Original Article

DOI: 10.7860/JCDR/2016/19268.8311

Dentistry Section

A Comparative Evaluation on Antimicrobial Effect of Honey, Neem Leaf Extract and Sodium Hypochlorite as Intracanal Irrigant: An Ex-Vivo Study

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ABSTRACT

Introduction: The major determinant of the success of root canal treatment depends on meticulous disinfection of the root canal using intracanal irrigants. The most commonly used root canal irrigant is sodium hypochlorite which has disadvantages of cytotoxicity and unpleasant taste. So there is a need to identify a more biocompatible root canal irrigant.

Aim: The aim of this ex-vivo study was to evaluate the efficacy of 40% honey, 100% neem leaf extract and 5.25% sodium hypochlorite as an intracanal irrigant against the isolated microorganisms from infected root canal.

Materials and Methods: The samples were collected from infected root canals of 60 primary molar teeth indicated for pulpectomy. Alpha hemolytic *Streptococci,* gram negative *bacilli*, *Candida*, *Staphylococci*, *Lactobacilli*, *Enterococci*, Spore bearing gram positive *bacilli* and *Micrococci* were the microorganisms isolated from the samples. The zone of inhibition against the microbial growth was measured by agar well diffusion method. Statistical analysis was done by Repeated Analysis of Variance (ANOVA) and Bonferroni method.

Results: Statistical analysis showed that the means of the zones of inhibition measured in this study were 18.56mm, 2.09mm and 1.62mm for sodium hypochlorite, 100% neem leaf extract and 40% honey respectively. The significance was greater between sodium hypochlorite and the other two agents as p-value was <0.001.

Conclusion: The results indicated that 5.25% sodium hypochlorite is more effective as root canal irrigant when compared with 100% neem leaf extract and 40% honey. It was also observed that 100% neem leaf extract has greater antimicrobial effect than 40% honey.

Keywords: Biocompatible materials, Ethnopharmacology, Root canal irrigant

INTRODUCTION

The outcome of root canal treatment depends on proper diagnosis, meticulous biomechanical preparation and thorough disinfection of the root canals. The recent imaging techniques revealed that necrotic tissues, microorganisms and their byproducts resulting in persistent periradicular inflammation remained in the pulpal space which was inaccessible to root canal instruments. Thus, chemomechanical debridement using intracanal medicaments and irrigants is essential in these areas [1].

The recent research has shown that various natural plant extracts have proven to be effective in dental treatment termed phytotherapeutics or ethnopharmacology. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant. It is well known for its antimicrobial activity and soft tissue dissolving capacity. The disadvantages of NaOCl include unpleasant taste, cytotoxicity and its inability to remove the smear layer. Thus, there is a need to identify a more biocompatible agent with better patient acceptance in taste and odour [2].

Honey has a wide spectrum of antimicrobial activity along with anti-inflammatory action and also increases the healing potential of damaged tissues [3]. Neem leaves, seeds and bark has a broad spectrum of antimicrobial action which is proved by many in vivo and in-vitro studies [4]. The efficacy of neem and honey as root canal irrigant against NaOCl is not yet substantiated.

The aim of this ex-vivo study was to evaluate the efficacy of 40% honey, 100% neem leaf extract and 5.25% sodium hypochlorite as an intracanal irrigant against the isolated microorganisms from infected root canal.

MATERIALS AND METHODS

The present study was an ex-vivo study conducted in the Department of Pedodontics, Government Dental College, Kozhikode, Kerala, India.

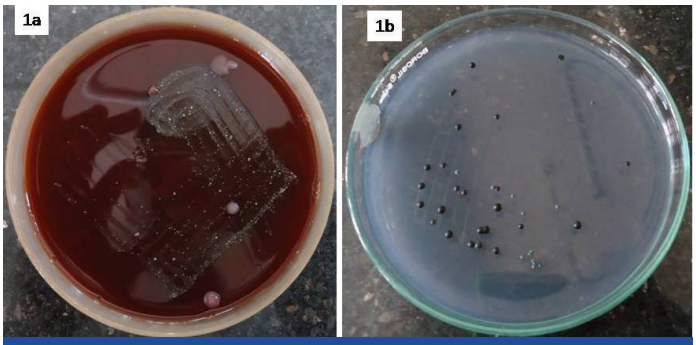
Collection of sample: The infected root canal specimen from 60 primary molar teeth indicated for pulpectomy were collected from children belonging to the age group of 5 to 7 years. A barbed broach with the infected pulp was transferred into a sterile vial with a screw cap containing 1ml saline and taken to microbiological laboratory within 2 hours.

Preparation of 100% neem leaf extract and 40% honey: 100% Neem leaf extract and 40% honey was prepared in the College of Pharmaceutical Sciences, Government Dental College, Kozhikode, India. Fresh neem leaves were collected and washed in sterile distilled water. Leaves were measured up to 25gms in sterile disposable cup to which 50ml of absolute ethanol was added. This mixture was macerated for 1-2minutes and the extract obtained was filtered using filter paper. The coarse residue obtained was again subjected to the same process using 25ml of ethanol. The extracts obtained in the above two steps were mixed together and filtered. The alcohol content in the extracts was removed by allowing the extract to boil on water bath till the volume was about 25ml. The prepared extract was stored in an air tight plastic container.

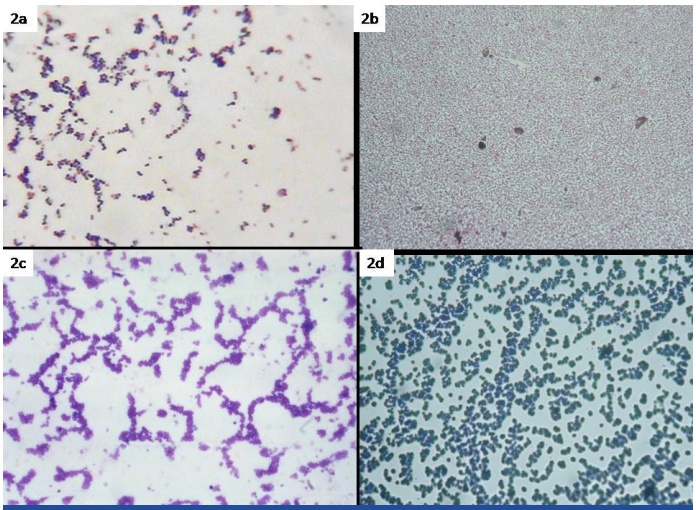
Pure honey was filled in the measuring jar up to 50ml. Aqueous solution of 40% honey was prepared by adding 20ml of distilled water to it and stirred well. The prepared 40% honey was stored in a glass container.

Microbiological procedure: The microbiological procedure was done in the Department of Microbiology, Government Dental College, Kozhikode, India. The infected sample was then streak cultured on Mitis Salivarius (MS) agar and blood agar which were incubated aerobically at 37^{0}C. After incubation for 24 hours, the agar plates were examined for microbial growth [Table/Fig-1].

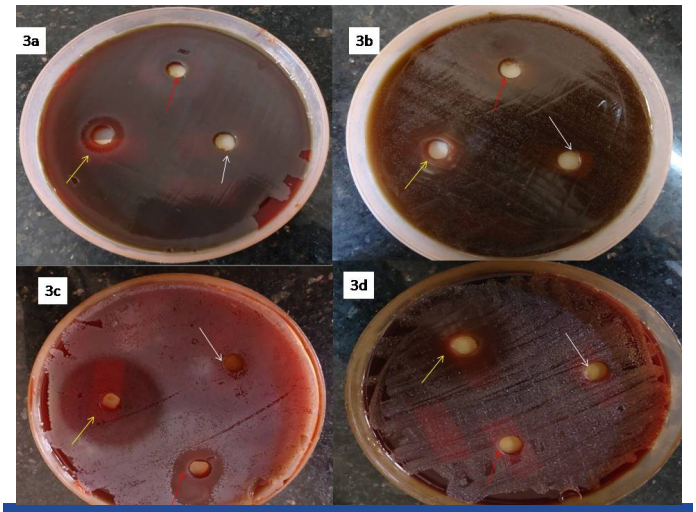
Microbiological analysis: The different microbial colonies were identified by gram staining [Table/Fig-2]. Pure culture of each microbial colony was obtained and was inoculated in glucose broth in test tube separately for 2 hours. To check the antimicrobial efficacy of 40% honey, 100% neem leaf extract and 5.25% NaOCl, agar diffusion method was performed. After 24 hours the largest diameter of the zones of inhibition of microbial growth around each well was measured using a transparent scale [Table/Fig-3].



[Table/Fig-1]: Microbial colonies in culture media.
1a: Microbial colonies in blood agar.
1b: Microbial colonies in Mitis salivarius agar.



[Table/Fig-2]: Identification of microbial colonies by gram staining.
2a: *Streptococci*, 2b: Gram negative *bacilli*, 2c: *Staphylococci*, 2d: *Micrococci*



[Table/Fig-3]: Zones of inhibition produced by (→) Neem leaf extract, (→) Honey and (→) Sodium hypochlorite.
3a: Zone of inhibition against Gram negative *bacilli*, 3b: Zone of inhibition against *Streptococci*, 3c: Zone of inhibition against stapylococci, 3d: Zone of inhibition against *micrococci*.

STATISTICAL ANALYSIS

Observations from the microbiological procedure were made and results were statistically analysed and tabulated. Statistical analysis was done by Repeated Analysis of Variance (ANOVA) and Bonferroni method.

Mean and standard deviation of the zones of inhibition produced by each irrigant against each microorganism were calculated and mean was compared by Repeated Analysis of Variance (ANOVA). Bonferroni test was done to find out which among the three had significant difference.

RESULTS

[Table/Fig-4] shows the means of the zones of inhibition produced by the three root canal irrigants used in this study. There was a significant difference among the groups as p-value was <0.001. Bonferroni test showed that the significance was greater between sodium hypochlorite and the other two agents as p-value was <0.001, whereas there was no significant difference between honey and neem leaf extract as p-value was 1.000. This indicates that sodium hypochlorite showed the maximum inhibition to growth of the microorganisms, neem leaf extract and honey showed only minimal inhibition to the growth of microorganisms.

Irrigant	N	Minimum (mm)	Maximum (mm)	Mean (mm)	Std. Deviation
NaOCl	78	0	30	18.56	5.397
Neem	78	0	11	2.09	3.626
Honey	78	0	11	1.62	3.244
Pairwise comparison by Bonferroni method					
Groups compared	p-value				
NaOCl vs. Neem	<0.001- Significant				
NaOCl vs. Honey	<0.001- significant				
Neem vs. Honey	1.000 - not significant				

[Table/Fig-4]: Mean of the zones of inhibition produced by 40% honey, 100% neem leaf extract and 5.25% sodium hypochlorite.
p -value<0.001*
*p-value assessed using repeated analysis of variance

[Table/Fig-5] shows zones of inhibition produced by 40% honey, 100% neem leaf extract and 5.25% sodium hypochlorite against each microorganism isolated. It indicates that sodium hypochlorite showed the maximum inhibition to growth of α hemolytic *streptococci*, gram negative bacilli, *Candida* and *Staphylococci*, neem leaf extract and honey showed only minimal inhibition to the growth of these microorganisms. The p-value was insignificant > 0.05 with the zones of inhibition produced by sodium hypochlorite, honey and neem leaf extract against *lactobacilli*, spore bearing gram positive bacilli, *Micrococci*, *Enterococci*. Thus, sodium hypochlorite, honey and neem were ineffective against *lactobacilli*, spore bearing gram positive bacilli, *Micrococci*, *Enterococci* according to this study.

DISCUSSION

The infective microorganisms are considered as the main etiologic factor in the development of pulp and periapical lesions. The root canal irrigants aid in the removal of pulpal tissue, dentinal debris and infective microorganisms especially in root canals with complex internal anatomy [5].

Sodium hypochlorite, the most commonly used root canal irrigant has broad spectrum of antimicrobial activity and excellent pulpal tissue dissolving potential. But its cytotoxicity due to its pH of 11-12 causes oxidation of proteins resulting in hemolysis and necrosis. Several NaOCl accidents such as damage to patient's clothing, patient's or operator's eye, air emphysema when injected into root canals and allergic reaction to the irrigant [6,7]. These disadvantages led to the need to identify a biocompatible and effective root canal irrigant.

Microorganism	Irrigant	N	Minimum	Maximum	Mean	Std. Deviation
α Hemolytic *Streptococci*	NaOCl	36	15	28	19.61	3.045
	Neem	36	0	11	3.19	4.139
	Honey	36	0	11	2.40	3.724
Gram Negative Bacilli	NaOCl	20	14	28	19.37	3.387
	Neem	20	0	9	1.60	3.299
	Honey	20	0	9	2.00	3.569
Candida	NaOCl	9	17	23	19.17	1.936
	Neem	9	0	0	0.00	0 .000
	Honey	9	0	0	0.00	0 .000
Staphyloccoci	NaOCl	7	14	30	20.14	5.757
	Neem	7	0	8	1.14	3.024
	Honey	7	0	0	0.00	0 .000
Others*	NaOCl	6	0	23	6.83	10.704
	Neem	6	0	8	1.33	3.266
	Honey	6	0	0	0.00	0.000

Microorganism	p-value*
α Hemolytic *Streptococci*	<0.001- Significant
Gram Negative Bacilli	<0.001- Significant
Candida	<0.001- Significant
Staphyloccoci	<0.001- Significant
Others*	0.180 - Not Significant

Multiple comparison by Bonferroni method

Microorganism	(I) well material	p value
α Hemolytic *Streptococci*	NaOCl vs. Honey	<0.001 Significant
	NaOCl vs. Neem	<0.001 Significant
	Neem vs. Honey	1.000 Not Significant
Gram Negative Bacilli	NaOCl vs. Honey	<0.001 Significant
	NaOCl vs. Neem	<0.001 Significant
	Neem vs. Honey	<0.001 Significant
Candida	NaOCl vs. Honey	<0.001 Significant
	NaOCl vs. Neem	<0.001 Significant
	Neem vs. Honey	1.000 Not Significant
Staphyloccoci	NaOCl vs. Honey	<0.001 Significant
	NaOCl vs. Neem	<0.001 Significant
	Neem vs. Honey	1.000 Not Significant
Others*	NaOCl vs. Honey	0.531 Not Significant
	NaOCl vs. Neem	0.531 Not Significant
	Neem vs. Honey	0.536 Not Significant

[Table/Fig-5]: Mean of the zones of inhibition produced by 40% honey, 100% neem leaf extract and 5.25% sodium hypochlorite against each microorganism.
*p-value assessed using repeated Analysis of variance.
Others±- *lactobacilli*, Spore bearing Gram positive *bacilli*, *micrococci*, *enterococci*

Honey has a broad-spectrum antibacterial activity suggested by several mechanisms. The hydroxyl ions present in honey as similar to hydrogen peroxide is considered to be responsible for its antibacterial activity. In addition, the osmotic pressure exerted by honey affects the survival of the microbes. The non peroxide components such as complex phenols and organic acids are also responsible for the antibacterial activity [3,8,9].

Neem tree [*Azadirachta indica*] is a tree in the mahogany family Meliaceae is well known for its antimicrobial and therapeutic effects. The neem bark extract has immunomodulatory activity. The extract from neem bark, leaves, fruits and flowers contain flavonoids, flavonoglycosides, dihydrochalocones and tannins [10-13].

The present study evaluated the effectiveness of 40% honey, 100% neem leaf extract and 5.25% sodium hypochlorite as intracanal irrigant. The neem leaf extract was prepared as suggested by Ghonmode et al., [14] in their study to evaluate the antimicrobial property of neem leaf extract, grape seed extract and sodium hypochlorite against *E.fecalis*.

The pure cultures of various microorganisms were obtained from the inoculation of the infected root canal specimens. Agar diffusion test is one of the most commonly used tests to study the antimicrobial activity of endodontic irrigants. In our study three wells of 6mm diameter were made in the blood agar. The different root canal irrigants to be evaluated were filled in the different wells. These zones of inhibition signify the presence of antimicrobial activity of the given chemical agent against the selected microorganisms. In the present study the largest diameter of the zone of inhibition was measured using a transparent ruler. Sodium hypochlorite showed the maximum inhibition to growth of the microorganisms followed by neem and honey which showed only moderate inhibition to the growth of microorganisms. The findings of the present study were compared with other experimental studies and tabulated in [Table/Fig-6]. The results of the present study show that NaOCl has

S.NO	Study	Root canal irrigants used	Effective against microorganisms	Zone of inhibition
1	Fidelgo et al., (2010) [15]	NaOCl	*Candida albicans*	28.5mm
			E.faecalis	8.3mm
			S.aureus	10mm
2	Prashant et al., 2007 [16]	Neem extract	*S.mutans*	3.8mm
			S.salivarius	2.9mm
			S.mitis	2.7mm
			S.sanguis	3.4mm
3	Hegde V et al., (2013) [17]	Neem extract	*E.faecalis*	21.33mm
		Propolis/Honey extract	*E.faecalis*	7.33mm
		NaOCl	*E.faecalis*	17.67mm
		Neem extract	*C.albicans*	15.33mm
		Propolis/Honey extract	*C.albicans*	8.33mm
		NaOCl	*C.albicans*	12.67mm
4	Ghonmode Wn et al., (2013) [14]	Neem	*E.faecalis*	19.57mm
		NaOCl	*E.faecalis*	16.34mm
5	Damre PG (2015) [18]	NaOCl	*E.faecalis*	3mm
		Honey	*E.faecalis*	7mm
		Neem	*E.faecalis*	4mm
6	Jerin Jose et al. (2015) [19]	Neem	*E.faecalis*	43mm
		NaOCl	*E.faecalis*	2.5mm
		Neem	*C.albicans*	7.6mm
		NaOCl	*C.albicans*	42mm
7	Kankariye et al., (2016) [20]	Neem	*S.mutans*	20.13mm
8	The present study	NaOCl	*Streptococci*	19.61mm
		Neem leaf extract		3.19mm
		Honey		2.40mm
		NaOCl	Gram negative *bacilli* (*E.faecalis*)	19.37mm
		Neem leaf extract		1.6mm
		Honey		2.0mm
		NaOCl	*Candida*	19.17mm
		Neem leaf extract		0mm
		Honey		0mm
		NaOCl	*Staphylococci*	20.14mm
		Neem leaf extract		1.14mm
		Honey		0mm

[Table/Fig-6]: Comparison of the results of the present study with other experimental studies.

maximum antimicrobial activity when compared with neem and honey which is in contrast with the results obtained by the authors shown in [Table/Fig-6]. Neem and honey though not as effective as NaOCl in their antimicrobial activity can still be considered as root canal irrigant due to their biocompatibility with the soft tissues and honey has an added advantage of sweet taste which would increase the patient acceptance.

LIMITATION

The limitations of the present study are that chemical agent's formulation (liquid or gel), its molecular size, diffusion property, viscosity of the agar medium, storage conditions and the incubation time might affect the reliability and reproducibility of the agar diffusion method. This indirectly affects the antimicrobial activity assessed against the test specimens.

CONCLUSION

This is an era of evidence based research, so in the near future clinical trials comparing the effectiveness of ethnopharmacological products with sodium hypochlorite might bring into limelight the antibacterial effectiveness of these products. The results obtained in this ex-vivo study indicated that sodium hypochlorite is an effective root canal irrigant whereas neem leaf extract and honey have only moderate antibacterial activity against the root canal pathogens. The disadvantage of sodium hypochlorite is that it is cytotoxic, causes tissue irritation, unpleasant taste and damages clothing. Neem leaf extract and honey have an added advantage in this aspect as they are biocompatible to the tissues. The sweet taste of honey further favors the patient acceptance and this plays an important role in case of pediatric patients. The success of endodontic treatment depends on the elimination of root canal pathogens. Sodium hypochlorite has a wide antimicrobial spectrum and it can be recommended as an effective root canal irrigant, but 100% neem leaf extract and 40% honey were not as effective as 5.25% sodium hypochlorite as root canal irrigants.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 03, 2016**
Date of Peer Review: **Apr 11, 2016**
Date of Acceptance: **May 10, 2016**
Date of Publishing: **Aug 01, 2016**