

A Comparative Evaluation of the Antibacterial Efficacy of Honey In Vitro and Antiplaque Efficacy in a 4-Day Plaque Regrowth Model In Vivo: Preliminary Results

S. Aparna,* S. Srirangarajan,* Veena Malgi,* Krishnanand P. Setlur,† R. Shashidhar,‡ Swati Setty,§ and Srinath Thakur§

Background: Honey has a potent broad-spectrum antibacterial action that may make it suitable for “anti-infective” treatment of periodontal disease. The aims of this study are as follows: 1) to evaluate the antibacterial efficacy of honey against oral bacteria and compare the same with 0.2% chlorhexidine; and 2) to compare antiplaque efficacy in vivo with chlorhexidine.

Methods: The study was conducted in two parts. In the in vitro part, the inhibitory effects of three test agents, 0.2% chlorhexidine gluconate, honey mouthwash, and saline, against six oral bacteria at concentrations of 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 $\mu\text{g/mL}$ were tested in duplicate. The minimum inhibitory concentration (MIC) was set as the lowest concentration of the agent that completely inhibited the growth of the test species. The in vivo part consisted of a double-masked parallel clinical trial based on a 4-day plaque regrowth model. Sixty-six volunteers, 20 to 24 years of age, participated in the study, and the plaque scores were compared at baseline and at the end of 4 days. The Kruskal-Wallis test was used for significance, and the Mann-Whitney U test was used for pairwise comparison of the groups. The mean plaque scores were 1.77 ± 0.86 , 1.64 ± 0.90 , and 3.27 ± 0.83 for groups 1, 2, and 3, respectively.

Results: The honey mouthrinse effectively inhibited the six tested microorganisms. The chlorhexidine gluconate rinse had the lowest MICs compared with honey and saline rinses for all test species examined. The in vivo results revealed that plaque formation was inhibited/reduced by chlorhexidine and honey rinses.

Conclusion: Honey has antibacterial action against tested oral microorganisms and also has antiplaque action. *J Periodontol* 2012;83:1116-1121.

KEY WORDS

Antibacterial agents; chlorhexidine; dental plaque; honey; mouthwashes, minimum inhibitory concentrations.

Periodontitis is an inflammatory disease, associated with pathogenic microorganisms colonizing tooth surfaces in a susceptible host. Understanding that specific oral microorganisms are the cause of periodontitis, antimicrobials have been used to treat periodontitis in the past and at present. Several reviews¹⁻⁴ have concluded that antimicrobial agents as adjuncts to mechanical therapy improve therapeutic outcome. However, the emergence of antibiotic-resistant microorganisms is compelling researchers to look for alternative means to destroy these microorganisms. Although some success has been reported with antibiotic therapy, several limitations have become evident. Most of these limitations are attributable to the fact that periodontal infections result from the formation of a biofilm.⁵

Mouthrinses have been used for centuries for medicinal and cosmetic purposes and have gained popularity worldwide. The advent of mouthrinses containing chlorhexidine was a major breakthrough in the search for a chemical means to prevent periodontal disease. Since then and especially in the past 10 years, the number of formulations that claim to have antiplaque, anticalculus,

* Department of Periodontics, Bangalore Institute of Dental Sciences and Post Graduate Research Centre, Bangalore, India.

† Department of Oral Microbiology and Oral Pathology, Syamala Reddy College of Dental Sciences, Bangalore, India.

‡ Department of Oral Microbiology and Oral Pathology, Coorg Institute of Dental Sciences, Virajpet, India.

§ Department of Periodontics and Oral Implantology, SDM College of Dental Sciences and Hospital, Sattur, Dharwad, India.

and anticaries activity has increased, and much emphasis has been placed on such substances as adjuncts to, or indeed to replace, conventional toothbrushing. Chlorhexidine is unequivocal in its effects on reduction of plaque and gingivitis, but major drawbacks lie in the taste and stain-producing problems.⁶

Complementary and alternative medicine (CAM) encompasses a diverse group of medical treatments, such as acupuncture, aromatherapy, massage therapy, meditation, hydrotherapy, and herbal therapy. The increasing interest in CAM by the public has encouraged dental professionals to investigate the existing science of CAM. An alternative medicine branch called apitherapy offers treatments for many diseases based on honey and other bee products.⁷⁻⁹

The first written reference to honey, a Sumerian tablet writing dating back to 2100 to 2000 B.C., mentions the use of honey as a drug and an ointment. In most ancient cultures, honey has been used for both nutritional and medical purposes.¹⁰ Honeys vary in taste and colors, depending on their botanical origin.¹¹

Honey has been shown to have potential for the treatment of periodontal disease, mouth ulcers, and other problems of oral health.¹² A trial has demonstrated a statistically significant difference between chewing gelled honey and plain chewing gum in decreasing the number of bleeding sites in patients with gingivitis.¹³ The remarkable antibacterial properties, easy availability, and economic feasibility make honey a prospective therapeutic agent. To the best of the authors' knowledge, there have been no studies thus far exploring the antibacterial efficacy of honey against putative periodontopathogens or antiplaque action, making this the first study of its kind aimed at evaluating the potential action of honey in vitro on oral bacteria, including key periodontopathic bacteria, and comparing the antiplaque effect of a honey mouthrinse versus a commercially available chlorhexidine mouthrinse in a 4-day plaque regrowth model in vivo.

MATERIALS AND METHODS

In Vitro Testing for Antibacterial Efficacy

In vitro testing was conducted to determine the efficacy of honey mouthrinse compared with 0.2% chlorhexidine and a negative control of saline as determined by the minimum inhibitory concentration (MIC) against reference strains of six predominant oral bacterial species: 1) *Eubacterium nodatum*, 2) *Streptococcus mutans*, 3) *Campylobacter rectus*, 4) *Streptococcus sanguinis*, 5) *Aggregatibacter actinomycetemcomitans*, and 6) *Porphyromonas gingivalis*.

All MICs were performed in duplicate, and an agar dilution method was used to assess the inhibitory ef-

fect of the three test agents. Serial dilutions of each agent to provide final concentrations of 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 $\mu\text{g/mL}$ were prepared, inoculated, and grown in blood agar plates under an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide at 35°C for 3 days. They were then transferred aseptically and suspended in 1 mL sterile mycoplasma broth. One hundred microliters of each strain suspension were placed in the wells of a sterile microtiter plate and incubated under an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide at 35°C and evaluated daily. The MIC was interpreted as the lowest concentration of the agent that completely inhibited the growth of the test species.

In Vivo Testing for Antiplaque Efficacy

The study was conducted in the Department of Periodontics, Bangalore Institute of Dental Sciences, Bangalore, India. Sixty-six of 70 screened individuals (40 males and 26 females, aged 20 to 24 years) were recruited for the study in October 2010. Written informed consent was obtained from the participants enrolled for the study, and approval was obtained from the Ethical Committee of the Institute. Participants had: 1) ≥ 22 natural teeth, 2) no removable or fixed prostheses or fixed and removable orthodontic appliances, and 3) no more than one full-coverage restoration. Participants were in good systemic health with no medical or pharmacotherapy histories that might influence the conduct of the study. Participants were randomly allocated to three groups and masked to the mouthrinse received. Rinsing preparations were filled in identical but coded bottles. Instruction for usage was written on the bottles. On day 1, the study participants received an oral soft tissue examination and then rendered plaque, calculus, and stain free by a thorough ultrasonic scaling and polishing of the teeth. Volunteers were then asked to refrain from all forms of tooth cleaning and to commence the rinsing regimen (i.e., 10 mL of respective mouthrinse twice daily for 30 seconds). The three test agents included the following: 1) 0.2% chlorhexidine mouthrinse^{||} (group 1); 2) processed honey (group 2); and 3) saline (group 3).

Processed honey was diluted with distilled water to a concentration of 1:1 (1.8 mg/mL honey) and dispensed as mouthrinse by a masked dispenser (SSr). The Turesky Gilmore Glickman modification of the Quigley Hein plaque index (PI)¹⁴ was used to verify the plaque status. All clinical examinations were performed by a single clinician (SA) who was masked to the study. At baseline, all participants had a score of 0, as confirmed by the use of a disclosing agent. On day 5, plaque scores were evaluated using disclosing solution, and results were tabulated.

|| Rexitin, Indoco Remedies, Mumbai, India.

Statistical Analyses

The statistical analysis for assessment of plaque was performed using the Kruskal-Wallis test for overall significance, and pairwise comparison was performed using the Mann-Whitney *U* test.

RESULTS

Table 1 summarizes the MICs with the three test agents.

MIC was determined as the lowest concentration of honey that inhibited the growth of the tested microorganisms. The honey mouthrinse effectively inhibited the growth of the tested microorganisms. Although 0.2% chlorhexidine was the most effective and saline showed no inhibition, the honey mouthrinse proved to be efficacious.

In Vivo Results

Table 2 depicts the results of the PI taken on day 5 of the study. Participants were divided into three groups of 22 each (group 1 = chlorhexidine; group 2 = honey; group 3 = saline). No adverse effects were reported by the participants in any of the groups.

In vivo comparison of antiplaque efficacy revealed chlorhexidine to be the most efficacious, followed by honey mouthrinse. Saline showed the least antiplaque action. Intergroup analysis between chlorhexidine and honey showed that both agents significantly reduced plaque formation. The results were statistically significant at a *P* value of <0.001. The mean plaque scores were 1.77 ± 0.86 , 1.64 ± 0.90 , and 3.27 ± 0.83 for groups 1, 2, and 3, respectively. Comparison between chlorhexidine and honey was not statistically significant (*P* = 0.670); comparison between groups 1 and 3 and between groups 2 and 3 were statistically significant (*P* < 0.001) (Table 2).

DISCUSSION

The multifloral processed honey used in this study is from *Apis mellifera* honey bees species with a pH of

3.84, 29.17 meq/kg acidity, 17.07 moisture content, 0.21 mS/cm electrical conductivity, 8.01% sucrose, 27.29 hydroxymethylfurfural, and diastase number of 14.20.

Honey has been found to be beneficial in treating gingivitis¹³ and oral mucositis in cancer patients undergoing radiation therapy.¹⁵ There is sufficient evidence to prove its effectiveness against various aerobic bacteria¹⁶⁻¹⁹; however, no literature on the use of honey as an antibacterial agent against key periodontopathogens *A. actinomycetemcomitans* and *P. gingivalis* is available to date. Thus, we aimed to evaluate the efficacy of honey against six oral bacteria, including *A. actinomycetemcomitans* and *P. gingivalis*. Subsequently, the MIC was determined for the selected oral bacteria with processed honey, chlorhexidine, and saline as the negative control. A comparative clinical trial was later conducted to evaluate the antiplaque action of the three test agents.

The in vitro results revealed that the tested organisms were susceptible to chlorhexidine and honey. Chlorhexidine gluconate exhibited the lowest MIC compared with honey or saline. Honey was also efficacious against the tested bacteria and particularly against *A. actinomycetemcomitans* and *P. gingivalis*, which are the putative pathogens implicated in periodontitis. The in vivo results revealed that plaque inhibition was the highest with chlorhexidine, followed by the honey rinse. Saline had minimal to no effect on plaque inhibition. Although there was no statistically significant difference between chlorhexidine and honey, it would be presumptuous at this point to say that honey is as efficacious as chlorhexidine in its antiplaque action.

The 4-day experimental period length was chosen because plaque accumulation reaches measurable volumes after 4 to 5 days of no oral hygiene.²⁰ As with other short-term plaque studies^{21,22} involving

Table 1.

MIC of Test Agents Against Oral Microorganisms

Microorganisms Tested	ATCC Reference No.	0.2% Chlorhexidine	Honey	Saline
<i>E. nodatum</i>	33099	1	1:2	—
<i>S. mutans</i>	25175	1:2	32	—
<i>A. actinomycetemcomitans</i>	29523	4	32	—
<i>P. gingivalis</i>	33277	1:16	1:32	—
<i>C. rectus</i>	33238	1:2	1:16	—
<i>S. sanguinis</i>	10556	1:16	1:512	—

ATCC = American Type Culture Collection.

Table 2.
Comparison of Antiplaque Efficacy in Three Groups

PI	Group 1 (n = 22)	Group 2 (n = 22)	Group 3 (n = 22)
No plaque	1 (4.5%)	2 (9.1%)	0
Separate flecks of plaque at the cervical margin of the tooth	7 (31.8%)	8 (36.4%)	1 (4.5%)
A thin, continuous band of plaque (≤ 1 mm) at the cervical margin	11 (50.0%)	8 (36.4%)	2 (9.1%)
A band of plaque wider than 1 mm but covering $\frac{1}{3}$ of the crown	2 (9.1%)	4 (18.2%)	9 (40.9%)
Plaque covering $\geq \frac{1}{3}$ but $\frac{2}{3}$ of the crown	1 (4.5%)	0	10 (45.5%)
Plaque covering $> \frac{2}{3}$ of the crown	0	0	0
Mean \pm SD	1.77 \pm 0.86	1.64 \pm 0.90	3.27 \pm 0.83
Overall significance (Kruskal-Wallis test)		$\chi^2 = 28.096; P < 0.001^*$	
Groups 1 to 2		Z = 0.426; P = 0.670	
Groups 1 to 3		Z = 4.473; P < 0.001*	
Groups 2 to 3		Z = 4.642; P < 0.001*	

Bonferroni-corrected P value for significance is 0.016.

* Pairwise significance by Mann-Whitney U test.

suspension of normal oral hygiene, the 4-day regrowth model provides important information in chemical plaque control methods. This model also enables the removal of the confounding variables, such as the Hawthorne effect, influence of pre-study prophylaxis, and the possible interaction between mouthrinse and toothpaste.^{23,24}

Since the original findings for plaque-inhibitory properties of chlorhexidine, this antiseptic has been used as positive control by which other potential plaque-inhibitory agents or formulations are compared.²⁵

From a professional medical perspective, honey used in wound care has proven antibacterial activity against many important pathogens, such as *Pseudomonas aeruginosa*,²⁶ *Staphylococcus aureus*,²⁷ and herpes simplex types 1 and 2.²⁸ A laboratory demonstration of its antibacterial activity was first performed by Dold et al.²⁹ Adock³⁰ first suggested the possibility that hydrogen peroxide was responsible for the antibacterial activity of honey. Non-dissociated organic acids also play a role in the antimicrobial activity of honey because they are very soluble in cell membranes and induce alterations in the cellular permeability and in oxidative phosphorylation. Honey is also known to contain flavanoids, which arrest bacterial growth.^{31,32}

Honey works differently from antibiotics, which attack the cell wall of the bacteria or inhibit intracellular metabolic pathways. Honey is hygroscopic, meaning

it draws moisture out of the environment and thus dehydrates bacteria. Its sugar content is also high enough to hinder the growth of microbes, but the sugar content alone is not the sole reason for the antibacterial properties of honey. When honey is diluted with water, reducing its high sugar content, it still inhibits the growth of many different bacterial species that cause wound infections.³³⁻³⁷ Thus, we can hypothesize that antibacterial and antiplaque actions of honey may possibly be attributable to the above-mentioned mechanisms.

Another mechanism may be related to the pH level of honey being low, 3.4 to 5.5; bacterial colonization or infection and recalcitrant wound healing situations are often accompanied by pH values > 7.3 in wound exudates.³⁸ It has been demonstrated that acidification of wounds speeds healing and is attributed to the low pH, increasing the amount of oxygen off-loaded from hemoglobin in the capillaries.³⁹ Honey also causes suppression of protease activity in wounds by getting away from the neutral pH that is optimum for the growth of microorganisms.⁴⁰

Cariogenicity and demineralization of tooth surface would be of concern with honey having a high content of fermentable sugars and an acidic pH. However, honey has been found to inhibit growth as well as acid production by cariogenic bacteria.⁴¹ The present study also found that honey inhibits *S. mutans* growth, which is a cariogenic microorganism. The effect of

acidic pH on human tooth enamel in vitro was observed by electron microscopy and microhardness measurements. No erosion of enamel by honey over a period of 30 minutes or deterioration of enamel structure was observed.⁴²

Thus, in summary, honey, although less potent than chlorhexidine, is effective against putative periodontopathogens and also reduces plaque formation. Other properties of honey, such as anti-inflammatory action that reduces edema and the amount of exudate by downregulating the inflammatory process,⁴³⁻⁴⁶ stimulation of tissue growth,^{43,47} and collagen synthesis and angiogenesis,^{31,48} merit investigation from a periodontal perspective. Honey has shown excellent cytocompatibility with healthy tissue cell cultures⁴⁹ and also inhibits fungal growth.⁵⁰ No adverse effects have been reported thus far in the literature.

The varied benefits of honey, such as economic feasibility, easy availability, antibacterial and anti-inflammatory properties, and healing, make honey a potential therapeutic agent in periodontal therapy.

In vitro testing of the antibacterial action against other plaque microorganisms, salivary counts of bacteria, a larger sample population, a commercially standardized formulation of honey mouthrinse, as well as application of honey as a local drug and in regenerative procedures would provide more conclusive evidence of the various properties and possible applications of honey in periodontal therapy.

CONCLUSION

Within the limitations of the study, honey is effective against oral bacteria and reduces plaque formation.

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- Correspondence: Dr. S. Aparna, Department of Periodontics, Bangalore Institute of Dental Sciences and Post Graduate Research Centre, Bangalore 560029, India. Fax: 080-41506025; e-mail: aparna_bds@yahoo.co.in.
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