ORIGINAL RESEARCH

Comparative Evaluation of Efficacy of Calcium Hydroxide, Propolis, and *Glycyrrhiza glabra* as Intracanal Medicaments in Root Canal Treatment

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Abstract

Aim: To evaluate and compare the antimicrobial efficacy of $Ca(OH)_2$, 25% propolis, and 25% *Glycyrrhiza glabra* as intracanal medicaments in root canal treatment.

Materials and methods: Total 60 freshly extracted permanent incisors were decoronated and chemomechanical preparation of root canal was performed. Samples were inoculated with a pure culture of *Enterococcus faecalis* and incubated for 21 days. Colony-forming units (CFUs) were recorded before medication. Incubated samples were randomly categorized into three groups, namely, $Ca(OH)_2$, propolis, and *G. glabra*, with 20 samples in each group. Antibacterial activity was assessed by evaluating the variance in the CFUs on Day 7. Paired "*t*" test and Post-hoc Tukey's test were applied to analyze the data.

Results: Reduction of CFUs was noticed in all the groups (p <0.001), however the reduction was more predominant in the propolis group.

Conclusion: Propolis is more effective against *E. faecalis*, when compared to *G. glabra* and $Ca(OH)_2$.

Clinical significance: Propolis could be used as an effective medicament in root canal treatment.

Keywords: Antimicrobial, Calcium hydroxide, *Glycyrrhiza glabra*, Propolis.

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Introduction

Endodontic therapy principally involves the procedures of biomechanical preparation, and microbial control followed by complete closure of the canal. Bacterial contamination inside the root canal system is considered as the primary etiological factor for oral infections. Enterococcus species are the most common species isolated in post-treatment evaluation of endodontic therapy.[1] The predominant goal of endodontic therapy is to restrict the bacterial proliferation in the radicular dentin which will eventually aid in preventing reinfection.[2] The morphological characteristic of the root canal system is intricate with many anatomical irregularities, which are unapproachable through chemomechanical procedures. Thus, intracanal medication is encouraged between the procedures for reducing bacterial proliferation, which also assists in providing a favorable environment for periapical tissue repair.[2]

In the field of endodontics, medicaments like chlorhexidine (CHX), iodine potassium iodide (IKI) and sodium hypochlorite (NaOCl) demonstrate wide-cut spectrum of antimicrobial activity. However, they never earned wide acceptance due to the plethora of adverse effects they possessed. In some *in vitro* studies, CHX is considered to be lethal to canine embryonic and gingival fibroblasts at bactericidal concentrations.[3,4] Sodium hypochlorite at concentrations greater than 0.5% possibly leads to the development of fragile teeth due to the dentine collagen damage. IKI has been shown to evoke an allergic reaction in some people.[3,4]

Conventionally, $Ca(OH)_2$ is frequently used for the eradication of microbes.[5] It shows the antimicrobial effect by inactivating membrane transport mechanisms of the organisms due to its high alkaline nature.[5] It also presents broad-spectrum antibactericidal activity, specifically targeting endodontic microorganisms. However its efficacy against *Enterococcus faecalis* is controversial.[5–7] Additionally, the increasing rate of unpredictable cytotoxic reactions and the inefficiency of commercially available medicaments to eliminate microflora effectively from the deeper layers of dentinal tubules have lead to a need for the researchers to explore a substitute.[8,9]

In recent times, utilization of alternate therapeutic agents derived from insects, florae, and microorganisms has been substantially increased.[10,11] Natural medicines have an imperative role in today's medicine, due to increased antibiotic-resistant strains and side effects produced by commercially available synthetic

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drugs. Several researches discovered that various natural products have antimicrobial and curative effects, suggesting its use as an intracanal medicament.[8–11]

Propolis is one such natural, flavonoid-rich, wax-cum resinous product of *Apis mellifera* more commonly known as honeybee. It predominantly comprises of resins, aromatic oils, minerals, vitamins, and flavonoids, among which flavonoids and hydroxyl cinnamic acid are found to be responsible for its biological activity.[12] In dentistry, it is widely used as a pulp capping agent,[13] storage medium for avulsed tooth,[14] sealant for dentinal hypersensitivity,[15] and as endodontic cavity disinfectant.[14] Propolis is efficacious against many resistant microbes including *E. faecalis* and *Candida albicans* and is also biocompatible with the periradicular tissues as compared to existing intracanal medicaments.[16]

Glycyrrhiza glabra, more commonly known as licorice, one of the traditional medicinal plants used in phytomedicine from past 4000 years.[17] In Ayurvedic formulations, its roots are used for bronchial-related therapies.[17] Glycyrrhizin, a diglucuronide derivative of glycyrrhetinic acid, is primarily accountable for its antibacterial properties.[17] *G. glabra* has been reported to be effective against *E. faecalis*. Moreover, it is also biocompatible with fibroblasts when compared to $Ca(OH)_2$.[5] However, when literature search was carried out, there were no studies comparing the efficacy of propolis and *G. glabra* with $Ca(OH)_2$ as an intracanal medicament against *E. faecalis*. So an attempt was made to carry out this research to evaluate the bactericidal effect of propolis, *G. glabra* and $Ca(OH)_2$ against *E. faecalis*.

Materials and Methods

The study was conducted in the Department of Conservative Dentistry and Endodontics. Ethical clearance was obtained from the Research and Ethical Committee of the Institution.

Sixty freshly extracted, single-rooted, and single canal incisors and canines were taken. Patients indicated for extraction due to periodontically compromised teeth and prosthetic rehabilitation were selected. Teeth with single patent canals, with no signs of internal or external resorption were included in the study. Teeth that were carious, restored, fractured, or with cracks were excluded. Additionally, teeth having calcified canals were also excluded from the study.

Preparation of Natural Extracts

Propolis dry powder was purchased from Hi-Tech Natural Products Ltd., India (Batch No. CRF/150/2016) and was certified from Central Bee Research and Training Institute, Ministry of MSM Enterprises, Govt. of India, Pune. *G. glabra* dry powder was collected and authenticated from the Institutional Ayurveda College.

Preparation of Carboxymethylcellulose

Carboxymethylcellulose (CMC) was obtained from sodium carboxymethylcellulose (SCMC; Coloron Industries, Goa, India). Two grams of SCMC was weighed and dispensed in 50 mL of distilled water obtained from Benzer Multitech Private Limited, Pune, India. The solution was continuously stirred with a magnetic stirrer for at least 120 minutes at room temperature. It was later kept for 24 hours to hydrate.

Preparation of Test Medicament

Twenty-five grams of propolis and *G. glabra* powder were weighed separately and amalgamated with the SCMC gel by constant stirring in a propeller at 400 rpm, until gel consistency was achieved. Weighed quantity of glycerin which is used as humectant (SD fine chemicals, Mumbai, India), and the gel mixture was mixed by stirring in a propeller at 400 rpm for 15 minutes. Sodium benzoate is used as a preservative (Balaji Chemicals, Mumbai, India) and the gel mixture was mixed and stirred for 30 minutes, to achieve an even distribution of gel ingredients. Sodium methylparaben and sodium propylparaben (SD fine chemicals, Mumbai, India) were weighed and dissolved in 10 mL of sterile distilled water.

Finally, the quantity of gel mixture was adjusted with plain sterile distilled water and stirred for 10 minutes. Gel was moved to previously sterilized plastic container and stored at room temperature (Patent Application No. 201841042422A and 201941054463A).[18] The prepared gel was subjected to minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) procedures.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Natural Extracts

The MIC and MBC were evaluated by broth dilution methods, followed by agar diffusion methods. MIC was assessed against ATCC strains of *E. faecalis*. Inoculum of standard strains of organisms was prepared as per 0.5 McFarland standard.

The powder was weighed and dissolved in 0.5 mL of dimethyl sulfoxide present in Eppendorf microcentrifuge tube. It was subsequently vortexed and used immediately. Ten autoclaved Eppendorf tubes were labeled with the serial numbers from 1 to 10. The standard operating protocols of vertical laminar flow were followed. In the vertical laminar flow, MIC procedure was carried out by adding 200 µL of plain brain heart infusion (BHI) broth from second to tenth tube by using a micropipette, followed by the addition of 200 µL of respective extract to first and second tubes. Broth and extract solution were thoroughly mixed using a micropipette. Further, 200 µL of diluted extract were pipetted and added to the third tube. The procedure was continued till the ninth tube and then the diluted extract from the ninth tube was pipetted and discarded. Serial dilution started from the second tube and continued till the ninth tube. Here, first tube was designated as the positive control and tenth tube as the negative control. Two hundred microliter of BHI broth was added to all the 10 tubes. Finally, 200 µL of *E. faecalis* inoculum was added to all the 10 tubes.

The Eppendorf tubes were kept for incubation in a CO_2 desiccator for 48 hours. The MIC was recorded, and the inhibitory concentration was confirmed by transferring each of these serial dilutions onto the blood agar culture plates under laminar airflow for propolis group. The culture plates were kept for incubation for 24 hours. Colony-forming units (CFUs) were logged after the respective clock times of incubation.

Preparation of Specimens

Samples were thoroughly cleansed and stored in compliance to the guidelines of Occupational Safety and Health Administration.[19] Before canal instrumentation, teeth decoronation was done using a high-speed bur along with water spray to attain standardized 16-mm longroots. Canal patency and working length were accomplished by placing 10# K file (Mani. Inc, Tochigi, Japan) to the terminus and deducting 1 mm from this measurement.[20] ProTaper rotary instrument size F5 was employed to prepare the canals. The canals were irrigated with 3% NaOCl after each instrument (Vishal Dentocare

Pvt. Ltd., Ahmedabad India), and last irrigation was done using 17% ethylenediaminetetraacetic acid (EDTA) (DEOR Deo Smear-Off, India). It was allowed to remain for 1 minute and then rinsed with 2 mL saline.[21] The specimens or samples were dried using paper points.

Contamination of Specimens

A pure culture of *E. faecalis* was isolated and grown for 24 hours in blood agar media. The culture was then suspended in 5 mL BHI broth and then incubated for 4 hours at 37°C. Samples were inoculated with 10 µL of *E. faecalis* and incubated for 21 days at 37°C.

Collection of Samples

After an incubation period of 21 days, the samples were evaluated for the microbiological analysis before placement of the medicaments (Premedicament sample S1). Each tooth was irrigated with 100 µL of sterile saline and then size-50 absorbent paper point was used for drying (Dentsply, India). Around three paper points were taken for each sample following the similar procedure as described above. After this, the paper points were shifted to a test tube containing 1 mL of sterile solution. Twenty-five microliter aliquots of each dilution were layered or plated on blood agar. The CFUs were counted after 24 hours using stereomicroscope. After this, the medicament was placed in 60 specimens which were divided randomly into three groups of 20 each. Group I included $Ca(OH)_2$, group II included 25% propolis, and group III included 25% *G. glabra*. The prepared pastes were carried into the canal using lentulo spiral (Mani Inc, Tachigi-ken, Japan) and were further condensed using hand pluggers (Sybron endo). The coronal openings of the root canals were sealed with a nonpermanent filling material to avoid any leakage.

The choice of irrigants in our study was 3% sodium hypochlorite (NaOCl), 17% EDTA, and 0.9% normal saline.[22] Sodium hypochlorite (3%) was used for irrigation first. Final irrigation was done with 2 mL of 17% EDTA and it was allowed to remain for 1 minute, followed by rinsing with 2 mL of saline.

The specimens were kept at 37°C in 100% humidity for 7 days to simulate clinical conditions. After 1 week, the medicaments were removed with saline and passive ultrasonic irrigation. A total samples of 60 were then sent for microbiological analysis (Postmedicament sample S2). The antibacterial efficacy was assessed by comparing the reduction in CFUs, before (premedicament sample S1) and after (postmedicament sample S2) placement of the medicament. The CFU was then converted to log CFU.

Results

Data analysis was done using Rv64 (3.5.1) software. Normality was checked using the Shapiro–Wilk test. The difference between the study groups was analyzed by the Kruskal–Wallis test. Box plots were used to compare all three groups. The paired data were derived using Wilcoxon signed-rank test and the correlation was done by Karl person's correlation coefficient. A *p*-value of ≤0.05 was considered as statistically significant.

Lesser CFUs were observed for propolis when compared with other groups. However, significant reduction of CFUs was observed in all three groups as indicated by a highly significant "*p*" value (p <0.001), before and after the medicament (Table 1). Comparison of CFUs within the groups showed significant difference in each of the three groups in both premedicament (p = 0.0017) and postmedicament (p <0.001) (Fig. 1).

Table 1: Comparison of three study groups at different time points with mean CFU counts

*Group**	*Premedicament sample*	*Postmedicament sample*	*p-value*
Calcium hydroxide group	5.06 ± 0.02	3.65 ± 0.05	<0.0001*
Propolis group	5.22 ± 0.01	1.25 ± 0.07	<0.0001*
***Glycyrrhiza glabra* group**	4.86 ± 0.02	2.82 ± 0.07	<0.0001*

*Wilcoxon signed-rank test

Table 2: Pairwise comparison to find the significant difference in colony-forming units between different groups

p-value		*Calcium hydroxide group*	*Propolis group*
Premedicament#	Calcium hydroxide group	—	
	Propolis group	0.257	—
	Glycyrrhiza glabra group	0.257	0.026*
Postmedicament*	Calcium hydroxide group	—	
	Propolis group	0.012	—
	G. glabra group	<0.0001	0.002

#Students *t*-test; *Wilcoxon test is used

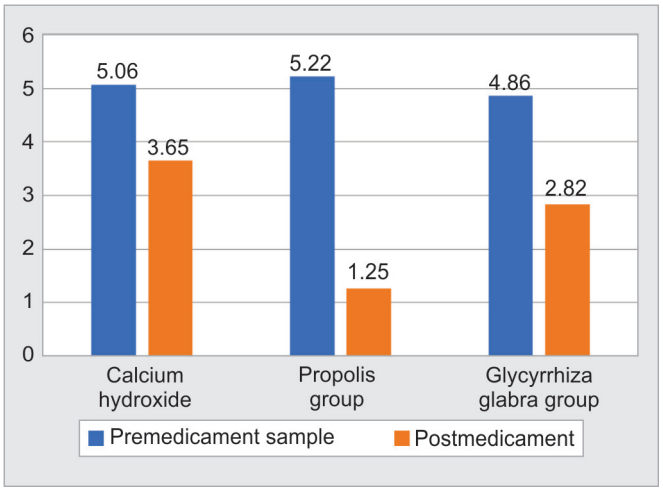
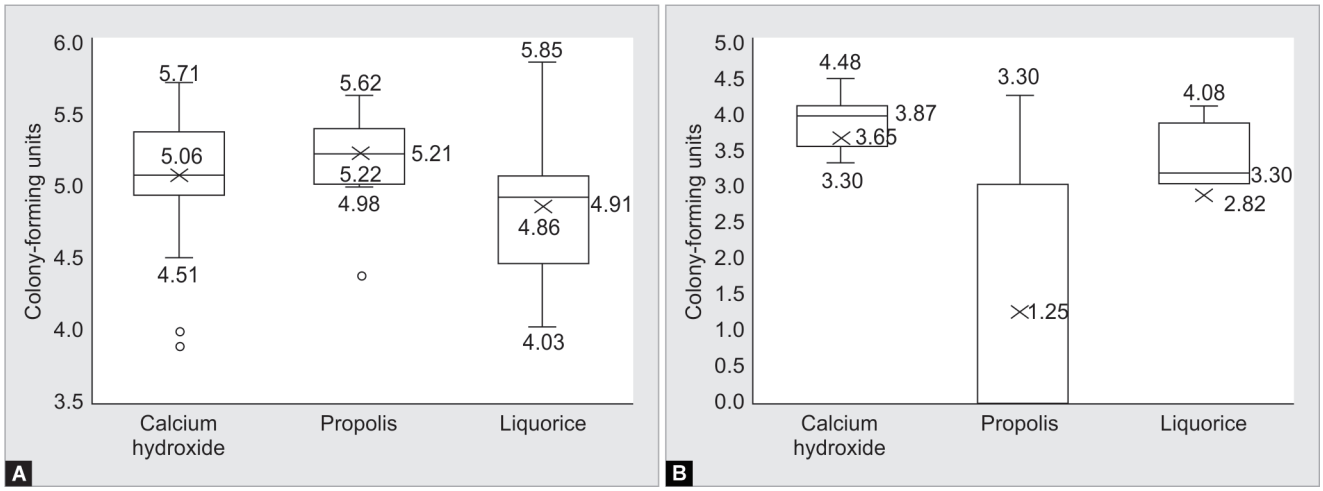


Fig. 1: Comparison of three study groups at different time points with mean CFU counts

Pairwise comparison of CFUs between groups has been demonstrated in Table 2. Highly significant results were observed when calcium hydroxide and *G. glabra* were compared along the pairs with p = 0.0001(p <0.001). Furthermore, when propolis and *G. glabra* were compared along the pairs, significant results were observed with p = 0.002 (p <0.05) Compared to other groups, propolis had larger variation in terms of CFUs and *G. glabra* had the least CFUs (Fig. 2A). Postmedicament, propolis showed the highest reduction in CFUs and a highly significant "*p*" value was obtained (p <0.001) as shown in Fig. 2B.

Discussion

Endodontic therapy prominently relies on proper elimination of bacterial growth from the pulp space.[2] To eradicate microorganisms in the complex root canal system, instrumentation plays a central



Figs 2A and B: Comparison of colony-forming units within the groups (A) premedicament (B) postmedicament

role in the cascade of treatment procedures. Proper and thorough instrumentation with effective irrigation removes most necrotic pulp tissue and a significant number of microbes by direct mechanical cleansing action.[2] Sometimes, even with thorough instrumentation, remnants can be localized in certain areas including isthmuses, dentinal tubules, and lateral canals or the dentinal walls of the root surface area where mechanical cleaning and irrigation are ineffective.[22] In such scenarios, antibacterial medication assists effectively in removing the remnant bacterias that are retained after canal preparation.[22] Thus, clinical disinfection along with intracanal irrigants and medications are essential cornerstone for a successful outcome of root canal treatment.

Calcium hydroxide is a commonly used intracanal medicament. The release of hydroxyl ions in an aqueous environment is responsible for the antimicrobial activity of calcium hydroxide. Their lethal effects on bacterial cells are probably caused by the mechanisms such as damage to the bacterial cytoplasmic membrane, protein denaturation, and damage to DNA.[22]

However, calcium hydroxide exerts antibacterial effects in the root canal system as long as a high pH is maintained. Several studies have attested to the ineffectiveness of $Ca(OH)_2$ in eliminating microbial cells. Two studies revealed that $Ca(OH)_2$ had no antibacterial effect as a paste or as the commercial preparation Pulpdent when used against *Streptococcus sanguis*. It was also shown that a $Ca(OH)_2$ paste failed to eliminate, even superficially, *E. faecalis* in the dentinal tubules.[23,24]

The reason for the limited antimicrobial effect of calcium hydroxide on facultative anaerobes could be attributed to the dentine buffering effect. The antibacterial activity of calcium hydroxide is related to its high pH (12.5) which has a destructive impact on bacterial cell membranes and protein structure. However, to be effective against bacteria located inside dentinal tubules, hydroxyl ions from calcium hydroxide must diffuse through the dentin and reach sufficient levels to be lethal. Dentin hydroxyapatite has a strong buffering property that must be overcome by hydroxyl ions leaving the root canal space. Proton donors in the hydroxyl layer of hydroxyapatite are responsible for the buffering of alkaline substances. It has been demonstrated that calcium hydroxide alkalinizes the dentin, but the pH values reached may be insufficient to kill some bacterial strains particularly *E. faecalis* which can survive a high pH of 11.5.[25]

Herbal products have been used since ancient times in folk medicine involving both eastern and western medical traditions. Antimicrobial agents of plant origin have enormous therapeutic potential. Propolis, renowned for its antimicrobial activity, was introduced into dentistry by Krell in 1996. It consists of highly active bioflavonoids which have antimicrobial, antioxidant, and anti-inflammatory properties. The antioxidant property of propolis is attributed to its radical scavenging ability and that the anti-inflammatory property is due to the presence of caffeic acid phenethyl ester.[26] The mechanism of antibacterial action for propolis may be attributed to its flavonoid content, various esters of caffeic acid, galangin (3,5,7-trihydroxyflavone), and its bioautogram components. Also, the ultraviolet-absorbing component of propolis has been shown to inhibit bacterial DNA-dependent RNA polymerase.[27,28]

G. glabra, the name given to the roots and stolons of Glycyrrhiza species, has been used since ancient times as a traditional herbal remedy. *G. glabra* contains several classes of secondary metabolites with which numerous human health benefits have been associated.[29] The antimicrobial effect of *G. glabra* extract against *E. faecalis* may be related to the glycyrrhizin. The mode of action of antibacterial effects of saponins seems to involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium, thus being influenced by microbial population density.[30]

The MIC was tested using the broth dilution method, and the MBC was detected by sub-culturing these on antibiotic-free media. In this study, the mean of MIC of propolis group was 25 µL, and *G. glabra* group was 12.5 µL against *E. faecalis*. The mean of MBC of propolis group was 25 µL and *G. glabra* was 25 µL.

Results of the present study were in accordance with the other authors who found that bactericidal effect of 57% $Ca(OH)_2$ is less effective in restricting bacterial growth compared to other medicaments.[31,32] These findings represent the low activity of $Ca(OH)_2$ in restricting bacterial growth. This might be ascribed to the *E. faecalis*'s capability of maintaining the pH homeostasis through ions penetrating the cell membrane and the cytoplasm's buffering capability along with proton pump action assisting pH homeostasis.[33]

In this study, propolis showed a significant reduction in *E. faecalis* in 7 days. Awadeh et al. also reported better bactericidal

efficacy of propolis against *E. faecalis* when compared to Ca $(OH)_2$,[34] which agrees with the findings of our study. Contrastingly, in a study conducted by Oncag et al., an insignificant difference between sodium hypochlorite, saline, EDTA, and propolis was reported at 48 hour period; on the 10th day, propolis showed greater bactericidal effect than others.[35] The antibacterial action of propolis can be attributed to its flavonoids, caffeic acid esters, galangin, and its bioactive components. Moreover, the ultraviolet absorbing component of propolis is reported to inhibit bacterial DNA-dependent RNA polymerase.[35]

In this study, a significant reduction of CFUs was observed for *G. glabra*. This finding is in line with the study conducted by Badr et al., who observed significant diminution in the CFUs with *G. glabra*. The reason attributed for the same is the presence of glycyrrhizin which is responsible for its antimicrobial effect against *E. faecalis*.[5] Based on the available evidence, propolis and *G. glabra* can be used as effective alternate intracanal medicaments in comparison with $Ca(OH)_2$ during a root canal treatment for eradicating endodontic microbes. However, *in vivo* studies are needed to support the findings.

Limitation of our study is that it is an *in vitro* study and an *in vivo* study needs to be carried out in future with larger sample size.

Conclusion

Within the limitations of this *in vitro* study, both propolis and *G. glabra* have demonstrated better antimicrobial efficacy against *E. faecalis* compared to $Ca(OH)_2$. The practice of herbal alternatives as intracanal medicaments may prove to be advantageous; however, more clinical studies are needed to explicate the efficacy of propolis and *G. glabra*. In future, more studies should focus toward other commonly detected microbes in root canal infections.

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