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ORIGINAL RESEARCH

Assessment of Antimicrobial Activity of Different Intracanal Medicaments against *Enterococcus faecalis* and *Candida albicans*: An *In Vitro* Study

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ABSTRACT

Aim: The aim of the present study was to compare the antimicrobial efficacy of various intracanal medicaments against *Enterococcus faecalis (E. faecalis)* and *Candida albicans (C. albicans).*

Materials and methods: Sixty single rooted, non-carious, permanent mandibular premolars with no developmental defects that were previously extracted for the orthodontic purpose were included in this study. *E. faecalis* and *C. albicans* strains were cultured on brain heart infusion (BHI) and sabouraud dextrose (SD) agar plates respectively. Sixty specimens were divided into two main groups and three subgroups with 10 teeth receiving a medicament; group I: *E. faecalis* (Subgroup Ia: BioPure MTAD, Subgroup Ib: propolis, Subgroup Ic: Triple antibiotic paste), group II: *C. albicans* (Subgroup IIa: BioPure MTAD, Subgroup IIb: propolis, Subgroup IIc: Triple antibiotic paste). The antimicrobial activity of the intracanal medicaments against E. faecalis and C. albicans were assessed at the end of 2nd and 7th day. The mean zones of inhibition were analyzed with a one-way ANOVA test.

Results: After 2 days, the mean zone of inhibition of *E. faecalis* and *C. albicans* was maximum for triple antibiotic paste (24.74 ± 0.622 mm, 28.22 ± 0.489 mm), followed by BioPure MTAD (19.58 ± 1.734 mm, 24.75 ± 0.954 mm) and propolis (13.10 ± 0.278 mm, 17.96 ± 0.163 mm). Similarly, the mean zone of inhibition of *E. faecalis* and *C. albicans* was maximum for triple antibiotic paste (26.86 ± 0.112 mm, 32.10 ± 0.908 mm), followed by BioPure MTAD (20.13 ± 1.842 mm, 27.22 ± 1.977) and propolis (14.11 ± 0.101 mm, 19.90 ± 0.742 mm) after 7 days. Statistically significant differences (p <0.0001) were found between the groups.

Conclusion: Present study concluded that the antimicrobial effectiveness of triple antibiotic paste was significantly more than BioPure MTAD and propolis against *E. faecalis* and *C. albicans* at the end of 2nd and 7th day.

Clinical significance: An endodontic treatment is considered successful only when the root canals are completely eradicated of microorganisms. As the morphology of the root canals is complex, mechanical instrumentation alone cannot completely debride them. Thus, the role of intracanal medicaments in complementing the mechanical instrumentation becomes important.

Keywords: *Candida albicans, Enterococcus faecalis,* Intracanal medicaments, Zone of inhibition.

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INTRODUCTION

The chief causative factors of pulpal and periapical inflammation are the microorganisms. Numerous studies have

shown that the elimination or reduction of microorganisms from the root canal system results in higher healing rates of periapical lesions. The main objective of root canal treatment is to remove or reduce the number of microorganisms residing within the infected root canal.[1]

A broad range of bacteria causes endodontic infections. Both strict and facultative anaerobes have been isolated from the root canals in endodontic cases with exacerbations and post-treatment disease. For the total eradication of bacteria from the root canals, intracanal placement of an effective antibacterial substance over a definite period of time is essential.[2] However, it is not easy to completely eradicate the bacteria. To ensure complete elimination of disease-causing bacteria in a predictable manner, the intracanal medicaments should remain in the root canals as inter-appointment medicaments and be able to pass through the dental tissues even in the presence of microorganisms to adequately reach a high concentration.[3]

A microorganism that is most commonly found in unsuccessful endodontic cases and exacerbations is E. faecalis. It has the ability to persist within the root canals as a lone organism without the support of other bacteria and can penetrate and survive within the dentinal tubules because of its small size.[4]

In addition to bacteria, fungi can also be involved in root canal infections. *C. albicans* is the most commonly isolated fungal species from the failed root canal-treated teeth. They possess collagen-degrading activity and thus, use dentin as a source of nutrition, and thus promote colonization in the root canal.[5]

The success of endodontic treatment depends upon the elimination or reduction of the microorganisms from the necrotic pulp. Although mechanical instrumentation provides effective cleaning of the root canals, it does not ensure the total removal of microorganisms from the root canal system.[6] The viable bacteria left in the root canal may proliferate between appointments or after obturation, often attaining a pathogenic level and result in failure.[7] Hence, it is important to disinfect the root canal system before obturation with a suitable medicament to promote healing and avoid recurrence. The present study assessed the antimicrobial efficacy of different intracanal medicaments against *E. faecalis* and *C. albicans.*

MATERIALS AND METHODS

This study was an in vitro study conducted in the Department of Conservative Dentistry and Endodontics, Educare Institute of Dental Sciences, Kerala.

Preparation of Sample

Sixty single rooted, non-carious, mandibular permanent premolars that had no developmental defects and were extracted for the orthodontic purpose were selected. The crowns of the collected teeth were separated from the roots at the CEJ with a diamond disc under saline irrigation. The inner diameter of the root canal was prepared and standardized using Gates Glidden (GG) drill, number 3 (Mani Inc., Japan). The working length was measured 1 mm shorter than the tip of the file that was visible at the apical foramen. The roots were cut to have a working length as 10 mm, so as to standardize the procedure. The canals were washed with 17% ethylene diamine tetraacetic acid (EDTA) for 5 minutes, followed by 5.25% sodium hypochlorite for another 5 minutes to remove the tooth shavings. The teeth were then dipped in distilled water for 5 minutes to flush out the irrigants. After drying, the teeth were autoclaved at 121° C for two cycles. The methodology followed in the present study was similar to the one that was used by Krithikadatta et al.[8]

Bacterial Strains and Media

Out of the 60 teeth, one half (n = 30) were immersed in 1 ml of brain heart infusion (BHI) broth, and the other half (n = 30) were immersed in Sabouraud's dextrose (SD) agar broth in individual microcentrifuge tubes. *E. faecalis* [American type culture collection (ATCC 29312)] and *C. albicans* (ATCC 90042) were subcultured on BHI and SD agar plates, respectively, and incubated aerobically at 37° C overnight. The organisms were also transferred into sterile test tubes containing sterile 1.5 mL BHI broth and SD agar. The test tubes were incubated at 37° C for 24 hours to cultivate a moderately turbid bacterial suspension. The bacterial suspension density was standardized and compared to 0.5 McFarland units of the barium-sulfate standard which is equivalent to 10^8 Colony Forming Units per milliliter (CFU/mL).

All the samples were placed in closed Eppendorf tubes and incubated at 37° C for 14 days. The canals were re-inoculated with fresh bacterial samples every third day. The levels of inoculation within the root canal were confirmed by streaking onto sterile BHI and SD agar plates.

Placement of Intracanal Medicaments

After 14 days, the canals were cleared of their contents and rinsed with 5 mL saline and dried. Sixty specimens were divided into two groups and further subdivided into three subgroups (n = 10) that received different medicaments.

Group I: *Enterococcus faecalis*

- *Subgroup Ia:* BioPure MTAD (Dentsply Tulsa Dental, Johnson City, TN)
- *Subgroup Ib:* Propolis (Stakich, Royal Oak, Michigan, USA)

- *Subgroup Ic:* Triple antibiotic paste (ciprofloxacin+ metronidazole+ clindamycin)

Group II: *Candida albicans*

- *Subgroup IIa:* BioPure MTAD (Dentsply Tulsa Dental, Johnson City, TN)
- *Subgroup IIb:* Propolis (Stakich, Royal Oak, Michigan, USA)
- *Subgroup IIc:* Triple antibiotic paste (ciprofloxacin + metronidazole + clindamycin)

All the canals were then sealed with temporary restorative material and incubated at 37°C for 48 hours.

Assessment of Antimicrobial Efficacy

The teeth were irrigated with 5 mL sterile saline to remove the medicament. Dentin debris present along the root canals at a depth of 200 µm was collected using sterile GG drills, number 4 and transferred to1 ml phosphate buffer saline solution. Diluted suspensions were then seeded on to their respective culture medium for the growth of microorganisms. Plates were incubated for 24 h at 37°C. At the end of the second and seventh days, the zone of inhibition (Figs 1 and 2) was measured in mm around the plate.

Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences version 18.0 (SPSS 18 developed by IBM, Chicago, USA). One-way ANOVA was done for multiple comparisons between the different groups. The level of significance was established at $p < 0.05$.

RESULTS

The mean zone of inhibition of *E. faecalis* and *C. albicans* with the three intracanal medicaments after 2 days is summarized in Table 1, and after 7 days is summarized in Table 2. After 2 days, triple antibiotic paste displayed the maximum zone of inhibition of *E. faecalis* (24.74 ± 0.622 mm) and C. albicans (28.22 ± 0.489 mm), followed by BioPure MTAD (19.58 ± 1.734 mm, 24.75 ± 0.954 mm) and propolis (13.10 ± 0.278 mm, 17.96 ± 0.163 mm). After 7 days, triple antibiotic paste displayed the maximum zone of inhibition of *E. faecalis* (26.86 ± 0.112 mm) and C. albicans (32.10 ± 0.908 mm), followed by BioPure MTAD (20.13 ± 1.842 mm, 27.22 ± 1.977) and propolis (14.11 ± 0.101 mm, 19.90 ± 0.742 mm).

Statistically significant differences were found in the antimicrobial activity within the groups and between the Intracanal medicaments after 2 days (ANOVA: $p < 0.0001$) (Table 3), and after 7 days (ANOVA: $p < 0.0001$) (Table 4).

DISCUSSION

The basis of root canal treatment is nonspecific eradication of intraradicular microorganisms. Although several studies have demonstrated the importance of

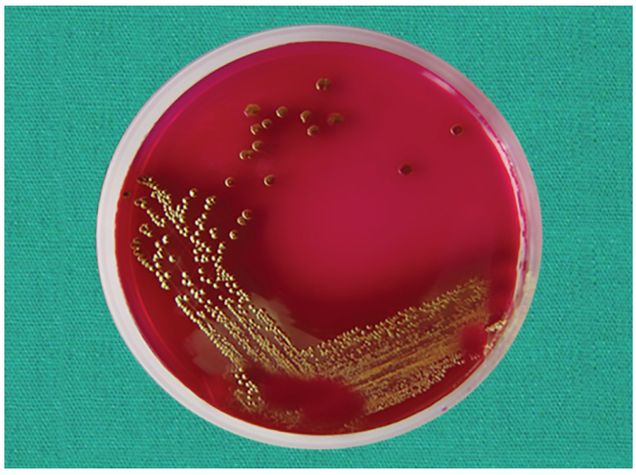

Fig. 1: Zone of inhibition of *Enterococcus faecalis*

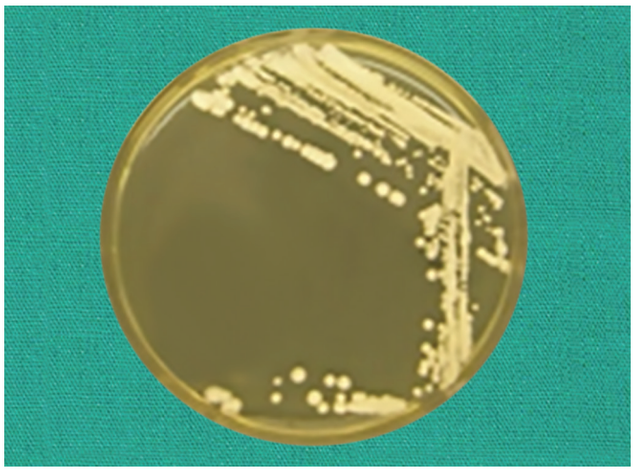

Fig. 2: Zone of inhibition of *Candida albicans*

Table 1: Mean zone of inhibition of *E. faecalis* and *C. albicans* with three intracanal medicaments after 2 days.

Intracanal medicaments	*Enterococcus faecalis (Mean ± SD)*	*Candida albicans (Mean ± SD)*
BioPure MTAD	19.58 ± 1.734	24.75 ± 0.954
Propolis	13.10 ± 0.278	17.96 ± 0.163
Triple antibiotic paste	24.74 ± 0.622	28.22 ± 0.489

Table 2: Mean zone of inhibition of *E.faecalis* and *C.albicans* with three intracanal medicaments after 7 days

Intracanal medicaments	*Enterococcus faecalis (Mean ± SD)*	*Candida albicans (Mean ± SD)*
BioPure MTAD	20.13 ± 1.842	27.22 ± 1.977
Propolis	14.11 ± 0.101	19.90 ± 0.742
Triple antibiotic paste	26.86 ± 0.112	32.10 ± 0.908

Table 3: Comparison of zone of inhibition after 2 days among Intracanal medicaments using ANOVA

	Sum of squares	*Df*	*Mean square*	*F*	*Sig.*
Between Groups	8278.621	3	1218.364	2810.770	0.0001
Within Groups	14.192	38	0.644		
Total	8292.813	41			

Table 4: Comparison of zone of inhibition after 7 days among intracanal medicaments using ANOVA

	Sum of squares	*Df*	*Mean square*	*F*	*Sig.*
Between Groups	9858.372	4	1424.132	2732.219	0.0001
Within Groups	16.998	42	0.844		
Total	9875.370	46			

inter-appointment intracanal medication in killing the microorganisms, some clinicians choose single-visit root canal treatment. In addition to the root canal lumen, intracanal medicaments also act inside the dentinal tubules and at apical resorption. The high resistance of E. faecalis to antibacterial substances is widely documented. During environmental stress, this bacterium can enter in a viable but non-culturable state.[9]

C. albicans is the most common fungi cultured from failed root canal-treated teeth.[10] Because of the collagenolytic activity, *C. albicans* can use dentin as a source of nutrition. This promotes its colonization in the root canal and increases its virulence.

In this study, the convention for instrumentation was the same for all the groups. In order to warrant adequate cleaning of the apical third, the canals were uniformly instrumented 1 mm shorter than the tip of the apex. The smear layer was removed using EDTA.[11]

Sodium hypochlorite causes disturbances in the metabolic functions in the microorganisms by irreversibly oxidizing the hydrosulfuric groups of essential enzymes and thus, acts as an effective root canal irrigant.[12] In the present study, 5.25% sodium hypochlorite was used to make the root canals sterile before inoculation with *E. faecalis* and *C. albicans*.

The least zone of inhibition against *E. faecalis* and *C. albicans* was shown by propolis in this study. A study by Bhandari et al.[13] that showed better antibacterial efficacy by propolis compared to different Intracanal medicaments but there was a statistically insignificant on days 3 and 5. The antibacterial effects of propolis may be due to its flavonoid contents like quercetin, galangin, pinocembrin, esters of caffeic acid, benzoic acid and cinnamic acid. In addition, propolis inhibits bacterial DNA dependent RNA polymerase due to its ultraviolet absorbing component.[14]

The present study showed a statistically significant zone of inhibition of *E. faecalis* and *C. albicans* by triple antibiotic paste after 2nd and 7th day. This was similar to the study done by Mehta et al.[15] which demonstrated significant inhibition of *E. faecalis* and *C. albicans* at 24, 48, and 72 hours by triple antibiotic paste. At all the time intervals, triple antibiotic paste achieved least optical density at 1:20 dilution (1.25 µg/mL) against *E. faecalis*; however, maximum inhibition of growth was seen only at highest concentration [(25 µg/mL at 24 h, 1:10 dilution, (2.5 µg/mL) at 48 hours, and 1:20 dilution (1.25 µg/mL) at 72 hours] for *C albicans*. Similar results were found by Hoshino et al.[16] and Sato et al.[17] where triple antibiotic paste was shown to be effective even at high dilutions, suggesting that low concentrations of antibiotics may be enough to attain the required antibacterial effect.

BioPure MTAD significantly inhibited E. faecalis and C. albicans. The efficacy of BioPure MTAD against pathogens was demonstrated by Davis et al.[18] states that BioPure MTAD showed significantly (p 0.05) more zones of microbial inhibition than 5.25% NaOCl, 2% CHX, and Dermacyn. The zone of inhibition between NaOCl and CHX was not significant ($p < 0.05$), and Shabahang and Torbinejad[19] state that teeth or dentin shavings were cultured to determine the presence or absence of the test bacteria. Fisher's exact test showed that the combination of 1.3% NaOCl as a root canal irrigant and MTAD as a final rinse was significantly more effective against *E. faecalis* than the other regimens.

Zone of inhibition and turbidity testing can signify the quantity of residual live bacteria within the root canals and so they were chosen to evaluate the antibacterial efficacy of intracanal medicaments. The effectiveness of root canal treatment is commonly assessed by culturing the microbes present within the root canal system. After evaluating the molecular and culture technique, Gomes et al.[20] established that the molecular technique cannot distinguish between viable and dead cultures. On the other hand, culture technique can isolate and clinically recognize the viable microorganisms, even when they are present in a minimum concentration.

Antibiotics inhibit bacterial infections. The elimination of microorganisms and adequate sterilization of the root canal cannot be achieved by a single antibiotic. Over the past decades, microorganisms have become resistant to antibiotics due to the repeated use of antibiotics against various infections. Therefore, to eliminate the various resistant microorganisms that are present in the complex root canal system, a combination of antibiotics or intracanal medications were used to chemically disinfect the root canals. This complete disinfection makes

nonsurgical endodontic therapy an efficient method of treatment of periapical lesions.

This investigation used differently in vitro conditions than the in vivo conditions of the infected root canal. Even though the infected root canal contains various pathogens, in the present study evaluated the efficacy of intracanal medications against only two microbial species, *E. faecalis*, and *C. albicans*. Additionally, the root canal contains necrotic and/or viable tissues and tissue fluids under *in vivo* conditions which may reduce the activity of the medications. Hence, future *in vivo* researches is required to assess the clinical efficiency of the tested intracanal medicaments.

CONCLUSION

The triple antibiotic paste was significantly more effective than BioPure MTAD, and propolis against E. faecalis and C. albicans at the end of 2nd and 7th days.

REFERENCES

1. Chua et al. Antifungal effectiveness of various intracanal medicaments against *Candida albicans:* an ex-vivo study. BMC Oral Health 2014;14:53.
2. Turk BT, Sen BH, Ozturk T. *In vitro* antimicrobial activity of calcium hydroxide mixed with different vehicles against *Enterococcus faecalis* and *Candida albicans*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108:297-301.
3. Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32:361-369.
4. Shaik J, Garlapati R, Nagesh B, Sujana V, Jayaprakash T, Naidu S. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste and calcium hydroxide using chitosan as carrier against *Candida albicans* and *Enterococcus faecalis*: An in vitro study. J Conserv Dent 2014;17:335-339.
5. Hagihara Y, Kaminishi H, Cho T, Tanaka M, Kaita H. Degradation of human dentin collagen by an enzyme produced by the yeast *Candida albicans*. Arch Oral Biol 1988;33:617-619.
6. Blome B, Braun A, Sobarzo V, Jepsen S. Molecular identification and quantification of bacteria from endodontic infections using real-time polymerase chain reaction. Oral Microbiol Immunol 2008 Oct;23(5):384-390.
7. Manzur A, González AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod 2007 Feb;33(2): 114-118.
8. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endod 2007;33:1473-1476.
9. Krittika Ravi. Antimicrobial Efficacy of Various Intracanal Medicaments against *Enterococcus faecalis*. J. Pharm. Sci. & Res 2017;9(10):1861-1863.
10. Waltimo TMT, Siren EK, Torkko HLK, Olsen I, Happasalo MPP. Fungi in therapy-resistant apical periodontitis. Int-Endod J 1997;30:96-101.
11. Somanath G, Samant PS, Gautam V, Singh Birring OJ. To comparatively evaluate the antimicrobial efficacy of chlorhexidine, nisin and linezolid as an intracanal medicament on *Enterococcus faecalis*: An *in vitro* study. Indian J Dent Res 2015;26: 613-618.
12. Siqueira JF Jr., Rôças IN, Paiva SS, Guimaraes-Pinto T, Magalhães KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:122-130.
13. Bhandari S, Ashwini T S, Patil R C. An in Vitro Evaluation of Antimicrobial Efficacy of 2% Chlorhexidine Gel, Propolis and Calcium Hydroxide Against *Enterococcus faecalis* in Human Root Dentin. Journal of Clinical and Diagnostic Research. 2014;8(11):ZC60-ZC63.
14. Madhubala MM, Srinivasan N, Ahamed S. Comparative evaluation of propolis and triantibiotic mixture as an intracanal medicament against *Enterococcus faecalis*. J Endod. 2011;37:376-381.
15. Mehta S, Verma P, Tikku AP, Chandra A, Bains R, Banerjee G. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste, calcium hydroxide, and a proton pump inhibitor against resistant root canal pathogens. Eur J Dent 2017;11:53-57.
16. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In-vitro antibacterial susceptibility of bacteria taken from infected rootdentine to a mixture of ciprofloxacin, metronidazole and minocycline.Int Endod J 1996;29:125-130.
17. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. Int Endod J 1996;29:118-124.
18. Davis M J, Maki J, and Bahcall J K. An In Vitro Comparison of the Antimicrobial Effects of Various Endodontic Medicaments on *Enterococcus faecalis*. J Endod 2007;33(5):567-569.
19. Torabinejad M, Shabahang S. Effect of MTAD on *Enterococcus faecalis*-contaminated root canals of extracted human teeth. J Endod 2003;29:576-579.
20. Gomes BP, Pinheiro ET, Gade-Neto CR, Sousa EL, Ferraz CC, Zaia AA, Teixeira FB, Souza-Filho FJ. Microbiological examination of infected dental root canals. Oral Microbiol Immunol. 2004 Apr;19(2):71-76.