ANTI MICROBIAL ACTIVITY OF HONEY AGAINST VARIOUS ENDODONTIC MICRO ORGANISMS-AN IN VITRO STUDY

Litik Mittal[1], Pooja Kakkar[2], Arun Verma[3], Kuldeep Kumar Dixit[4], Mridul Mehrotra[5]*

1. Dr. PG student, Department of Conservative Dentistry and Endodontics, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India.
2. Prof, MDS Department of Conservative Dentistry and Endodontics, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India.
3. Reader, MDS, Department of Conservative Dentistry and Endodontics, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India.
4. Prof. and Head, MDS, Department of Conservative Dentistry and Endodontics, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India.
5. Prof and Head, MD, Department of Microbiology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India.

Abstract

To evaluate the antimicrobial effect of honey as root canal medicament against common endodontic microflora in comparison to some common antimicrobials/ standard drugs.

Different concentrations of honey were studied in vitro against Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 25853 and Pseudomonas aeriginosa ATCC 27853 - and its activity compared with commonly used antimicrobials – Ampicillin and Gentamycin. The experiment was done using disc diffusion test. Culture plates were incubated. Zones of inhibition were measured after 24 hours. Results were analysed statistically using Anova test.

The antibacterial action of honey was observed at 50% as well as the neat concentration. Neat honey showed greater activity than 50% honey regardless of the organism. However honey showed greatest activity against S. aureus and least against P. aeriginosa. Both the antimicrobials showed better activity than honey at all concentrations. Ampicillin (Penicillin) exerted a greater potency than Gentamycin (Aminoglycoside), except with Pseudomonas aeriginosa.

The study showed that honey, like antibiotics, has certain organisms sensitive to it, and provides alternative therapy against certain bacteria and is also known to have antimicrobial action against a broad spectrum of bacteria.

Experimental article **(*J Int Dent Med Res 2012; 5: (1), pp. 9-13*)**

Keywords: Honey, antimicrobials, sensitivity, microorganisms, intracanal medicament.

Received date: 22 April 2011 **Accept date: 10 January 2012**

Introduction

Endodontic infections are polymicrobial in nature dominated by obligate anaerobic bacteria. Complete elimination of microbial flora from the root canal system is next to impossible. Following thorough instrumentation of an infected root canal, there is a significant reduction in number of microbes but it is well documented that instrumentation alone cannot clean all the internal surface of the root canal walls, within the dentinal tubules and in the lateral canals(2004)[1].

Antibacterial irrigants and the inter appointments intracanal medicaments are needed to destroy the remaining microorganisms

The use of honey as a medicine is referred to in the most ancient written records. From the ancient Egyptians to the Greeks and Romans, all used honey in combination with other herbs and on its own to treat wounds and diseases of the gut (1989)[2].

Corresponding author:
Dr. Pooja Kakkar
Department of Conservative Dentistry and Endodontics
Sardar Patel Post Graduate Institute of Dental and Medical Sciences,
Rai Bareilly Road Lucknow, 226025 India.
Cell.+919235597565

E-mail: poojakakkar11@yahoo.com

Honey as a medicine has continued into present day folk medicine for example in India lotus honey is said to be a panacea for eye diseases (1945)[3]. Another example of current usage of honey in folk medicine are treatment of infected leg ulcers in Ghana (1992)[4].

The integration of traditional and modern medicine is gaining increased recognition globally. In the medical profession honey has been accepted as an antibacterial agent for the treatment of some diseases and infections resulting from wounds and burns (2001)[5].

Its effectiveness as an antimicrobial agent is widely reported and in many cases it is used with success on infections not responding to standard antibiotic and antiseptic therapy (2000)[6].

Some of the prerequisites of an intra canal medicament are antimicrobial action in the root canal system, control or prevention of post operative pain and inflammation, neutralization of the canal remnants control of exudation and to induce hard tissue formation (2003)[7].

Honey has a potent broad spectrum antibacterial activity along with a soothing effect resulting from the very effective anti inflammatory action and finally stimulatory action of honey on the growth of granulation tissue and epithelial cells would also benefit in hastening the repair of damaged tissues [8].These therapeutic benefits has put a new light on the possibility of honey being of benefit to oral health.

The purpose of this study was to screen for antimicrobial spectrum and efficacy of honey using common bacteria found in the root canal against standard drugs .

The aim of the study was to,

1) To screen for the antimicrobial spectrum and efficacy of honey using a few selected gram positive and gram negative organisms.
2) To compare the antimicrobial effect of honey against standard drugs.

Materials and methods

Honey sample and dilution: The honey used in this study was the commercially available Honey (Dabur Pharmaceuticals). It was collected in sterile container and checked for purity on blood agar by streaked on blood agar plate by streaked on blood agar plate and incubated overnight.

The honey sample was diluted by physiological saline to 50% and non diluted honey (100%) referred to as neat. The study was done in Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Department of Microbiology and Pathology, Lucknow.

Microorganisms: *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeriginosa* were obtained and maintained in nutrient agar and subcultured in Brain heart Infusion broth.

Microorganisms	Source	Morphotype
Staphylococcus aureus	ATCC 6538	Gram positive cocci
Pseudomonas aeruginosa	ATCC 27853	Gram negative bacilli
Escherichia coli	ATCC 25853	Gram negative bacilli

ATCC- American Type Culture Collection

Dilution of standard drugs: A concentration of 0.2% (2mg/ml) ampicillin and gentamycin were used.

Preparation of filter discs: Whatman No 1 filter paper was obtained. Round discs of 6 mm diameter were cut from the filter paper. They were dried and sterilized in hot air oven at 60 degree C. Each of these discs were then soaked with 0.1 ml of each of the honey suspensions and the control antimicrobials.

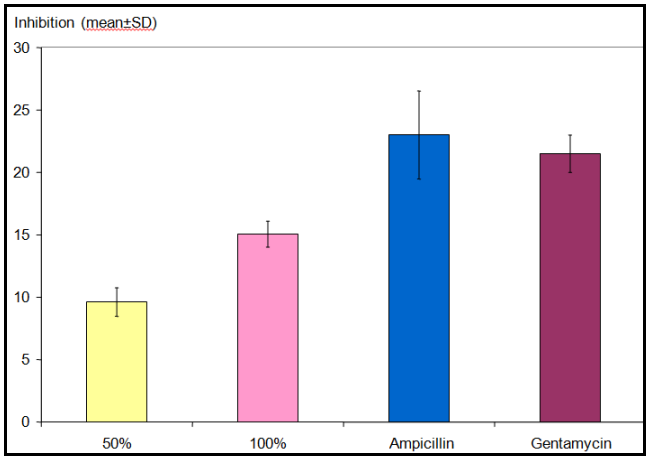
Pure culture of microorganisms was grown on nutrient agar. Five colonies of each organisms were picked using inoculation loop into the Brain Heart infusion broth, incubated for 4 hr at 37 degree C. Then the previously soaked discs with various concentrations of honey were added as well as discs with standard antimicrobials, which served as the controls.

The plates were incubated at 37 degree C for 24 hours under aerobic condition and were thereafter examined for zones of inhibition and again at 48 hour.

The examiner was calibrated and the intra examiner reliability coefficient was found to be 0.84.

Results

In the present study Ampicillin group had the maximum mean bacterial inhibition irrespective of species while 50% honey group had minimum(Graph 1).



Graphic 1. Ampicillin group had the maximum mean bacterial inhibition irrespective of species while 50% honey group had minimum.

(I) Honey	(J) Honey	Mean Difference (I-J)	SE	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
50% Honey	100% Honey	-5.000	0.773	**0.001**	-7.475	-2.525
	Ampicillin	-16.500	0.773	**<0.001**	-18.975	-14.025
	Gentamycin	-12.333	0.773	**<0.001**	-14.808	-9.859
100%Honey	50% Honey	5.000	0.773	**0.001**	2.525	7.475
	Ampicillin	-11.500	0.773	**<0.001**	-13.975	-9.025
	Gentamycin	-7.333	0.773	**<0.001**	-9.808	-4.859
Ampicillin	50% Honey	16.500	0.773	**<0.001**	14.025	18.975
	100% Honey	11.500	0.773	**<0.001**	9.025	13.975
	Gentamycin	4.167	0.773	**0.003**	1.692	6.641
Gentamycin	50% Honey	12.333	0.773	**<0.001**	9.859	14.808
	100% Honey	7.333	0.773	**<0.001**	4.859	9.808
	Ampicillin	-4.167	0.773	**0.003**	-6.641	-1.692

Table 1. shows multiple comparisions between the 4 groups against S. Aureus.

Table 1: Both the concentrations of honey had significantly lower mean microbial inhibition as compared to the antibiotics Ampicillin and Gentamycin (p<0.001). Comparison between 50% and 100% honey revealed the mean zone of inhibition in 100% honey to be higher as compared to that of 50% honey. On comparing the two antibiotics *i.e.* ampiciliin and gentamycin, a statistically significant difference was observed between two was observed with gentamycin showing significantly lower value as compare to ampicillin (p=0.003). On the basis of the observations made herein above, the following order of efficacy could be seen in different test materials : **50% Honey < 100% Honey < Gentamycin < Ampicillin**

Table 2: Both the concentrations of honey had significantly lower mean microbial inhibition as compared to the antibiotics Ampicillin and Gentamycin (p<0.001). Comparison between 50% and 100% honey revealed the mean zone of inhibition in 100% honey to be higher as compared to that of 50% honey. On comparing the two antibiotics *i.e.* Ampiciliin and gentamycin, no statistically significant difference was observed between the two (p=0.104). On the basis of the observations made hereinabove, the following order of efficacy could be seen in different test materials: **50% Honey < 100% Honey < Ampicillin ~ Gentamycin.**

(I) Honey	(J) Honey	Mean Difference (I-J)	SE	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
50% Honey	100% Honey	-5.667	0.745	**<0.001**	-8.054	-3.280
	Ampicillin	-12.500	0.745	**<0.001**	-14.887	-10.113
	Gentamycin	-10.500	0.745	**<0.001**	-12.887	-8.113
100% Honey	50% Honey	5.667	0.745	**<0.001**	3.280	8.054
	Ampicillin	-6.833	0.745	**<0.001**	-9.220	-4.446
	Gentamycin	-4.833	0.745	**0.001**	-7.220	-2.446
Ampicillin	50% Honey	12.500	0.745	**<0.001**	10.113	14.887
	100% Honey	6.833	0.745	**<0.001**	4.446	9.220
	Gentamycin	2.000	0.745	0.104	-0.387	4.387
Gentamycin	50% Honey	10.500	0.745	**<0.001**	8.113	12.887
	100% Honey	4.833	0.745	**0.001**	2.446	7.220
	Ampicillin	-2.000	0.745	0.104	-4.387	0.387

Table 2. Shows multiple comparisions between the 4 groups against E.coli**.**

(I) Honey	(J) Honey	Mean Difference (I-J)	SE	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
50% Honey	100% Honey	-5.667	0.745	**<0.001**	-8.054	-3.280
	Ampicillin	-12.500	0.745	**<0.001**	-14.887	-10.113
	Gentamycin	-10.500	0.745	**<0.001**	-12.887	-8.113
100% Honey	50% Honey	5.667	0.745	**<0.001**	3.280	8.054
	Ampicillin	-6.833	0.745	**<0.001**	-9.220	-4.446
	Gentamycin	-4.833	0.745	**0.001**	-7.220	-2.446
Ampicillin	50% Honey	12.500	0.745	**<0.001**	10.113	14.887
	100% Honey	6.833	0.745	**<0.001**	4.446	9.220
	Gentamycin	2.000	0.745	0.104	-0.387	4.387
Gentamycin	50% Honey	10.500	0.745	**<0.001**	8.113	12.887
	100% Honey	4.833	0.745	**0.001**	2.446	7.220
	Ampicillin	-2.000	0.745	0.104	-4.387	0.387

Table 3. shows multiple comparisions between four groups against Pseudomonas.

Table 3: Both the concentrations of honey had significantly lower mean microbial inhibition as compared to the antibiotics Ampicillin and Gentamycin (p<0.001). Comparison between 50% and 100% honey revealed the mean zone of inhibition in 100% honey to be higher as compared to that of 50% honey. On comparing the two antibiotics *i.e.* ampiciliin and gentamycin, a statistically significant difference was observed between two was observed with gentamycin showing significantly higher value as compared to ampicillin (p=0.042). On the basis of the observations made hereinabove, the following

order of efficacy could be seen in different test materials: **50% Honey < 100% Honey < Ampicillin< Gentamycin.**

Discussion

The therapeutic features of honey seen in its usage in wound care elsewhere on the body indicate that it has the potential to be useful for prevention or treatment of infections related to oral health.

It has been observed that honey clears infection, removes malodour, reduces inflammation and pain, causes edema and exudation to subside, and increases the rate of healing by stimulation of angiogenesis, granulation and epithelialisation. And, unlike other antiseptics, honey is not cytotoxic so it does not slow healing, nor does it have any adverse side-effects like antibiotics do (2003)[7].

Honey has a potent broad-spectrum antibacterial activity effective against aerobic, anaerobic Gram-positive and Gram-negative bacteria, and a variety of fungi, that rapidly clears infection from wounds when applied topically, which may make it suitable for "anti-infective" treatment of periodontal disease as well as for clearing infection in mouth ulcers and wounds from oral surgery (1999)[8].

Several mechanisms have been suggested to explain the antimicrobial actions of honey. Presence of “Inhibine”, factor in honey, which is Hydrogen Peroxide (1963)[9,](1966)[10]. Hydrogen peroxide is a well known antimicrobial agent and its harmful effects when added in isolation is not noticeable with honey since the latter sequesters and inactivates the free iron which catalyses formation of oxygen free radicals produced by hydrogen peroxide Its antioxidant components help to mop up free radicals (1966)[10].

Honey being a super- saturated sugar exerts an osmotic pressure which makes little or no water available for the micro-organisms to survive (1999)[8,] (1991)[11]. Recent studies showed that the proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentration as low as 0.1% and phagocytes are also activated by honey at such low concentrations. Furthermore, honey stimulates monocytes in cell culture to release cytokines, tumour necrosis factor (TNF) –alpha, interleukines (IL) -1 and (IL) –6, which stimulate the immune response to infection (1991)[12,](2001)[13].

Honey supplies glucose which is essential for the ‘respiratory burst’ in macrophages that is an essential part of their mechanism of destroying bacteria In addition, the glucose content of honey provides substrates for glycolysis which is the major mechanism for energy production in the macrophages and thus allows them to function in damaged tissue and exudates where the oxygen supply is often poor. The acidic pH of honey(typically between 3 and 4) may assist in the bacterial destroying action of macrophages as an acidic pH inside the phagocytic vacuole is involved in killing ingested bacteria (1999)[12,] (2001)[13].Lastly, non- peroxide component: Among these are complex phenols and organic acids often referred to as flavonoids (1988)[14].

This study was undertaken to investigate in vitro antimicrobial activity of honey against certain microbial isolates found in the root canal against standard drugs.

The bacteria selected for this study are commonly found isolates from the root canal system. Staphylococcus Aureus is a gram positive anaerobic bacterium that is most commonly isolated in pus from the oral cavity. This bacteria along with Pseudomonas Aeruginosa, another common gram negative aerobic isolate from chronically infected root canals are of particular clinical significance as both these bacteria show a tendency for antibiotic resistance to conventional therapy. The clinical significance of the antibacterial activity of honey can be seen in reports of honey being effective on wounds not responding to routine therapy of antibiotics and antiseptics (2000) [6].

Escherichia Coli, a gram negative anaerobic bacteria is more commonly found in the gut as a harmless strain that is part of the normal flora. It is a model organism for the study of antibiotic resistant bacteria.

The Efficacy of honey on these bacteria can contribute to better clinical management of chronically infected root canal systems and could lead to development of new intracanal medicaments.

In the study, honey sample showed antimicrobial activity and our result were in agreement with Willix et al (1992)[15] who found that honey inhibited the growth of S.aureus, E.coli and Pseudomonas sp. And also in

agreement with Bilal et al (1998)[16] who found that honey exhibited a fairly good antimicrobial activity against both gram positive and negative bacteria. In the study it was found that 100% honey had higher zone of microbial inhibition as compared to that of 50% honey which is in accordance with Raied Taha Al-Naama (2009)[17].

However honey showed greatest activity against S. Aureus and least against P. Aeriginosa which is in contrast with Abd-el-et al (2007)[18] who showed that honey have a greater inhibitory effect on isolated gram-negative bacteria(P.aeruginosa and E.coli) . Ampicillin showed the highest zone of microbial inhibition irrespective of the species.

To conclude, The study showed that honey, like antibiotics, has certain organisms sensitive to it, and provides alternative therapy against certain bacteria and is also known to have antimicrobial action against a broad spectrum of bacteria.

Conclusions

The study reveals that pure/neat honey has antimicrobial effect against common endodontic microflora. However, more research is required to evaluate the feasibility of honey to be considered for use as an intracanal medicament.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

References

1. Meneze MM, Valera MC, Jorge AO, Kogacy Camargo CH, Manicini MN. In vitro evaluation the effectiveness of irrigants and intracanal medicament. Int Endod J. 2004; 37 (5):311-319.
2. Zumla A; Lulat A. Honey - a remedy rediscovered. Journal of the Royal Society of Medicine 1989; **82:** 384-5.
3. Fotidar M R; Fotidar S N . 'Lotus' Honey. Indian Bee Journal 1945; 7: 102.
4. Ankra – Badu GA. Sickle cell leg ulcers in Ghana. East African Medical Journal. 1992; 69 (7): 366-9.
5. Molan PC . Why honey is effective as a medicine. 2. The Scientific explanation of its effects. Bee World 2000; 82(1): 22-40.
6. Dunford C; Cooper R; White RJ; Molan PC. The use of honey in wound management. Nursing Standard 2000; 15(11); 63-68.
7. Ehrmann ET, Messer HH, Adams GG. The relationship of intracanal medicament to post operative pain in endodontic.Int Endodo J. 2003; 36: 868-875.
8. Molan PC. Why honey is effective as a medicine. 1. Its use in modern medicine. Bee World 1999; 80(2): 80-92.
9. White JW, Subers MH, Schepartz AI. The identification of inhibine. The antibacterial factor in honey as hydrogen peroxidase and its origin in a honey glucose oxygen system. Biochem Biophys Acta. 1963;73:57-70.
10. Baur AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing a standardized single disk method. Am J Clin Pathol 1966;45:493-96.
11. Molan PC. Honey as a topical antibacterial agent for treatment of infected wounds.11th conference of the European Wound Managemant Association, Dublin, 1991;70.
12. Abuharfeil N, Al-Oran R, abo-Shehada M. the effect of bee honey on the proliferative activity of human B and T lymphocytes and the activity of phagocytes. Food Agric immunol 1999;11:169-77.
13. Tonks A, Cooper RA, Price AJ, Molan PC, Jones KP. Stimulation of TNF-alpha release in monocytes by honey. Cytokine 2001;14:240-42.
14. Molan PC, Russel KM, Non-peroxide antibacterial activity in some New Zealand honeys. J apic Res 1988; 27:62-67.
15. Willix DJ, Molan PC, Harfoot CG. A comparision of the sensitivity of wound-infecting infecting species of bacteria to the antibacterial activity of Manka honey and other honey. J. appl. Bacteriol 1992;73(5):388-94.
16. Bilal AN, Molan PC, Sallal AK. Antimicrobial activity of honey on selected microorganisms: A preliminary study. Biomed. Res. 1998;9:51-54.
17. Raied Taha Al Naama. Evaluation in vitro inhibitory effect of honey on some microbial isolate. Journal of Bacteriology Research.2009;1(6):064-67.
18. Abd- El Aal AM, El-Hadidy MR, El-Mashad NB, El-Sebaie AH. Antimicrobial effect of bee honey in comparision to antibiotic on organisms isolated from infected burns. Annals of Burns and fire disasters 2007;2.