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ORIGINAL RESEARCH

Antibacterial Efficacy of Neem (*Azadirachta indica*) Extract against *Enterococcus faecalis*: An *in vitro* Study

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ABSTRACT

Introduction: Debridement and disinfection of the root canal is a crucial step in the success of endodontic treatment. Several antimicrobial agents alone or in combination are used to achieve this. The objective of this *in vitro* study was to assess the antimicrobial efficacy of neem (*Azadirachta indica*) extract against *Enterococcus faecalis*.

Materials and methods: Neem leaf extract, 2% chlorhexidine, 3% sodium hypochlorite were used to assess the antimicrobial efficiency. Agar well diffusion test was used to study the antimicrobial efficacy with saline as control. The zone of inhibition was recorded, tabulated, and analyzed statistically with the help of IBM Statistical Package for the Social Sciences statistics version 20 using analysis of variance test.

Results: All the three medicaments showed well-defined and comparable zones of inhibition around their respective wells. All values were significantly higher than the control group. Analysis of variance showed significant difference between zone diameters of chlorhexidine, neem leaf extract, and 3% sodium hypochlorite against *E. faecalis* ($p < 0.05$).

Conclusion: From the present study, it can be concluded that neem leaf extract shows comparable zones of inhibition with that of chlorhexidine and sodium hypochlorite.

Clinical significance: Neem leaf extract has significant antimicrobial activity against *E. faecalis* and thus opens the perspectives for the use of neem extract as an intracanal medication.

Keywords: Antimicrobial activity, Chlorhexidine, *Enterococcus faecalis*, Intracanal medications, Neem extract, Sodium hypochlorite.

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INTRODUCTION

Eradication of bacteria from the root canal system is important in ensuring the long-term success of root canal therapy. The microorganisms are the primary etiological agent in endodontic infections, and failure to eradicate them affects the outcome of endodontic therapy.[1] Studies have shown that the bacterial flora in endodontic infections is polymicrobial, with a predominance of anaerobic species.[2,3] This is mainly achieved through the reduction of bacteria by debridement of the root canal system utilizing mechanical preparation of the canals combined with chemical interventions. Several areas of the root canal walls, specifically in the apical third, are difficult to clean mechanically.[4-7]

Enterococcus faecalis is part of the human normal flora and an important pathogen in opportunistic infections in humans. *E. faecalis* is rarely present in primary apical periodontitis, but it is the dominant microorganism in root canal-treated teeth presenting with posttreatment apical periodontitis.[8] Eradication of *E. faecalis* from the root canal remains a challenge, since it is resistant to a variety of antimicrobial agents. Studies have also shown that *E. faecalis* may be one of the reasons of failure of endodontic treatments.[9] The control and suppression of *E. faecalis* in these dental procedures are of primarily importance in decreasing the penetration of bacteria inside the dentinal tubules and also limiting the formation of any relationship with other microorganisms, as in virulence factors, environment, and the biofilms.[10-12]

Elimination of bacteria can be achieved with mechanical preparation combined with the use of antimicrobial irrigants.[13] The most commonly used irrigant is sodium hypochlorite (NaOCl), introduced by Dakin.[14] Some of the drawbacks of NaOCl are toxicity, unpleasant odor

and taste, inability to completely remove the microorganisms, and smear from the root canal.[15-17] Due to the side effects of these agents and the development of resistant strains, research is still ongoing to find ideal root canal disinfection. Herbal extracts have also been tried as root canal irrigants.[18-21]

Neem (*Azadirachta indica*) has a wide range of various therapeutic properties based on its characteristics, such as antifungal, antibacterial, antioxidant, antiviral, anti-inflammatory, analgesic, antipyretic, and immune stimulant activity.[22] *Azadirachta indica* (*neem*) leaf extract is a commonly used antibacterial agent and little information is available on its usage as a potential agent in root canal irrigation.[23,24] Hence, the present study was conducted to evaluate the antimicrobial activity of neem leaf extract and to compare it with 2% chlorhexidine gluconate and 3% NaOCl.

MATERIALS AND METHODS

The study was approved by the departmental review board. A single species of *E. faecalis* American Type Culture Collection no 29212 was used to test the sensitivity. The bacterial culture was prepared in sterile brain heart infusion (BHI) broth (Oxoid, England) and adjusted spectrophotometrically to an optical density of 625 nm corresponding to match the turbidity of a McFarland 0.5 scale. The sensitivity assay was done using Neem leaf (*A. indica*) extract 2% chlorhexidine and 3% NaOCl.

Preparation of Neem Leaf Extract

Fresh neem leaves were used for the study. Neem leaves were thoroughly cleaned in distilled water and then weighed and recorded. About 25 gm of fresh neem leaves was mixed with 50 mL of absolute alcohol and the mixture was macerated for 1 to 2 minutes and the separation of the course residue was done using muslin cloth filtration. Same separation procedure was repeated for the coarse residue using alcohol. Both these extracted parts were later pooled together and then again filtered using fast filter paper. Total volume of 25 mL was taken after separation of the alcohol from the extract and then the remaining extract was stored in amber-colored air-tight bottle.[25,26]

Agar-diffusion Test

The cultures of *E. faecalis* were made on BHI agar. The agar was incubated at 37°C for overnight duration on a rotary shaker, and the growth of the bacteria was checked for changes in turbidity after a period of 24 hours. Agar well diffusion method was used for assessing the antimicrobial efficacy of the three irrigating materials used in the study.

Preparation of the BHI agar plates was followed by spread of the cultures on these prepared plates. After this, 6 mm diameter circles were made in these agar plates; 3% NaOCl, chlorhexidine, neem leaf extract, and normal saline were added to the respective wells. These agar plates were kept in an incubator for 24 hours at a temperature of 37°C. Normal saline was used as a control. Agar plates were removed after the period of incubation, and the zones of inhibition were recorded. Similar experiment was also done simultaneously on five BHI agar culture plates.

Recording of the zones of inhibition was done for all the culture plates and the results were analyzed statistically with the help of IBM Statistical Package for the Social Sciences statistics version 20 using analysis of variance test.

RESULTS

All the three medicaments showed well-defined zones of inhibition around their respective wells except the control well (Fig. 1). Analysis of variance showed significant difference between zone diameters of 3% NaOCl, chlorhexidine, and neem leaf extract against *E. faecalis* ($p < 0.05$). Maximum antimicrobial activity was shown by 2% chlorhexidine (20.45), followed by 3% NaOCl (19.22) and neem leaf extract (17.19). Neem leaf extract showed comparable zones of inhibition to 2% chlorhexidine and 3% NaOCl (Table 1 and Graph 1).

DISCUSSION

Chemomechanical preparation is of paramount importance in successful endodontic treatment. Completely eliminating intracanal bacterial populations or reducing them to levels that are compatible with periradicular tissue healing are the main microbiologic goals of the chemomechanical preparation of infected root canals.[27] Bacteria persisting after chemomechanical procedures

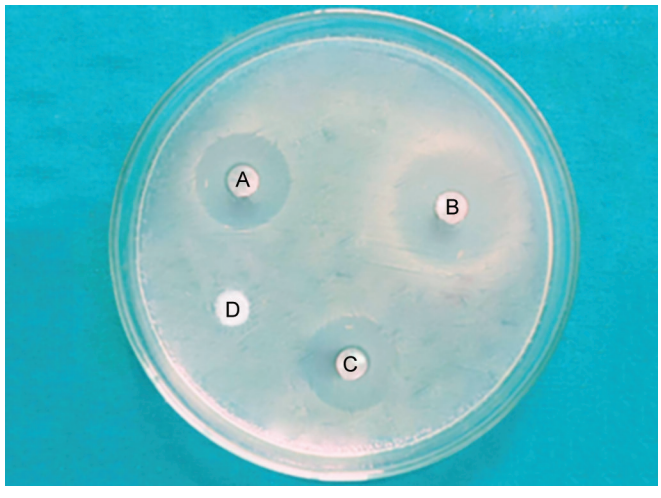


Fig. 1: Zones of inhibition: (A) 2% chlorhexidine; (B) 3% NaOCl; (C) neem leaf extract; and (D) saline

Table 1: Comparison of the zones of inhibition of the root canal irrigants against *E. faecalis*

Group	*Zone of inhibition (Mean ± SD)*	*Significance*
Neem leaf extract	17.70 ± 2.54	p < 0.05*
2% Chlorhexidine	20.30 ± 1.57	
3% NaOCl	19.50 ± 1.90	
Saline	0.00 ± 0.00	

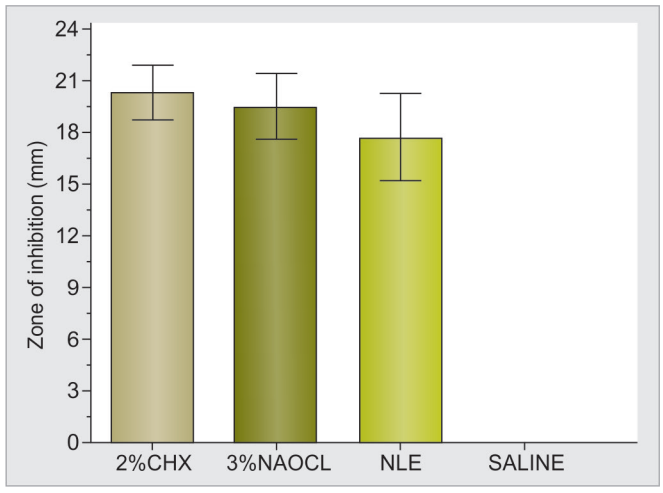
SD: Standard deviation, *Significant

at levels detectable by culturing techniques might negatively influence the treatment outcome.[28] The most frequently isolated microorganisms before root canal treatment include Gram-negative anaerobic rods, Gram-positive anaerobic cocci, Gram-positive anaerobic and facultative rods, *Lactobacillus* species, and Gram-positive facultative *Streptococcus* species.[29] During root canal treatment, the obligate anaerobes are easily eradicated. Facultative anaerobes are more likely to survive chemo-mechanical instrumentation and root canal medication.[30]

The irrigants that are currently used as disinfectant in root canal therapy are NaOCl, chlorhexidine, ethylene-diaminetetraacetic acid, and a mixture of tetracycline.[31] Even though NaOCl is widely used, extrusion to the tissue may cause severe pain, burning sensation, inflammation, and possible delayed healing.[32]

The increased resistance of microbial strains to antimicrobial agents and adverse side effects have raised the essentiality for the search of new herbal alternatives. Recently, herbal compounds attracted greater attention because of their useful beneficial effects.[33] The observations from the present study showed that neem leaf extract has comparable antimicrobial effects to NaOCl and chlorhexidine. Neem (*A. indica*) is predominantly found in the Indian subcontinent. The bark, leaves, and seeds are used to make medicine and less frequently, the root, flower, and fruit are also used. Neem leaf is used for leprosy, eye disorders, bloody nose, intestinal worms, stomach upset, loss of appetite, skin ulcers, diseases of the heart and blood vessels, fever, diabetes, gingivitis, and liver problems.[34]

In this study, the antimicrobial efficacy of neem has been compared with that of the chlorhexidine gluconate and NaOCl, and it was found that neem efficacy was comparable to that of other commonly used gold standard compounds. This observation is in agreement with earlier study by Dubey et al[35] who found the antimicrobial efficacy of neem leaf extract to that of the Biopure MTAD and 2.5% NaOCl. Further studies are required to establish the antimicrobial effects of neem extract against wider spectrum of microorganisms responsible for the periapical pathology.



Graph 1: The zones of inhibition of the three root canal irrigants against *E. faecalis* (CHX: Chlorhexidine, NaOCl: Sodium hypochlorite, NLE: Neem leaf extract)

CONCLUSION

In this study, it was shown that the zone of inhibition in the agar diffusion test showing the antimicrobial efficiency of the neem extract was comparable to that of 2% chlorhexidine and 3% NaOCl. Therefore, it can be concluded that neem leaf extract could be used as an alternative agent in root canal disinfection. However, further *in vitro* studies on its toxicological effects and optimal concentration against a wider spectrum of microorganisms have to be established.

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