ORIGINAL RESEARCH

Comparative Evaluation of Intracanal Smear Layer Removal by Different Root Canal Irrigants: A Scanning Electron Microscope Study

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Abstract

Aim: The purpose of the study is to compare and evaluate the efficacy of different root canal irrigants—100, 75, 50, and 25% *neem* extract, 100, 75, 50, and 25% apple cider vinegar (ACV), a combination of 5.25% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid (EDTA), and saline on smear layer removal using a scanning electron microscope (SEM).

Materials and methods: A total of 80 freshly extracted single-rooted teeth were collected and divided into 10 groups—group I: normal saline (negative control), group II: NaOCl with EDTA (positive control), group III: 100% *neem* extract, group IV: 75% *neem* extract, group V: 50% *neem* extract, group VI: 25% *neem* extract, group VII: 100% ACV, group VIII: 75% ACV, group IX: 50% ACV, and group X: 25% ACV. The samples were irrigated with a specific group of irrigants, then split in a longitudinal axis and processed for analysis in an SEM. Microphotographs were obtained and scored according to Torabinejad et al.

Results: Microphotographs were assessed and showed that 100% *neem* extract was similar to NaOCl with EDTA, followed by 75% *neem* extract and 100% ACV.

Conclusion: This study showed that 100% *neem* extract removed the smear layer, similar to the NaOCl with EDTA.

Keywords: Apple cider vinegar, *Neem* extract, Root canal irrigants, Sodium hypochlorite.

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Introduction

Microorganisms and their toxic byproducts are the common factors causing pulpal and periapical diseases in dentistry. The endodontic procedure is aimed toward the complete removal of microorganisms from the root canal system and the prevention of reinfection, which emphasizes the need for chemomechanical preparation.[1]

The objective for optimal instrumentation is continuous tapering preparation facilitating efficient delivery of antimicrobial irrigant and creating resistance form for compact root filling and maintenance of the original canal path.[2]

Irrigating solutions have a major role in the successful endodontic treatment because:

- Mechanical preparation cannot effectively eliminate bacteria from dentinal tubules and other irregularities in the root canal.
- Remnant microorganisms can multiply between appointments, often reaching the same level as that of the previous session.[3]

Sodium hypochlorite (NaOCl) has a particular ability to dissolve pulp tissue, necrotic debris, and organic smear layer components. It has broad-spectrum antimicrobial action, is a great lubricant, and is widely accessible.[4] It is, however, corrosive to tissue and should be used with caution. It is a transparent, straw-colored reducing agent solution containing around 5% accessible chlorine.[5]

Ethylenediaminetetraacetic acid (EDTA) was introduced and patented by Munz in 1935. It functions by forming a calcium-chelate solution with the calcium ion of dentin; the dentin thereby becomes more friable and easier to instrument. Chelating agents are available in both liquid and paste form.[6]

Neem (*Azadirachta indica*) is one of the most commonly used traditional medical plants in India. Each part of the tree has been explored in phytotherapy. Around >140 biologically active compounds that exhibit immunomodulatory, anti-inflammatory, anti-fungal, antibacterial, antiviral, antioxidant, antimutagenic, or anticarcinogenic properties have been isolated from this plant. Its antimicrobial properties are due to the presence of alkaloids, tetranortriterpenoid, glycosides, saponins, flavonoids, steroids, anthraquinone, tannic acid, and active constituents such as nimbidin and cyclic trisulfide. The isoprenoid group (nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid) of *nee*m has a broad range of therapeutic and antimicrobial effects suggesting its potential as an endodontic irrigant. *Neem* is an excellent antioxidant with very high biocompatibility, and thus there is no risk of tissue

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toxicity. *Neem* extract can be considered an efficient smear layer removal irrigant in the apical third of the root canal.[6]

Apple cider vinegar (ACV) is mostly made of malic acid, which has anti-inflammatory and antibacterial characteristics.[7] The mineral content of ACV combats free radicals that harm the immune system and may have a role in the periapical healing process. Researchers in the field of dentistry are continually testing it as a chelating agent. It also aids in the binding of calcium ions and softening of the root canal walls, making debris removal easier.[8] However, few investigations on its antimicrobial properties have been conducted. Therefore, this study aims to evaluate the use of *neem* extract and ACV as root canal irrigants for smear layer removal by using a scanning electron microscope (SEM) at various concentrations.

Materials and Methods

The study was approved by the Institutional Ethical Committee [F. No. SU/2021/1832(16)]. A total of 80 freshly extracted, single-rooted, noncarious premolar teeth with closed apex were collected from the Department of Oral and Maxillofacial Surgery, Santosh Dental College, and processed in the Department of Pediatric and Preventive Dentistry, Santosh Dental College, Ghaziabad, NCR Delhi. The teeth were extracted due to compromised periodontal health and also for orthodontic purposes. Exclusion criteria are open apex, periapical pathology, internal or external resorption, and obstruction of the root canal. Samples were washed and stored in 10% formaldehyde at room temperature till further use.

Access cavity preparation was done in all samples using round bur (Mani Dia-Burs), and pulp was extirpated using #15 size k-file (Densply India Pvt. Ltd), and working length was determined using #10 size k-file (Densply India Pvt. Ltd) with 1 mm short of the apex. Samples were divided into 10 groups, with eight samples in each. Group I: normal saline (negative control), group II: NaOCl with EDTA (positive control), group III: 100% *neem* extract, group IV: 75% *neem* extract, group V: 50% *neem* extract, group VI: 25% *neem* extract, group VII: 100% ACV, group VIII: 75% ACV, group IX: 50% ACV, and group X: 25% ACV. Biomechanical preparation was done by crown down technique till F3 using ProTaper files (Dentsply India Pvt. Ltd). During the procedure, all the specimens were kept moist by holding them in moist gauze. After each instrumentation, canals were irrigated by a 30G side vented irrigating needle with 2 mL of irrigating solution for 1 minute and again rinsed with 5 mL of irrigating solution of the respective group. A final flush with distilled water was performed to eliminate the irrigating solution's overreaction with the root canal walls. After irrigation, the root canals were dried using paper points.

All tooth samples were sliced from the crown with a diamond disc placed on a low-speed micromotor handpiece. Then, along the buccal and lingual surfaces of each tooth, longitudinal grooves were made that did not enter the root canal. The specimens were then mounted on coded stubs, air dried, and then kept in a vacuum chamber where a 300 Å gold coating was sputtered onto them. The exposed surface of each specimen was then viewed under an SEM at 2500× magnification. The microphotographs were obtained to assess the presence and removal of the debris and smear layer in and around dentinal tubules, as shown in Figures 1 and 2 . The microphotograph assessment was done by using scoring criteria given by Torabinejad et al. in 2003, as described in Table 1.[9]

Scores given from microphotographs were evaluated with one way analysis of variance test and *post hoc* test. Statistical evaluation was done as shown in Figures 3 and 4.

Result

Microphotographs from SEM were assessed and showed that 100% *neem* extract removed the smear layer, similar to NaOCl with EDTA, followed by 75% *neem* extract and 100% ACV. There was no significant difference seen in the smear layer removal capacity of 100% *neem* extract and NaOCl. Microphotographs of 50% *neem* extract, 25% *neem* extract, 75% ACV, 50% ACV, and 25% ACV do not have the efficacy to remove the smear layer.

Discussion

Dental caries is a polymicrobial disease that is one of the most common human infectious diseases in various parts of the world.[10] According to Newbrun, the dimension of the time factor, along with the tooth, microflora, and suitable local substrate, causes dental caries, which is termed caries tetralogy.[11]

Enterococcus faecalis (E. faecalis) is an anaerobic bacterium that is responsible for over 90% of *Enterococcus* infections. Endodontic therapy fails when bacteria enter dentinal tubules. *Enterococcus* are natural gastrointestinal system resident bacteria that keep the gut healthy. They have been discovered to be resistant to a variety of antimicrobials and antibiotics.[12]

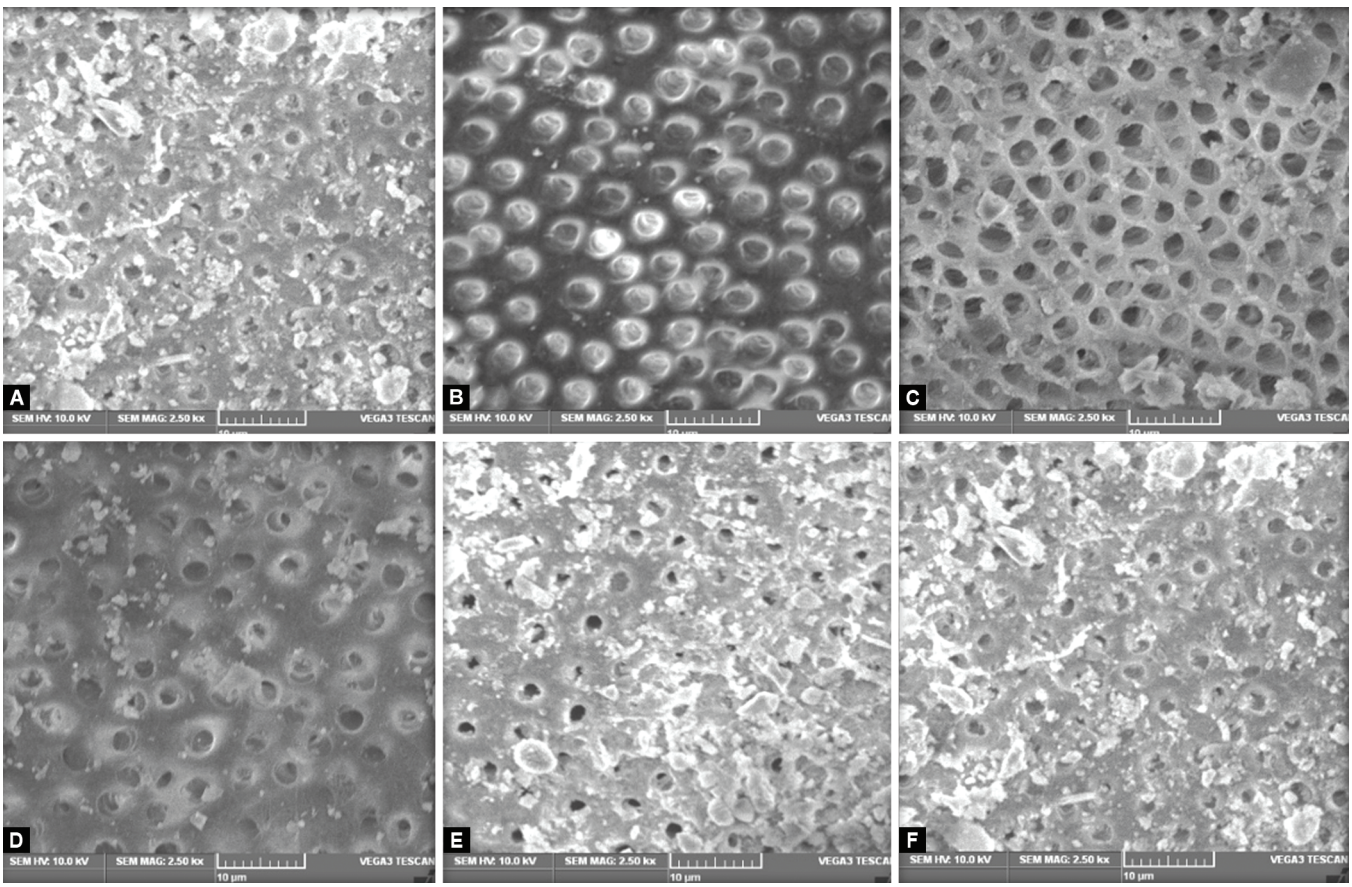
Three-dimensional disinfection is difficult to attain, especially when germs populate the isthmus, fins, cul-de-sacs, and intercanal communication.[13]

Over the years, NaOCl and EDTA, as chelating agents, became the most often employed agents for disinfecting the root canal. NaOCl is a nonspecific proteolytic agent with antibacterial characteristics dissolving the remaining pulp tissue and also organic dentin components.[14] A contact time of at least 40 minutes with 5.25% NaOCl, has shown to be more effective than a lower concentration for the same period, which means bacteriostatic at low concentration and bactericidal at high concentration.[15] But, when extruded beyond the periapical region, it may lead to pain, swelling, ecchymosis, hemorrhage, and severe tissue reaction.[16] Clarkson concluded that irrigation with the solution of EDTA followed by NaOCl is most effective in the removal of the smear layer. However, it has certain disadvantages; it damages all living tissues except keratinized epithelium and also erodes the metal.[17] Karatas et al. stated that NaOCl is used for the final irrigation of root canals in teeth with asymptomatic apical periodontitis. Preheating of NaOCl has no additional antibacterial benefit and causes more postoperative discomfort compared to cold usage.[18]

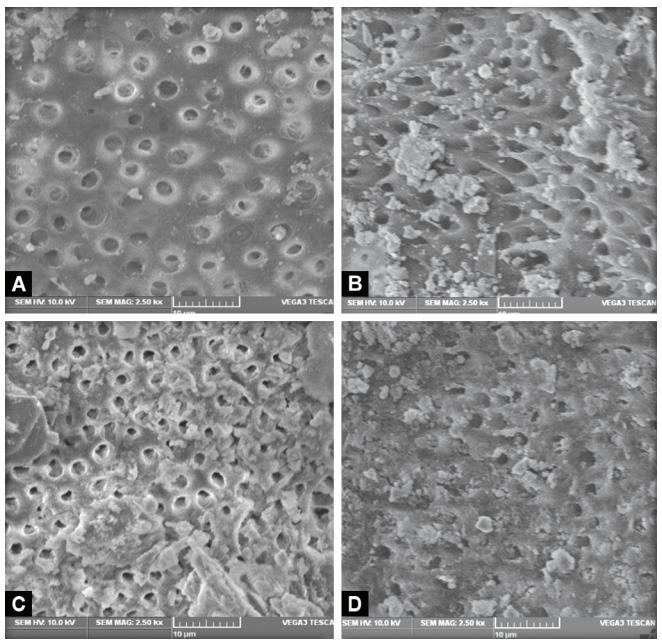
Many authors, like Babaji et al., in 2016,[13] and Panchal et al. in 2020[19] recommended the use of herbal agents as an alternative to or as an addition to the traditional root canal irrigants to avoid the complications related to the use of NaOCl. Due to a significant rise

Table 1: Torabinejad et al. scoring criteria 2003

Score	*Interpretation*
1	No smear layer (no smear layer on the surface of the root canals with all tubules clean and open)
2	Moderate smear layer (no smear layer on the surface of root canals, but tubules contain debris)
3	Heavy smear layer (smear layer covers the root canal surface and the tubules)



Figs 1A to F: Scanning electron microscope (SEM) microphotographs: (A) Group I (negative control) showing canal walls are filled with debris and smear layer, all tubules are closed; (B) Group II (positive control) showing canal walls are free of debris, all tubules are open; (C) Group III showing canal walls are free of debris, all tubules are open; (D) Group IV showing tubules are partially open; (E) Group V showing canal walls are full of debris, all tubules are closed; (F) Group VI showing canal walls are full of debris, all tubules are closed



Figs 2A to D: SEM microphotographs: (A) Group VI showing canal walls are partially filled with debris and smear layer; all tubules are partially closed; (B) Group VII showing canal walls are full of debris, all tubules are closed; (C) Group III showing canal walls are full of debris, all tubules are closed; (D) Group IV showing canal walls are full of debris, all tubules are closed

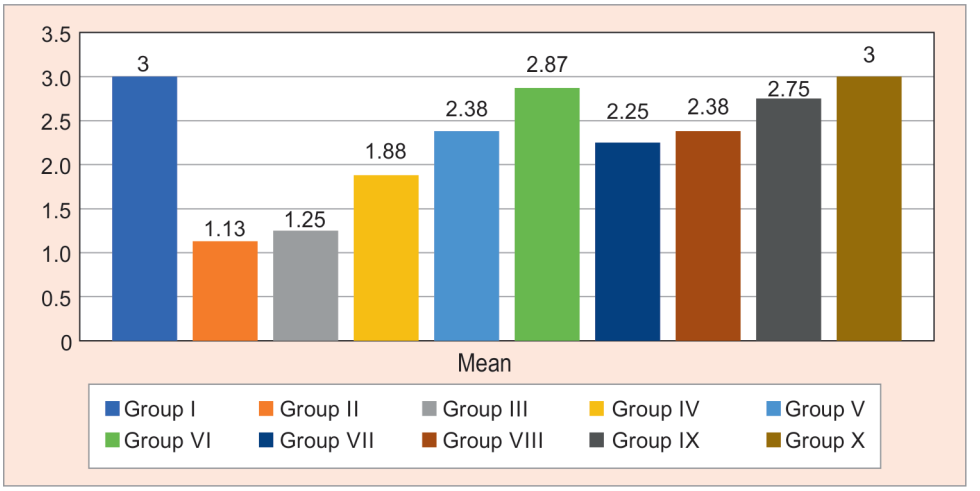


Fig. 3: Median grading for removal of smear layer using different irrigants

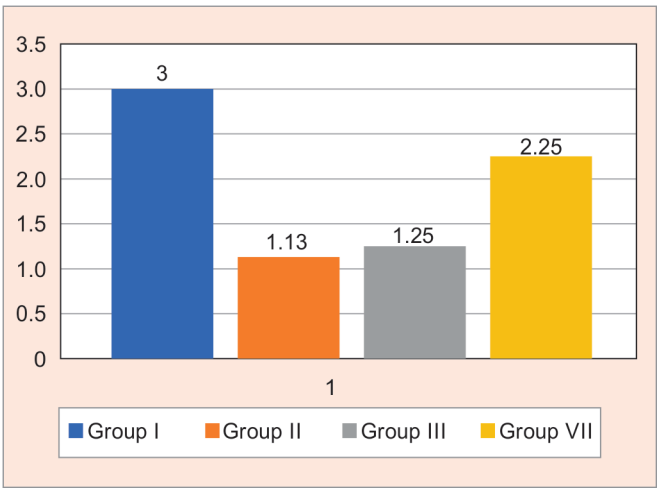


Fig. 4: Comparison of saline (group I), NaOCl with EDTA (group II), 100% *neem* extract (group III), and ACV (group VII)

in bacterial strains resistant to antibiotics, it has become necessary to explore herbal medications and their antibacterial properties that help to improve the results of biomechanical preparations.[20] Endodontic irrigants may be made from a variety of plant extracts that have antibacterial, anti-inflammatory, and therapeutic properties. The following are some of the most significant herbal irrigants: *neem*, tulsi, aloe vera, ACV, *Morinda citrifolia (M. citrifolia)*, and *Curcuma longa*. In addition to having lower toxicity and fewer side effects, plants with antibacterial properties are advantageous to use as root canal irrigants.[21]

Scanning electron microscope (SEM) was used in this study in accordance with the study conducted by Patil et al.,[22] Vemuri et al.,[23] and Demirel et al.[24] SEM helps to study the surface character of the material in higher magnification. In 1948, Charles Oatley and his students from the Engineering Department of Cambridge University made the first SEM.[25] Traditional SEM can only provide monochrome color images; however, with recent improvements, SEM can also produce colored images. SEM is used in a variety of industries, including medicine, chemistry, and engineering. It allows us to view many things that we would not be able to notice with our naked eyes.[26]

There are various scoring criteria by which we can evaluate the amount of smear layer removal and debris removal. Due to various limitations in different scoring criteria, Torabinejad et al.'s scoring criteria[9] is very simple and easy to differentiate in photomicrographs.

The selection of *neem* and ACV as irrigants lies in the fact that they are readily available, known for their medicinal value, and also exhibit less toxicity.

Neem (*Azadirachta indica*) is well-known for its therapeutic properties of >140 chemicals. *Neem* constituent isoprenoid group that has anti-inflammatory, immune-modulating, antibacterial, anti-fungal, antiviral, antioxidant, and anti-carcinogenic activities. It also helps in glucan inhibition and some other virulence factors, which promote plaque and smear layer formation.[27] Wolinsky et al. confirmed the inhibitory effects of aqueous extract from *neem* upon bacterial aggregation, growth, adhesion to hydroxyapatite, and production of insoluble glucan, which reduces the smear layer formation.[28]

In this study, groups III (100% *neem* extract) (1.25 + 0.46) and IV (75% *neem* extract) show better smear layer removal in comparison to group V (50% *neem* extract) and group VI (25% *neem* extract).

This result coincides with the result of Sebatni and Kumar's study, in which they concluded that *neem* extract shows the highest efficacy of smear layer removal than green tea extract and orange oil.[29] Ranjitha et al. concluded *neem* extract is a more efficient irrigating solution for smear layer removal in the apical third of the root canal.[30]

Afshan et al. concluded that *neem* extract shows better inhibition than *M. citrifolia* against *E. faecalis*.[31] Lakshmi et al. concluded that both aqueous and ethanolic *neem* extract shows better antimicrobial property that inhibits *E. faecalis* in root canal failure and is a better alternative for NaOCl.[10] A study by Prashanth et al. concluded that the maximum antibacterial activity of *neem* extract at 50% against *Streptococcus mutans*.[11] Another study done by Rajasekaran et al. demonstrated the comparison of antimicrobial activity of *neem* extracts (aqueous and organic solvents), showing that the organic extracts were more effective than aqueous extract.[32] Maragathavalli et al. concluded that both ethanolic and methanolic *neem* extract showed better antimicrobial efficacy against *E. faecalis, Escherichia coli*, and *Streptococcus aureus*.[33]

Apple cider vinegar (ACV) is composed mainly of malic acid with high mineral content (potassium, phosphorus, magnesium, sulfur, calcium, fluoride, and silicon). It also contains other substances like pectin, β-carotene, enzymes, and amino acids that fight off free radicals that harm the immune system and may play a beneficial role in the periapical repair process.[1] It is constantly being tested by researchers in the dentistry field as a chelating agent. The apical third of apple vinegar removes the smear layer with noticeably greater efficiency. Thus, after examining its antibacterial effectiveness and impact on sealing ability, it is conceivable to utilize diluted apple vinegar as an irrigant.[19]

In our study, group VII 100% ACV (2.25 + 0.46), shows better smear layer removal in comparison to groups VIII (75% ACV), IX (50% ACV), and X (25% ACV).

This result is concomitant with the study by Alyamany et al., who concluded that ACV exhibited better antibacterial activity with a decrease in *E. faecalis* count when used immediately and 48 hours by loss of cell integrity due to the presence of acetic and malic acid presence.[34] Mohanty et al. showed that ACV had better antimicrobial activity against *E. faecalis* as similar to 5% NaOCl but not effective against *Candida albicans*.[35]

It was observed that the use of traditional Indian plants and their extracts prevent the failure of endodontic treatment caused by *E. faecalis*. Moreover, these extracts are locally available in almost all parts of India. The antimicrobial efficacy of these extracts has a wider range against various bacteria and fungi. The analysis of the literature supports the antibacterial activity along with (1) better smear layer removal; (2) inhibits the adherence of bacterial cells to the canal surfaces; and (3) directly against *E. faecalis*. In the marketing of plant extracts as medicinal agents, several clinical demonstrations of the efficacy of their activity are employed.

The current study compared the efficacy of various root canal irrigants in smear layer removal by using SEM. It should be noted that the usage of plant-based extracts with appropriate percentages showed a better effect on smear layer removal, similar to synthetic irrigants.

The observation from the present study provides insight into the use of plant-based extracts as root canal irrigants for smear layer removal and action against *E. faecalis*.

Conclusion

This study concludes that 100% *neem* extract shows a similar result as that of 5.25% NaOCl. Hence, 100% *neem* extract can be used as a substitute for NaOCl as a root canal irrigant.

Clinical Significance

The current study suggested that in endodontic treatment, herbal extracts have the potential to be employed as root canal irrigants because they are effective in reducing microbial infections in infected root canals. When used in pediatric endodontics, they can be regarded as a helpful substitute for NaOCl since it does not produce hypersensitivity or pain.

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