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Antibacterial Efficacy of Different Irrigants Used During Endodontic Surgery: A Comparative Study

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ABSTRACT

Background: Endodontic surgery is often required in cases of persistent periapical infections where conventional root canal treatment fails. Effective irrigation plays a crucial role in bacterial elimination, thereby improving surgical outcomes. Various irrigants, including sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), and newer biocompatible agents, have been evaluated for their antibacterial efficacy.

Objective: The present study aimed to compare the antibacterial efficacy of different irrigants used during endodontic surgery to determine the most effective solution for bacterial elimination in periapical infections.

Methodology: Extracted human teeth with standardized periapical lesions were inoculated with *Enterococcus faecalis* and divided into groups based on the irrigation solution used: (1) 5.25% NaOCl, (2) 2% CHX, (3) 17% EDTA, (4) Herbal irrigants (e.g., propolis or neem), and (5) Sterile saline as a control. The samples underwent surgical endodontic procedures, followed by microbiological analysis using colony-forming unit (CFU) counts and confocal laser scanning microscopy to assess bacterial viability.

Results: The findings suggested that NaOCl demonstrated the highest antibacterial efficacy, followed by CHX, while herbal irrigants showed promising results with fewer cytotoxic effects. EDTA exhibited limited direct antibacterial action but enhanced biofilm removal. The control group (saline) showed persistent bacterial growth.

Conclusion: The study highlighted the importance of selecting an appropriate irrigant in surgical endodontics. While NaOCl remained the gold standard, alternative irrigants such as CHX and herbal solutions offered effective antibacterial properties with improved biocompatibility. Further research was recommended to assess their long-term clinical outcomes.

Keywords: *Endodontic surgery, antibacterial efficacy, sodium hypochlorite, chlorhexidine, irrigants, periapical infection.*

1. INTRODUCTION

The ultimate goal of endodontic treatment is to eliminate infection from the root canal system and to prevent reinfection, thereby preserving the natural dentition. However, in some cases, conventional root canal therapy fails due to persistent periapical pathology, often necessitating endodontic surgery as a corrective approach. Endodontic surgery—especially apicoectomy combined with retrograde filling—has been widely accepted as an effective solution for treating persistent periapical lesions that do not respond to orthograde therapy [1]. A key determinant of surgical success in such cases is the thorough disinfection of the periapical tissues and the root-end cavity. Therefore, the choice of irrigant becomes a crucial factor influencing the elimination of microbial biofilms and, consequently, the healing outcome.

The microbial etiology of periapical disease has been well-established, with *Enterococcus faecalis* identified as a predominant pathogen in failed endodontic treatments [2]. This gram-positive, facultative anaerobic bacterium exhibits high resistance to environmental stress, intracanal medicaments, and antimicrobial agents, which makes its eradication particularly challenging [3]. As a result, developing effective irrigation protocols targeting *E. faecalis* has become a significant focus in endodontic research.

Sodium hypochlorite (NaOCl), in concentrations ranging from 0.5% to 6%, has been extensively used in endodontics due to its potent antimicrobial and tissue-dissolving properties [4]. It disrupts microbial cell walls through oxidative action and effectively removes organic debris from the root canal system [5]. However, its high cytotoxicity to periapical tissues, especially during surgical procedures, is a limitation [6].

Chlorhexidine gluconate (CHX), commonly used in a 2% solution, offers broad-spectrum antimicrobial activity and substantivity, meaning it binds to dentin and releases slowly over time [7]. It is less cytotoxic than NaOCl and has shown effectiveness against *E. faecalis*. However, CHX lacks tissue-dissolving capability and may form potentially harmful precipitates when used sequentially with NaOCl [8].

Ethylenediaminetetraacetic acid (EDTA), primarily employed as a chelating agent to remove the smear layer and inorganic debris, also demonstrates limited antimicrobial action [9]. It is commonly used in combination with other irrigants to enhance penetration and biofilm removal, although on its own, it does not achieve complete disinfection [10].

Given the limitations of synthetic irrigants, attention has turned to herbal alternatives that may offer antimicrobial efficacy with greater biocompatibility. Natural products such as propolis, neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), and green tea polyphenols have shown promising antibacterial, antifungal, and anti-inflammatory properties [11]. For example, neem exhibits bactericidal effects by disrupting microbial cell walls and inhibiting essential enzyme activity [12]. Propolis, a resinous substance derived from bees, contains flavonoids and phenolic compounds that exert significant antibacterial and antioxidant actions [13]. These herbal agents offer the added benefit of reduced cytotoxicity, which may be advantageous in periapical surgery where close proximity to vital tissues is inevitable.

While previous studies have assessed the antibacterial effects of various irrigants in conventional root canal therapy, limited evidence exists regarding their comparative performance during surgical endodontic procedures. Surgical irrigation is fundamentally different from orthograde irrigation in that the exposure of the periapical tissues to the irrigants is more direct and potentially more hazardous. Hence, understanding how different irrigants perform under surgical conditions is essential.

Moreover, the efficacy of irrigants should be assessed not only by bacterial culture methods but also by advanced imaging techniques such as confocal laser scanning microscopy (CLSM). CLSM enables three-dimensional visualization of live and dead bacteria within biofilms and provides more accurate insights into the antimicrobial performance of irrigants [14]. By employing both colony-forming unit (CFU) counts and CLSM, researchers can gain a comprehensive understanding of the irrigants' efficacy and their ability to disrupt mature biofilms.

In this context, the present study was designed to evaluate and compare the antibacterial efficacy of commonly used irrigants—5.25% NaOCl, 2% CHX, 17% EDTA—as well as selected herbal irrigants (e.g., neem and propolis), in an ex vivo surgical model using extracted human teeth inoculated with *Enterococcus faecalis*. A sterile saline solution served as a negative control to determine baseline bacterial survival. The findings of this study aim to provide clinically relevant data to guide endodontic surgeons in selecting irrigants that offer both efficacy and safety in surgical environments. By focusing on a comparison of both traditional and novel irrigants under surgical conditions, this study seeks to address a key gap in the current endodontic literature and support the advancement of biocompatible, effective clinical protocols in surgical endodontics.

2. MATERIALS AND METHODS

The present in vitro experimental study was conducted in the Department of Conservative Dentistry and Endodontics at a private dental college in India between January and March 2025. The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC Approval No.: IEC/2025/021), and all procedures were performed in accordance with the ethical standards of the institutional research committee and the 1964 Helsinki declaration and its later amendments.

A total of 50 freshly extracted human mandibular premolar teeth with single straight canals were selected for the study. The sample size was calculated based on previous literature, assuming an effect size of 0.40, a significance level of 5%, and a power of 80%, resulting in 10 specimens per group. Teeth with visible cracks, caries, root resorption, or previous endodontic treatment were excluded.

All teeth were cleaned of soft tissue and calculus using ultrasonic scalers and stored in 0.1% thymol solution at 4°C until use. The teeth were decoronated at the cementoenamel junction using a diamond disc under water cooling to obtain a standardized root length of 15 mm. Access cavities were prepared using Endo Access burs, and the working length was established by inserting a #10 K-file until visible at the apical foramen, then subtracting 1 mm.

Biomechanical preparation was carried out using ProTaper Universal rotary files (Dentsply Sirona) up to size F3. During instrumentation, canals were irrigated with sterile saline to avoid any antimicrobial bias. The canals were then autoclaved to ensure sterility before bacterial inoculation.

A standard strain of *Enterococcus faecalis* (ATCC 29212) was rehydrated and cultured in brain heart infusion (BHI) broth. Each canal was inoculated with 20 µL of the bacterial suspension (adjusted to 0.5 McFarland standard) and incubated for 21 days at 37°C in 100% humidity to allow for mature biofilm formation. The culture medium was refreshed every 3 days.

After incubation, specimens were randomly assigned into five groups (n = 10 per group) based on the irrigation solution used:

Group 1: 5.25% Sodium Hypochlorite (NaOCl)

Group 2: 2% Chlorhexidine Gluconate (CHX)

Group 3: 17% Ethylenediaminetetraacetic Acid (EDTA)

Group 4: Herbal irrigant (standardized neem extract)

Group 5: Sterile saline (control group)

A simulated endodontic surgery was performed by resecting 3 mm of the root apex using a diamond disc under water cooling. Retrograde cavities were prepared to a depth of 3 mm using ultrasonic surgical tips. Each cavity was irrigated with 5 mL of the assigned irrigant using a 30-gauge side-vented needle over a duration of 1 minute. This was followed by a final flush with 2 mL of sterile saline and drying with absorbent paper points.

Microbiological sampling was performed using sterile paper points inserted into each canal for 60 seconds. The paper points were transferred into Eppendorf tubes containing 1 mL of BHI broth and vortexed for 30 seconds. Serial dilutions were prepared and plated on BHI agar, which were incubated at 37°C for 48 hours. Colony-forming units (CFUs) were counted manually and recorded.

Additionally, three samples from each group were analyzed using confocal laser scanning microscopy (CLSM) to assess bacterial viability. The root segments were stained with the LIVE/DEAD BacLight bacterial viability kit (Molecular Probes, USA), which stains live bacteria green and dead bacteria red. Samples were examined at 10× magnification, and images were analyzed using image analysis software to determine the percentage of live and dead bacteria.

All collected data were statistically analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to compare the mean CFU counts across groups, followed by Tukey's post hoc test for intergroup comparisons. A *p*-value < 0.05 was considered statistically significant.

Tables:

Table 1. Comparison of Mean CFU Count (×10³) Among Groups (n = 10)

Group	Irrigant	Mean CFU Count (×10³)	SD	P-Value
Group 1	5.25% NaOCl	1.2	±0.35	**0.001***
Group 2	2% CHX	2.1	±0.42	**0.004***
Group 3	17% EDTA	4.8	±0.51	**0.005***
Group 4	Herbal (Neem extract)	2.9	±0.39	**0.04***
Group 5	Saline (Control)	6.2	±0.48	**0.001***

***P-value significant at 0.05 levels, Test used: One-way ANOVA with Post hoc Tukey's test**

Table 2. CLSM Analysis: Mean Percentage of Dead Bacteria

Group	Irrigant	% Dead Bacteria	SD	P-value
Group 1	5.25% NaOCl	92.3%	±3.1	**0.001***
Group 2	2% CHX	84.5%	±4.2	**0.01***
Group 3	17% EDTA	41.2%	±5.6	**0.002***
Group 4	Herbal (Neem extract)	75.4%	±3.9	**0.04***
Group 5	Saline (Control)	26.7%	±4.8	**0.001***

***P-value significant at 0.05 levels, Test used: One-way ANOVA with Post hoc Tukey's test**

RESULTS

The antibacterial efficacy of various irrigants was assessed using colony-forming unit (CFU) analysis and confocal laser scanning microscopy (CLSM). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test for pairwise comparisons. A p-value < 0.05 was considered statistically significant.

As shown in **Table 1**, the mean CFU counts ($\times 10^3$) differed significantly among the five groups (ANOVA, F = 95.47, $p < 0.001$). Group 1 (5.25% NaOCl) demonstrated the lowest bacterial count (1.2 ± 0.35), indicating the highest antibacterial efficacy. Group 2 (2% CHX) and Group 4 (Neem extract) also showed lower CFU counts, while Group 3 (EDTA) exhibited moderate growth. Group 5 (saline control) had the highest bacterial load (6.2 ± 0.48). Tukey's test confirmed that Group 1 was significantly more effective than all others ($p < 0.001$).

CLSM results in **Table 2** revealed statistically significant differences in bacterial viability across groups (ANOVA, F = 134.82, $p < 0.001$). Group 1 showed the highest percentage of dead bacteria (92.3 ± 3.1%), followed by Group 2 (84.5 ± 4.2%) and Group 4 (75.4 ± 3.9%). Group 3 (EDTA) demonstrated moderate antibacterial activity (41.2 ± 5.6%), while Group 5 (saline) showed the least efficacy (26.7 ± 4.8%). Tukey's test confirmed that Groups 1, 2, and 4 were significantly more effective than the control ($p < 0.001$).

3. DISCUSSION

The present study evaluated and compared the antibacterial efficacy of five different irrigating solutions—5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), 17% ethylenediaminetetraacetic acid (EDTA), a herbal irrigant (neem extract), and sterile saline (control)—in the context of endodontic surgery. The results demonstrated that NaOCl exhibited the highest antibacterial activity in both CFU analysis and CLSM evaluation, followed by CHX and neem extract. EDTA showed limited direct antibacterial efficacy, while saline was least effective.

The superior performance of NaOCl aligns with several previous studies that confirmed its potent antimicrobial properties and tissue-dissolving ability. Mohammadi et al. (2008) emphasized that NaOCl disrupts bacterial biofilms and denatures bacterial proteins through its strong oxidative action, thereby reducing microbial load significantly during root canal procedures [15]. Similar outcomes were reported by Gomes et al. (2001), who demonstrated that NaOCl was most effective in eliminating *Enterococcus faecalis*, a common pathogen in persistent periapical infections [16]. In the current study, the significantly lower CFU counts and higher percentage of dead bacteria in the NaOCl group affirm these findings.

Chlorhexidine (CHX), the second most effective irrigant in this study, acts primarily by disrupting bacterial cell membranes and precipitating cytoplasmic contents [17]. Although it lacks tissue-dissolving capabilities, its substantivity and broad-spectrum antimicrobial action justify its use as an adjunct or alternative to NaOCl. Basrani et al. (2004) supported its efficacy, particularly in retreatment cases and apical surgeries [18]. However, our results indicate that while CHX is effective, it is still inferior to NaOCl, corroborating findings by Vianna and Gomes (2005), who reported similar comparative efficacy [19].

Interestingly, the herbal irrigant (neem extract) showed promising antibacterial efficacy, performing better than EDTA and close to CHX. Neem (Azadirachta indica) has been shown to possess strong antibacterial, antifungal, and anti-inflammatory properties due to the presence of compounds like nimbidin and azadirachtin [20]. According to Prashanth et al. (2010), neem-based irrigants exhibit effective *E. faecalis* inhibition with favorable biocompatibility [21]. The current results suggest its potential as an alternative in patients sensitive to conventional irrigants or where cytotoxicity is a concern. However, variability in extract preparation and lack of standardized formulations remain barriers to its mainstream clinical application.

EDTA, although commonly used for smear layer removal due to its chelating effect, demonstrated limited antibacterial efficacy in this study. This result supports the findings of Zehnder (2006), who described EDTA as having negligible antimicrobial properties when used alone [4]. However, its role in facilitating the penetration of antibacterial agents by removing inorganic debris and smear layer is well documented and valuable in combination irrigation protocols.

The control group irrigated with sterile saline predictably showed the highest bacterial survival rates. This reaffirms the critical need for chemical adjuncts during surgical debridement to eliminate resistant pathogens like *E. faecalis* [2].

Our findings not only support the continued clinical use of NaOCl as the gold standard but also emphasize the need to explore alternative irrigants that combine effectiveness with lower cytotoxicity. The use of CLSM in this study offered a precise evaluation of bacterial viability post-irrigation, consistent with studies by George et al. (2005), who validated CLSM as a reliable tool for biofilm and microbial assessment in endodontics [14].

4. LIMITATIONS AND FUTURE SCOPE

The study was conducted on extracted human teeth under controlled laboratory conditions, which may not fully replicate the complexities of in vivo environments. Further in vivo studies and randomized controlled trials are required to validate these findings. Long-term clinical outcomes, tissue compatibility, and effects on healing should also be assessed, especially for herbal alternatives.

5. CONCLUSION

The findings of the present study underscore the critical role of irrigation solutions in enhancing bacterial elimination during endodontic surgery, particularly in cases involving persistent periapical infections. Among the tested irrigants, 5.25% sodium hypochlorite demonstrated the highest antibacterial efficacy against *Enterococcus faecalis*, followed by 2% chlorhexidine, which showed substantial, albeit slightly lower, effectiveness. Neem extract, used as a herbal alternative, displayed promising antimicrobial potential with added benefits of biocompatibility, positioning it as a viable adjunct or substitute, especially for patients sensitive to synthetic irrigants. While 17% EDTA exhibited limited direct antibacterial properties, its chelating action remains valuable for smear layer removal and enhancing the effectiveness of other irrigants. In contrast, sterile saline failed to offer significant antimicrobial effects, highlighting the necessity of using active chemical agents during surgical debridement. Overall, this study reaffirms NaOCl as the gold standard irrigant while opening avenues for the clinical integration of herbal alternatives like neem. Further in vivo studies are warranted to evaluate long-term outcomes and biocompatibility in clinical settings.

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