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Comparative Evaluation of Antibacterial and Smear Layer Removal Efficacy of Two Different Herbal Irrigants: An In Vitro Study

ARVIND KUMAR[1], A SHEERIN SARTHAJ[2], S MARIA ANTONY[3]

ABSTRACT

INTRODUCTION: Irrigation during root canal therapy is especially needed for teeth with complex internal anatomy. Biomechanical preparation of root canal produces "smear layer" which gets embedded with debris and microorganisms. The major side effects of these widely used chemical irrigants such as Sodium hypochlorite for antibacterial efficacy and EDTA for smear layer removal has led to the search of a more biocompatible irrigant.

MATERIALS AND METHODS: Azadirachta indica(neem) and Ocimum sanctum(tulsi) extract were used in this study as herbal irrigants to evaluate the above mentioned properties by the q PCR method and SEM analysis.

RESULTS: In antimicrobial efficacy, the Mean value of NaOcl showed higher antimicrobial efficacy followed by Neem leaf extract and the lower antimicrobial efficacy was recorded with Tulsi extract. Similarly, the mean smear layer removal by different irrigants at different root levels, EDTA showed the maximum smear layer removal, followed by Neem leaf extract and Tulsi extract showed the least smear layer removal. Hence Tulsi has poor smear layer removal efficacy and comparable antimicrobial efficacy when compared with Neem extract

STATISTICAL ANALYSIS: The collected data were analyzed by one-way analysis of variance to compare the mean of the groups. The post-hoc test (Tukey) was performed to find the interrelationship between different groups for significant difference ($P < 0.05$; confidence interval 95%).

CONCLUSION: From this study, these two herbal irrigants are proved to be safe and effective. It can be concluded that neem leaf extract has a significant antimicrobial efficacy against E. faecalis and significant smear layer removal efficacy compared to 17% EDTA.

KEYWORDS: Antibacterial, Smear Layer, Herbal Irrigants, qPCR, Scanning Electron Microscopy

INTRODUCTION

Pulpal and peri-radicular infections are caused by a variety of microorganisms in the root canal. The aim of the root canal therapy is to eliminate microorganisms from the root canal to provide an appropriate environment for tissue healing.[1] Success rate of endodontic treatment depends upon adequate biomechanical preparation, irrigation, and obturation of the root canal.[2] Irrigation during root canal therapy is especially needed for teeth with complex internal anatomy. Even though Sodium hypochlorite is the most effective irrigating solution because of its dissolution property of the organic content, it also has several undesirable characteristics such as toxic and potential irritant to the periapical tissues and its unpleasant smell and taste.[3]

Biomechanical preparation of root canal produces "smear layer" which gets embedded with debris and microorganisms. The smear layer may interfere with the penetration of intracanal medicaments and sealers into the dentinal tubules. Ethylene-Diamine Tetra Acetic acid (EDTA) is effective for smear layer removal. The major drawback of EDTA is that it reacts with calcium ions in dentine, produces calcium chelation, it promotes dentine decalcification at depths of 20–30 μm within 5 minutes. EDTA decreases the dentin microhardness and its increased with increased time of application.[1,3]

The major side effects of these widely used chemical irrigants such as Sodium hypochlorite and EDTA led to the search for a more biocompatible irrigant. Among the various herbs one such herbal alternative is Azadirachta indica (Neem). Not just one but all parts of medicinal herbs and plants can be used therapeutically.[3] Another one is Ocimum sanctum (Tulsi), which is native to the Indian subcontinent, consisting mostly of Eugenol (70%), Aerosol acid, Oleanolic acid, β- element(11%), β-caryophyllene and germacrene . The constituents of these widely used herbal irrigants are well known for anti-inflammatory; immune-modulatory, antibacterial, antifungal, antiviral, antioxidant, and anti-carcinogenic properties.[4] Hence these two herbal irrigants were chosen for this study.

Hence, the present study was undertaken to evaluate the efficiency of 17% EDTA, Neem leaf extract and Tulsi extract endodontic irrigants in

smear layer removal through scanning electron microscopic image analysis and the Antimicrobial efficacy of herbal alternatives (Azadirachta indica extract, Ocimum sanctum) and to compare it with 3% sodium hypochlorite against E. faecalis by qPCR.

MATERIALS AND METHOD

Preparation of Azadirachta indica and Ocimum sanctum extract

100 g of neem and tulsi leaves were tied in a cloth and soaked in 800 ml of distilled water. To make the solution attain a 25% concentration, the beaker was boiled under low flame. It was filtered using a filter paper. Herbal irrigants were subjected to phytochemical study for the presence of its appropriate active constituents.

Grouping

120 maxillary central incisors were categorized into two groups, and 60 samples were allocated for each group; Group 1: Antimicrobial efficacy(n=60), Group 2: smear layer removal efficacy (n=60).

Group I was divided into three sub-groups:
Group IA: NaOCl(n=20)
Group IB: Neem extract(n=20)
Group IC: Tulsi extract(n=20)

All the 120 teeth were decoronated at the level of CEJ to standardize the length of 12 mm. E. Faecalis was inoculated into the root canal for about 21 days. In the E. Faecalis inoculated canal, Cleaning and shaping were done by using K-files(Mani, K-files) apically up to 30 K-size and coronal up to 50 K-size files inside the UV-Chamber. To prevent taper lock, the needle was kept passively at 1 mm from the working length. The specification of the needle was 25 gauge, followed by irrigation with 6 ml of the irrigants at a rate of approximately 2 ml/15 seconds. Drying of canals was ensured with paper points. Contact time was 20 minutes. Samples were obtained by using Headstoerm files(Mani H– files). Samples were analyzed by qPCR (Mastercycler x50) to obtain the threshold cycle (CT) value of samples.

Real-Time quantitative polymerase chain reaction

The universal primers used amplified enterococcal DNA sequence. The PCR evaluation was done as follows, initial denaturation at 94°C for 15 seconds, annealing at 55°C and extension at 72°C for 45 seconds and then cooled to 4°C until removed. The thermo cycler (7900 HT Real-time PCR system).

In Group II, For analyzing smear layer removal efficacy, Samples were further divided into 3 subgroups
Group IIa: EDTA(n=20),
Group IIb: Neem extract(n=20),
Group IIc: Tulsi extract(n=20).

After decoronation, Cleaning and shaping were done by using K-files (Mani K-files) 30 size apically and 60 sizes coronally, 25-G needle tip was placed to a depth of 1 mm short of working length, followed by respective irrigation with 6 ml of the irrigants at a rate of approximately 2 ml/15 seconds. Deep grooves were made on the buccal and palatal aspect of the root. Longitudinal sectioning was done by using chisel and mallet. Samples were examined under Scanning electron microscope. Scanning electron microscopy (Zeiss), which can assess the effectiveness of various irrigants in the removal of smear layer:

Smear layer removal scores (Rome et al.: 1985)

Score	Interpretation
0	No smear layer
1	Smear layer present only in aperture of dentinal tubules
2	Thin smear layer covering the root canal surfaces and dentinal tubules openings
3	Heavy smear layer masking dentinal tubule apertures

STATISTICAL ANALYSIS

The collected data were analyzed by one-way analysis of variance to compare the mean of the groups. The post- hoc test (Tukey) was performed to find the interrelationship between different groups of significant difference ($p<0.05$; confidence interval 95%).

RESULTS

In qPCR, threshold cycle values were obtained. Threshold cycle values are inversely proportional to DNA population. In antimicrobial efficacy, the Mean value of NaOcl showed higher antimicrobial efficacy followed by Neem leaf extract and the lower antimicrobial efficacy was recorded with

Tulsi extract. In the inter group comparison showed significant differences for all the three groups. Table 1 also depicts that Tulsi extract had the least antimicrobial efficacy among three irrigants.

Similarly, the mean smear layer removal by different irrigants at different root levels, 17% EDTA showed the maximum smear layer removal (1.20) followed by Neem leaf extract(1.9). Tulsi extract (2.7) showed the least smear layer removal. Hence Tulsi has poor smear layer removal efficacy and comparable antimicrobial efficacy when compared with Neem extract. The smear layer removal efficacy of the three groups are depicted in Table 2.

DISCUSSION

Elimination of microflora in the root canal is not achieved only by cleaning and shaping of the root canal. Because the areas which are inaccessible in the root canal by the cleaning and shaping lead to endodontic failure. Proper irrigation is essential to eliminate the microflora in the inaccessible areas in the root canal. Young tender branches of Neem are used as "chewing sticks" to keep the teeth and gum clean and healthy. Toothpaste containing neem is also available in India and Europe.Limited clinical trials have shown neem toothpaste have a potential to cure gingivitis.[5,6] For over centuries, Tulsi termed as " The Queen of herbs, " and it has been known for its remarkable healing properties.[7] Because of the easy availability and its Known medicinal values of Neem and Tulsi, these two herbs were chosen for this study.

In this study Maxillary central incisors were selected to have single straight canals. Enterococcus faecalis is a persistent organism and plays a vital role in root canal infections. It is commonly found in failed root canals. E. Faecalis is inoculated into the root canal for about three weeks Because the 3-week inoculation of E.Faecalis in the root canal reaches a depth of 200 µm to 400 µm into the dentinal tubules.[6] Hence E. faecalis was chosen for this study.[8]

In this study, Quantitative Real-time Polymerase chain reaction was used to evaluate the Antimicrobial activity of an irrigant. In qPCR, during each amplification round fluorescent dye was released by the amplified DNA to cycles which allows the products to be detected and analyzed and measured in real-time when the amplification is the first detected.[9]

Removal of smear layer is mandatory because it does not permit a hermetic seal between the Gutta-percha and the sealer.[7] For evaluating smear layer removal Scanning electron microscope is one of the most commonly used technique and hence it was used in the present study.[10]

From this study, 2 herbal extracts used in this study are proven to be safe, containing active constituents that have an antimicrobial, antioxidant and anti-inflammatory activity. Because of these Antimicrobial, Anti-inflammatory, and therapeutic effects, These two potential extracts would appear promisingly to replace the traditional root canal irrigant. In this study, Neem leaf extract showed Significant Antimicrobial activity and smear layer removal efficacy because of its active constituents such as Nimbin, nimbidin and nimbidol and tulsi showed comparable antimicrobial efficacy because of its active ingredients such as Eugenol, Ursolic acid, carvacrol and oleanolic acid. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in Ocimum sanctum L., has been found to be largely responsible for the therapeutic potentials of Tulsi.

CONCLUSION

From this study, these two herbal irrigants are proven to be safe and effective. It can be concluded that neem leaf extract has a significant antimicrobial efficacy against E. faecalis and significant smear layer removal efficacy compared to 17% EDTA.

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AUTHOR AFFILIATIONS
1. Professor
2. PG Student
3. PG Student
Department Of Conservative Dentistry & Endodontics, Rajas Dental College & Hospital

Corresponding Author:
Dr. Sheerin Sarthaj
PG Student
Department Of Conservative Dentistry & Endodontics
Rajas Dental College & Hospital
Kavalkinaru, Tamil Nadu-627105

For manuscript enquiry/author contact details, e-mail at:
maunscriptenquiry.ihrj@gmail.com

LEGENDS

IRRIGANTS	No.	MEAN	INTERGROUP COMPARISION	p-value
NaOcl	20	34.20	Neem leaf extract Tulsi extract	0.513 0.000
Neem	20	33.93	Naocl Tulsi extract	0.513 0.000
Tulsi	20	31.86	Naocl Neem leaf extract	0.000 0.000

Table 1. Antimicrobial efficacy of the three groups

IRRIGANTS	No.	MEAN	INTERGROUP COMPARISON	p-VALUE
EDTA	20	1.20	Neem leaf extract Tulsi extract	0.074 0.000
Neem leaf extract	20	1.90	EDTA Tulsi extract	0.074 0.037
Tulsi extract	20	2.70	EDTA Neem leaf extract	0.000 0.037

Table 2. Smear layer removal efficacy of the three groups