Honey as a Potential Antimicrobial Agent against *P. gingivalis*

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ABSTRACT

Introduction: Periodontitis is a chronic inflammation that occurs in response to the presence of sub gingival bacteria. *Porphyromonas gingivalis*, an anaerobic gram-negative bacterium plays crucial role in the pathogenesis; hence the anti-infective regimen is an essential part of therapy. Development of resistance against antibiotics implicates search for alternatives. Honey is a known ancient remedy which can be an alternative to the commonly used antimicrobials in periodontitis treatment. This in-vitro study was aimed to determine the antimicrobial efficacy of honey.

Material and methods: One processed (commercially available) honey sample and one unprocessed (collected from native honey comb) sample was selected for the study. These samples were tested against *P. gingivalis* strains and assessed for their anti bacterial potency using Minimum Inhibitory Concentration (MIC). Once the anti-microbial property was determined, the concentrations at which the samples are most effective were analyzed by Disc diffusion test.

Results: MIC showed *P. gingivalis* strains resistant to unprocessed honey till the concentration 0.8 µg/ml. As the concentration increased to 100 µg/ml, the strains were sensitive to unprocessed honey. Processed honey was resistant to all the strains of *P. gingivalis*. Unprocessed honey showed resistance to the strains at 5 µg/ml and 10 µg/ml. Zones of inhibition were seen at concentrations of 25, 50 and 75 µg/ml for both.

Conclusion: Honey acts as growth inhibitory on P. gingivalis as a major periodontopathogen. Therefore an addition of honey or its compounds to oral health-care products may have potential in prevention and treatment of periodontitis.

Keywords: Antibacterial, Disc diffusion, Honey, MIC, *P.gingivalis*

INTRODUCTION

Human kind has been the victim of periodontal disease since ages. Various complex micro-organisms have been associated with periodontitis. The progress of this destructive form of ailment seems to be as a result of specific infection.

According to the Consensus report of the World Workshop on Clinical Periodontics (1996), human periodontitis is considered to be initiated by a small group of bacteria that gain colonization in the sub gingival region, namely gram-negative, anaerobic or microaerophilic bacteria. It was further deduced that most cases of periodontal disease are caused by *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans*.[1]

Periodontitis is an infectious disease where it is practically impossible to target and eliminate all the associated micro-organisms by mechanical means. Additionally, it has to be taken into consideration that not all patients respond to conventional mechanical therapy. Hence, the need to prescribe antimicrobial agents as an adjuvant to periodontal treatment arises. Development of resistance against antibiotics and side effects of the drugs implicate search for alternatives.

From the age of Charaka, honey is considered as an efficient wound therapy and was brought back into modern medical therapy because of its antimicrobial and wound-healing promoting efficacy.[2] Honey derives its anti- infective properties from its potency to fight broad-spectrum bacteria. It acts by clearing the suggested wound in minimum time on topical application. As periodontitis has a varied microbial etiology, honey can prove a beneficial anti-bacterial agent for treatment.

Honey is a natural derivative of nectar of a wide array of plants and bulbs that is collected by bees. It is a form a saturated or supersaturated solution of sugars that is prepared by loss of water from the nectar collected. Its constitution is 17% water, 38% fructose, 31% glucose, 10% other sugars, and a wide range of micronutrients like vitamins, amino acids and minerals, with a pH of below 4. The honey bees add a few enzymes in the process of ripening of the nectar, namely invertase and glucose oxidase. Invertase enzyme converts the sucrose found in the nectar into glucose and fructose. Hence, the quantity of sucrose content in honey is approximately only 1% of the total sugar content. The second enzyme, glucose oxidase forms gluconic acid and hydrogen peroxide from the residual glucose.[3] This enzyme is in an inactive state in ripened honey. It gets reactivated to its full potency when honey is diluted thus imparting the essential antibacterial property to honey.[4] Miniscule levels of hydrogen peroxide produced also suffice enough to make honey quite potently antibacterial.

In patients with gingivitis and plaque, the Manuka honey was able to reduce bleeding and the amount of plaque. The mechanism of action was projected to be inhibition of polysaccharide formation during plaque maturation.[6] Hence, the aim of this study was to determine the effect of two types of honey (processed and unprocessed) for their antimicrobial property on P. gingivalis strains.

Honey is also considered as an efficient immune modulatory agent by stimulating release of pro-inflammatory cytokines from various cells like monocytes. It is proposed to up regulate mRNA expression of TGF-β which is an important cytokine that promotes wound healing and repair.[7]

MATERIAL AND METHODS

It was conducted by the department of Periodontology in

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MGV, KBH Dental College and Hospital, Nashik during the time period of November 2015 to December 2015. Samples of the honey were labeled as Test group 1 (unprocessed honey) and Test group 2 (processed honey) and sent to the Department of Oral Microbiology, (Maratha mandal dental college, Belgaum) for evaluation.

***Porphyromonas gingivalis* strains**

P. gingivalis strains were included in the following experiments from the bank of strains in the Laboratory of Oral Microbiology (Maratha mandal dental college, Belgaum). It was used to determine the Minimal Inhibitory Concentration (MIC) values of the processed and unprocessed honey test groups. Two laboratory strains (ATCC 33277, W83) were included in the study which were frozen in the strain bank and used for the analysis. They were transferred and an anaerobic culture was made in 10% H_2, 5% CO_2, and 85% N_2 and 8% sheep blood on Schaedler agar plates for 24 hours. Experiments were conducted at room temperature.

Honey samples

A local (Maharashtra, India) multifloral blossoms honey from a beekeeper and a processed honey (Dabur pharmaceuticals, India) were selected for the experiments.

Disc Diffusion Procedure

Brain Heart Infusion agar was chosen as a media. Agar plates were brought to room temperature before use. Using a loop or swab, colonies were transferred to the plates. Turbidity was adjusted visually with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Within 15 minutes, a sterile cotton swab was dipped into the inoculum. It was then rotated above the liquids against the wall of the tube in order to remove excess inoculum. The overall surface of agar plate was swabbed three times, rotating plates approximately 60º between streaking to ensure even distribution. Inoculated plate was consequently allowed to stand for 3 minutes at the least but no longer than 15 min before making wells. A 5mm diameter hollow tube was heated. It was pressed above inoculated agar plate prepared and removed immediately, thus making an impression of a well in the plate.

Similarly, five wells of different concentrations were made on each plate. 75µl, 50 µl, 25 µl, 10 µl and 5 µl of compound were added into the respective wells on each plate. Plates had to be incubated within 15 min of application of the sample, inverted and stacked no more than five high. They were incubated for 18-24 hours at 37 ºC in incubator. Diameter of inhibition zone was measured to nearest whole millimeter by holding the measuring device.

Minimum Inhibitory Concentration (MIC) procedure

9 dilutions of both honeys were done with BHI (brain heart infusion) for MIC separately. 380microliter of BHI broth was taken in the initial tube and 20microliter of honey was added into it. For further dilutions, 200microliter of BHI broth was added into successive 9 tubes separately. The initial tube was taken and 200microliter was transferred to the first successive tube that contained 200microliter of BHI broth. This was considered as 10^{-1} dilution for the concerned sample in this experiment. From 10^{-1} diluted tube, 200microliter was transferred to second tube which was considered as 10^{-2} dilution. This serial dilution was then repeated up to 10^{-9} dilution for each sample of both the test groups. 5microliter of the stock cultures of the micro-organism was then taken to be added into 2ml of BHI broth. In each serially diluted successive tube, 200 microliter of above culture suspension was added. The tubes were underwent incubation for 24 hours and were observed for turbidity.

STATISTICAL ANALYSIS

The results obtained for minimum inhibitory concentration (MIC) were evaluated by observation and quantitative percentage analysis. Disc diffusion test results of zones of inhibitions were measured using a manual caliper with accurate calibrations.

RESULTS

MIC Results

P. gingivalis strains were found resistant to unprocessed honey till the concentrations of 0.8 µg/ml. As the concentration increased till 100 µg/ml, the strains were sensitive to unprocessed honey. It was found that processed honey was resistant to all the strains of *P. gingivalis* as illustrated in Table-1. Figure 1a and 1b shows the different concentration at which the test was done.

Disc diffusion Results

Unprocessed honey showed resistance to the strains at 5 µg/ml and 10 µg/ml. zones of inhibition were seen at concentrations of 25, 50 and 75 µg/ml as shown in Table-2. Processed honey too showed zones of

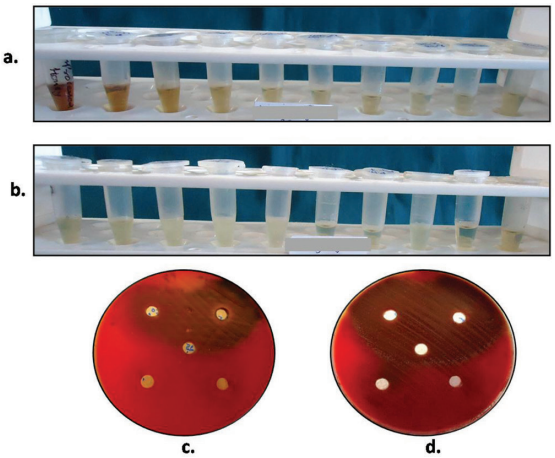

Figure-1: (a). Minimum inhibitory concentration of unprocessed honey, turbidity was seen at the concentration of 1.6µg/ml. Turbidity increased with serial dilutions till 100 µg/l; **(b)**. Minimum inhibitory concentration of processed honey was 6.25 µg/ml. Turbidity increased with serial dilutions till 100 µg/l **(c,d)** Disc diffusion results of unprocessed honey and processed honey respectively

Table-1: MIC results

P. gingivalis	100 ug/ml	50 ug/ml	25 ug/ml	12.5 ug/ml	6.25 ug/ml	3.12 ug/ml	1.6 ug/ml	0.8 ug/ml	0.4 ug/ml	0.2 ug/ml
Unprocessed Honey	S	S	S	S	S	S	S	R	R	R
Processed Honey	R	R	R	R	R	R	R	R	R	R

P.gingivalis	75 ug/ml	50 ug/ml	25 ug/ml	10 ug/ml	5 ug/ml
Unprocessed Honey	14mm	12mm	10mm	R	R
Processed Honey	16mm	12mm	10mm	R	R

Table-2: Disc diffusion results

inhibition at the same concentrations.

DISCUSSION

The effectiveness of honey as a therapeutic agent has been unequivocally demonstrated in previous studies against both gram positive and gram negative rods as well as cocci, in yeasts and fungi.[8] It suggests that honey has a broad spectrum of antimicrobial activity. The present study explains the effect of honey on one of the most important periopathogen *Porphyromonas gingivalis.* The results of this study are in tandem with previous studies on *P. gingivalis*[9] which essentially prove honey can be used against *P.gingivalis* in an in-vitro set-up.

In a study against the strains of *Pseudonmonas* collected from an infected wound, the mean value of MIC was found to be 6.9% (v/v) with a range of 5.5% to 8.7% for the unprocessed honey derived from a local beekeeper and 7.1% (v/v) (range 5.8% to 9.0%) for the honey derived from other species of honeybees.[10] A similar study with a variety of clinically isolated strains of *S. aureus* found that MIC was calculated between 2% and 3% (v/v) for the local unprocessed honey and 3% and 4% (v/v) for the a purified version of the same honey. Several enterobacteriacae and staphylococci growth was inhibited by a minimum concentration of 10-50 %. Despite the use of different kinds of honey, all types showed only low differences in antimicrobial efficacy.

Due to the changing concepts of host-microbial interaction, the genetic constituency of microorganisms and the resistance against antibiotics, there was a failure to treat the infestation of these pathogens in the oral cavity. Research has been undertaken to decipher the etiopathology of the diseases in the past several years and is still going on. In the current study, the use of low concentrations like 1.6 mg/l of unprocessed honey had a high potency antibacterial activity for periodontopathogen *P. gingivalis*, which would be generally useful in a clinical situation in the etiotrophic phase of the periodontal treatment plan.

Honey acts antibacterial against P. gingivalis strains, the effect is being more pronounced for the unprocessed honey compared to a processed honey. The MIC of honey was found within the range of 1.8% -10.8%, i.e. the honey had sufficient antibacterial potency to still be able to inhibit bacterial growth if diluted successively for at least nine times. The antibacterial efficacy for other species was found where 10 – 50% honey was growth inhibitory against several enterobacteriacae and staphylococci.[11] Up to 56 times dilutions were seen effective for *Staphylococcus aureus* which is the most common pathogen found in infected wounds. Different kinds of honey by processing the varieties by a professional showed only low differences in the anti-infective efficacy.[12] The obtained MIC values in this study are in the range of this study for processed and unprocessed honey.

In a study, MICs of values 12.5 - 25% were found against oral streptococci which are one of the abundant microorganisms in the oral cavity.[13] Another study concluded 0.1% growth-inhibitory against oral streptococci and anaerobe.[14] This study focused basically on periodontopathogens like *P. gingivalis* and found similar results.

A potential antimicrobial compound in the honey is the sugar which exerts no or limited antibacterial effect. Antibiotics follow the mechanism of action of attacking the cell wall of the bacteria or inhibition of intracellular metabolic pathways. Honey is draws moisture out of the environment and is thus hygroscopic. This property dehydrates the bacteria, making it void of all the water required for its survival. It has a high sugar content that helps in hindering the growth of microbes. Additionally, when honey is diluted with water in specific concentrations, its high sugar content is reduced but it still inhibits the growth of many different bacterial species that are responsible for wound infections.[15-17]

Another mechanism for the anti-bacterial effect may be related to the pH level of honey being as low as 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. It has been exhibited that the low pH increases the amount of oxygen off-loaded from hemoglobin in the capillaries near the wounds. It acidifies the wounds which in turn speeds the healing process.[18] Low pH also causes suppression of protease activity in wounds by moving away from the neutral pH which is optimum for the growth of pathogenic microorganisms.

Cariogenicity and demineralization of tooth surface would be of concern with honey having a high content of fermentable sugars and an acidic pH. However, it has been found to reduce the growth as well as acid production by cariogenic bacteria. A study also found that honey inhibits S. *mutans* growth, which is a cariogenic microorganism.[19] The effect of acidic pH on human tooth enamel in vitro has been observed by electron microscopy and microhardness measurements. Honey does not cause any erosion of enamel over a period of 30 minutes or deterioration of enamel structure was observed.[20]

In the application of the findings of this study in a clinical analysis, studies have shown that the anti-bacterial potency of honey is sustained when it comes in contact with saliva. Chewable form of delivery of honey extracts were found effective against *S. mutans*, *P. gingivalis,* and *L. acidophilus.*[21] Hence, further scope for research in preventive treatment of periodontitis can be explored using the results of this study.

CONCLUSION

Honey has proved to have a inhibitory effect on the growth of *P.gingivalis* in-vitro. As many other periodontopathogens are collectively responsible for the pathogenesis of periodontitis, further studies and research can be carried out for the evaluation of the efficacy of honey and its compounds. Inclusion of honey or its components and derivatives as natural products can be done in mouth rinses, tooth pastes as a beneficial and preventive treatment option for periodontal diseases.

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