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Comparative Evaluation of Anti-Microbial Efficacy of Manuka Honey and Chlorhexidine on Red, Orange and Green Complex of Periodontal Pathogens – An *In-Vitro* Study

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Research Article

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ABSTRACT

Aim: To comparatively evaluate the antimicrobial efficacy of manuka honey on gram negative periodontal pathogens of Red, Orange and Green Complex (*Porphyromonas Gingivalis* (P.G), *Prevotella Intermedia* (P.I), *Forshythus Nucleatum* (F.N), *Aggregatibacter Actinomyecetemcommitans* (A.A) in *in-vitro* conditions.

Materials and Methods: Nutrient agar plates were inoculated by rubbing sterile cotton swabs dipped into bacterial suspensions of *P. Gingivalis*, *P. Intermedia*, *F. Nucleatum*, *A. Actinomyecetemcommitans* (over night cultures grown at 37°C on nutrient agar) over the entire surface of the plate. After inoculation, 10 mm diameter five wells were cut into the surface of the agar using a sterile cork borer for each sample. Honey and chlorhexidine, was added into wells in four different plates containing above mentioned four different bacterias. Plates were then incubated at 37°C for 24 hours. The diameter of zones of inhibition were measured using a Vernier calipers on all the plates. The mean score of zones of inhibition was calculated. Similar procedure for all different concentration of manuka honey and chlorhexidine was performed.

Results: 0.2% chlorhexidine had edge over the manuka honey in inhibiting the growth of micro-organisms. *Aggregatibacter actinomyecetemcomitans* showed the greatest zone of inhibition by manuka honey of 18 mm as compared to chlorhexidine getting 20 mm. Manuka honey is most effective against *A. Actinomyecetemcommitans* followed by *F. Nuecleatum*, *P. Gingivalis*, *P. Intermedia* as compared with 0.2 % chlorhexidine.

Conclusion: The new horizons in the field of chemical agents that can be used as an adjunct to mechanical periodontal therapy can be explored and compared with 0.2% Chlorhexidine.

INTRODUCTION

In ancient Egyptian text honey was shown to be beneficial as natural dietary substance which provides health benefits. In acute or chronic disease stage, many investigators have studied effect of honey on various micro-organisms and on wound healing. Interest amongst biomedical researchers is increasing in honey due to its anti-oxidative, antimicrobial properties and safety. A recent studies showed molecules of honey increases release of TNF α , IL-6, IL-1- β and TGF β through stimulation of peripheral monocytes which promotes wound healing^[1].

Honey neutralized bacterial ammonia production by releasing oxygen from haemoglobin in damaged tissue. High osmolar honey forms hydrogen peroxide through absorbing water out from the surrounding bacteria in which glucose oxidase is activated in more diluted honey^[2].

Till date many researchers have reported various remedial use of mauka honey both *in vitro* and *in vivo*. It has been confirmed that honey has wide range of activity against various micro-organisms. Newly determined honey may have slight edge over manuka honey due to its augmented antimicrobial activity.

Periodontal diseases are considered to be caused by specific micro-organism as suggested in specific plaque hypothesis [3] Socransky et al. showed that there are few groups of micro-organisms which cause severe periodontal infections, they classified these micro-organisms into colour coded complexes out of which Orange and Red Complex were the most destructive for the periodontal tissues [4].

Some important pathogens in the Orange Complex are *Prevotella Intermedia*, *Fusobacterium Nucleatum*, *Camphylobacter Rectus*, *Camphylobacter Showae*. Whereas, Red complex consists of *Poryromonas Gingivalis*, *Teneralla forshythus*, *Teneralla Denticola* [5]. It would be of great interest of the current trend in periodontal treatment modality if manuka honey with its nutraceutical properties can act in inhibition or even better, destruction of the periodontal pathogens. Before including Manuka Honey in periodontal treatment modalities, it becomes mandatory to evaluate its anti-bacterial efficacy for periodontal pathogens and to compare the same with established local anti-microbial used that is 0.2% chlorhexidine.

The present study was planned to evaluate effect of manuka honey on orange complex microorganisms.

MATERIAL AND METHODOLOGY

Preparation of microbial colonies

Sterile cotton swabs were dipped into bacterial suspension of *Porphyromonas Gingivalis*, *Prevotella Intermedia*, *Fusobacterium Nucleatum*, *Aggregatibacter* which were rubbed to inoculate sterilized nutrient agar plates.

The micro-organisms used were cultured and made available from the strains present with the institute. Strict precautions were followed in order to avoid any contamination of the surrounding area. The inoculation of the plates was performed in the laminar air flow unit. The strains were allowed to grow in strict anaerobic condition. The plates were allowed to culture over night at 37 °C.

Disc diffusion method and measurement of zone of inhibition

After inoculation, five wells with 10 mm diameter were cut into the surface of the agar using a sterile cork borer for each sample. Manuka Honey and 0.2% chlorhexidine, were added into wells in four different plates containing above mentioned four different bacteria. Plates were then again incubated at 37 °C for 24 hours. The diameters of zones of inhibition were measured using Digital Vernier callipers of all the plates. The mean score of zones of inhibition was calculated.

Preparation of different concentration of mauka honey

The concentration of manuka honey was obtained by Volume by Volume dilution. 100% manuka honey had 1 ml of manuka honey. 75% manuka honey had 0.75 ml manuka honey and 0.25 ml distilled water. 50% manuka honey had 0.50 ml of manuka honey and 0.50 ml of distilled water. Similarly 25% manuka honey had 0.25 ml of manuka honey and 0.75 ml of distilled water. 12.5% manuka honey had 0.125 ml of manuka honey and 0.875 ml of distilled water.

RESULTS

100% mauka honey shows comparable ZOI for all four evaluated micro-organisms. In reduced concentration effect decrease and the lowest concentration (12.5%) shows effect against only *F. nucleatum* (ZOI is 8 mm).

Manuka honey showed most equivalent zone of inhibition against *Aggregatibacter Acetenomyecetemcommitans* as compared to chlorhexidine. Manuka honey showed 18 mm of zone of inhibition and compared to 20 mm of 0.2% chlorhexidine. 75%, 50% and 25% manuka honey wells showed 11 mm, 10 mm and 08 mm respectively. *Aggregatibacter acetenomyecetemcommitans* were resistant against 12.5% manuka honey (Table 1).

Table 1. Comparison of zone of inhibition by manuka honey and chlorhexidine on various periodontal pathogens (R = Resistant).

Organisms	MANUKA HONEY					Chlorhexidine (0.2%)
	100%	75%	50%	25%	12.50%	
<i>P.Gingivalis</i>	13 mm	10 mm	08 mm	R	R	18 mm
<i>P. Intermedia</i>	12 mm	08 mm	08 mm	R	R	20 mm
<i>A. Actinomyecetemcomitans</i>	18 mm	11 mm	10 mm	08 mm	R	20 mm
<i>F. Nucleatum</i>	14 mm	12 mm	12 mm	10 mm	08 mm	20 mm

DISCUSSION

The basic reason behind antimicrobial property might be due to the osmotic effect that is; hygroscopic nature of manuka honey pulls out the water content of the microorganism which leads to death of the microorganism. The acidic Ph of manuka honey also acts as a bacteriocidal property against microbes, but the most important property of manuka honey can be said to

be production of hydrogen peroxide, to which microorganisms are very sensitive and which leads to the cell death of the microbes. This study was undertaken to investigate *in vitro* antimicrobial activity of manuka honey against certain microbial isolates like *Aggregatibacter actenomyecetamcommitans*, *Porphyromonas gingivalis*, *Prevotella Intermedia*, *Fusobacterium nucleatum*.

The consensus reports of American academy of periodontology proved and suggested that the micro-organisms responsible for the periodontitis as follows *Aggregatibacter actenomyecetamcommitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* [6]. According to specific plaque hypothesis, by eliminating above mentioned species, periodontal disease would regress [7]. Thus periodontal health of the individual can be established with the help of the agents like manuka honey along with the use of mechanical plaque control therapy. Even after surgical periodontal therapy manuka honey can be used for application on the wound. *In-vitro* as well as *in vivo* studies has showed microbes inhibition and better wound healing as compared to placebo. Manuka honey has shown promising results in faster healing and less complications in healing in the studies which has compared manuka honey with other variety of honey [8].

A study compared manuka oil obtained from honey, tea tree oil, lavandula oil, romarilus oil and eucalyptus oil *in vitro* against periodontopathic bacterias. The results showed significant bactericidal as well as adhesion inhibiting activity of manuka oil against periodontopathic bacterias like *Aggregatibacter actenomyecetamcommitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* [9].

Through stimulation of monocytes Manuka honey promote synthesis of cytokines that have the potential to modulate the immune response. Neutrophils and macrophage mediate the activity of monocytes and platelets by employing free radicals such as superoxide and hydroxyl and thus respond to challenges such as infected wounds. This leads to production of specific cytokines which signal activation of other cells. In chronically infected wound such stimuli give rise to the exaggerated response and ability to dampen free radicals which can contribute to the complicated interaction to resolve chronic inflammatory state illustrating these wounds [8].

Other natural products like Neem tree extracts, Tea tree oil, eucalyptus oil are also tested against periodontopathic bacterias along with manuka honey both in *in-vitro* and *in-vivo* studies [10].

A study showed that the amount of chlorhexidine digluconate required to achieve an equivalent growth inhibition against the biofilm cultures was reduced 4–10-fold in combination with manuka honey as compared with chlorhexidine digluconate alone [11].

To arrest the progression of the periodontal disease, the periodontal pathogens must be curbed first [12,13]. The efficacy of 0.2% chlorhexidine is well established in inhibition of periodontopathic bacterias, till date it serves as gold standard in chemical plaque control along with the mechanical periodontal therapy [14]. Chlorhexidine is also used as prophylactic mouthwash, gel and spray in different concentrations before and after periodontal surgery. The chlorhexidine mouthwash also plays a pivotal role in the non-surgical periodontal therapy.

Manuka honey also over comes the disadvantage of development of resistance to various pathogens however it has to be tested in the clinical as well as microbial studies. Activity of chlorhexidine is dose-dependent, which entails the clinician to increase the dosage of chlorhexidine with time of usage.

However, as manuka honey is a natural product it can be used for long duration, whereas chlorhexidine cannot due to its various disadvantages like staining, bilateral parotid gland swelling, altered taste sensation, increased supragingival calculus formation.

Manuka honey when compared with chlorhexidine could not show better inhibition of periodontopathic microbial species. However, it was effective in inhibiting the growth of the pathogens.

Manuka honey can be used for the periodontal treatment in various forms like mouthwash, gel and even direct application can be done. Manuka honey can be used as adjunct to use of chlorhexidine in the treatment of periodontal disease. It can be used as Pre-emptive as well as prophylactic medication.

There may be few improvements made in this study like inclusion of more periodontopathic bacterias, minimum inhibitory concentration can also be found for the different periodontopathic bacterias.

Clinical study may also be conducted by direct application of manuka honey as a massaging agent at different concentrations and compared with 0.2% chlorhexidine. A mouthwash or gel can be formulated to test the efficacy of manuka honey against periodontal pathogens as compared with commercially available chlorhexidine mouthwash and gels.

Manuka honey can also be used as local drug delivery agent in non-surgical periodontal therapy by direct placement of manuka honey in the periodontal pocket. Its results can be compared with the commercially available chlorhexidine gels which can be used as local drug delivery agents.

CONCLUSION

Based on our present findings we can conclude that mauka honey does indeed possess antimicrobial activity against the

periodontal pathogens *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*, however its ideal concentration and clinical efficacy need to be addressed in future clinical trials of short and long duration, along with its action on the periodontal biofilm. Thus, from this study a wide plethora of new dimension in nonsurgical periodontal therapy can open up, if manuka honey is used as adjunct to nonsurgical periodontal therapy.

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