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An in vitro study to compare antibacterial efficacy of cinnamon extract, neem extract and sodium hypochlorite as root canal irrigants

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***Abstract*---**Background: E. faecalis is the predominant micro-organism recovered from root canal of the teeth where previous endodontic treatment has failed. Thorough debridement and complete elimination of micro-organisms are objectives of an effective endodontic treatment. For many years, intracanal irrigants have been used as an adjunct to enhance antimicrobial effect of cleaning and shaping in endodontics. Aim: The aim of the study is to compare the antibacterial effectiveness between cinnamon extract as irrigant with neem extract irrigant and sodium hypochlorite against *E. fecalis*. Methodology: The present study is a randomized controlled *in vitro* study conducted on 90 extracted permanent single rooted teeth. The teeth were divided into three groups- Group 1 was irrigated with

cinnamon extract, Group 2 was irrigated with neem extract irrigant and Group 3 with 3% sodium hypochlorite. The colonies in the reduction of *E. feacalis* was noted as CFU values per ml. Wilcoxon signed ranked test was used to compare in between the three groups. Mann- Whitney's test was used for inter group comparison between the three groups. Results: Our results showed that the Cinnamon extract had the maximum reduction in the CFU colonies followed by sodium hypochlorite. Neem extract irrigant had the least reduction in the colonies of *E. feacalis*. ($P < 0.05$). Conclusion: Cinnamon extract irrigant show better reduction in *E. fecalis* as compared to 3% sodium hypochlorite and neem extract irrigant.

***Keywords*---**cinnamon extract, endodontic irrigant, Enterococcus feacalis, neem extract, sodium hypochlorite.

Introduction

The objective of root canal treatment is to thoroughly clean the root canal system debride it, so that it can be sealed without microleakge. This process is also called as chemico-mechanical process in which the canal is derided with the help of chemical irrigants and mechanical instruments.[1]Biomechanical preparation involves removal of infected dentin and debridement of the root canals. Instrumentation of the root canals shapes and prepares the canal to aid in better debridement.The ideal requirements of irrigants include its ability to dissolve pulp along with removal of smear layer and an extended antimicrobial action.[2-6]Sodium hypochlorite fulfils most of the requirements of the ideal endodontic irrigant and is widely used in dentistry. It has been the choice of irrigant due to its pulp dissolving capacity, antimicrobial action and ability to remove smear layer. However, cases have been reported of allergic reaction due to the use of sodium hypochlorite.[7-10] There have been cases of tissue toxicity and accidental mishaps associated with sodium hypochlorite. A bad smell and taste is also associated with irrigation using sodium hypochlorite. To overcome this disadvantage, other herbal alternatives have been considered over time.Neem also known as *Azadirachta indica* is widely used in clinical dentistry due to it antibacterial and antiviral properties.Studies have been done to evaluate the antibacterial effectiveness of neem against wide variety of micro-organism.[11-15]The advantage of using neem as an endodontic irrigant is due to its antibacterial and antioxidant properties. It shows a wide spectrum of action against gram positive and gram negative oraganism.Cinnamon also known as *C. zeylanicum* has gained popularity in recent time due its antibacterial activity.The antibacterial action of cinnamon is due to the presence of vanillic, caffeic, gallic, protocatechuic, p-coumaric, and ferulic acids in the extracts.[16-20] It has shown effectiveness against *S. mutans* and *E. fecalis* organisms.It is also known to show a wide spectrum of action on gram positive and gram negative organism.Therefore, the aim of the present study is to compare neem extract, cinnamon extract and sodium hypochlorite as irrigant. The comparison is based on the effectiveness of these irrigants against *E. fecalis* in extracted permanent teeth.

Methodology

The present study followed an *in vitro* study design. The study design was approved by the institutional review board of the dental institution.The present study uses ethanolic extract of neem and cinnamon to prepare the irrigation solution.The ethanolic extract of neem was obtained from neem leaf ground into coarse and medium coarse particles. These particles were mixed with 50% ethanol and this suspension was placed on the magnetic stirrer for 4 hours. Whitman's filter paper was used to obtain the filtrate which was then dried to obtain the extract. The extract was dried to evaporate the solvent to prevent the influence of extracting agent (ethanol) on the final extract. Cinnamon extract was obtained using cinnamon bark which was pulverized into medium coarse granules. The extracting agent and the procedure to obtain the final extract was similar to that for neem extract. These extracts were stored in dark coloured bottles at 4 degree Celsius until use.The irrigating solution was prepared my mixing the crude extract in DMSO (dimethyl sulfoxide) solvent. Previous studies have noted MIC for neem extract to be 25% and for cinnamon extract to be 20%. Neem extract was prepared by dissolving 25 grams of crude extract in 100 ml DMSO solution. Similarly, cinnamon extract was prepared by dissolving 20 grams of crude extract in 100 ml of DMSO. The final solution were refrigerated at 4 degree Celsius.The present study includes a total of 90 single rooted extracted teeth for periodontal or orthodontic purpose. Any tissue tags, calculus or debris present on the teeth were removed to obtain a clean external surface. These teeth were stored in normal saline until use to prevent desiccation of the tooth surface.
The extracted teeth were mounted on individual block of modelling wax. Access opening was done with No. 4 round carbide bur (DENTSPLY Maillefer, OK, USA). The working length was determined using radiograph and was kept 0.5 mm short of the radiographic apex. After initial instrumentation up to no 30 stainless steel hand files, rotary instrumentation was done using ProTaper (DENTSPLY Maillefer, OK, USA) up to F3. After complete instrumentation the tooth were scheduled for the inoculation of *E. Fecalis*

A pure culture of test strain of *E. fecalis* ATCC 29212 was prepared in sterile nutrient broth. The extracted teeth placed in the nutrient broth containing *E. fecalis* and incubated for 21 days at 37 degree Celsius. The level of the nutrient broth was checked daily, and new broth inoculated with *E. fecalis* was added to maintain all the extracted teeth completely submerged in the inoculated broth for 21 days.The inoculated teeth were divided into three groups for the irrigation protocol. 30 teeth were randomly assigned to each group. Each tooth was assigned a serial number. The allocation of each sample into individual groups was done using block randomization using lots. Group 1 was irrigated using 3% sodium hypochlorite, group 2 was irrigated using neem extract irrigant and group 3 was irrigated using cinnamon extract irrigant. The solution was irrigated using flexible tip attached to a 5 ml irrigating syringe. Each tooth was irrigated with 5 ml of irrigating solution. The presence of *E. fecalis* was verified using an eosinophilic and gentian violet staining to verify gram positive organism.The data obtained were entered in the spreadsheet and and statistical analysis was done using SPSS software version 22. (IBM Corp, Armont, NY, USA). Kruskal-Wallis test was used to compare the CFU values per ml between the three groups. Mann-

Whitney's test was used was used in multiple pairwise comparison between the groups.

Results

A total of 90 permanent single rooted teeth are included in the study. The mean CFU values obtained using neem as irrigant was 0.72 × 10^3 having a standard deviation of 0.34. The mean CFU values obtained using cinnamon extract as irrigant was 0.25 × 10^3 having a standard deviation of 0.08. The mean CFU values obtained using sodium hypochlorite as irrigant is 0.40 × 10^3 having a standard deviation of 0.30 [Table 1].

Table 1: Comparision of colony forming units values between cinnamon extract irrigant, neem extract irrigant and sodium hypochlorite

Group	Number of Samples	Mean	SD	*P*
Sodium Hypochlorite	30	0.43 x 10^3	0.32	0.008*
Neem Extract	30	0.29 x 10^3	0.27	
Cinnamon Extract	30	0.80x 10^3	0.79	

*Significant: *P<0.05,*Kruskal – Wallis test . SD = Standard deviation

Kurskal-Wallis test was used to compare CFU values between the three groups. Kruskal Wallis test showed a significant difference between the three groups with cinnamon extract irrigant having the least CFU values (*P* = 0.008). Mann-Whitney's test was used for multiple pairwise comparison between the three groups. Cinnamon extract irrigant showed significantly less CFU values as compared to neem extract irrigant (*P* = 0.004). However, cinnamon extract irrigant as compared to sodium hypochlorite showed lower CFU values but the results was not statistically significant (*P* = 0.189) On the other hand sodium hypochlorite irrigant showed statistically significant lower CFU values as compared to neem extract irrigant (*P* = 0.032). Thus, cinnamon extract showed statistically significant difference to reduce the *E. fecalis* colonies as compared to other two groups.

Discussion

Chemical irrigants have been in practice over a long time in the disinfection of infected root canal.Due to its antibacterial action and pulp dissolving ability, sodium hypochlorite is being used as an ideal irrigant for root canal treatment. Other irrigants with increased increased antibacterial effectiveness such as chlorhexidine has also been advocated in necrotic teeth.However, there has been an increased allergic reaction and adverse effects associated with the use of chemical irrigants.To overcome this, the use of herbal alternatives having comparable antibacterial effectiveness should be considered.

Neem extract is the most common herbal extract used as an irrigant due to its antioxidant properties which aid in antibacterial action against *E. fecalis*. Dutta and Kundabala[20] in 2014 showed similar antibacterial efficacy of neem extract as irrigant with sodium hypochlorite and chlorhexidine irrigant against *E. fecalis*. Cinnamon extract has recently gained a limelight as an irrigant in

endodontics due its antibacterial properties. Gupta-Wadhwa *et al.*[22] have demonstrated comparable antibacterial effectiveness of cinnamon than other herbal irrigants.Gupta *et al.* [21]also shows higher antibacterial effectiveness of cinnamon extract as compared to other herbal agents.

The present study shows cinnamon extract irrigant to have better antibacterial effectiveness followed by sodium hypochlorite. The neem showed to have least antibacterial effectiveness. These results are contradictory to previous study by Gupta-Wadhwa *et al.*, [22] where sodium hypochlorite showed higher effectiveness as compared to cinnamon extract.This can be attributed to a lower concentration of sodium hypochlorite used in the present study as compared to other study.[15-21] The present study showed similar results in relation to comparison of neem and sodium hypochlorite.

The present study compares the antibacterial effectiveness of only two herbal agents. Further improvement in the study can be done by including more herbal agents in the comparison. The present study evaluates the colony forming units per ml to determine the antibacterial efficacy. However, further improvement in the study can done by evaluation of the antibacterial efficacy by more standardized methods like PCR. This can aid in achieving more standardized results. This study thus acts as a preliminary study to compare cinnamon extract, neem extract irrigant and sodium hypochlorite irrigant. This study recommends further studies at a molecular level to understand the exact mechanism of the action of pharmacological agents.

Conclusion

The present study shows cinnamon extract to have better effectiveness followed by sodium hypochlorite and neem extract irrigant. This study thus acts as a preliminary study to compare the antibacterial effectiveness of cinnamon extract irrigant and neem extract irrigant with sodium hypochlorite.

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