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Superior Antibacterial Efficacy of Calcium Hydroxide–Propolis Paste against Enterococcus faecalis in Infected Root Canals: A Randomized Controlled In Vitro Study

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ABSTRACT

Enterococcus faecalis plays a major role in root canal infections. This investigation sought to compare the ability of two intracanal pastes—one containing calcium hydroxide with saline and the other containing calcium hydroxide with propolis—to eradicate E. faecalis from contaminated canals. Twenty extracted first premolars with single roots and single canals were randomly divided into two groups of 10 teeth. Each canal was inoculated with a prepared bacterial suspension grown aerobically at 37 °C for 7 days. Following infection, canals were irrigated using NaOCl. Group 1 received a dressing of calcium hydroxide + saline, whereas Group 2 received calcium hydroxide + propolis. Specimens were then incubated in a humid environment for another 7 days. Colony-forming units were recorded at three time points: T0 (post inoculation), T1 (after NaOCl irrigation), and T2 (after application of the paste). A T-test was used for statistical evaluation, with significance set at $P \leq 0.05$.

At T2, the mean E. faecalis count in Group 2 was 0.030, which was lower than that of Group 1 (0.363). The difference between groups at this stage was 2.192, with a P-value of 0.042, confirming a statistically significant distinction. The propolis-containing formulation produced a notable reduction in E. faecalis counts within treated canals.

Keywords: Calcium hydroxide, Enterococcus faecalis, Propolis, Saline, Sodium hypochlorite

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Introduction

Microbial activity and its byproducts are central factors in the onset and continuation of endodontic and periodontal conditions [1]. Consequently, a successful endodontic procedure must target the removal and long-term suppression of these organisms [2–5].

Recent research has emphasized strategies aimed at thoroughly removing microorganisms and their metabolites from the canal system [6]. Enterococcus species, which contribute to intestinal tract sepsis [7], are also frequently identified in cases where endodontic therapy fails [8], prompting the use of medications as supportive treatments [9].

The purpose of canal shaping and irrigation is to eradicate or deactivate residual microbes and to alter any remaining bacterial structures. When complete elimination cannot be achieved, these steps should create favorable conditions for applying an intracanal medicament between sessions to improve disinfection [10].

Sodium hypochlorite (NaOCl), a disinfectant known for its ability to break down organic tissues, remains the standard irrigant in root canal therapy due to its strong antimicrobial performance [11]. Calcium hydroxide—introduced by Hermann between 1920 and 1930 [12]—is one of the most extensively used intracanal dressings because of its antimicrobial actions and its role in supporting healing. It is now considered the preferred temporary medicament in many clinical situations [13].

This material has no odor, dissolves poorly in water, and releases slowly—features that enhance its clinical relevance [14]. Its high pH (12.5–12.8) contributes to its biocompatibility [15]. The compound behaves as a strong base, releasing calcium and hydroxyl ions upon contact with aqueous fluids [14].

Hydroxyl ions are largely responsible for the extreme alkalinity of calcium hydroxide, and many harmful microorganisms cannot survive in such conditions. Thus, in infected canals, bacteria are destroyed primarily through direct exposure to this alkaline environment [13].

In addition to traditional medicaments, propolis has gained prominence as a natural antimicrobial agent. The global shift toward biologically derived therapies has encouraged further investigation into its medicinal potential. Propolis has a long-standing history in traditional medicine and has been applied in dentistry to manage oral microbial communities [12].

Its antibacterial activity is well established, and it was first introduced into dental applications in 1996 by Krell [16]. Propolis is a resin-like product generated by bees—often referred to as "bee glue"—and used to protect hive structures. Its composition varies according to the flora in the region [17]. Typically, raw propolis consists of 50 % resins, 30 % wax, 10 % essential oils, 5 % pollen, and 5 % miscellaneous organic substances [18, 19].

Given these characteristics, the present study aimed to evaluate how effectively two intracanal dressings—calcium hydroxide with saline versus calcium hydroxide with propolis—could eliminate E. faecalis from previously infected root canals.

Materials and Methods

Study design

This investigation utilized 20 fully formed apical roots taken from single-rooted, single-canal mandibular permanent first premolars extracted for orthodontic reasons.

Only teeth with no internal resorption, calcific deposits, fractures, or structural/pathologic abnormalities were included. Specimens could not have undergone prior endodontic intervention and were excluded if removed because of periodontal issues.

All procedures adhered to the ethical standards of the World Medical Association's Declaration of Helsinki, and approval was granted by the biomedical ethics committee under reference DN-290424-227. Written informed consent was obtained for the use of human material.

Sample size calculation

Sample estimation was completed using G∗Power 3.1.7 (Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany), assuming 90 % power, a 95 % confidence level, and a 5 % significance threshold. Based on pilot data — group 1 (mean 0.2, SD 0.748) and group 2 (mean 1, SD 0.5) — the computed effect size D was 1.25. Anticipated attrition was N1 = n/(1 − d) = 16.6 %, mainly due to failure to meet eligibility requirements. Consequently, 20 teeth were required to populate both groups.

Study groups

The specimens were randomly allocated, via computer-based selection, into two equal sets of 10, determined by the intracanal medicament used.

Group 1

Calcium hydroxide 1 g mixed with 1.5 mL saline.

Group 2

Calcium hydroxide 1 g combined with 2 mL propolis.

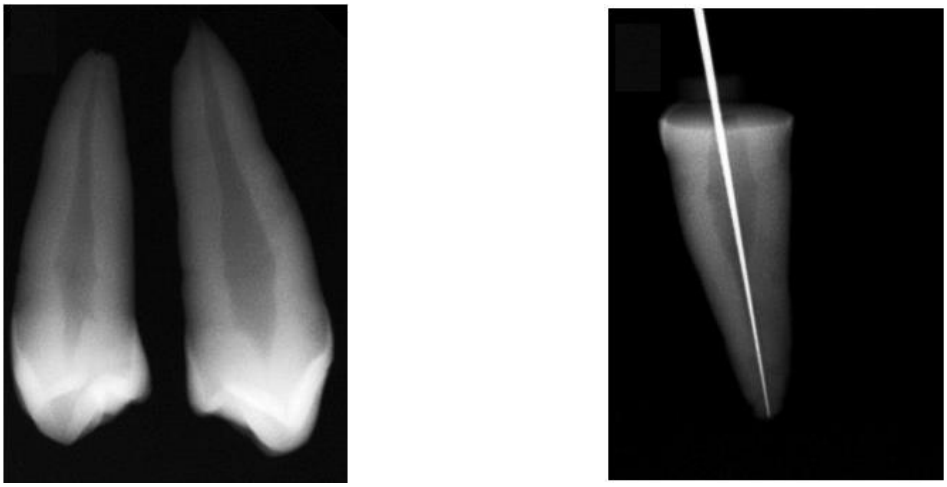
Methodology

Initial radiographs were taken in mesiodistal **(Figure 1a)** and buccolingual **(Figure 1b)** projections to confirm the presence of a single canal. Root surfaces were debrided with a U15 manual scaler (MEDESY, Italy) to clear soft-tissue remnants. Samples were submerged in 5.25 % NaOCl (Aphamia, Syria) for 1 h, then transferred into sterile serum for 2 min before further handling.

Crowns were sectioned horizontally with diamond discs so that each root measured 17 mm. Canal enlargement was initiated with 15–20 K-Flexo files (DENTSPLY Maillefer, Switzerland) under sterile saline (Serda, Syria) perfusion until the file tip emerged 0.5 mm beyond the apex, confirming the working length.

Subsequent shaping employed SC Pro motor files (COXO, China) with saline irrigation in the sequence: SX, S1, S2, F1, F2, advancing to full working length.

Irrigation at the end of preparation consisted of 40 mL of 5.25 % NaOCl alternated with 10 mL of 17 % EDTA (Alpharespharma, Syria) to remove the smear layer, with a total perfusion time of 10 min per tooth [20]. Final sterilization was performed using a class-B autoclave (MIC, China) at 121 °C for 15 min.



a) b)

Figure 1. Radiograph displaying a tooth with a single canal.

E. faecalis isolation

Clinical isolates of E. faecalis were used to prepare a suspension adjusted to 10^8 CFU/mL, standardized using the McFarland scale (HARDY, USA) within the Department of Bacteriology and Parasitology.

Sample contamination protocol

Each canal was inoculated with the bacterial suspension using a MicroPipette (HUMAN, Germany). The teeth were then incubated aerobically at 37 °C for 7 days, permitting penetration of bacteria into dentinal tubules. A baseline swab was collected at T0 to verify comparable microbial loads among all specimens.

Perfusion protocol

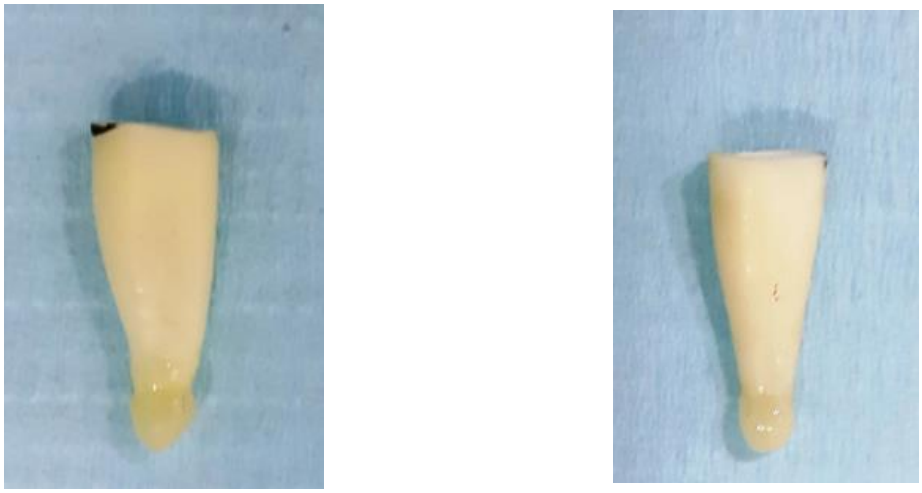
Irrigation was executed for 40 s at room temperature using 2 mL of 5.25 % NaOCl delivered through a 27-gauge Endo-Eze needle (Ultradent, USA), positioned 2 mm from the apical foramen. The solution remained within the canal for 30 s, then was flushed with 10 mL sterile saline to remove any NaOCl remnants. A second swab was taken at T1 to determine the updated bacterial count.

Dressing application protocol

Group 1 received a calcium hydroxide–saline paste (prepared from 1 g calcium hydroxide and 1.5 mL saline) [2]. Group 2 was treated with a calcium hydroxide–propolis mixture (1 g calcium hydroxide + 2 mL propolis) [21].

A paste carrier bur (DENTSPLY Maillefer, Switzerland) was used to fill each canal fully. Both apical and coronal openings were sealed with wax (BMS, Italy) **(Figures 2a and 2b)**. Each root was wrapped in aluminum foil (Serda, Syria), placed in an acrylic holder (Serda, Syria), and stored in a humid container for 7 days **(Figure 3)**.

After one week, wax seals were removed, and the medicaments were evacuated with Hydstrom (DENTSPLY Maillefer, Switzerland) using 5 mL saline. The canals were left for 15 s, then dried using paper points (DIADENT, Korea). Circumferential filing with a sterile H-file (DENTSPLY Maillefer, Switzerland) simulated routine debridement, followed by sampling at T2.



a) b)

Figure 2. Depicts the tooth specimens following crown removal with their apical ends sealed using wax.

Figure 3. Illustrates the samples positioned inside the acrylic holder.

Bacterial swab technique

Microbial samples were taken at T0, T1, and T2 using paper cones matching the dimensions of the F2 preparation instrument. Each cone was inserted to the full working length and kept in place for 10 s. Afterwards, it was transferred into a sterile Eppendorf tube containing 2 mL of sterile saline solution. The tubes were agitated for 1 min with a Bio Vortex mixer (BOECO, Germany) to ensure complete homogenization.

A micropipette (WLD, China) was used to withdraw an aliquot from each tube, which was subsequently spread onto pre-prepared Petri dishes following the Muller–Hinton agar protocol for aerobic culture. Plates were placed in the incubator under the same environmental conditions used earlier. After 24 h, they were removed, and colony numbers were recorded with a digital colony counter (560) after converting raw values to their logarithmic form for statistical processing **(Figure 4)**.

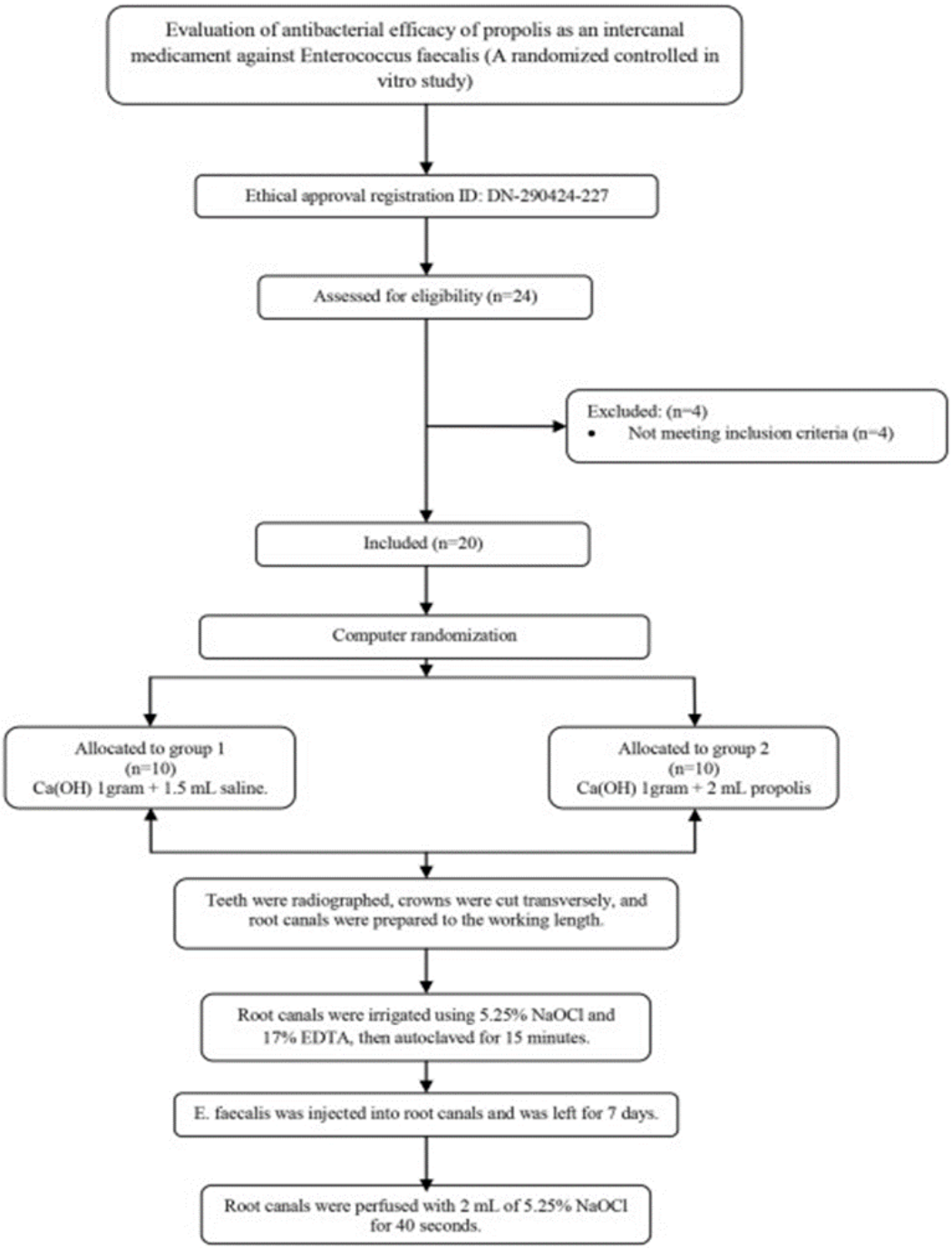


Figure 4. CONSORT flow diagram.

Statistical analysis

All calculations were carried out in SPSS Version 27 (IBM Co., Chicago, IL, USA). A significance level of $P < 0.05$ was adopted.

Between-group comparisons of E. faecalis counts over time were evaluated with the independent-sample T-test, whereas temporal changes within each group were analyzed using the paired-sample T-test.

Results and Discussion

Comparison of E. faecalis **counts between group 1 (calcium hydroxide + saline) and group 2 (calcium hydroxide + propolis)**

The independent-sample T-test evaluated differences in bacterial counts between the two groups at T0, T1, and T2. These findings are summarized in **Table 1**.

According to **Table 1**, no significant differences were present at T0 (P = 0.573). Similarly, no meaningful distinction was observed at T1 (P = 0.994). However, the comparison at T2 yielded a statistically significant result (P = 0.042). The lower mean value in group 2 (0.030) compared with that of group 1 (0.363) indicates a superior reduction in bacterial load for the propolis-based dressing.

Table 1. Results of the independent T-test for differences in E. faecalis counts between groups 1 and 2 at each sampling time.

Time Point	Group	Sample Size (n)	Mean Bacterial Count (log_{10} CFU/mL)	Standard Deviation	t-statistic	p-value	Statistically Significant ? ($p \leq 0.05$)
T0	Group 1	10	3.347	0.033	0.573	0.573	No
	Group 2	10	3.340	0.020			
T1	Group 1	10	2.502	0.880	0.008	0.994	No
	Group 2	10	2.499	0.878			
T2	Group 1	10	0.363	0.471	2.192	0.042	Yes
	Group 2	10	0.030	0.095			

Differences in E. faecalis count within group 2 across T0, T1, and T2

Paired-sample T-tests were used to evaluate how bacterial load shifted within group 2 during each interval. Outcomes are provided in **Table 2**.

A significant reduction was found between T0 and T1 (P = 0.013), with the mean decreasing from 3.340 to 2.499. A more pronounced drop occurred between T0 and T2 (P = 0.000), as the mean declined to 0.030. Additionally, T1 versus T2 (P = 0.000) showed a further reduction, again favoring T2, since 0.030 was substantially lower than 2.499.

Table 2. Paired-sample T-test results showing intra-group variations in group 2 across T0, T1, and T2.

Comparison pair	Sample size (n)	Mean	Standard Deviation	t-statistic	p-value	Statistically significant ($p < 0.05$)?
Baseline (T0) vs T1	10	3.340	0.020	3.066	0.013	YES
T1	10	2.499	0.878			
Baseline (T0) vs T2	10	3.340	0.020	121.678	0.000	YES
T2	10	0.030	0.095			
T1 vs T2	10	2.499	0.878	8.959	0.000	YES
T2	10	0.030	0.095			

Differences in E. faecalis count within group 1 across T0, T1, and T2

Paired-sample T-tests were also applied to group 1, as summarized in **Table 3**.

Statistical significance was present between T0 and T1 (P = 0.013), with a drop in mean from 3.347 to 2.502. Significant reductions also occurred from T0 to T2 (P = 0.000), with T2 showing a mean of 0.363. Likewise, comparisons between T1 and T2 (P = 0.000) indicated an additional decrease, as 0.363 was notably lower than 2.502.

Table 3. Paired-sample T-test results for changes in E. faecalis levels within group 1 over the three evaluated time points.

Pairwise Comparison	Time point	Sample size (n)	Mean	Standard Deviation	t-statistic	p-value	Statistically significant? ($p < 0.05$)
T0 vs. T1	T0	10	3.347	0.033	3.097	0.013	YES
	T1	10	2.502	0.880			
T0 vs. T2	T0	10	3.347	0.033	21.353	0.000	YES

	T2	10	0.363	0.471			
T1 vs. T2	T1	10	2.502	0.880	7.852	0.000	YES
	T2	10	0.363	0.471			

Bacteria are central contributors to pulpal and periapical disease, and the primary aim of endodontic therapy is to diminish the microbial load inside the canal system as thoroughly as possible [22, 23]. E. faecalis is frequently cited as one of the most persistent organisms associated with these infections [24]. Although mechanical shaping and chemical irrigation form essential components of canal disinfection, they do not completely eradicate microorganisms. For this reason, intracanal medicaments are required to suppress any surviving microbes [25].

Enterococcus faecalis is particularly challenging to eliminate. It can establish itself early in infection and frequently persists after failed endodontic procedures. Its ability to penetrate dentinal tubules deeply, generate robust biofilms, and resist both mechanical and irrigating procedures contributes to its survival. In addition, its tolerance to highly alkaline environments explains why calcium hydroxide [$Ca(OH)_2$] does not always eliminate it [26, 27].

Research examining the biofilm-forming capacity of E. faecalis and the responsiveness of these biofilms to 5.25 % NaOCl has shown that cells in the starvation phase generate dentin biofilms that are more difficult for NaOCl to remove than those formed in the stationary phase [28].

Calcium hydroxide, first introduced by Hermann in 1920, has remained a common medicament in endodontics due to its pH of 12.5, its antibacterial properties, its ability to deter root resorption, and its role in promoting repair through hard tissue induction [29].

Among newer medicaments, propolis has drawn attention because of its antimicrobial potential. Interest in natural therapeutic materials has led to expanded evaluation of propolis in dentistry, where it has been used for oral microbial control for centuries [12].

In the current study, the baseline bacterial loads were equivalent across all specimens prior to the application of the 5.25 % NaOCl rinse in an aerobic environment. Following irrigation, the logarithmic bacterial counts were reduced relative to pre-irrigation levels.

Additionally, the data indicated that post-irrigation bacterial counts remained comparable between the two investigated groups and within the overall sample.

After placement of the intracanal dressings, the decimal logarithm values of E. faecalis counts decreased in both groups, independent of the specific medicament used.

Nevertheless, the reduction achieved by the calcium hydroxide–propolis mixture exceeded that produced by the calcium hydroxide–saline preparation. The study also demonstrated a significantly higher incidence of complete bacterial elimination in the propolis-containing group.

These observations align with Rezende's findings [30], which showed that two calcium hydroxide–propolis formulations—regardless of the solvent—exhibited antimicrobial properties.

They are also consistent with the study by Ahangari, Eslami, and collaborators [31], who reported that propolis demonstrated stronger antibacterial action against E. faecalis than calcium hydroxide, suggesting that propolis may serve as a natural adjunct or alternative during canal disinfection.

Comparable conclusions were drawn by Jahromi, Toubayani, and colleagues in 2012 [32], who compared CFUs and MICs of calcium hydroxide with those of propolis used as an intracanal dressing. Their results showed significantly lower MIC and CFU values for propolis. They emphasized that E. faecalis is difficult to eradicate in periapical infections and noted that while calcium hydroxide shows only moderate activity, propolis displayed marked effectiveness.

However, the present study differed from that of Marickar and co-workers [33], who observed no meaningful difference between propolis–calcium hydroxide mixtures and calcium hydroxide alone in killing E. faecalis. Differences in the methodological design and root canal anatomy may account for the discrepancy.

Conclusion

Taking the study's limits into consideration, the calcium hydroxide–propolis dressing demonstrated a more pronounced ability to reduce E. faecalis counts than the calcium hydroxide–saline dressing.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: This study was performed in compliance with the World Medical Association Declaration of Helsinki on medical research ethics and approved by the biomedical research ethics committee under the reference number DN-290424-227. Also, informed consent was obtained for experimentation with human subjects.

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