Antimicrobial activity of herbal medicines (tulsi extract, neem extract) and chlorhexidine against *Enterococcus faecalis* in Endodontics: An *in vitro* study

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Abstract

Background: Successful endodontic treatment depends on effective disinfection and complete sealing of root canal. Various medicaments are advised for disinfecting root canal, such as herbal and non-herbal medicaments. This study was done to assess the antimicrobial activity of herbal medicines (neem extract, tulsi extract) and chlorhexidine against *Enterococcus faecalis* in Endodontics. Materials and Methods: Agar diffusion method was used to evaluate the antimicrobial action of different medicines. Sixty samples were segregated into four groups with 15 samples in each: Group I: chlorhexidine 2%, Group II: neem extract, Group III: tulsi extract, and Group IV: distilled water. The inhibition zones against *E. faecalis* were recorded and statistically assessed using one-way analysis of variance (ANOVA) test (\(P < 0.05\)). Results: Significant antibacterial effect against *E. faecalis* was observed with chlorhexidine followed by neem extract and tulsi extract. Conclusion: Herbal medicines seemed to be effective against *E. faecalis* compared to 2% chlorhexidine gluconate.

Key words: Antimicrobial, endodontic, Enterococcus faecalis, herbal medicine, neem extract, tulsi extract

INTRODUCTION

Successful endodontic treatment depends on effective disinfection and complete sealing of root canal. Elimination of microorganisms from the root canal space is important in pulpal disease management. Isolated microorganisms from the infected root canals have serious implications on oral and systemic health. It has been observed from several studies that intracanal instrumentation does not remove all the microorganisms, but use of intracanal medicament helps in removal of remaining bacteria after cleaning and shaping. *Enterococcus faecalis* is the commonly found microorganism in failed/infected root canals of both

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Chandrappa, et al.: Antimicrobial activity of herbal medicines and chlorhexidine against Enterococcus faecalis in Endodontics

Tulsi (Ocimum sanctum) is a holy plant of Indian origin. It is known as the mother medicine of nature. It is an easily available and economical material without side effects. It has antimicrobial properties and is most commonly used for treating variety of diseases such as arthritis, bronchitis, diabetes, and skin diseases. Tulsi has been tested for its antimicrobial properties against Escherichia coli, Klebsiella, Candida albicans, Staphylococcus aureus, Enterococcus faecalis, and Proteus. O. sanctum is mainly responsible for the therapeutic effect of tulsi. Antimicrobial activity of tulsi is due to its constituents, ursolic acid and carvacrol.

It is well known that neem leaves (A. indica or AI) have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, and analgesic effects without any side effect. AI has several active constituents like nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone, and cycloltrisulfide which are responsible for its antibacterial action. AI causes maximum reduction in adherence of E. faecalis to dentin.

There are very few studies on the antibacterial efficacy of herbal medicines (neem and tulsi extracts) on E. faecalis in Endodontics. Hence, our study was undertaken to assess the antimicrobial efficacy of herbal medicines (tulsi extract, neem extract) and chlorhexidine against E. faecalis in Endodontics.

MATERIALS AND METHODS

Agar diffusion microbiological method was used to evaluate the antimicrobial action of different medicines. Sixty samples were categorized into four groups with 15 samples in each: Group I: chlorhexidine gluconate 2% as positive control, Group II: neem extract, Group III: tulsi extract, and Group IV: distilled water as negative control group.

Extract preparation

Tulsi extract for the study was obtained by finely powdering the dried leaves. Then the powder was macerated with 100% ethanol followed by filtration. Eighteen grams of tulsi extract (residue 6% w/w) was obtained by dissolving 300 g of tulsi powder in 1 l of ethanol.

Neem extract (A. indica) was prepared by washing fresh mature neem leaves in sterilized water and adding them to 50 ml ethanol. This was thoroughly mixed for 1–2 min and then the extract was filtered. The alcohol part of the extract was separated in a water bath to obtain 25 ml of extract.

Chlorhexidine gluconate 2% solution was used as the positive control for the study and distilled water as the negative control.

Agar diffusion test

Brain heart infusion (BHI) broth and agar were used to maintain cultures of E. faecalis. Cultures were grown on BHI broth on a rotary shaker at 150 rpm. Changes in turbidity help in assessing bacterial growth. Agar diffusion method was performed to assess the antibacterial efficacy of medicaments/irrigants (chlorhexidine, tulsi extract, neem extract, and distilled water). Four cultures (200 μm) were spread over BHI broth agar plates. Wells of 6 mm diameter were made in these agar plates. Chlorhexidine gluconate, tulsi extract, neem extract, and distilled water were added to specific wells and the agar plates were incubated at 37°C for 24 h. Zones of inhibition were checked after incubation of each plate against E. faecalis. They were recorded and statistically assessed using one-way analysis of variance (ANOVA) test and SPSS software version 20 (IBM) (P < 0.001).
RESULTS

Table 1 and Graph 1 show the mean values of antibacterial actions of chlorhexidine gluconate (2%), tulsi (4%), neem, and saline medicaments against \textit{E. faecalis} in millimeters. Graph 1 shows the mean inhibition zone against \textit{E. faecalis} from highest to lowest: chlorhexidine (26.4) > neem (20.5) > tulsi (16.9) > distilled water (0). One-way A NOVA results indicate statistically significant mean zone of inhibition of the three groups from each other at F = 1438.276, \( P \leq 0.001 \).

DISCUSSION

Nowadays use of natural extracts in dentistry is gaining popularity because of their lesser side effects. Hence, several studies have been undertaken to evaluate herbal medicines in Endodontics.[1]

\textit{E. faecalis} was taken in the present study because it has been identified as the most common species in root canal diseases.[1] The present study has shown maximum inhibitory effect against \textit{E. faecalis} by chlorhexidine followed by neem extract and then tulsi extract and no effect with distilled water. Similar results were observed by Prabhakar \textit{et al.}[7] Arora \textit{et al.}[10] and Rani \textit{et al.}[11] found highest antimicrobial effect with 0.2% chlorhexidine compared to herbal medicament (\textit{Morinda citrifolia}, garlic and turmeric), whereas Vinthukumar \textit{et al.}[4] found that neem extract is more effective than sodium hypochlorite 5.25% against \textit{E. faecalis}.[4]

Bhardwaj \textit{et al.} observed in their study 100% inhibition of bacterial growth (\textit{E. faecalis}) with chlorhexidine followed by \textit{M. citrifolia} gel, aloe vera, papin gel, and calcium hydroxide.[1] Agarwal \textit{et al.} found that 4% tulsi extract has potential antimicrobial properties. Similarly, our results showed acceptable zone of inhibition against \textit{E. faecalis}.[9] Vinthukumar \textit{et al.}[4] and Ghonmode \textit{et al.}[12] found that neem was highly effective against \textit{E. faecalis} compared to 5.25% sodium hypochlorite and other extracts. This is similar to our results.

Bazvand \textit{et al.}, observed that herbal medicines (propalis, aloe vera) were effective against \textit{E. faecalis} compared to chlorhexidine.[3] Similar results were observed by Lakshmi.[3] Several studies showed that herbal extracts are relatively effective as irrigants and help in removal of smear layer, such as amla, neem, and Triphala.[13] Zakarea \textit{et al.} found that castor detergent 20% and papain enzyme 4% (MCP) has the ability to completely eradicate \textit{E. faecalis} bacteria from the infected root canal \textit{in vitro} in 5 min.[14]

CONCLUSION

Herbal extracts (neem, tulsi) have shown significant inhibitory effects against \textit{E. faecalis} compared to 2% chlorhexidine gluconate. Hence, these can be used alternatively as endodontic irrigants/medicaments.

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Conflicts of interest

There are no conflicts of interest.

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