma prostaglandin concentrations in normal individuals. *J Med Food* 2003;6:129-33. [12935324] [doi:10.1089/109662003322233530]
3. Ooshima T, Osaka Y, Sasaki H, Osawa K, Yasuda H, Matsumura M, Sobue S, Matsumoto M. Caries inhibitory activity of cacao bean husk extract in in-vitro and animal experiments. *Arch Oral Biol* 2000; 45:639-45. [10869475] [doi:10.1016/S0003-9969(00)00042-X]
4. Radcliffe CE, Akram NC, Hurrell F, Drucker DB. Effects of nitrite and nitrate on the growth and acidogenicity of *Streptococcus mutans*. *J Dent* 2002;30:325-31. [12554114] [doi:10.1016/S0300-5712(02)00046-5]
5. Leitão DP, Filho AA, Polizello AC, Bastos JK, Spadaro AC. Comparative evaluation of in-vitro effects of Brazilian green propolis and *Baccharis dracunculifolia* extracts on cariogenic factors of *Streptococcus mutans*. *Biol Pharm Bull* 2004;27:1834-9. [15516733] [doi:10.1248/bpb.27.1834]
6. Koo H, Rosalen PL, Cury JA, Ambrosano GM, Murata RM, Yatsuda R, Ikegaki M, Alencar SM, Park YK. Effect of a new variety of *Apis mellifera* propolis on *Streptococcus mutans*. *Curr Microbiol* 2000; 41:192-6. [10915206] [doi:10.1007/s0028400101170]
7. Koo H, Pearson SK, Scott-Anne K, Abranches J, Cury JA, Rosalen PL, Park YK, Marquis RE, Bowen WH. Effects of apigenin and *trans*-farnesol on glucosyltransferase activity, biofilm viability and caries development in rats. *Oral Microbiol Immunol* 2002; 17:337-43. [12485324] [doi:10.1034/j.1399-302X.2002.170602.x]
8. Xiao J, Liu Y, Zuo YL, Li JY, Ye L, Zhou XD. Effects of *Nidus Vespa* extract and chemical fractions on the growth and acidogenicity of oral microorganisms. *Arch Oral Biol* 2006;51:804-13. [16723116] [doi:10.1016/j.archoralbio.2006.03.014]
9. Basson NJ, du Toit II, Grobler SR. Antibacterial action of honey on oral streptococci. *J Dent Assoc S Afr* 1994;49:339-41. [9508952]
10. Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *J Ethnopharmacol* 1999;64:235-40. [10363838] [doi:10.1016/S0378-8741(98)00131-7]
11. Menezes H, Bacci JR M, Oliveria SD, Pagnocca FC. Anti bacterial properties of propolis and products containing propolis from Brazil. *Apidologie* 1997;28:71-6. [doi:10.1051/apido:19970203]
12. Park YK, Koo MH, Abreu JA, Ikegaki M, Cury JA, Rosalen PL. Antimicrobial activity of propolis on oral microorganisms. *Curr Microbiol* 1998;36:24-8. [9405742] [doi:10.1007/s002849900274]
13. Abu-Tarboush HM, Al-Kahtani HA, El-Sarrage MS. Floral-type identification and quality evaluation of some honey types. *Food Chem* 1993; 46:13-17. [doi:10.1016/0308-8146(93)90068-Q]
14. Al-Wailli N, Lootah A, Shaheen W. Mixture of crude honey and olive oil in natural wax to treat chronic skin disorders. *FASEB J* 1999;13:A846.
15. Asadi-Pooya AA, Pnjehshahin MR, Beheshti S. The antimycobacterial effect of honey: an in vitro study. *Riv Biol* 2003;96:491-5. [15055885]
16. White JW JR, Subers MH, Schepartz AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochim Biophys Acta* 1963;73:57-70. [14000328] [doi:10.1016/0006-3002(63)90359-7]
17. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol* 1983;32:1141-8. [6342623] [doi:10.1016/0006-2952(83)90262-9]
18. Mundo MA, Padilla-Zakour OI, Worobo RW. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int J Food Microbiol* 2004; 97:1-8. [15527912] [doi:10.1016/j.ijfoodmicro.2004.03.025]
19. In Vitro Assessment of Anti-*Streptococcus mutans* Potential of Honey. J Ghabanchi, A Bazargani, M Daghigh Afkar, S Balady Foroshan, S Dad Ayeen Department of Oral Medicine, Shiraz Dentistry School, Department of Bacteriology and Virology, Shiraz Medical School, Shiraz Dentistry School, Shiraz University of Medical Sciences, Shiraz, Iran