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Comparative Evaluation of Neem, Cinnamon and its Combination as an Intracanal Medicament on *E. Faecalis* - An Invitro Real-time Polymerase Chain Reaction Study

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ABSTRACT

Introduction: Intracanal medicaments play a vital role in disinfecting the root canals. Considering the ineffectiveness, potential side effects of synthetic drugs, the herbal alternatives for endodontic usage prove to be advantageous. Currently, very few pieces of literature are available on which combination of herbs are used as an intracanal medicament. Hence in this study, the effectiveness of neem, cinnamon and its combination against *E. faecalis* is evaluated.

Aim: The objective of this study is to evaluate the antibacterial efficacy of neem, cinnamon and its combination and to compare these with chlorhexidine and calcium hydroxide as intracanal medicaments against *Enterococcus faecalis* (*E. faecalis*).

Methodology: A total of 75 freshly extracted teeth specimens (n=15) were inoculated with *E. faecalis* for 21 days. Specimens were divided into five groups (Group 1: Neem paste, Group 2: Cinnamon paste, Group 3: Combination of Neem and Cinnamon paste, Group 4: Calcium hydroxide; Group 5: 2% chlorhexidine gel (CHX). The intra-canal medicaments were packed inside the tooth. After 7 days, the remaining microbial load was determined by using real-time PCR.

Results: The threshold cycle (Ct) values of Neem, Cinnamon, Neem with Cinnamon, Calcium hydroxide & 2% CHX were found to be 21.51, 20.48, 22.11, 22.46 & 23.42 respectively. The mean bacterial load of these samples was 8.56, 12.45, 7.24, 0.83 & 0.13 respectively.

Conclusion: Combination of Neem with Cinnamon has better antibacterial efficacy against *E-Faecalis* when compared to individual pastes of Neem and Cinnamon, the efficacy of the combination was comparable to that of calcium hydroxide. However, 2% Chlorhexidine had the highest antibacterial property.

Key Words: Calcium Hydroxide, Chlorhexidine, Cinnamon, *E-Faecalis*, Neem, RT-PCR

INTRODUCTION

Anatomical complexities and microbiological factors often constitute serious threats to adequate root canal disinfection.¹

Irrigants and intracanal medicaments play a pivotal role in reducing the number of residual bacteria. Calcium hydroxide has a wide range of antimicrobial activity against common endodontic pathogens but is less effective against *Enterococcus faecalis* and *Candida albicans*.²

Studies reported that 2% CHX gel had greater activity against *E. Faecalis* by causing cell membrane damage. However few literature state the toxic effects of CHX on human neutrophils.³

Herbal alternatives have the least ability to develop resistance against microbes and also have the least side effects, considering these two properties the herbal extracts will pave a pioneer disinfection strategy in the domain of endodontics.^{4,5}

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Several studies have shown neem^{6,7} and cinnamon to possess antibacterial properties, but there is currently very little literature available on which combination of herbs are used as an intracanal medicament.⁸ Hence in this study the effectiveness of neem, cinnamon and combination of neem-cinnamon paste targeted against *E. faecalis* is evaluated.

Currently, there is a need for a material that would completely disinfect the root canal. Herbal medicaments are gaining keen interest due to their good antibacterial property with the least side effects.⁹

Hence Neem and Cinnamon with known antibacterial properties were chosen for this study. This study can pave the way for the development of newer herbal agents which can also be manipulated and incorporated in various endodontic materials like irrigants, sealers and coated on gutta-percha as disinfecting agents.

Amongst the currently available methods of microbial growth assessment, PCR is the most rapid and best suited molecular method for studying microbial count and gene expression. In our study, Real-time PCR was used to assess the gene expression of *E. faecalis* after using the herbal intracanal medicaments. The results of the current study will aid in evaluating the efficacy of newer herbal intracanal medicaments against *E. faecalis*, thus improving the prognosis of root canal therapy.¹⁰

MATERIALS AND METHODS:

Preparation of the block

Fifty freshly extracted mandibular premolars were collected and the middle third of the root was sectioned using a diamond disk under copious irrigation to be used for the study. The sectioned portion of the dentin was standardized to its internal diameter with GG drill no.3 (Mani Inc, Tachigiken, Japan). Following the routine irrigation protocol using 3% sodium hypochlorite and 17% EDTA for smear layer removal and a final rinse with saline, the samples were autoclaved at 121°C for 15mins.

Contamination of the dentin blocks:

The samples were contaminated overnight by the laminar flow method using a pure strain of *Enterococcus faecalis*, cultured in a brain heart infusion broth at 37 degrees Celsius for 24 hours. 1ml of the inoculum was transferred to sterile microcentrifuge tubes containing the samples and broths and fresh inoculum was transferred after 48 hours.¹¹

Preparation of Extracts

The fresh leaves of neem and barks of cinnamon were dried under shade for 7 days. The herbs were spread flat in a single layer to ensure even drying. The dried leaves were then

ground into a powder with the grinding time for each grinding operation kept constant at 30 seconds.¹² The powdered herbs were taken on a sterile glass slab and mixed with 2-3 drops of methylcellulose. 0.5 g of neem and cinnamon powder were mixed with 0.5 mL of a 1.66% solution of carboxymethylcellulose to form an aqueous paste.¹³

Anti-microbial assessment

After the incubation period, saline (5ml) was used as the final irrigant and the dentin blocks were divided into 5 groups (n=10) as follows:

Group 1: Neem extract with methylcellulose

Group 2: Cinnamon extract with methylcellulose

Group 3: Combination of Neem with Cinnamon extract with methylcellulose

Group 4: Calcium hydroxide

Group 5: 2% chlorhexidine gel

After treatment with intracanal medicament, all the samples were sealed and placed in the aerobic incubator at 37°C for 24 hours. Then the samples were rinsed with saline. Using a GG no.5, the dentinal debris was harvested at a depth of 400 um. The debris was collected in 1 ml of PBS buffer solution and incubated at 37C for 24 hours. After this, the samples were evaluated by real-time PCR.

RESULTS

The study revealed the highest antibacterial efficacy by 2% Chlorhexidine and the least efficacy in the cinnamon group. The following chlorhexidine, Ca (OH)₂ showed the highest antibacterial (Table1) property which was equipotential to that of the combination paste of Neem with Cinnamon. Table 2 showed the multiple comparisons of the mean threshold cycle (Ct) among groups. 2% Chlorhexidine had the highest antibacterial efficacy followed by Ca (OH)₂ and Neem with Cinnamon (Table 3). Table 4 shows that the percentage of bacterial loads was reduced in all the tested groups.

TABLE 1: Comparison of mean threshold cycle (Ct) among the groups

*One-way analysis of variance (ANOVA), p<0.05

S. No.	Group	Mean ± SD	p- value
1.	Neem	21.51 ± 0.26	
2.	Cinnamon	20.48 ± 0.31	
3.	Neem+ Cinnamon	22.11 ± 0.46	0.00
4.	Calcium hydroxide	22.46 ± 0.42	
5.	Chlorhexidine	23.42 ± 0.81	

TABLE 2: Multiple comparisons of mean threshold cycle (Ct) among groups#Tukey's honestly significant difference (HSD), $p < 0.05$

S. No.	Groups	Mean difference	p-value
1.	Neem * Cinnamon	1.10	0.01
2.	Cinnamon * Neem with Cinnamon	-1.63	0.00
3.	Neem with cinnamon * Calcium hydroxide	-0.35	0.62
4.	Calcium hydroxide * Chlorhexidine	-0.96	0.00
5.	Neem * Neem with Cinnamon	-0.60	0.18
6.	Neem * Calcium Hydroxide	-0.95	0.00
7.	Neem * Chlorhexidine	-1.91	0.00
8.	Cinnamon * Calcium Hydroxide	-1.98	0.00
9.	Cinnamon * Chlorhexidine	-2.94	0.00
10.	Neem with Cinnamon * Chlorhexidine	-1.31	0.00

Table 3: Comparison of mean number of copies of bacteria (bacterial load) among the groups *One-way analysis of variance (ANOVA), $p < 0.05$

S. No	Group	Mean±SD	p value
1.	Neem	8.56±0.36	
2.	Cinnamon	12.45±0.57	
3.	Neem with Cinnamon	7.24±0.51	0.00
4.	Calcium Hydroxide	0.83±0.24	
5.	Chlorhexidine	0.13±0.12	

Table 4: Multiple comparison of mean number of copies of bacteria (bacterial load) among groups #Tukey's honestly significant difference (HSD), $p < 0.05$

S. No	Groups	Mean difference	p-value
1.	Neem * Cinnamon	-3.89	0.00
2.	Cinnamon * Neem with Cinnamon	5.21	0.00
3.	Neem with cinnamon * Calcium hydroxide	6.41	0.00
4.	Calcium hydroxide * Chlorhexidine	0.70	0.45
5.	Neem * Neem with Cinnamon	1.32	0.00
6.	Neem * Calcium Hydroxide	7.73	0.00
7.	Neem * Chlorhexidine	8.43	0.00
8.	Cinnamon * Calcium Hydroxide	11.62	0.00
9.	Cinnamon * Chlorhexidine	12.32	0.00
10.	Neem with Cinnamon * Chlorhexidine	7.11	0.00

DISCUSSION

The root canal treatment aims to eliminate microorganisms from the root canal space to provide a good environment for tissue healing. *E. faecalis* is chosen for this study because it is the major cause of treatment failure. Its virulence factors and survival mechanisms make it an important pathogen in post-treatment diseases. It is the most common micro-organism found in secondary root canal infection and the major cause of treatment failure.¹⁴

Synthetic intracanal medicaments like calcium hydroxide cause collagen breakdown and hence weakening of radicular dentin. 2% Chlorhexidine is effective against *E. faecalis*, but its effectiveness is questionable hence there is a search for a better alternative.

The development of antibiotic-resistant strains and side effects of chemical irritants and intracanal medicaments has led to exploration for alternative herbal medicaments. Herbal alternatives have gained attention due to their antioxidant, antimicrobial, sedative, anxiolytic, and anti-inflammatory properties, thereby making them ideal for root canal disinfection.^{15,16,17}

Neem also called "Indian neem / Margosa tree / Indian lilac" and tetranortriterpenes extracted from neem are nimbidin, Nimbin, nimbolide, Azadirachtin, gallic acid, epicatechin, catechin, and margolone. All these exhibit potent antibacterial activities. The chief active constituent of *neem* is azadirachtin, which has antimicrobial action against many gram-positive and gram-negative microorganisms. The study by Bohra et al. revealed that the aqueous and ethanolic extract of neem leaf has potential action against *E. faecalis*.^{18,19,20}

Cinnamon (Lauraceae family) includes more than 250 species among which 2 are the most studied types- 1) *Cinnamomum verum* or 2) *Cinnamomum zeylanicum*. The main components of cinnamon extracts are cinnamaldehyde, eugenol, phenol, and linalool. Cinnamon bark has a higher cinnamaldehyde content (65–80%) and a low eugenol content (5–10%).²¹

Panchal et al. revealed ethanolic extract of *C. zeylanicum* was found to be even more effective against *E. faecalis* ATCC 29212 when compared to Neem, whereas in our study neem was found to be more effective, this may be due to difference in the synthesis parameters of the extracts.^{22,23,24,25}

In the current study, the combination of herbs was found to be more effective than the individual herbs which were similar to the study conducted by Mathew et al. in which Endopam a combination of herbs (Ingredients: *Clove, Eucalyptus, Cinnamon* and *Peppermint*) showed the better antibacterial property.⁸

CONCLUSION

From the results of our study, we conclude that 2% chlorhexidine has the highest antibacterial efficacy. However, the combination of Neem with Cinnamon extract in Methylcellulose base also possesses better antibacterial activity than the individual herbal component. Thus, it can be considered as an alternative intracanal medicament. Further in-vivo studies are essential to validate the results of the current study.

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Conflict of Interest: Nil

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