

Original Article

COMPARATIVE EVALUATION OF THE EFFICACY OF THREE ANTI-OXIDANTS VS NaOCl AND EDTA: USED FOR ROOT CANAL IRRIGATION IN SMEAR LAYER REMOVAL-SEM STUDY

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ABSTRACT

Objective: The aim of this study was to compare the efficacy of 3 Anti-Oxidants versus NaOCl and EDTA: used for root canal irrigation in smear layer removal by SEM analysis.

Methods: Root canal treatment was performed on 100 single rooted teeth and the smear layer removing abilities of Neem, Triphala, Amla EDTA and Saline were checked by using them as the last irrigant.

Results: EDTA and Amla showed the best smear layer removing ability followed by Neem and Triphala.

Conclusion: Neem, Triphala and Amla showed the potential to remove the smear layer. EDTA showed the maximum efficacy in removing the smear layer.

Keywords: Anti oxidants, Smear layer Neem, Triphala, Amla.

INTRODUCTION

Successful endodontic therapy requires shaping and cleaning of root canal systems [1]. During canal preparation of infected teeth, special attention must be given to the elimination of bacteria, their toxins and smear layer from the root canal system [2].

Scanning electron microscope studies of cavity preparations by Brannstrom & Johnson (1974) demonstrated a thin layer of grinding debris [3]. They estimated it to be 2-5 μ m thick, extending a few micrometers into the dentinal tubules. The first researchers to describe the smear layer on the surface of instrumented root canals were McComb & Smith (1975) [4].

The term 'Smear layer' is used most often to describe the grinding debris left on dentin by cavity preparation. However, the term applies to any debris produced iatrogenically by the cutting, not only of dentin, but also of enamel, cementum and even the dentin of the root canal [5].

Cengiz *et al.* (1990) proposed that the penetration of smear material into dentinal tubules could be caused by capillary action as a result of adhesive forces between the dentinal tubules and the material [6]. This hypothesis of capillary action may explain the packing phenomenon observed by Aktener *et al.* (1989) who showed that the penetration could increase up to 110 μ m when using surface-active reagents in the canal during endodontic instrumentation [7].

The thickness may also depend on the type and sharpness of the cutting instruments and whether the dentine is dry or wet when cut [7]. In the early stages of instrumentation, the smear layer on the walls of canals can have a relatively high organic content because of necrotic and/or viable pulp tissue in the root canal. Increased centrifugal forces resulting from the movement and the proximity of the instrument to the dentine wall formed a thicker layer which was more resistant to removal with chelating agents like EDTA [7].

The most common chelating solutions are based on EDTA which reacts with the calcium ions in dentine and forms soluble calcium chelates. It has been reported that EDTA decalcified dentine to a depth of 20-30 μ m [8].

Sodium hypochlorite (NaOCl), in a 1±5.25% concentration is an irrigating solution used widely in root canal treatment because of its bactericidal properties and ability to dissolve organic tissues [9] but NaOCl has not been shown to be effective in removing the smear

layer [10]. The organic tissue-dissolving activity of NaOCl is well known and increases with rising temperatures. However, the capacity to remove the smear layer from the instrumented root canal walls have been found to be insufficient. Many authors have concluded that the use of NaOCl during or after instrumentation produces superficially clean canal walls with the smear layer present [11].

The use of herbal alternatives as a root canal irrigant might prove to be advantageous considering the several undesirable characteristics of NaOCl. Cytotoxic and genotoxic effects on human peripheral lymphocytes have been observed with the usage of NaOCl. Also the extensive use of antibiotics can generate drug-resistant bacteria and it is necessary to develop new materials in order to attack this problem.

Various alternative irrigants that are currently being worked upon are Triphala, Green Tea Polyphenols, Neem, *Morinda citrifolia* and Propolis [12,13]. But their efficacy in removal of smear layer is not well known. Evaluation of the capability of alternative irrigants to remove the smear layer, is the need of the hour, particularly as they are increasingly replacing the conventional irrigants. Any residual smear layer that remains shall impair the lateral penetration of resin based sealers that are so popular these days [14]. Alternative irrigants are proven to be safe, containing active constituents that have beneficial physiologic effect apart from its curative property such as anti-oxidant, anti-inflammatory and radical scavenging activity and may have an added advantage over the traditional root canal irrigants.

The aim of this study is to compare the efficacy of 3 Anti-Oxidants vs NaOCl and EDTA: used for root canal irrigation in smear layer removal by SEM analysis.

MATERIALS AND METHODS

This study was conducted in the Department of Bioinformatics and Biotechnology at University of Pune, Pune after obtaining approval from the Director of the Department.

Creation of the smear layer

- The study was conducted on 100 extracted mature human teeth. After extraction, the teeth were conserved in a solution of physiologic saline. Each individual tooth was then radiographed to visualize the root canal anatomy for making sure of a single canal.

- After cutting a four-wall access cavity, the full length of the canals was determined after #08 K-type file could be visualized at the apical foramen. The roots were sealed with melted wax to close the apical foramen. The aim was to prevent the irrigants from escaping through the apex in order to simulate *in vivo* conditions.

- The samples were then divided into five experimental groups. DMSO (Dimethyl Sulfoxide) is added to improve the efficiency of herbal products. This was stirred for 2 min and then passed through fast filter paper. The strained liquid was collected and used samples were prepared. All groups will consist of 20 teeth each, assigned as:

Group 1-Neem (n=20) (60 mg/ml in 10% DMSO)

Group 2-Triphala (n=20) (60 mg/ml in 10% DMSO)

Group 3-Amla (n=20) (60 mg/ml in 10% DMSO)

Group 4-5.25% Sodium Hypochlorite+EDTA (Positive Control, n=20)

Group 5-5.25% Sodium Hypochlorite+Normal Saline (Negative Control, n=20)

- The root canals were initially prepared using 2% hand files (upto #20 K file) followed by the Protaper rotary files system. All the canals were prepared in such a way that the finished size of each apical foramen was of 0.30 mm in diameter and 9% in taper (F3).

- After the use of each instrument, the canal was flooded with the 0.2 ml irrigant of the respective group. A total of 10 ml of the selected irrigant was used per canal. After instrumentation was complete the canal was irrigated with 2 ml of the respective irrigant for 2 mins for the removal of the smear layer. The final wash of the canal was done with 2 ml of saline.

- Group 4 served as the positive control group. In this group, the root canal was irrigated with 0.5 ml of EDTA as the final rinse.

- Group 5 served as the negative control group. In this group the root canal was irrigated with 0.5 ml of saline as final rinse.

Sectioning of the teeth and preparation for SEM

- The teeth were decelerated at the level of the CEJ for all the samples. Using a double ended carborundum disks, the roots were sectioned into two halves; for further study.

- Two horizontal grooves were made using a diamond-cutting disk mounted on a straight dental handpiece to split the root longitudinally.

- The objective was to avoid any intrusion of the cutting disc into the canals, which would pollute the samples by splattering cutting debris into the root canal system. The canal was then sectioned in the longitudinal plane with a precision diamond bur.

- A continuous supply of air was delivered to improve vision and cutting precision, which eliminated the potential of introducing debris into this region of the canal. Each root was longitudinally split by applying slight pressure to an enamel chisel into the longitudinal groove.

- The teeth were transferred to distilled water for 24 h. The specimens were dehydrated using a series of graded ethanol solutions (70, 90, 100%) and then vacuum dried. The specimen were Each specimen was sputter-coated with 35 nm of gold and examined using a scanning electron microscope.(ESEM, Carl Zeiss)

SEM Evaluation

Image acquisition of the middle third of the root canal of the sample was performed at a magnification of (1000 X) to assess the presence of the smear layer. The images were blindly evaluated by three blinded observers.

Statistical analysis

The difference in smear layer removal of Neem, Triphala, Amla, saline and sodium hypochlorite was analyzed using One way Analysis of Variance (ANOVA) followed by post hoc Tukey’s test for multiple pair wise comparisons.

All the statistical analyses were carried out using SPSS version (19.0). The data is expressed as mean±SD. P<0.05 was considered as significant and P>0.05 as not significant.

Evaluation criteria

The scores were given to the images according to the scoring criteria of Torabinejad *et al.* (2003) [15].

Table 1: It shows the evaluation criteria for smear layer removal

| Scores | Criteria |
|---------|--|
| Score 1 | No smear layer. No smear layer on the surface of the root canal; all tubules were clean and open. |
| Score 2 | Moderate smear layer. No smear layer on the surface of the root canal, but tubules contained debris. |
| Score 3 | Heavy smear layer. Smear layer covered the root canal surface and the tubules. |

RESULTS

The results showed that NaOCl+EDTA (1.28±0.05) (fig. 4) showed the best smear layer removing ability and NaOCl+Saline (2.7±0.05) (fig. 5) showed the worst smear layer removing ability.

Of the alternative irrigants used Amla (1.38±0.07) (fig. 3) showed the best results. There was no significant difference in the smear layer removing ability of NaOCl+EDTA and Amla. This was followed by Neem (1.7±0.13) (fig. 1) and Triphala (2.08±0.05) (fig. 2).

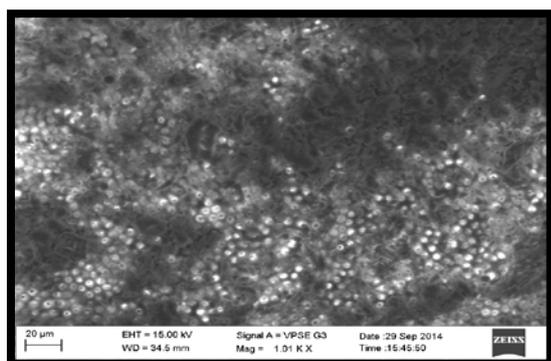


Fig. 1: It shows an SEM image of the dentinal tubules and smear layer removal viewed after irrigation with Neem at 1000x magnification

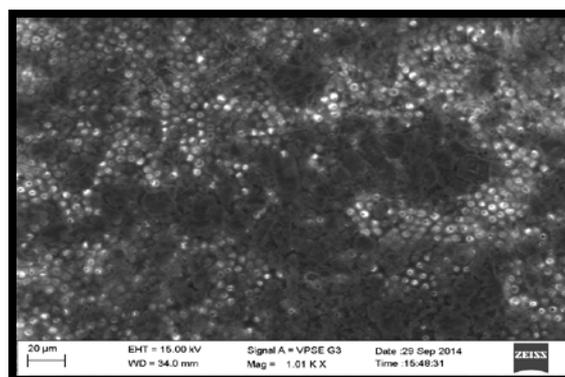


Fig. 2: It shows an SEM image of the dentinal tubules and smear layer removal viewed after irrigation with Triphala at 1000x magnification

There was a significant difference between the efficacy of Amla and Neem/Triphala ($p < 0.05$). There was a significant difference between the efficacy of Neem and Triphala ($p < 0.05$).

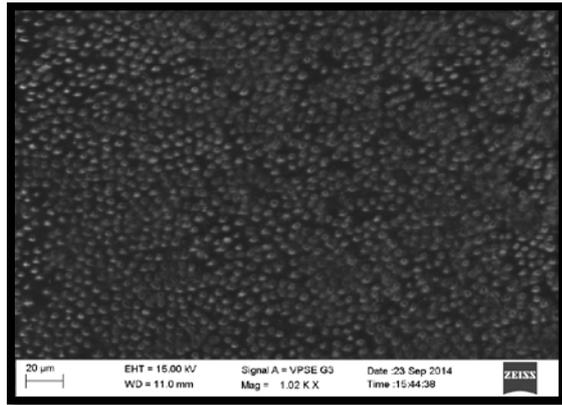


Fig. 3: It shows an SEM image of the dentinal tubules and smear layer removal viewed after irrigation with Amla at 1000x magnification

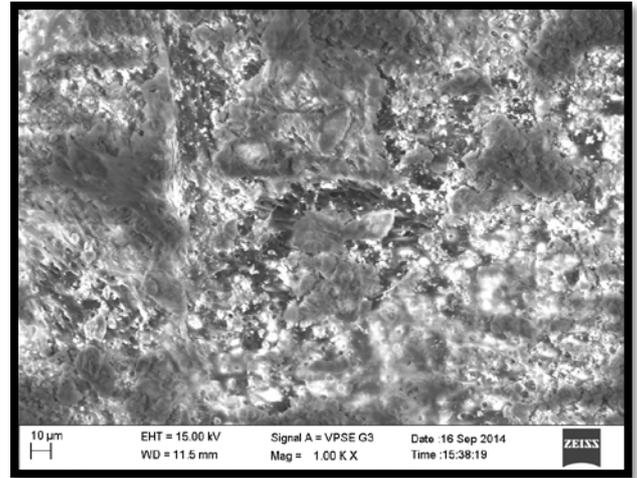


Fig. 5: It shows an SEM image of the dentinal tubules and smear layer removal viewed after irrigation with saline at 1000x magnification

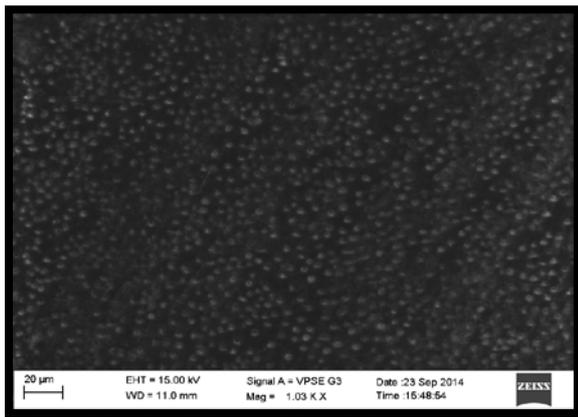
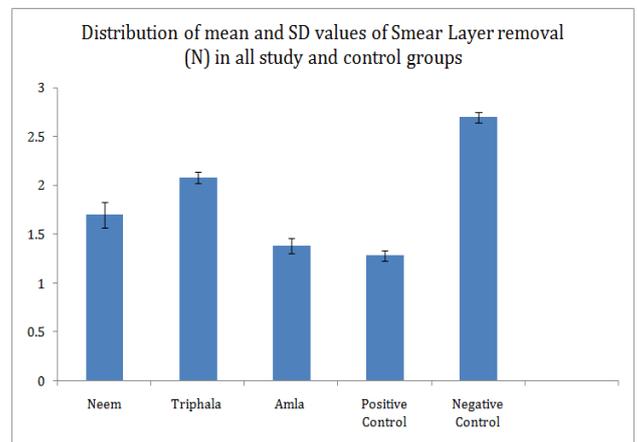


Fig. 4: It shows an SEM image of the dentinal tubules and smear layer removal viewed after irrigation with EDTA at 1000x magnification



Graph 1: Showing the Distribution of mean and SD values of Smear layer removal in all study and control groups

Table 2: Distribution of mean and SD values of Smear layer removal in all study and control groups

| Smear layer removal (n) | | | | |
|-------------------------|-------------------|-------------------|-------------------------|-------------------------|
| Neem (n=20) | Triphala (n=20) | Amla (n=20) | Positive control (n=20) | Negative control (n=20) |
| mean±SD | mean±SD | mean±SD | mean±SD | mean±SD |
| 1.7±0.132288 | 2.083333±0.057735 | 1.383333±0.076376 | 1.283333±0.057735 | 2.7±0.0543896 |

Table 3: This table shows comparison of scores given by different evaluators for Neem by One way ANOVA

| Neem | Sum of square | Standard deviation | Mean square | F | Sig. |
|----------------|---------------|--------------------|-------------|-------|-------|
| Between groups | 0.700 | 2 | 0.350 | | |
| Within groups | 21.900 | 57 | 0.384 | 0.911 | 0.048 |
| Total | 22.600 | 59 | | | |

Table 4: This table shows comparison of scores given by different evaluators for Triphala by One way ANOVA

| Triphala | Sum of square | Standard deviation | Mean square | F | Sig. |
|----------------|---------------|--------------------|-------------|-------|-------|
| Between groups | 0.143 | 2 | 7.15E-02 | | |
| Within groups | 24.026 | 56 | 0.429 | 0.167 | 0.847 |
| Total | 24.169 | 58 | | | |

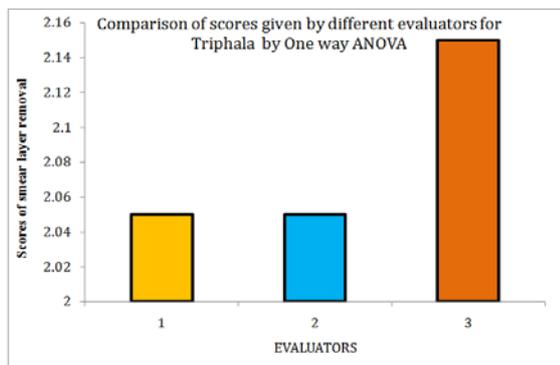
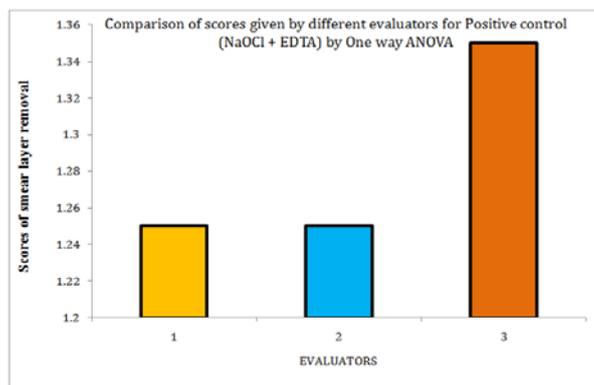
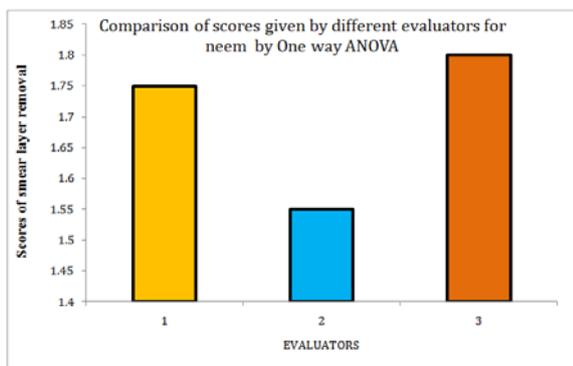


Table 7: This table shows comparison of scores given by different evaluators for Negative control (NaOCl+Saline) by One way ANOVA

| NaOCl+Saline | Sum of square | Standard deviation | Mean square | F | Sig. |
|----------------|---------------|--------------------|-------------|-------|-------|
| Between groups | 0.000 | 2 | 60667E-02 | | |
| Within groups | 12.600 | 57 | 0.221 | 0.000 | 1.000 |
| Total | 12.600 | 59 | | | |

Table 5: This table shows Comparison of scores given by different evaluators for Amla by One way ANOVA

| Amla | Sum of Square | Standard Deviation | Mean Square | F | Sig. |
|----------------|---------------|--------------------|-------------|-------|-------|
| Between groups | 0.233 | 2 | 0.117 | | |
| Within groups | 17.950 | 57 | 0.315 | 0.370 | 0.692 |
| Total | 18.183 | 59 | | | |

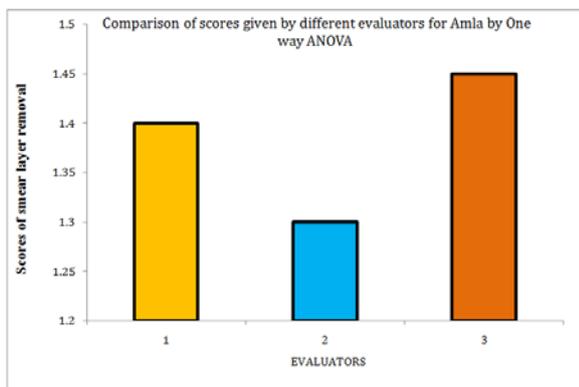
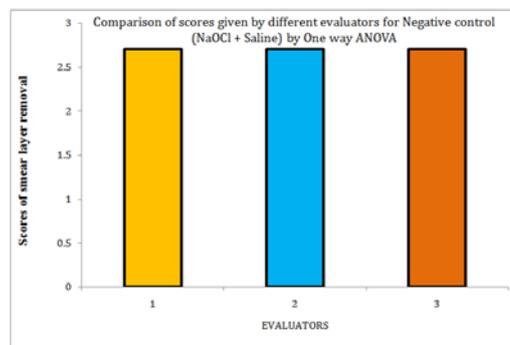


Table 6: This table shows Comparison of scores given by different evaluators for Positive control (NaOCl+EDTA) by One way ANOVA

| NaOCl+EDTA | Sum of Square | Standard Deviation | Mean Square | F | Sig. |
|----------------|---------------|--------------------|-------------|-------|-------|
| Between groups | 0.133 | 2 | 60667E-02 | | |
| Within groups | 12.050 | 57 | 0.211 | 0.315 | 0.731 |
| Total | 12.183 | 59 | | | |

By Applying One way ANOVA test, it is shown that there is no significant difference between evaluators of Neem.

By Applying One way ANOVA test, it is shown that there is no significant difference between evaluators of Triphala.

By Applying One way ANOVA test, it is shown that there is no significant difference between evaluators of Amla

By Applying One way ANOVA test, it is shown that there is no significant difference between evaluators of positive control.

By Applying One way ANOVA test, it is shown that there is no significant difference between evaluators of negative control.

DISCUSSION

A predominant trend in modern dentistry has been to search for biocompatible agents, especially those to be used in direct contact with tissues. In this context, phytotherapy has evolved as a science, and there has been growing interest in evaluating plant extracts with a potential therapeutic application in dentistry [16].

Although research is on the rise in this field but there have been no studies done to evaluate the efficacy of herbal irrigants on the removal of endodontic smear layer.

In the present study, the use of sodium hypochlorite with EDTA did not result in significantly superior cleaning of the root canals, when compared to Amla extracts analyzed. A comparison of the three solutions used in this study shows that the Amla solution presented

the best results in terms of root surface cleaning. This may have resulted from the tonic and astringent properties of the solution.

Amla (*Emblica officinalis*) contains chemical ingredient Vitamin C, carotene, nicotinic acid, riboflavin, and tannins. A major constituent of Amla is also gallic acid. It is reported to possess hepatoprotective and antioxidant activity. Fruit juice of *Emblica officinalis* (EO) contains the highest vitamin C (478.56 mg/100 ml) content. The pH of gallic acid is 2.5. The fruit when blended with other fruits boosted their nutritional quality in terms of vitamin C content. Vitamin C in EO (*Emblica officinalis*) accounts for approximately 45- 70% of the antioxidant activity [17].

As the pH increases, the availability of calcium ions from hydroxyapatite for chelation decreases. At the same time, a greater dissociation of the acidic irrigant produces an increased attraction for calcium ions.

On the other hand, the solution showing the least efficacy in smear layer removal was Triphala that did not clean the dentinal surface smear layer accumulation in all the teeth evaluated. Of the alternative irrigants Amla was the group which showed maximum removal of smear layer. This could be because of the lower pH of Amla (2.8-4.5) as opposed to slightly alkaline pH of Triphala (3-6) [13]. It is safe to conclude that the superior efficacy of smear layer removal with Amla could be a result of its low pH. Triphala is one of the well-known Indian Ayurvedic herbal formulations consisting of dried and powdered fruits of three medicinal plants namely *Terminalia Bellerica*, *Terminalia Chebula* and *Emblica officinalis* [18].

The canals in this investigation were prepared with a combination of the passive step-back technique and rotary nickel-titanium instruments. This technique is an effective method to prepare root canals with rotary instruments [19]. In addition, the use of the rotary files creates a significant amount of smear layer [20]. The apical portion of each canal was enlarged to a size 30 file to allow adequate cleaning and penetration of the solution to the apical third of each root canal.

According to Mathew et al. (1995) an apical preparation of 0.35 mm with a 4% taper is sufficient for thorough irrigation of the canal and as the taper increases there is sequential increase in the volume of irrigation. According to Khademi et al. (2006) minimum instrumentation size needed for penetration of irrigants to the apical third of the root canal is a #30 file. Therefore, 0.30 mm apical size was chosen for the study [21].

DMSO was used as a solvent for Neem, Triphala and GTP, although they were readily soluble in water. DMSO is a clean, safe, highly polar, aprotic solvent that helps in bringing out the pure properties of all the components of the herb being dissolved [22, 23]. Antibacterial inertness of 10% DMSO was confirmed with the disc diffusion test.

The independent variable for this study, contact time, was chosen based upon the findings of Calt and Serper (2000) in that 1 min were sufficient for smear layer removal. In their study, 10 ml of EDTA was the only volume of irrigation used [24].

In this study, the SEM has been used to determine the effectiveness of various irrigants to remove the smear layer. Using the SEM also allowed an examination of the morphologic details of the surfaces of prepared root canals [11].

In a study done by Rosaline et al., it was found that Neem was effective in preventing adhesion of *E. faecalis* to dentin [25]. Vinothkumar et al., found that Neem was more efficient than 5.25% NaOCl in reducing *Enterococcus faecalis* and *Candida albicans* within the root canals [26].

The authors found only 1 another study on the use of herbal irrigants for smear layer removal. It was done by Sadr and colleagues' where chamomile was more effective than NaOCl in removing the smear layer but less effective than EDTA [27].

Given the fact that the three alternative irrigants used in this study showed potential of smear layer removal this was comparable to EDTA, which acted as a positive control, incorporation of alternative irrigants in routine root canal disinfection protocol could be

considered. Further trends of study needs to investigate the effect of these alternative irrigants on radicular dentin.

It is possible that these irrigants could exhibit substantivity with the root dentin. This could be extremely beneficial in maintaining the bacteriostatic environment of the root canal. However, the interaction between these irrigants and root canal sealers also needs to be investigated in subsequent works.

CONCLUSION

Neem, Triphala and Amla all showed the potential to remove the smear layer. EDTA showed the maximum efficacy in removing the smear layer. Since the smear layer removing abilities of Amla were found to be as good as EDTA, and it is a biocompatible agent, it can be considered for use in the root canal however further investigations are necessary to confirm its efficacy as an endodontic irrigant.

CONFLICT OF INTERESTS

Declared None

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