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Original Article

Dentistry Section

Comparison of the Antibacterial Efficacy of Manuka Honey Against *E.faecalis* and *E.coli* – An In vitro Study

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ABSTRACT

Aim: To compare the antibacterial efficacy of Manuka honey against *E.faecalis* and *E.coli*.

Materials and Methods: *Escherichia coli* (ATCC-25922) and *Enterococcus faecalis* (ATCC-29212) were separately inoculated in the nutrient broth and incubated at 37°C for 24-48 hrs. Bacterial samples were kept in contact with each disinfectant solution for varying intervals of time. Once the test time had elapsed 10µL of the bacterial dilutions were plated on Mueller–Hinton agar and incubated for 24-48 hrs at 37°C to estimate the density. Study of the disinfection process with respect to time and Modeling was done.

Results: The mean value of the antimicrobial activity of Manuka honey against *E.coli* and *E.faecalis* are 1.55 and 0.36 respectively and are relatively higher. It shows that there is a significant difference among the various root canal disinfectant groups against *E. coli* and *E. feacalis*. ($p<0.001$)

Conclusion: Manuka honey is shown to be a potential root canal disinfectant against gram positive and gram negative bacterial pathogens.

Keywords: Antibacterial efficacy, Death kinetics, Manuka honey

INTRODUCTION

During the past 20yrs endodontics has began to appreciate critically the important role of irrigation in successful endodontic treatment. The objective of the endodontic treatment is to remove the infection within the root canal. Over the years research and clinical practices have concentrated on instrumentation, irrigation, medication of root canal system followed by filling of the root canal space, it's truly said 'Instruments shape, irrigants clean'. Following cleaning and shaping of the infected root canal the number of bacteria are reduced, but instrumentation only cannot clean all the surfaces of root canal. Bacteria can be found on the root canal walls and lateral canals within the dentinal tubules. Antibacterial disinfectants and intracanal medicaments are needed to kill the remaining microorganisms [1].

History of honey in medicine is related to stoneage paintings in several locations dating 6000 BC. It is indicated that honey has a role as therapeutic agent in oral disease [2]. It has been often assumed that it is entirely due to osmotic effect of its high sugar control. The fact that the antibacterial properties of honey were increased when diluted [3]. Manuka honey is a monofloral honey obtained from the species Leptospermumscorpium and has a long standing reputation in New Zealand for its antiseptic property [4].

Hence, the present study was to compare the antibacterial efficacy of Manuka honey against *E.faecalis* and *E.coli* with some of the routine root canal disinfectants used in endodontics. They are Calcium Hydroxide ($Ca(OH)_2$), 5.25% Sodium Hypochlorite (NaOCl), 2% Chlorhexidine (CHX), 0.2% Chlorhexidine and Saline.

MATERIALS AND METHODS

Direct Contact Test (DCT)

Nutrient broth was prepared and sterilized. *Escherichia coli* (ATCC-25922) and *Enterococcus faecalis* (ATCC-29212) were inoculated separately and incubated at 37°C for 24-48 hrs.

One milliliter (mL) of the bacterial suspensions (10^9CFU/mL–1) was centrifuged in micro tubes. Routinely used root canal disinfectants 5.25% NaOCl, Net Manuka honey, 2% CHX, 0.2% CHX, $Ca(OH)_2$, Honey 1:2 dilution, Honey 1:4 dilution, Saline were added to the sediment (pellet), vortexed and stored for different time intervals before 50 µL aquilots were transferred to 5mL of Nutrient broth. Bacterial samples were kept in contact with each disinfectant solution for varying intervals of time; Group1-Immediately, Group2-5 min, Group 3-10 min, Group 4-15 min, Group 5-20 min, Group 6-25 min, Group 7-30 min. Once the test time had elapsed 10 µL of the bacterial dilutions were plated on Mueller–Hinton agar and incubated for 24-48 hrs at 37°C to estimate the density. All tests were repeated 10 times for each irrigating solution. Each irrigant solution without the bacterial strains was kept as negative control.

Modelling equation of Disinfectants

$dx/dt = -K_d x$

dx/dt=Death rate

X=microbial density

K_d =death rate constant

RESULTS

Direct contact test of *E.coli* and *E. faecalis* with various root canal disinfectants were tabulated in [Table/Fig-1,2].

Death kinetics analysis of *E. coli* and *E. faecalis* showed reduction in the density of pathogens at various time intervals. The observation data from various time intervals shows that 5.25% NaOCl, 2% CHX, 0.2% CHX, Net Manuka honey are bactericidal while $Ca(OH)_2$, Honey 1:2 dilution, Honey 1:4 dilution are bacteriostatic based on the death rate constant values.

Mean and Standard deviation were estimated from the sample for each study group. The mean values were compared by one-way ANOVA. In the present study, the level of significance was set at p = 0.05. The obtained p-value is less than 0.001and there was a significant difference among the groups.

Groups	N	Mean	SD	p-value
1	1	0.00	0.00	p<0.001
2	3	1.07	0.01	p<0.001
3	3	1.59	0.01	p<0.001
4	3	0.48	0.01	p<0.001
5	3	1.55	0.01	p<0.001
6	3	1.04	0.01	p<0.001
7	3	0.67	0.58	p<0.001
Total	19	1.01	0.51	p<0.001

[Table/Fig-1]: Effect of root canal disinfectants on *E.coli*

Groups	N	Mean	SD	p-value
1	3	1.00	0.200	p<0.001
2	3	0.49	0.025	p<0.001
3	3	0.53	0.051	p<0.001
4	3	0.12	0.020	p<0.001
5	3	0.36	0.020	p<0.001
6	3	0.18	0.026	p<0.001
7	3	0.95	0.020	p<0.001
Total	21	0.52	0.032	p<0.001

[Table/Fig-2]: Effect of root canal disinfectants on *E.faecalis*

DISCUSSION

Direct contact test was performed in this study to quantitatively measure the effect of physical direct contact between the tested material and bacteria [5].The microorganisms used in this study were facultative bacteria, which are predominant in persistent periapical lesions of teeth subjected to periapical surgery. *E.faecalis* is a robust microorganism that may infect root canal and are more likely to be found in root canal failure cases than in cases of primary infection. *E.coli* is sometimes recovered from root canals and represents a standard organism used in antimicrobial testing [6,7].

5.25%NaOCl, 2% CHX, 0.2% CHX are widely used in endodontics as root canal irrigants at different concentrations, but some of them are causing irritation to the periapical tissues. To overcome the side effects of root canal irrigants like sodium hypochlorite, chlorhexidine previous studies have investigated the effects of natural compounds mainly extracted from teas, cranberrie or propolis for antimicrobial purpose [8,9].

The antibacterial properties of honey have recently reviewed in different aspects. In ripe honey the glucose oxidase is inactive and it contains less peroxide amount which is not sufficient to inhibit bacterial growth. When it is heated and diluted peroxide is formed which is having antibacterial property. The non peroxide antibacterial activity is not sensitive to heat and light and remains intact after storage of honey for longer periods. Sugar is the main constituent of honey. The osmotic effect of these sugars exerts an antimicrobial action. Honey contains an enzyme lysozyme, which is a well known antibacterial agent [10]. The antibacterial flavanoid pinocembrim is present in honey, but its concentration and contribution to honey's non peroxide antibacterial activity is small. In New Zealand Manuka honey, several aromatic acids with antibacterial activity have been isolated [11]. Another investigation claimed, the low ph and high osmolarity of honey was responsible for the antibacterial activity [12].

In some studies, they have isolated volatile substances with antibacterial activity [13,14] but their quantitative contribution to the antibacterial action of honey was not examined. Some authors postulated that non peroxide activity of honey is by organic solvents, but the exact chemical nature of these substances is not known [15,16]. The chemical identity, the quantitative contribution and origin of the different honey antimicrobial substances remain to a greater extent unknown [17].

Dold and Witzenhainen coined the term inhibine number to describe the degree of dilution to which the honey will retain its antibacterial activity.

The present work proved that Manuka honey was active against gram positive and gram negative bacterial pathogens. Besides hydrogen peroxide, which is produced on dilution in most "conventional" honeys by the endogenous enzyme glucoseoxidase several other "non peroxide factors" were discussed to be responsible for the unique antibacterial activity of Manuka honey on dilution but the chemistry beyond the phenomenon remained unclear for decades. Nevertheless, the so called "unique manuka factor" (UMF) was introduced some years ago for marketing purposes, leading to the classification of premium products based on microbiological assays. A UMF of 10 used in this study has the same antibacterial activity to a 10% solution of phenol (Allen et al., 1991).

Bilal et al., found that honey exhibited good antimicrobial activity against gram positive and gram negative bacteria [18]. Raied T ahe-Al-Naama reported that 100% honey concentration showed higher zone of microbial inhibition when compared to that of 50% honey concentration [19].

In a study conducted by Litik Mittal et al., 50% and 100% honey concentrations showed greater antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25853 and least antimicrobial activity against *Pseudomonas aeriginosa* ATCC 27853 and concluded that 50% honey and 100% honey had significant antimicrobial action against broad spectrum of bacteria [20].

The results of this study showed decreased antibacterial activity on dilutions. So the increased antibacterial activity of net Manuka honey suggest that osmotic disturbance, leakage of cellular materials, floral origin, phytochemical component could be the possible mechanism behind the antibacterial activity of net Manuka honey used in this study. Ahmadi et al., reported that 5%, 10%, 20%, 50% and 100% honey concentrations prepared in sterile solutions showed significant antibacterial activity against *Streptococcus mutans* PTCC 1683 and *Lactobacillus* PTCC 1643 and concluded that greater than 20% honey concentrations had significant antibacterial activity on *Streptococcus mutans* and 100% honey concentration on *Lactobacillus* [21]. The role of H_2O_2 and nonperoxide are not promising in this study as they showed decreased antibacterial activity of Manuka honey on dilutions and this should be confirmed in further studies and research on this particular statement.

Manuka honey can be used as an alternative endodontic disinfectant to sodium hypochlorite and chlorhexidine as it shows good antimicrobial properties and as a substitute in patients who are allergic to NaOCl [22]. This study also shows that non peroxide and hydrogen peroxide components alone does not play active role in the antibacterial property of honey. The known safe use of honey without toxic effects suggests that honey could be used to treat infections arising from bacterial pathogens [23].

CONCLUSION

This study suggests Manuka honey to be a potential root canal disinfectant against gram positive and gram negative bacterial pathogens in the search for a biocompatiable agent, but clinical trials need to be carried out to what extent this is true.

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