Comparative Evaluation of Anti Microbial Efficacy of Root Canal Sealers Modified With Different Herbal Extracts against E Faecalis: An In Vitro Study

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Abstract

Introduction: In endodontic diseases microbes are considered the primary etiological factor. Ways of reducing the microbial load is by root canal debridement and antibacterial materials. In order to determine the success of endodontic treatment, sealing root canals with various materials possessing potent bactericidal effect has proven to be useful. The use of herbal extracts have improved due to cytotoxic reactions of sealers and its ineffectiveness to eliminate bacteria completely from dentinal tubules.

Aim: To compare antimicrobial activity of endodontic sealer modified with different herbal extracts against E faecalis.

Materials and methods: In the present study, the antimicrobial efficacy of commercially available AH Plus sealer mixed with 3 herbal extracts i.e. Azadirachta indica (Neem), Ocimum tenuiflorum (Tulsi), Black seed extract are evaluated against E faecalis. An agar well diffusion method was used to determine the capability of the root canal sealer against E. faecalis (ATCC 29212). After every 24 hrs zone of inhibition of E faecalis was recorded. The differences between groups were analyzed by one-way ANOVA and Tukey’s post hoc tests for intergroup analysis.

Conclusion: AH-Plus sealer and neem extract combination exhibited larger zone of inhibition than tulsi oil and black seed oil against E. faecalis at 24hrs.

Keywords: E faecalis, herbal extracts, antibacterial efficacy, sealer.
Introduction

Microorganisms and their products are the main aetiological factors in dentinal, pulpal and periapical pathosis (Kakehashi et al. 1965, Brannstrom & Nordenvali 1978, Fabricus et al. 1982, Barnett et al. 1990, Sundqvist 1992). The main goal of root canal treatment is the elimination of bacteria from the infected root canal and prevention of secondary reinfection. This is mainly achieved by thorough irrigation and biomechanical preparation of the root canal, followed by a canal filling that should seal the canal system from bacterial ingress from the oral cavity and periradicular tissues. (1) Long-lasting sealing ability and adaptation to the root canal walls are one of the prime requisites for a root canal sealer.

The presence of bacteria in dentinal tubules and cementum even after treatment has been reported. (Dalton et al. 1998, Molander et al. 1999, Shupinget al. 2000, Chavez De Paz et al. 2003, Sundqvist & Figdor 2003). Microbial load and its persistence is seen in dentinal tubules, lateral canals and apical ramifications have also been demonstrated. A well-adapted sealer will only hinder the release of bacteria entrapped within the root canal system.(2,3). But, for eradication of the remaining microorganisms, particularly when pulpal necrosis and apical periodontitis are present, the choice of a sealer with substantial antimicrobial activity could play an important role (Spangberg et al. 1973).

An ideal sealer should have the ability to destroy the residual bacteria present on the dentinal walls of root canals along with those present deep inside the dentinal tubules. In order to achieve the antimicrobial activity of sealer, it should diffuse inside the dentinal tubules. In order to gain this, the sealer should have both antimicrobial properties and good flow properties. Enterococcus faecalis, facultative Gram-positive cocci, is present in over one third of the canals of teeth with persisting periapical lesions. It is one of the most frequently isolated species, recovered in over one-third of the canals of root-filled teeth with persisting periapical infection and has been used as the test organism in this study.

Materials and Methods

In the current study, the following root canal sealers were tested:

Group 1 – AH PLUS sealer, Group 2-AH PLUS sealer and neem extract, Group 3 - AH-Plus sealer and tulsi extract, Group 4-AH PLUS and black seed extract.

**Preparation of the Medium for Enterococcus Faecalis**

ATCC 29212 the standard strain of E. faecalis was used in the current study and was subcultured in blood agar plate and was incubated at 37°C for 24 h. E. faecalis colony was isolated from the cultured plate and to confirm its growth Gram staining was performed. The growth of the organism was checked under oil immersion microscope and then inoculated with a brain-heart infusion (BHI) broth. The BHI-broth was incubated at 37°C for 24 h period and checked for bacterial growth by changes in turbidity. A drop of BHI-broth containing E. faecalis was placed into saline solution and checked for correct bacterial concentration with a spectrophotometer. By analyzing the broth at a density associated to the barium sulfate standard of 0.5 McFarland units, which was equal in value to $1.5 \times 10^8$ CFU/ml, the density of the bacterial suspension is standardized.
Antimicrobial activity by agar diffusion test Mueller-Hinton agar was used to prepare petri plates. The fresh inoculums of E. faecalis of 0.5 McFarland standard suspensions were formulated and the sterility of the plates was checked. The plate was turned at 60° angle between each streaking. For 5–15 min the inoculums were allowed to dry with lid in place. Four wells of size 8 mm was created with the help of sterile cork borer. The amount of the root canal sealer was placed the well and then it was checked against E. faecalis. Diffusion of 10-15 min was allowed for the medicament in agar and then was incubated immediately at 35 ± 2°C for 24 h. The whole experiment was carried out under aseptic conditions and was repeated twenty times to ensure reproducibility.

Measurement of inhibition zones Zones of bacterial growth inhibition were measured at the end of 24 and 48 h using inhibition zone measuring scale.

Result

Based on the mean diameters, AH Plus and neem extract combination had the highest zone of inhibition followed by Tulsi extract and then the AH Plus. Black seed extract and AH Plus combination had the least zone of inhibition.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH Plus</td>
<td>9.78 mm</td>
</tr>
<tr>
<td>AH Plus + Neem Extract</td>
<td>11.07 mm</td>
</tr>
<tr>
<td>AH Plus + Tulsi Extract</td>
<td>10.51 mm</td>
</tr>
<tr>
<td>AH Plus + Black Seed</td>
<td>0 mm</td>
</tr>
</tbody>
</table>

Discussion

The main purpose of endodontic sealers in root canal is to fill the gap between the core material and root canal
wall, and help minimize leakage to reduce the possibility of infection by residual microbes. Good antimicrobial property is considered to be highly desirable for an ideal root canal sealer. (11, 13)

AH Plus sealers releases formaldehyde in the polymerization process which stated the antimicrobial effect of resin-based sealers. Kangarlou et al demonstrated that freshly mixed AH Plus had strong antimicrobial activity against E. faecalis, while no antimicrobial activity was found after 1, 3 and 7 days setting.

The Azadirachta indica (Neem) extracts has undergone various antimicrobial screening. The constituents responsible for antimicrobial activities of neem were nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone and cyclictrisulphide. Its anti-adherence activity by altering bacterial adhesion and the ability of organism to colonize has resulted in AI having the maximum reduction in adherence of E. faecalis to dentin.

Ocimum sanctum (Tulsi) is a holy plant of Indian origin. It is known as the mother medicine of nature. Tulsi has been tested for its antimicrobial properties against Escherichia coli, Klebsiella, Candida albicans, Staphylococcus aureus, Enterococcus faecalis, and Proteus. Ursolic acid and carvacrol are responsible for the antimicrobial activity of tulsi.

Nigella sativa (Black seed) is of considerable interest nowadays. The antibacterial activity of black seed oil is related directly to its active constituent thymoquinone and its related compounds thymohydroquinone and thymol. These active volatile constituents are present in low percent in cold pressed oil and sensitive to light, heat, depends on source of seed and extraction method.

Conclusion

Almost negligible side effects are seen with the herbal products. These medicinal plants have anti-inflammatory, antibacterial, and antioxidant properties that inhibit pathogens greatly without effecting the dental tissues. They not only heal but also protect by increasing immunity. More tests and research need to be done to discover better treatments and usage of these herbs and to analyze their side effects if any. The usage of natural extracts have gained popularity in dental products.

References


