RESEARCH ARTICLE

Antimicrobial Efficacy of Triple Antibiotic Paste and Propolis as an Intracanal Medicament in Young Permanent Teeth: An *In Vivo* Study

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Abstract

Aim and objective: To evaluate the antimicrobial efficacy of triple antibiotic paste and propolis extracts as an intracanal medicament in young permanent teeth.

Materials and methods: A total of 30 single-rooted non-vital young permanent teeth with open apex were selected randomly from the children aged between 7 years and 14 years with no systemic complications. Group I—triple antibiotic paste and group II be propolis allocating 15 teeth in each group. After access opening, the first sample (S1) was collected by inserting paper point into the root canal, the second sample (S2) was collected immediately after irrigation, and the third sample (S3) was collected after post-intracanal medication after 3–4 weeks. Samples were sent for microbiological analysis to assess the bacterial count, and for the obtained data, statistical analysis was done.

Results: The mean colony count among the triple antibiotic paste group was 1906.75. After access opening, which was reduced to 315.12 after irrigation, and after 3–4 weeks, it was 817.25. There was a significant difference between sample 1, sample 2, and sample 3 (p value = 0.008). The mean colony count among the propolis group was 1427.87 after access opening, which was reduced to 436.00 after irrigation, and after 3–4 weeks, it has reduced to 252.37. There was a significant difference between sample 1, sample 2, and sample 3 (p value = 0.032). Intergroup comparison between the groups showed no statistical difference between the samples.

Conclusion: Propolis exhibited similar antimicrobial efficacy, which is comparable to triple antibiotic paste. So, propolis can be utilized as an intracanal medicament in young permanent teeth with an open apex.

Clinical significance: Propolis is a naturally occurring flavonoid-rich resinous product with antibacterial, antifungal, antiviral, immunomodulatory, and antioxidant effects. It is safe without any drug allergies and bacterial sensitivity and is a promising alternative to triple antibiotic paste for disinfecting non-vital young permanent teeth.

Keywords: Propolis, Sodium hypochlorite, Triple antibiotic paste, Young permanent teeth.

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Introduction

Endodontic treatment for non-vital young permanent teeth poses a substantial challenge due to thin dentinal walls and the lack of apical constriction.[1] The apexification procedure has been used to treat these teeth for decades in which calcium hydroxide ($Ca(OH)_2$) is introduced into the root canals to induce the calcific barrier formation at the root apex. Despite the use of $Ca(OH)_2$ apexification technique, the lengthy treatment duration, which may necessitate multiple visits and the replacement of the medicament, unpredictable nature of apical closure, and the risk of cervical root fracture upon constant exposure to calcium hydroxide have all raised serious concerns about the treatment's drawbacks.[2] The conventional apexification technique has been altered by introducing the mineral trioxide aggregate (MTA) as an artificial apical barrier.[1] Though this treatment might considerably improve patient compliance, shortens the treatment time, and results in satisfactory periapical tissue healing, it is still unable to stimulate the dentin thickness and does not enhance the apical closure.[1] On this basis, the prognosis of this apexification procedure for non-vital immature teeth in the future appears to be uncertain.[3]

The regenerative endodontic approach can enable continuous root development, and it may be a viable treatment option for treating immature permanent teeth having compromised tooth structure. In revascularization, using a combination of antibiotics has recently gained more attention in treating the young permanent teeth with apical periodontitis. This procedure involves the root canal disinfection with sodium hypochlorite (NaOCl) irrigating solution followed by the placement of antibiotic paste (ciprofloxacin, metronidazole, and minocycline).[4] After disinfection, the triple antibiotic paste is withdrawn, and apical bleeding into the canal is stimulated to produce a blood clot. Finally, the canal orifice is secured with MTA, followed by permanent restoration. Various clinical studies and case reports have stated that the regeneration potential of this procedure is indicated by enhanced root length,

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root wall thickening, and varying degrees of apical closure.[5] But, minocycline in TAP has been associated with discoloration; also, there is evidence of bacterial sensitivity and drug resistance.[6]

To minimize these side effects caused by these antibiotics, the current trend has been shifted to natural products to disinfect the root canals. Among the various natural products used, propolis, a flavonoid-rich resinous substance of honeybees. In dentistry, it is widely used as an anticaries agent,[7] an avulsed tooth storage medium,[8] capping agents for pulp,[9] and a dentinal hypersensitivity sealant.[10] Propolis is 10 times less toxic than calcium hydroxide[11] and contains antibacterial,[12] antiviral, antifungal,[13] immunomodulatory,[14] and antioxidant properties.[15] The purpose of this study was to evaluate the antimicrobial efficacy of triple antibiotic paste and propolis as an intracanal medicament in young permanent teeth.

Materials and Methods

The present study is a single-blinded randomized clinical trial conducted in the outpatient Department of Pediatric and Preventive Dentistry, Sri Venkata Sai Institute of Dental Sciences, Mahabubnagar, Telangana, with no history of systemic disease or hospitalization. Written informed consent was taken from the parents of all the children included in the study. The study was approved by the Institutional human ethical committee (SVSIDS/PEDO/3/2017). The children cannot be blinded to the procedure as the two materials are visible to the naked eye. An independent investigator manually counted the bacterial colonies without the knowledge of the allocation of the subjects.

Sample Size Calculation

A sample size determination was performed using a sample size calculator (World Health Organization). With 90% power and β at 10%, the sample size turned out to be 30 teeth (15 in each group).

Patient Selection and Randomization

A total of 30 single-rooted young permanent teeth that met the inclusion criteria were selected randomly from the children aged between 7 years and 14 years. The teeth were randomly distributed into two groups, group I-triple antibiotic paste and group II-propolis allocating 15 teeth in each group. Teeth were selected irrespective of the maxillary or mandibular single-rooted teeth. Inclusion criteria for teeth selection include non-vital tooth with open apex, history of trauma, presence of clinical/radiographic periapical pathology, and restorable teeth. Exclusion criteria for teeth selection include grade III tooth mobility and medically compromised children.

Study Materials

Group I

Triple antibiotic paste preparation was done by using three drugs, namely Ciprofloxacin (CIFRAN 500 mg, Ranbaxy laboratories limited, India), Metronidazole (METROGYL 400 mg, J.B Chemicals and Pharmaceuticals Ltd. India), and Minocycline (MINOZ 100 mg, Ranbaxy laboratories limited, India).

The enteric coating of the three drugs was removed, followed by pulverization of each drug under aseptic conditions, which was later mixed in the ratio of 1:1:1 weight by volume, and this mixture was suspended into capsules (Fig. 1A).

Group II

Commercially available Propolis powder (Hi-tech Natural Products, India) (Fig. 1B), mixed with saline.

Methodology

After anesthetizing the tooth [lignocaine hydrochloride solution–Lignox A 2% (Indico remedies)] and isolation with a rubber dam (Hygiene Coltene, Whaledent, Germany), access cavity preparation was done using a high-speed airotor (NSK Pana Max, Japan) with round bur (Diaburs, prime dental products, India) and the pulp chamber was exposed.

Collection of the First Sample (S1)

The S1 was collected by placing no. 35 paper point into the canal and was left for 1 minute (Fig. 1C) and transferred into the sterile test tube comprising brain heart infusion (BHI) nutrient broth and sealed the test tube tightly.

Collection of the Second Sample (S2)

According to American Association of Endodontics (AAE 2016) guidelines, irrigation of the root canal was done using 20 mL of 1.5% NaOCl for 5 minutes using double side vented needle (Fig. 1D). The later canal was disinfected with 20 mL of saline for 5 minutes with the same needle. After that, S2 was collected, similar to the procedure done for the first sample.

Labeled test tubes (Fig. 2A) containing samples were sent for microbiological analysis.

Finally, the root canals were dried with paper points, and teeth were randomly allocated into two groups.

Group I

Triple antibiotic paste (ciprofloxacin, metronidazole, minocycline in 1:1:1 ratio) was mixed with 0.3 mL of saline (Fig. 1E), placed into the canal with the help of lentulospiral (Dentsply, India) followed by placement of interim restorative material (Dentsply, India).

Group II

Propolis powder (Hi-tech Natural Products, India) was combined with saline (Fig. 1F) in a ratio of 1.5:1 (wt/vol), later placed into the canal with the help of lentulospiral followed by placement of interim restorative material.

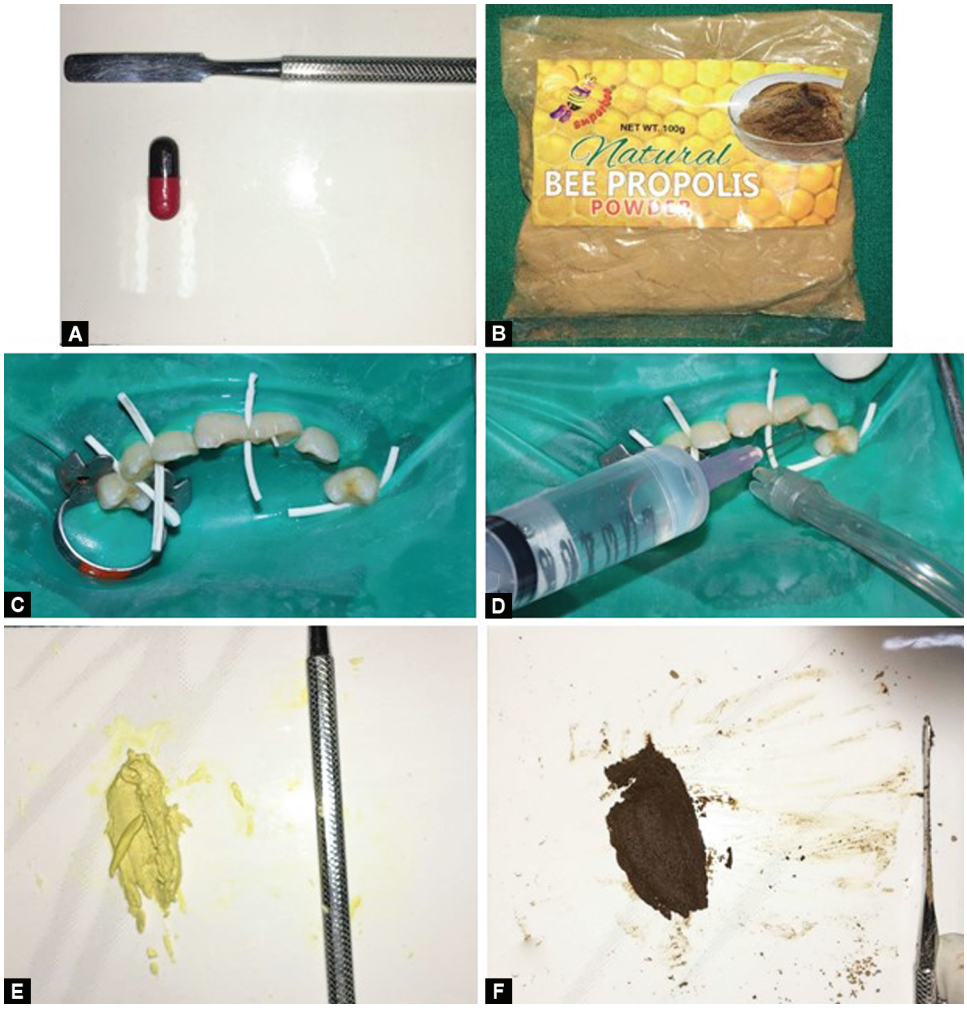
Patients were recalled after 3–4 weeks for the evaluation of these test materials and the collection of 3rd sample.

Collection of the Third Sample (S3)

In this appointment, temporary restorative material was removed. After regaining access, irrigation of the root canal was done using saline with double side vented needle till the intracanal medicament has washed off. After that, the third sample (S3) was collected, similar to the procedure done for the first sample. A labeled test tube containing the sample was sent for microbiological analysis (Fig. 2A).

Microbiological Analysis

All the samples were transferred to Petri plates containing tryptone soya agar with 5% of blood through the pour plate method and were incubated at 37°C for 48 hours. The obtained bacterial colonies were manually counted (Figs 2C to F).



Figs 1A to F: (A and B) Study materials—triple antibiotic paste and propolis; (C and D) Collection of sample and 1.5% NaOCl irrigation; (E and F) Mixing of triple antibiotic paste and propolis

Results

Kruskal–Wallis test was performed to obtain the mean colony count in the triple antibiotic paste and propolis groups. Intergroup comparison was done using Wilcoxon signed ranks test. Statistical analysis was conducted using SPSS software version 21.0. All the *p* values <0.05 are considered statistically significant ($p < 0.05$).

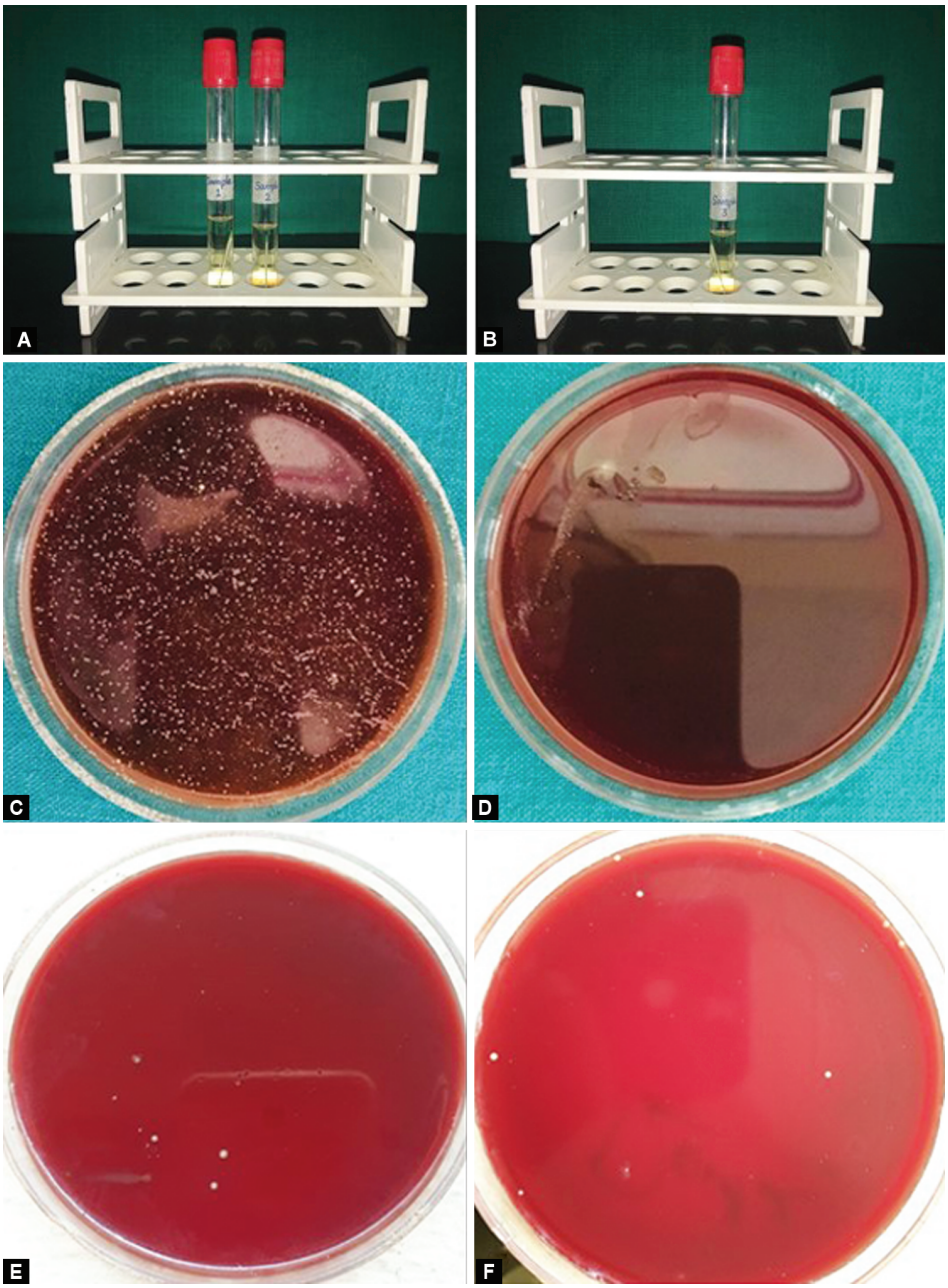
Table 1 represents the mean colony count of the TAP group at three different time intervals. A statistically significant difference was observed between samples 1 and 2, samples 1 and 3 ($p = 0.008^{a}$). Table 2 displays the mean colony count of the propolis group at three different intervals. There is a statistically significant difference between samples 1 and 2, samples 1 and 3 ($p = 0.032$*). Table 3 represents the intergroup comparison between TAP and propolis groups at three different time intervals; the difference between the triple antibiotic paste and propolis groups was not statistically significant.

Discussion

Conventional root canal treatment of young permanent teeth with apical infection and necrotic pulp has several treatment challenges. The mechanical cleaning using dentin removing instruments is not recommended because it causes additional thinning of the root canal walls, which are already weak. Root canal obturation without extending the filling material into periapical tissues is a challenge, even with the experienced clinician; the large open apex might have a divergent shape, which does not offer the mechanical stop required to support the filling material confined within the root canal. Although a calcium hydroxide apexification method or an apical plug of MTA can be employed to address most of the challenges associated with traditional root canal therapy,[16] but the possibility of root fracture remains.

In the early 1960s, Nygard Ostby suggested that in endodontically treated teeth with necrotic pulps and apical lesions, a new vascularized tissue might be produced in the apical third of the root canal.[17] It is done by creating a blood clot in the apical third of the root canal by extending the file apically before filling the root canal. This vascularized tissue is due to clot formation (scaffold), which helps in the development of new tissue in the root canal. Usually, three factors influence the effectiveness of pulp revascularization: root canal disinfection, the existence of a scaffold (blood clot), and tight coronary filling.[18]

In root canal treatment, 5.25% sodium hypochlorite solution is widely used as an antiseptic irrigating agent. Studies stated that it effectively prevents biofilm formation among five different bacterial strains in the root canal.[19] Sodium hypochlorite with different concentrations ranging from 1 to 6% has been studied in regenerative endodontic treatment (RET).[20] The American



Figs 2A to F: (A and B) Collection of first, second and third samples; (C and D) Agar plates showing the bacterial colonies in first and the second samples; (E) Agar plates showing the bacterial colonies in the third sample in one of the triple antibiotic paste group; (F) Agar plates showing the bacterial colonies in the third sample in the propolis group

Table 1: Mean colony count of all three samples in triple antibiotic paste group

Sample (n = 15)	*Mean ±*	*Std. deviation*	*p value*
S1	1906.75	1291.38	0.008* Significant
S2	315.12	704.08	
S3	817.25	1663.04	

Table 2: Mean colony count of all three samples in the propolis group

Sample (n = 15)	*Mean ±*	*Std. deviation*	*p value*
S1	1427.87	1616.58	0.032* Significant
S2	436.00	1135.78	
S3	252.37	417.35	

Table 3: Comparison of triple antibiotic paste and propolis

Sample	*Medicament*	*Mean*	*Std. deviation*	*p value*
1	TAP	1906.75	1291.38	0.401 Not significant
	P	1427.87	1616.58	
2	TAP	315.12	704.08	1.000 Not significant
	P	436.00	1135.78	
3	TAP	817.25	1663.04	0.779 Not significant
	P	252.37	417.35	

Association of Endodontics recommended using 1.5% sodium hypochlorite followed by 17% EDTA in RET. This is due to the cytotoxic action of sodium hypochlorite on the viability of stem

cells from apical papilla.[21] In the present study, 1.5% NaOCl was used as an intracanal irrigant 20 mL for 5 minutes according to the AAE guidelines given for the revascularization procedure. Kalhoro et al.[22] stated that the open-ended beveled needle had a greater extrusion incidence (62%) than the closed-ended side vented needle (24%) in apical extrusion of NaOCl, and it is dependent on the irrigation needle utilized for irrigation. A close-ended needle with side vents appeared to be safe over open-ended beveled needles. Irrigation with double side vented needles have the advantage of removing the microorganisms from the walls when compared to single vented needles. This justifies the use of double side vented needles of 27 gauges in this study to improve the availability of NaOCl to the root canal and also decrease its toxic effects by reducing the extrusion to the periapical structures, which was similar to the studies done by Chen et al.[23] and El Ashiry et al.[24]

The most widely used intracanal medicaments in RET procedures are calcium hydroxide, chlorhexidine, and triple antibiotic paste. Antibiotics are intended to selectively target infection-causing bacteria instead of the host's normal tissue. Grossman et al. was the first person to report the application of a topical antimicrobial agent to disinfect an affected root canal. Later, Hoshino et al.[4] and Sato et al.[25] developed a triple antibiotic paste to disinfect the root canals *in vitro*. To minimize the stem cell injury from the apical papilla, AAE in 2016 suggested using triple antibiotic paste at a dose of no more than 1 mg/mL (0.1–1 mg/mL) in RET.[26] Unfortunately, antimicrobial combinations can potentially increase the side effects, antagonistic interactions, and bacterial resistance.[10] To reduce these side effects caused by antibiotics, the trend has been shifted to the use of natural products to disinfect the root canals. Propolis is well-known for its antibacterial, antifungal, antiviral, immunomodulatory, and antioxidant effects. Propolis is widely used in dentistry as an irrigating solution for the removal of *Candida albicans* and *Enterococcus faecalis*.[27] The chemical components in propolis, such as chrysin, terpenoid, volatile compounds (coumaric acid), and protocatechuic acid, are thought to be responsible for its antibacterial properties.[28] When sodium hypochlorite is compared with propolis as a root canal irrigant, both the agents have similar antibacterial properties.[29] Calcium hydroxide and propolis possess similar physical characteristics as intracanal medication. However, propolis is 10 times less toxic to periodontal ligament fibroblasts and dental pulp than calcium hydroxide, and it is easy to remove from the canals.[30]

In the animal studies, Pagliarin et al.[31] compared propolis and triple antibiotic paste for root canal disinfection and evaluated the tissue repair in immature canine teeth with apical periodontitis and suggested that the disinfection of root canals in immature necrotic permanent teeth with propolis paste might be a viable option for triple antibiotic paste. Another study by El-Tayeb et al.[32] evaluated the antibacterial properties and tooth regeneration potential of propolis in immature permanent non-vital canines teeth and stated that propolis could be equivalent with TAP as a root canal disinfection in RET. An *in vivo* study done by Parolia and Kundabala[33] describes a case of successful non-surgical endodontic retreatment where propolis was placed as an intracanal medicament. They suggested that propolis as an intra-canal medicament is very useful, which gives a new direction in the management of retreatment cases. Bose et al.[5] performed a study on various endodontic regeneration treatments using calcium hydroxide, TAP, and formocresol and stated that TAP and calcium hydroxide as an intracanal medicament in young permanent teeth which aids in the development of pulp dentin complex. The mean time period considered necessary for the disinfection of the root canals, leaving the triple antibiotic until the tooth has become asymptomatic, varies between 1 and 4 weeks, while Ding et al.[34] (in their clinical study with humans) suggest waiting for 1 week, Windley et al.[35] and Kousedghi et al.[27] in experimental research, suggested 2 weeks; Asgary et al.[36] suggested leaving the triple antibiotic paste for 3 weeks, and Chen et al.[23] have proposed that it be left for 4 weeks. Neelamurthy et al.[37] conducted an *in vivo* study where the canals were treated with TAP in a 1:1:1 ratio and restored with IRM and stated that increase in root development, lateral wall thickness, apical closure, and positive pulpal response was appreciated to the pulp sensibility test in both immature and mature non-vital teeth. In the present study, triple antibiotic paste in a ratio of 1:1:1, which was mixed with saline and placed in the root canal for 3–4 weeks. The findings of the present study showed a reduction in the bacterial count.

Commercially available Propolis powder (Hi-tech Natural Products, India) was used as an intracanal medicament by mixing with saline in a 1.5:1 ratio (wt/vol) to attain a paste-like consistency, which was similar to an *in vitro* study done by Chua et al.[38] in which 95% of propolis (Stakich, Royal Oak, Michigan, USA) was mixed with saline in a ratio of 1.5:1 (wt/vol) to obtain a paste-like substance. In this study, triple antibiotic paste and propolis showed no statistical difference between the samples, which was similar to the previous study done by El-Tayeb et al.[31] A total of three samples were collected by placing paper points into the canals to assess the bacterial count in the present study, i.e., after access opening, after irrigation, and after removal of the medicament similar to the study performed by Sabharwal et al.[39] After that, samples were transferred to Petri plates containing tryptone soya agar with 5% of blood through pour method and were incubated at 37°C for 48 hours. In the present study, propolis exhibited equal antimicrobial activity to TAP when used as an intracanal medicament in necrotized young permanent teeth.

Limitations of the study: A similar study should be conducted in larger samples and different ethnic groups; the amount of the intracanal medicament placed into the root canal was not quantifiable.

Conclusion

The following conclusions can be drawn from the study:

- Intracanal medicament using propolis had a similar impact in reducing the antimicrobial efficacy compared with triple antibiotic paste.
- In young permanent teeth with an open apex, propolis can be used as an intracanal medicament.
- Thus, propolis can be suggested as a safe intracanal medicament along with TAP in immature permanent non-vital teeth.

Ethical Approval

The institutional human ethical committee (SVSIDS/PEDO/3/2017) at Sri Venkata Sai Institute of Dental Sciences, Mahabubnagar, Telangana.

Informed Consent

All the study participants provided their informed consent.

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