ORIGINAL RESEARCH

Comparative Evaluation of Antimicrobial Efficacy of Triple Antibiotic Paste Herbal Combination and Camphorated Monochlorophenol as Intracanal Medicaments against *Enterococcus faecalis* in Deciduous Molars: An *In Vivo* Study

Mayuri M Tawde[1], Laxmi Lakade[2], Smita Patil[3], Amol Kamble[4], Alok Patel[5], Shweta S Jajoo[6]

Abstract

Aim: Compare the efficacy of triple antibiotic paste (TAP), herbal extracts, and camphorated monochlorophenol (CMCP) as intracanal medicaments against *Enterococcus faecalis* (*E. faecalis*) in deciduous molars.

Materials and methods: A total of 60 samples were collected from canals of first and second molars of 4–10-year-old children, with more than two-thirds root length, and fitting the inclusion criteria. Samples were collected at three intervals—S1 was collected just after access opening, S2 was collected after biomechanical preparation (BMP) and irrigation, and just before placement of medicament. Randomization was done to place the medicaments into three groups: group I—CMCP, group II—TAP, and group III—herbal combination. Sample S3 was taken 48 hours after removal of medicament from the canals. The collected samples were transported *via* Amies media to the laboratory, where they were anaerobically incubated for 24 hours. Growth of *E. Faecalis* was observed, and manual counting of the colony-forming unit (CFU) was done. The change in CFU in all samples was calculated, and the results were statistically analyzed.

Results: The results show that there is a change from S1 (TAP = 118.67 ± 122.48, herbal = 109.07 ± 106.43; CMCP = 110.73 ± 120.53) to S2 (TAP = 34.13 ± 63.47; herbal = 27.67 ± 39.39; CMCP = 16.40 ± 26.32) and S3 (TAP = 12.33 ± 24.82; herbal = 4.73 ± 12.78; CMCP = 3.40 ± 7.12). It is seen that there is a significant difference seen from S1 to S2 in all three groups ($p \leq 0.05$) using repeated measure analysis of variance (ANOVA) test. This shows that all three medicaments were effective in reducing bacterial counts of *E. Faecalis* from sample S1 (pre) to S3 (post) significantly after exposure to root canal bacterial flora for 48 hours (2 days). The pairwise comparison of the change in CFU within each group, S1–S3, also shows significant changes. There is a significant decrease in CFU seen from S1 to S2 and S1 to S3 but not from S2 to S3 for all three groups, which was evaluated using the *post hoc* Bonferroni test. It was also observed that in between the canals, although there was a change from S1 to S3 in terms of the CFU, there was no significant difference in the decrease in the bacterial count when intercanal comparison was made. There was, however, a change that was seen to be significant when values from each canal were compared from S1 to S3.

Conclusion: All three medicaments have successfully shown a decrease in the numbers of *E. faecalis*, which the study aimed at checking. Although the effect varied intergroups, it was mild, so herbal alternatives could be used instead of antibiotics and CMCP. Also, because the local application is effective in controlling interappointment flare-ups, the medicaments can be successfully given without having to prescribe systemic antibiotics.

Keywords: Aloe vera, Camphorated monochlorophenol, Cucurmin oil, *Enterococcus faecalis*, Herbal combination, Intracanal medicaments, Intracanal medicaments in deciduous molars, Ocimum sanctum oil, Triple antibiotic paste, Tulsi oil, Turmeric oil.

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Introduction

Dental caries, an infectious process that involves the breakdown of tooth enamel, is a multifactorial disease caused by a complex interaction between cariogenic acid-producing bacteria in combination with fermentable carbohydrates and other dietary, genetic, behavioral, social, and cultural factors.[1–3] Dental caries is a progressive and polymicrobial disease involving enamel, dentin, and eventually the vascular tissue pulp. The inflammation of the pulp involves endodontic intervention as a treatment, which aims at decreasing and eliminating microbial flora with chemical and mechanical measures in single or multiple visits. Root canal flora consists of obligatory anaerobes, particularly black-pigmented gram-negatives, which produce signs and symptoms; aerobes and facultative anaerobes, such as *Enterococcus*, *Candida*, and alpha *Streptococcus*, though in low proportion, are considered

[1-6]Department of Pediatric and Preventive Dentistry, Bharati Vidyapeeth Dental College and Hospital, Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra, India

Corresponding Author: Mayuri M Tawde, Department of Pediatric and Preventive Dentistry, Bharati Vidyapeeth Dental College and Hospital, Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra, India, Phone: +91 9820196115, e-mail: mayuritawde93@gmail.com

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one of the most resistant species and possible cause of root canal treatment failures.[4] If the root canal is not dressed properly with antiseptic medicaments between the visits, the residual bacteria may increase and cause interappointment flare-ups, for which the use of intracanal medicaments is primarily indicated. The most commonly used medicaments are calcium hydroxide [$Ca(OH)_2$], chlorhexidine (CHX) 2%, either alone or in combination with $Ca(OH)_2$, triple antibiotic paste (TAP) have been experimented in primary necrotic pulp canals with different degrees of success. However, there is a paucity of literature related to the efficacy of various combinations of medications used in primary teeth. The use of $Ca(OH)_2$ in primary teeth is debated because the response to its medicament is osteoclastic resorption of the roots.[5] Apart from these, other materials used as intracanal medicaments are TAP, double antibiotic paste, herbal oils, and extracts of *Azadirachta indica*, *Ocimum sanctum*, *Curcuma longa*, propolis, and many others.

Chemomechanical removal of pulp from roots of primary teeth with more focus on disinfection of canals using intracanal medicaments plays an important role in primary teeth owing to the tortuous nature of the root canals, where only biomechanical preparation (BMP) and irrigation is insufficient to remove pulp from the root canals. The reason for carrying out the current research is to check whether the herbal alternatives could be locally equally effective in decreasing bacterial flora because of the growing concerns about the injudicious use of antibiotics in children. The use of CMCP and TAP is present in the literature as an effective intracanal medicament. The herbal counterparts in the form of essential oil as intracanal medicaments are fairly recent, and their ability to eradicate bacterial flora in root canals has shown promising results when compared to their counterparts.

Aim

The aim of the present study is to assess the antimicrobial efficacy of TAP, CMCP, and an herbal combination [tulsi, turmeric essential oil, and aloe vera) against *Enterococcus faecalis* (*E. faecalis*)].

Materials and Methods

After approval from the research and ethical committee, on the suggestion of the ethical committee, an animal study was carried out to check the safety of all materials to be used.

Methodology in Sample Collection in Patients

A total of 60 samples were taken from 4 to 10-year-old children who were selected from the Outpatient Department of Pediatric and Preventive Dentistry, Bharati Vidyapeeth Dental College and Hospital, Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra, India.

Samples were taken from patients who fulfilled the following selection criteria. Canals of deciduous first and second maxillary and mandibular molars with chronic irreversible pulpitis, restorable teeth, and caries involving enamel dentin and pulp with at least two-thirds of root length seen radiographically. The exclusion criteria consisted of teeth showing excess bone resorption, medically compromised and children with a history of drug allergies.

All potential participants explained the treatment that was indicated, that is, pulpectomy, after a thorough clinical and radiographic examination, with a full history of the associated tooth and the requirement for the use of intracanal medicaments. A detailed information sheet was provided for the patients and the parents to read regarding the procedure to be conducted. A preformulated consent and assent form was signed by the parents and the patient.

Study Methodology

A randomized control design was selected for the study. Samples were collected from all canals meeting the inclusion criteria. Each tooth was randomly allocated to one of the three medicament groups.

- Group I: Triple antibiotic paste (TAP).
- Group II: Herbal combination (tulsi oil, turmeric oil, and aloe vera gel).
- Group III: Camphorated monochlorophenol (CMCP).

Materials Used in the Study

- For pulpectomy procedure: Rubber dam isolation, local anesthesia, diamond burs, high-speed aerator handpiece, 5% hypochlorite solution, normal saline, 5 mL syringe, and needle, 21 mm K files (15–25), 21 mm short barbed broach (#1, #2, and #3) and suction (Figs 1 and 2).
- Collection of samples: Sterilized paper points and amies transport media.
- For medicaments: Triple antibiotic paste (TAP) with propylene glycol, essential oil of ocimum sanctum, curcuma longa, aloe vera gel, CMCP (Fig. 3).

Method of Sample Collection and Patient Preparation

For pulpectomy, local anesthesia (1.8–2 mL) was administered to the patient. The rubber dam was then applied following the selection of an appropriate clamp size. Using a high-speed aerator and suction, the access opening was carried out. Coronal pulp was removed using a spoon excavator until the orifices of the canals were visible. Sterile 10 K 21 mm files were inserted into each canal separately to ensure the patency of the canal. After this, sterile paper points were inserted into each canal using a sterile tweezer and removed after 60 seconds (Fig. 4). This collected sample was the baseline or S1 sample. Following this, they were transferred to a sterile transport media (Amies medium) (Figs 5 and 6) and stored under appropriate conditions till they were processed. Baseline samples were collected individually from each canal of the concerned tooth and stored in separate transport tubes. Each canal was treated as an independent sample, as the variations in microflora are also seen to be different in individual canals.

Post this, the removal of necrotic pulp was carried out using barbed broaches, K files, and H files till 25 K files were up to the estimated working length. Irrigation protocols include the use of saline and 5% hypochlorite, alternatively using a 26 gauge needle and syringe. The second sample (S2) was taken just before placing the medicament in the canals. The canals were dried, and the prepared medicaments were placed using a reamer, which thoroughly coated the canal with the medicament.

Medicament Preparation

Group I, that is, CMCP, is available as a volatile liquid in an amber-colored bottle, which is to be dispensed on a sterile cotton pellet and placed in the pulp chamber (Fig. 7).

Group II, that is, TAP, has three components, namely, ciprofloxacin 500 mg, metronidazole 400 mg, and minocycline

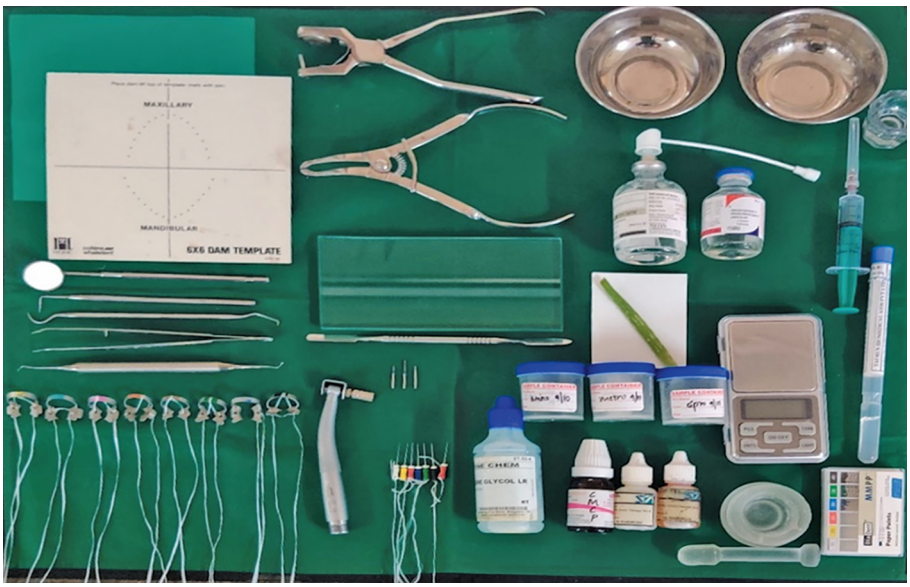


Fig. 1: Armamentarium required for carrying out the research project

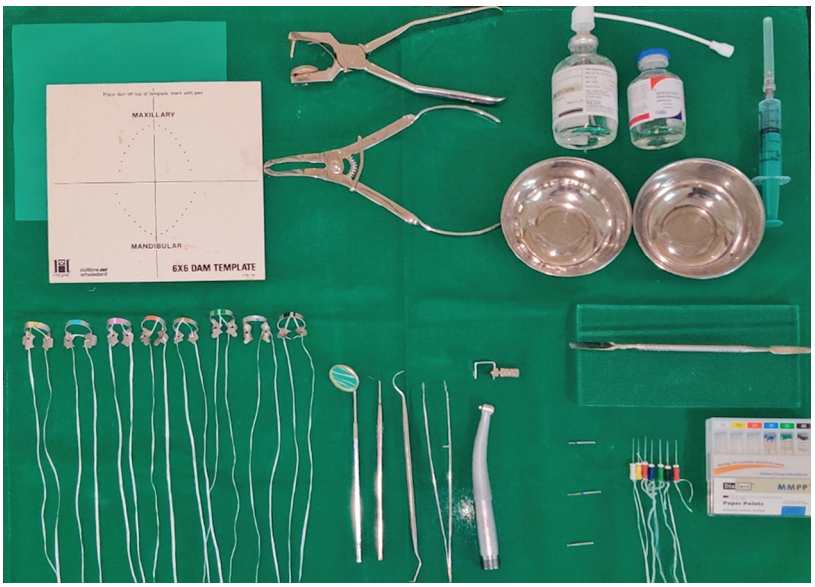


Fig. 2: Armamentarium for endodontic restoration and rubber dam equipment for isolation

Fig. 3: Materials and medicaments of all three groups

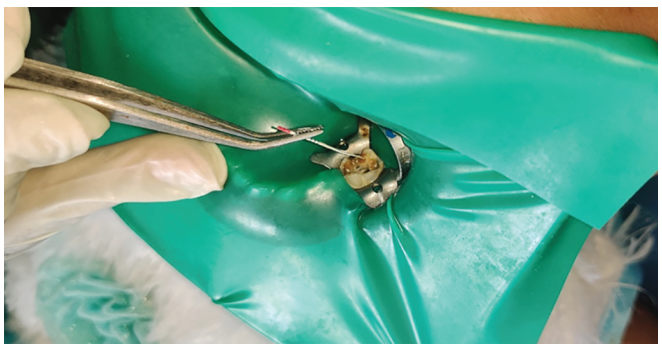


Fig. 4: Collection of samples from root canals by placing the paper point in the canals for 60 seconds

Fig. 5: Amies media for transport of collected paper points to the laboratory

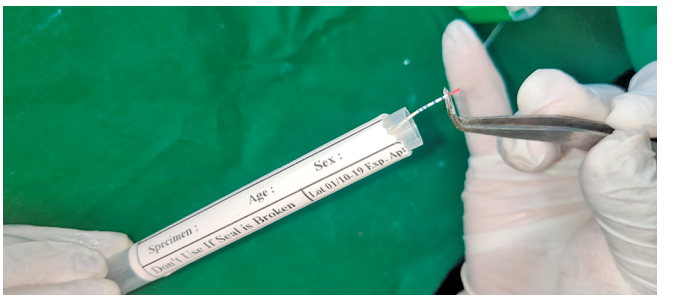


Fig. 6: Collected sample on paper point transferred to sterile Amies media for transport to laboratory

Figs 7A and B: (A) Camphorated monochlorophenol (CMCP) in an amber-colored bottle; (B) Dispensing of volatile CMCP on a cotton pellet for intracanal dressing

Fig. 8: Commercially available tablets used for TAP preparation—ciprofloxacin 500 mg, metronidazole 400 mg, minocycline 50 mg

Fig. 9: Trituration of these tablets in mortar pestle

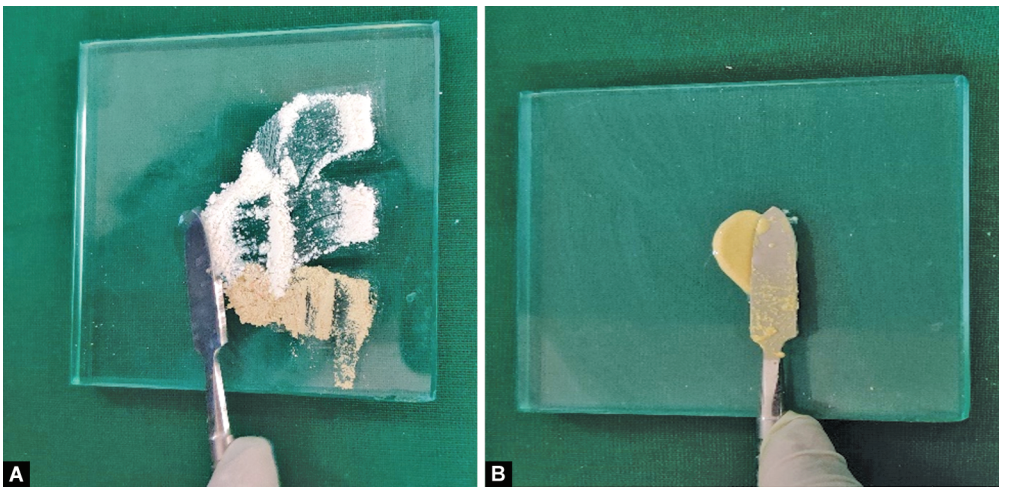
50 mg (Fig. 8). The enteric coatings are removed using a sterile handpiece and burs and individually ground into fine powder using a mortar and pestle (Fig. 9), preweighed and stored in airtight, sterile containers (Fig. 10). Propylene glycol is added to mixture of 1:1:1 of each component by weight to prepare a paste (Fig. 11).

Group III, that is, the herbal combination, was prepared using three components (Fig. 12)—essential oil of *Ocimum sanctum*, *Curcuma longa*, and aloe vera gel (freshly extracted) and mixed in a proportion of 1:1:1. Fresh aloe vera leaf was taken (Fig. 13), cut using sharp scissors, fresh gel is extracted and one portion in a scoop was taken on a glass slab (Fig. 14). One drop each of essential oil of tulsi and turmeric were dispensed on to the glass slab (Figs 15 and 16)

All three were thoroughly mixed to form a fine paste using a cement spatula. The selected medicaments were delivered into the canal using a K-reamer, 2 mm short of working length, to ensure it did not enter the periapical area. Temporary restoration (zinc oxide eugenol) was placed into the cavity, and the tooth was sealed from the influences of the oral cavity. The patient was recalled after a follow-up period of 2 days (48 hours). Sample S3, the last sample,

Fig. 10: Propylene glycol and triturated TAP powders stored in airtight containers

Fig. 12: Materials of the herbal group—essential oil of tulsi (holy basil), turmeric, and fresh aloe vera leaf



Figs 11A and B: (A) Mixing of TAP on a sterile glass slab; (B) Mixed TAP on a sterile glass slab

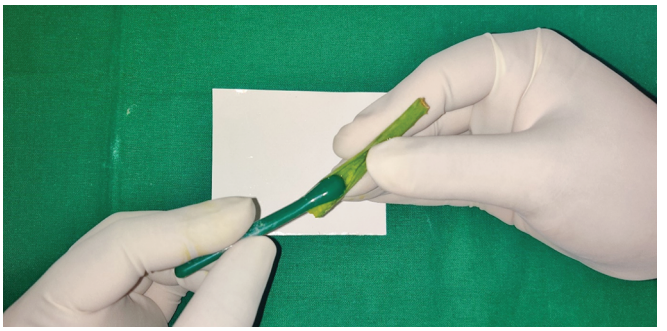


Fig. 13: Removal of fresh aloe vera gel

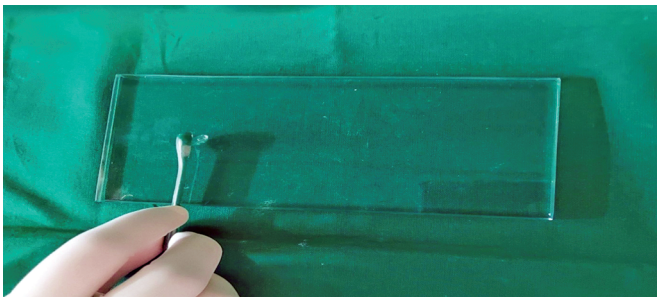


Fig. 14: Placing aloe vera gel on the glass slab for medicament preparation

was taken after 48 hours in the same manner. If the symptoms had resolved, the tooth was obturated using an appropriate obturating material.

Samples were processed in microbiological laboratories by rinsing the transport media tube containing the paper point sample with 1 mL of sterile normal saline and plating 0.1 mL (dilution factor of 10^1 of the resultant solution on a Bile Esculin Agar plate using the pour plate method. Plates were anaerobically incubated at 37°C in an anerobic Gas Pak Jar, and growth of *E. faecalis* was observed after 48 hours (Fig. 17). Results were calculated on the basis of manual colony counting of the units (Fig. 18).

Discussion

The invasion of bacteria from enamel to dentin and eventually the pulp leads to pulpal inflammation, the treatment for which is indicated in primary teeth is pulpectomy or pulpotomy, depending on the extent of pulpal involvement. The reduction of the number of microorganisms can be achieved by root canal preparation,

Fig. 15: Dispensing of tulsi oil onto the glass slab

Fig. 16: Dispensing of turmeric oil onto the glass slab

Fig. 17: Bile esculin agar plate being prepared in the laboratory

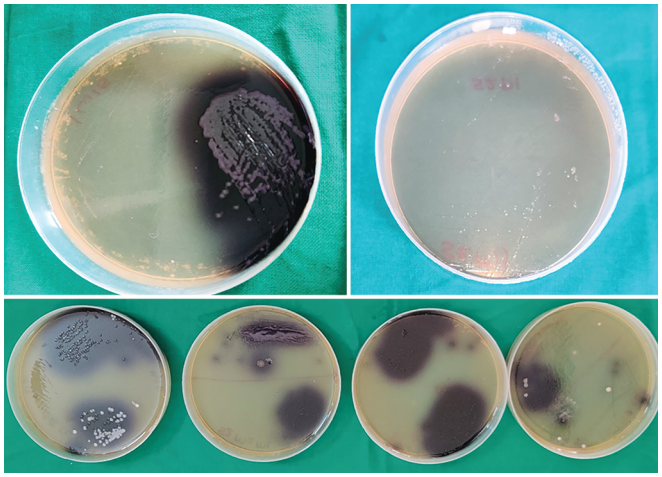


Fig. 18: Growth of *E. faecalis* seen on bile esculin agar of various samples

irrigation, and administration of sterilizing medications and root canal filling materials. The administration of irrigating agents and drugs to the root canal plays an important role in decreasing the amount of infected tissue and eliminating the microorganisms from the root canal.[6] *E. faecalis*, a gram-positive, facultatively anerobic cocci, is a commensal of the gastrointestinal tract and is found in the oral cavity in root canal-treated and retreated cases. Molander et al.[7] found *E. faecalis* to be present in 30–90% cases. *E. faecalis* is one of the most resistant microorganisms in the root canal systems[8–10] and is the most prevalent bacterial strain isolated from teeth with endodontic treatment failure. The presence of *E. faecalis* in primary teeth has been corroborated by studies conducted by various authors like Silva et al.,[10] Cancio et al.,[11] Siqueira JF et al.[12] Ledezma-Rasillo et al.,[13] Ruviere et al.,[14] where predominantly facultative anaerobic bacteria are seen to be associated with infections in primary teeth.

The rationale for the application of medicament is to maintain asepsis or prevent microbial recolonization in the root canals, for which the irrigating solutions and medicaments need to be antibacterial in action to prevent the microorganisms from multiplying in the canals. The injudicious use of antibiotics in dentistry is very well known, and their action in the healing of pulpal infection is through a systemic route, whereas the action of intracanal medications acts more *via* a local route. The duration of action of intracanal medicaments has been shown to vary from a minimum of 24 hours to a few weeks for an effective antimicrobial action. Studies conducted by Sjögren in 1991[15] have shown that $Ca(OH)_2$ applied for 7 days in the root canal exerts action effectively for up to 5 weeks, and Bystrom in 1985[16] confirmed that even at 1 month of treatment, the efficacy of $Ca(OH)_2$ is good, requiring at least 1 day to exert effect. Not only $Ca(OH)_2$ but also herbal medicaments have been under research as intracanal medicaments in primary teeth. Various studies have been conducted to test the efficacy of materials used as intracanal medicaments specifically against *E. faecalis* by Oncag Gogulu [$Ca(OH)_2$ and 1% CHX], Ramamurthy [aloe vera, *Ricinus communis*, $Ca(OH)_2$], Maryam (propolis, aloe vera), Abbas Zegden [aloe vera, *Zataria multiflora*, $Ca(OH)_2$], Chandrashekar [neem, aloe vera, CHX, $Ca(OH)_2$], and Datta Prasad [neem, aloe vera leaf, sodium hypochlorite (NaOCl), 2% CHX]. Studies have shown that extracts from various herbal alternatives have a significant bacteriostatic action against many aerobic and anaerobic bacteria.[17–22]

In the current study, three material groups are compared, namely, TAP, CMCP, and an herbal combination, as intracanal medicaments in primary teeth. As pulp therapy in primary teeth focuses more on chemomechanical ways of disinfecting the canals, intracanal medicaments play a vital role in multivisit pulpectomy to decrease root canal flora.

Going through systemic antibiotic therapy depends on so many factors, including the patient's compliance in taking a specific dosage regimen, the absorption of these drugs by the gastrointestinal system, the transportation *via* the blood circulatory system in order to get to the infected area which implies the medication-required area having a proper blood supply which is no longer available in teeth with necrotic pulps, a pulpless and infected canal or a root-filled tooth that has become infected. As a result, local application of antibiotics within the canal may be a more effective mode for delivering the drug.

Triple antibiotic paste (TAP) has been used for a long time as an intracanal medicament in nonhealing periapical lesions, chronically affected teeth, lesion sterilization and tissue repair therapy, and regenerative endodontics. Although systemic antibiotic therapy has proven useful in dental surgical and nonsurgical procedures, it also comes with some complications, such as various side effects (allergic reactions or toxicities) and the development of resistant strains of microbes.[23–25]

Considering the polymicrobial nature of tooth infection, single empirical antibiotics are not able to provide a bacteria-free zone in the canal. Tetracycline, including tetracycline hydrochloride, minocycline, demeclocycline, and doxycycline, are a group of broad-spectrum antibiotics effective against a wide range of microorganisms. Tetracyclines possess various unique properties except their antimicrobial action, including the inhibition of mammalian collagenases, which prevent tissue degeneration, and the inhibition of clastic cells, which result in antiresorptive activities. The newer tetracyclines like doxycycline and minocycline have better absorption, greater antimicrobial action,[26,27] lipophilic with better tissue penetration, and it has been seen to chelate calcium to a lesser extent than the older tetracyclines, putting the developing dentition at a lesser risk for staining.

Metronidazole is a nitroimidazole compound that exhibits a broad-spectrum of activities against protozoa and anerobic bacteria. Since it is famous for its effective antimicrobial activities against anerobic cocci as well as gram-negative and gram-positive bacilli, it has been used widely in both systemic and local forms. Metronidazole destroys bacteria cells by permeating their membrane and then binding to the DNA, disrupting the helix structure and causing a very rapid death. It has been shown that metronidazole is effective against anaerobic bacteria but not in aerobic bacteria, and it prevents the growth of all obligate anaerobes tested.[28,29]

Ciprofloxacin and other fluoroquinolones are being used for this wide broad-spectrum of activities since they target DNA gyrase (gram-negative bacteria) and topoisomerase (gram-positive bacteria), their availability in both oral and intravenous formulations, and their excellent tissue penetration.[30] It also has excellent activity against Enterobacteriaceae.

The herbal combination used in the study consists of naturally occurring *Ocimum sanctum*, *Curcuma longa*, and aloe vera, which, although they have been used individually, the use of combination has been done in very few studies.

The herbal combination used in the study consists of naturally occurring *Ocimum sanctum*, *Curcuma longa*, and aloe vera, which, although they have been used individually, the use of combination has been done in very few studies. Essential oil of tulsi leaves have significant inhibitory effects against *E. coli*, *Bacillus subtilis* (*B. subtilis*), *B. anthracis*, *Staphylococcus aureus* (*S. aureus*), *Pseudomonas vulgaris* (*P. vulgaris*), and *P. aeruginosa*, etc. These essential oils include major constituents of leaves such as caryophyllene, eugenol, and methyl eugenol, which are effective against even the most resistant bacteria. Grover and Rao[31] in 1977 stated that eugenol is the most therapeutically effective constituent of tulsi. The essential oil has activity against both gram-positive and gram-negative bacteria. For enteric pathogens, aqueous extract and alcoholic extract are beneficial, while tulsi seed oil yields considerable antimicrobial properties.[32]

Aloe vera (*Aloe barbadensis* Miller) is a kind of plant that is well known for its numerous biological and therapeutic functions, such as wound healing, hypoglycemic effects, anti-inflammatory and immunomodulation features, and also antimicrobial properties. It has been proven in several studies that aloe vera shows considerable antimicrobial activity against various species, such as *S. pyogenes*, *E. faecalis*, *Candida albicans*, and *S. aureus*.[6]

Curcuma longa, or turmeric oil, is also known to have a good antimicrobial and anti-inflammatory spectrum. Curcumin achieved 100% killing of bacteria, albeit at different time intervals. Turmeric (curcumin) is able to eliminate the Extracellular Polymeric Substances (EPS) matrix and the bacteria.

The CMCP is the oldest known intracanal medicament given interappointment to manage the growth of microorganisms. Various authors like Menezes et al. and others have conducted studies on CMCP and found it to be an effective agent against root canal pathogens.[33]

In the current study, following the sample collection protocol as followed by Pratibha Ahirwar et al.,[34] sample collection was done at three intervals for groups I, II, and III, that is, at access opening, postirrigation, and the third sample was taken 48 hours after placement of the medicament.

This was more or less based on sample collection as described by Cohen for studies of bacteria occurring in the root canal after treatment approaches should involve three basic conditions—(1) postinstrumentation samples, (2) postmedication samples, and (3) postobturation samples.

Table 1 lists the values in CFU detected of *E. faecalis* in all samples collected for S1 (baseline), S2 (intermediate), and S3 (post) for the group of TAP. Table 2 lists the values in CFU detected of *E. faecalis* in all samples collected for S1 (baseline), S2 (intermediate), and S3 (post) for the group of herbal intracanal medicament (aloe vera gel, tulsi oil, turmeric oil). Table 3 lists the values in CFU detected of *E. faecalis* in all samples collected for S1 (baseline), S2 (intermediate), and S3 (post) for the group of CMCP.

The results how that there is a change from S1 (TAP = 118.67 ± 122.48, herbal 109.07 ± 106.43; CMCP = 110.73 ± 120.53) to S2 (TAP = 34.13 ± 63.47; herbal = 27.67 ± 39.39; CMCP = 16.40 ± 26.32) and S3 (TAP = 12.33 ± 24.82; herbal = 4.73 ± 12.78; CMCP = 3.40 ± 7.12). It is seen that there is a significant difference seen from S1 to S2 in all three groups ($p \leq 0.05$) using repeated measure analysis of variance (ANOVA) test. (Table 4). This shows that all three medicaments were effective in reducing bacterial counts of *E. faecalis* from sample S1 (pre) to S3 (post) significantly after exposure to root canal bacterial flora for 48 hours (2 days) (Fig. 19). This is similar to the results by studies carried out by Ahirwar et al.,[34] Lele and Subba,[35] Reddy et al.[36] where they had compared the antimicrobial action of individual components like *Ocimum sanctum*, aloe vera, and CMCP with TAP and other intracanal medicaments.

In Table 5, which shows the pairwise comparison of the change in CFU within each group, S1–S3 also shows significant changes. There is a significant decrease in CFU seen from S1 to S2 and S1 to S3 but not from S2 to S3 for all three groups, which was evaluated using the *post hoc* Bonferroni test.

Table 1: The values of CFU from baseline samples (S1), intermediate sample (S2), and postmedicament placement sample (S3) for the group TAP

Sample number	*S1 (CFU)*	*S2 (CFU)*	*S3 (CFU)*
1	13×10^1	Nil	Nil
2	3×10^1	1×10^1	Nil
3	1×10^1	Nil	Nil
4	240×10^1	49×10^1	1×10^1
5	17×10^1	4×10^1	Ni
6	45×10^1	5×10^1	Nil
7	20×10^1	5×10^1	Nil
8	300×10^1	75×10^1	53×10^1
9	300×10^1	235×10^1	81×10^1
10	285×10^1	20×10^1	13×10^1
11	100×10^1	10×10^1	Nil
12	80×10^1	7×10^1	Nil
13	40×10^1	Nil	Nil
14	56×10^1	1×10^1	Nil
15	280×10^1	100×10^1	37×10^1

#, 10^1 is the dilution factor for the samples

Table 2: The values of CFU from baseline samples (S1), intermediate sample (S2), and postmedicament placement sample (S3) for the group herbal (aloe vera gel, tulsi oil, and turmeric oil)

Sample number	*S1 (CFU)*	*S2 (CFU)*	*S3 (CFU)*
1	54×10^1	12×10^1	4×10^1
2	50×10^1	9×10^1	Nil
3	41×10^1	20×10^1	50×10^1
4	78×10^1	17×10^1	5×10^1
5	20×10^1	1×10^1	Nil
6	52×10^1	2×10^1	Nil
7	5×10^1	Nil	Nil
8	46×10^1	6×10^1	Nil
9	206×10^1	51×10^1	Nil
10	285×10^1	52×10^1	8×10^1
11	100×10^1	45×10^1	Nil
12	300×10^1	29×10^1	4×10^1
13	54×10^1	8×10^1	Nil
14	45×10^1	6×10^1	Nil
15	300×10^1	157×10^1	Nil

#, 10^1 is the dilution factor for the samples

Table 3: The values of CFU from baseline samples (S1), intermediate sample (S2), postmedicament placement sample (S3) for the group CMCP

Sample number	*S1 (CFU)*	*S2 (CFU)*	*S3 (CFU)*
1	41×10^1	5×10^1	Nil
2	1×10^1	Nil	Nil
3	51×10^1	8×10^1	1×10^1
4	21×10^1	6×10^1	2×10^1
5	53×10^1	10×10^1	Nil
6	48×10^1	20×10^1	2×10^1
7	1×10^1	2×10^1	3×10^1
8	5×10^1	1×10^1	Nil
9	300×10^1	83×10^1	10×10^1
10	275×10^1	75×10^1	27×10^1
11	300×10^1	23×10^1	Nil
12	300×10^1	5×10^1	Nil
13	157×10^1	1×10^1	Nil
14	80×10^1	6×10^1	Nil
15	28×10^1	1×10^1	6×10^1

#, 10^1 is the dilution factor for the samples

Table 4: Comparison of change in CFU within each group

Medicament	*S1*	*S2*	*S3*	*F-value*	*p-value*
TAP	118.67 ± 122.48	34.13 ± 63.47	12.33 ± 24.82	13.989	0.001*
Herbal	109.07 ± 106.43	27.67 ± 39.99	4.73 ± 12.78	13.483	0.001*
CMCP	110.73 ± 120.53	16.40 ± 26.32	3.40 ± 7.12	12.214	0.003*

Repeated measure ANOVA test; *indicates significant difference at $p \leq 0.05$

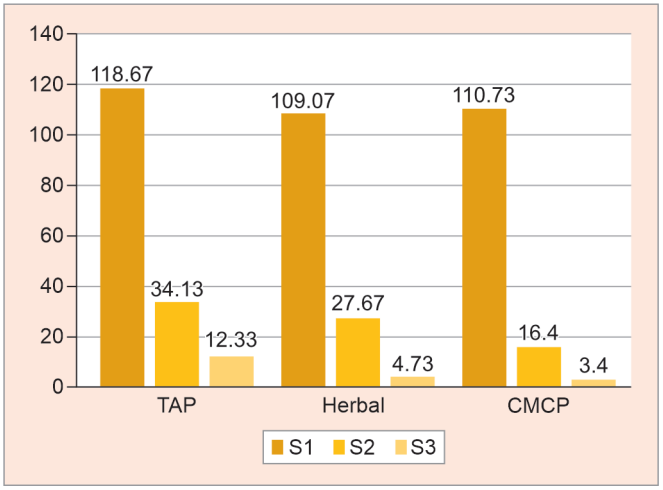


Fig. 19: Graphical representation of the change of CFU within each group

Table 5: Pairwise comparison of the change in CFU within each group

Medicament	*Interval*	*Difference*	*p-value*
TAP	S1–S2	84.53	0.007*
	S1–S3	106.33	0.005*
	S2–S3	21.80	0.177 (NS)
Herbal	S1–S2	81.40	0.005*
	S1–S3	104.33	0.007*
	S2–S3	22.93	0.166 (NS)
CMCP	S1–S2	94.33	0.011*
	S1–S3	107.33	0.010*
	S2–S3	13.00	0.095 (NS)

Post hoc Bonferroni test; *indicates significant difference at $p \leq 0.05$; NS, nonsignificant

Table 6: Intergroup comparison of change in CFU among study groups

Interval	*TAP*	*Herbal*	*CMCP*	*F-value*	*p-value*
S1–S2	84.53 ± 87.42	81.40 ± 80.88	94.33 ± 104.86	0.081	0.922 (NS)
S1–S3	106.33 ± 104.73	104.33 ± 108.26	107.33 ± 117.66	0.003	0.997 (NS)
S2–S3	21.80 ± 41.07	22.93 ± 42.49	13.00 ± 21.10	0.338	0.715 (NS)
% reduction	85.53 ± 14.76	88.61 ± 27.36	95.82 ± 31.55	0.640	0.532 (NS)

One way ANOVA test; NS, nonsignificant; % reduction–total reduction from S1 to S3

One reason for this could be the wide selection criteria of inclusion of teeth in the study were all chronically infected teeth; it included all teeth with a draining sinus as well as the history of previous infection and swelling as an acute exacerbation of a chronic abscess. The CFU varied significantly from individual patients because of the wide selection criteria. The change that was seen to be significant from S1 to S2 could be due to the instrumentation using K, H, and broaches for removal of infected necrotic pulp, along with chemical agents like 5% hypochlorite and NS were used alternatively (for irrigation) to flush out the debrided tissue and for dissolution of the organic debris. NaOCl is the most used irrigation solution in endodontics, and because of its mechanism of action, it causes a biosynthetic alteration in cellular metabolism and phospholipid destruction, a formation of chloramines that interferes in cellular metabolism, an oxidative action with irreversible enzymatic inactivation in bacteria, and a lipid and fatty acid degradation. Chemomechanical methods play a significant role in the reduction of bacterial counts. These results support the current rationale of instrumentation being the mainstay of endodontic treatment, along with irrigation and intracanal medicaments as adjuncts.

The change in S2–S3, although not significant, just reveals that although there has been a reduction in bacterial count from the previous sample (S2), the change is not significant because of the presence of bacteria in the S3 sample. The results indicate that although the medicaments could not achieve complete eradication of the bacteria, they have been successful in reducing counts from the previous sample(S2) and significantly from the baseline sample (S1–S3). The results, hence show that the intracanal medicaments act as an adjunct to the chemomechanical means of debriding the root canals in primary teeth.[36]

Also, interappointment flare-ups are reduced, as no case was reported that they have come with pain, swelling, or abscess after placement of medication in any of the study groups.

Although there was an equal reduction seen from S1 to S3 in all groups (Table 6), which is without a significant difference, the percentage reduction was seen to be greater for CMCP (95.82 ± 31.55) followed by herbal (88.61 ± 27.36) followed by TAP (85.53 ± 14.76). The strong antimicrobial action of CMCP is attributed to camphor oil, which exerts potent antimicrobial activity. The herbal combination being more effective than TAP is in contrast with the study conducted by Ahirwar et al.,[34] where TAP was seen to be more effective in the reduction of bacteria than *Ocimum sanctum*. It is possible that these effects are due to the combination of herbal agents used, which could accentuate each other's efficacy. Individually, all three components of the herbal combination have been proven to have strong antimicrobial action against root canal flora.[35,36] More studies are needed to support the efficacy of the herbal combination.

It was also observed that in between the canals, although there was a change from S1 to S3 in terms of the CFU (Fig. 20), there was no significant difference in the decrease in the bacterial count when intercanal comparison was made (Table 7). There was, however, a change that was seen to be significant when values from each canal were compared from S1 to S3 (Table 8 and Fig. 21). Tables 9 and 10 show the values of S1, S2, and S3 at different intervals in different canals, but no difference in intercanal is encountered. Although there was a variation seen in the baseline values between all the canals, the probable reason could be due to the selection criteria of cases, partially necrosed, or vital pulp in a few canals as compared to the others. Since the selection criteria were such, there is a difference seen in the baseline values (S1) in both intergroup and intergroup, since teeth that are chronically affected, with a draining sinus, intraoral swelling, or might have a partially vital pulp, all were included.

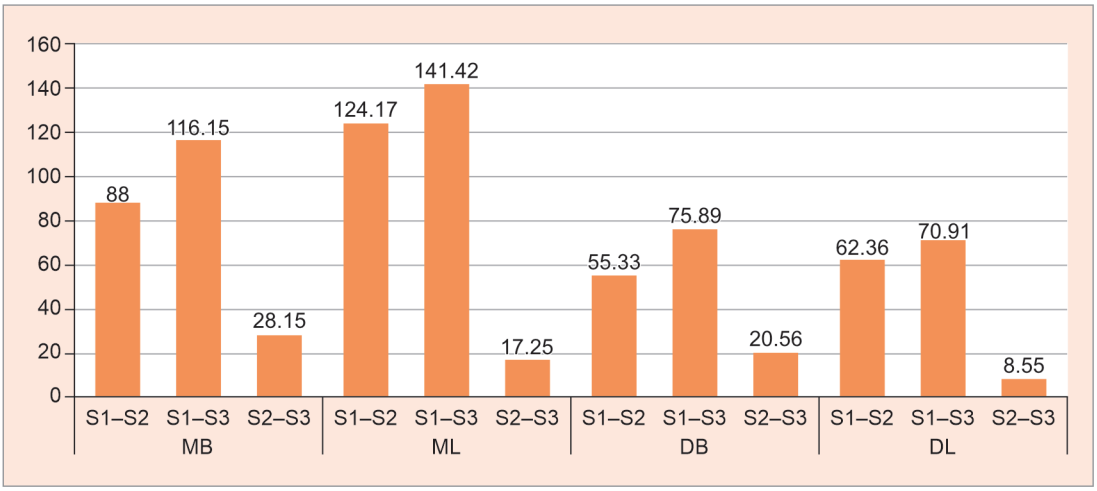


Fig. 20: Pairwise comparison of the change in CFU within each canal

Table 7: Pairwise comparison of the change in CFU within each canal

Canal	*Interval*	*Difference*	*p-value*
MB	S1–S2	88.00	0.007*
	S1–S3	116.15	0.003*
	S2–S3	28.15	0.107 (NS)
ML	S1–S2	124.17	0.010*
	S1–S3	141.42	0.010*
	S2–S3	17.25	0.063 (NS)
DB	S1–S2	55.33	0.070 (NS)
	S1–S3	75.89	0.204 (NS)
	S2–S3	20.56	0.892 (NS)
DL	S1–S2	62.36	0.115 (NS)
	S1–S3	70.91	0.100 (NS)
	S2–S3	8.55	0.216 (NS)

Post hoc Bonferroni test; *, indicates significant difference at $p \leq 0.05$; NS, nonsignificant

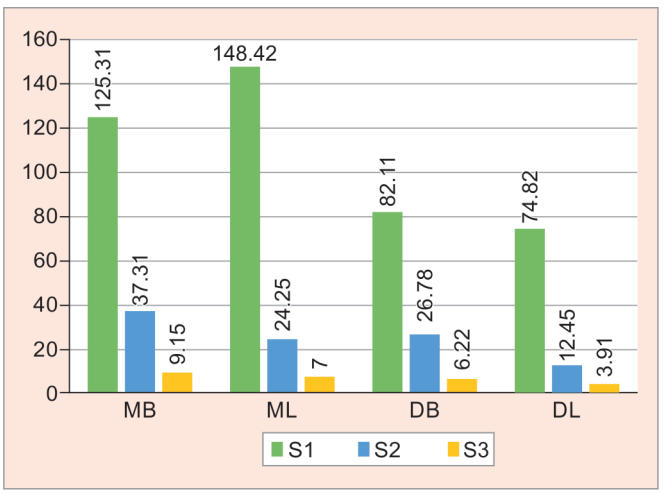


Fig. 21: Comparison of change in CFU within each canal

Table 8: Comparison of change in CFU within each canal

Canal	*S1*	*S2*	*S3*	*F-value*	*p-value*
MB	125.31 ± 110.70	37.31 ± 65.81	9.15 ± 23.88	15.751	0.001*
ML	148.42 ± 137.15	24.25 ± 29.56	7.00 ± 14.86	13.873	0.003*
DB	82.11 ± 103.82	26.78 ± 51.60	6.22 ± 16.54	4.564	0.027*
DL	74.82 ± 103.13	12.45 ± 22.02	3.91 ± 8.57	5.873	0.035*

Repeated measure ANOVA test; *, indicates significant difference at $p \leq 0.05$

Table 9: Intergroup comparison of CFU among different canals at each interval

Interval	*MB*	*ML*	*DB*	*DL*	*F-value*	*p-value*
S1	125.31 ± 110.70	148.42 ± 137.15	82.11 ± 103.82	74.82 ± 103.13	1.034	0.387 (NS)
S2	37.31 ± 65.81	24.25 ± 29.56	26.78 ± 51.60	12.45 ± 22.02	0.579	0.632 (NS)
S3	9.15 ± 23.88	7.00 ± 14.86	6.22 ± 16.54	3.91 ± 8.57	0.187	0.904 (NS)

One-way ANOVA test; NS, Nonsignificant

Table 10: Pairwise comparison of CFU among study groups

Interval	*Pair*	*Difference*	*p-value*
S1	MB vs ML	−23.11	0.959 (NS)
	MB vs DB	43.20	0.824 (NS)
	MB vs DL	50.49	0.711 (NS)
	ML vs DB	66.31	0.567 (NS)
	ML vs DL	73.60	0.431 (NS)
	DB vs DL	7.29	0.999 (NS)
S2	MB vs ML	13.06	0.894 (NS)
	MB vs DB	10.53	0.953 (NS)
	MB vs DL	24.85	0.561 (NS)
	ML vs DB	−2.53	0.999 (NS)
	ML vs DL	11.80	0.928 (NS)
	DB vs DL	14.32	0.901 (NS)
S3	MB vs ML	2.15	0.989 (NS)
	MB vs DB	2.93	0.979 (NS)
	MB vs DL	5.25	0.879 (NS)
	ML vs DB	0.78	1.000 (NS)
	ML vs DL	3.09	0.973 (NS)
	DB vs DL	2.31	0.991 (NS)

Post hoc Tukey test; NS, nonsignificant

All three medicaments have successfully shown a decrease in the numbers of *E. faecalis*, which the study aimed at checking. Although the effect varied intergroups, it was mild, so herbal alternatives could be used instead of antibiotics and CMCP. Also, because the local application is effective in controlling interappointment flare-ups, the medicaments can be successfully given without having to prescribe systemic antibiotics.

The CMCP has recently been advocated to be potentially cytotoxic on long-term use; therefore, it can be substituted with the herbal counterparts, which subsequently have lesser side effects. The advantage of carrying out this study is that since the animal study was conducted, all the materials used are safe for use in the root canals of teeth. In addition to this, there is no direct contact with the periapical tissues; although there might be some leakage of materials in the periapical space, no adverse reaction, either local or systemic, is seen. There were no side effects or allergic reactions in the subjects who were enrolled in the study. Very few *in vivo* studies have been done on intracanal medicaments, specifically in primary teeth, and comparing this combination of medicaments.

The current study had a sample size of 45 canals to test the three groups of medicaments. The results showed the eradication of bacteria, but more studies with a greater sample size should be carried out *in vivo* so that the efficacy of herbal alternatives could be tested and validated. Since the bacteria in root canals of primary teeth are polymicrobial, the efficacy of the materials used in the study could also be extended to check if they are equally efficacious in inhibiting other bacteria.

In accordance with the results of the study, where three intracanal medicaments were tested, they hinted toward an equal efficacy of all three intracanal medicaments. There are very few studies that have been conducted that have determined the presence of *E. faecalis* in primary teeth, but its presence as a root canal pathogen is well established in primary teeth. Its presence in permanent teeth has been well established and is responsible for the failure of root canal procedures. Although the counts of *E. faecalis* are lower in primary teeth than in permanent teeth, its presence has been noted in primary teeth. In the current study, a selective media, that is, Bile Esculin Agar, was taken, which ensure selective growth of *E. faecalis*, and also as a differentiating media. *E. faecalis* was found in all samples of S1, the initial sample, many samples of S2 (postirrigation and BMP), and some samples of S3. The complete eradication of *E. faecalis* was not possible for all cases, using different medicaments, owing to the fact that the degree of microbial infection varied from case to case and also the fact that elimination of *E. faecalis* is quite difficult and is known to be a persistent root canal pathogen. Considering all the above factors, all three medicaments used in the research have been shown to be effective in decreasing the counts of *E. faecalis*, which is noteworthy considering all three are distinct from each other, one being a phenolic compound, the other an antibiotic combination and third being a herbal combination.

In chronic cases, unless there is significant extraoral swelling, antibiotic medications are not advocated. This could be avoided if the local placement of an intracanal medication could be done to prevent interappointment in cases of multivisit pulpectomy. Since the results have shown that the herbal combination of aloe vera gel along with tulsi and turmeric oil has shown similar effects to TAP and CMCP, it could effectively used as an alternative agent as an intracanal medicament. It will greatly reduce the cost of treatment and can also help prevent the injudicious use of antibiotics, which is a cause of concern today. Since the use of CMCP raises concerns about its long-term dose-related effects with use, the herbal could be used as an effective alternative. More studies should be carried out to explore the potential of intracanal medicaments for local application instead of administering systemic antibiotics. Also, incorporating herbal extracts in treatment for routine dental procedures should be considered, keeping in mind their safety spectrum and minimal side effects.

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