Comparative Evaluation of Antimicrobial Efficacy of MTAD, 3% NaOCl and Propolis Against E Faecalis

Asha Nara, Dhanu, Prakash Chandra, Latha Anandakrishna, Dhananjaya

Postgraduate Student, Department of Pedodontics and Preventive Dentistry, MS Ramiah Dental College and Hospital Bengaluru, Karnataka, India

Professor, Department of Pedodontics and Preventive Dentistry, MS Ramiah Dental College and Hospital Bengaluru, Karnataka, India

Professor and Head, Department of Pedodontics and Preventive Dentistry, MS Ramiah Dental College and Hospital Bengaluru, Karnataka, India

Reader, Department of Pedodontics and Preventive Dentistry, MS Ramiah Dental College and Hospital Bengaluru, Karnataka, India

Correspondence: Asha Nara, Postgraduate Student, Department of Pedodontics and Preventive Dentistry MS Ramiah Dental College and Hospital, C/o Dr HS Kadlimatti, Nagenhalli, Bengaluru, Karnataka, India e-mail: ashanara@yahoo.com

Abstract

Aim: The present study sought to compare the antimicrobial efficacy of 3% NaOCl, Biopure MTAD (Tulsa Dentsply, Tulsa, OK) and Brazilian ethanolic extract of propolis (EEP) against Enterococcus faecalis (E. faecalis).

Methodology: The study utilized 55 extracted human permanent teeth with single root canal. The samples were decoronated, instrumented and sterilized. The teeth were infected with E faecalis for 48 hours. The teeth were divided randomly into 3 groups according to the irrigants used and kept in contact with the respective irrigant for 5 minutes. All the samples were incubated in brain heart infusion (BHI) broth for 96 hours. Disinfection of the samples was determined based on presence or absence of turbidity in the BHI broth 96 hours later. Statistical analysis was done using Chi-square test.

Results: All the samples treated with MTAD showed complete absence of turbidity, while all the 15 teeth treated with propolis showed presence of turbidity, 8 out of 15 teeth treated with NaOCl showed presence of turbidity. Statistical analysis of the data using chi-square test showed significant difference between the groups (P < 0.05).

Conclusion: The study concluded that MTAD was more effective than 3% NaOCl and propolis against E. faecalis.

Keywords: BHI broth, Enterococcus faecalis, MTAD, 3% NaOCl, propolis.

INTRODUCTION

Microorganisms play fundamental role in etiology of pulp and periradicular lesions.1 Successful root canal therapy relies on triad of instrumentation, disinfection and obturation.2 Disinfection of the root canal is major determinant in the healing of periapical tissues.1 Though the chemomechanical preparation and use of antimicrobials are effective in reducing the bacterial load, some bacteria can still persist.3 Enterococcus faecalis is one among the facultative organism which is persistently found in root canal failures,4 and is resistant to various intracanal medicaments.5 The microorganisms found in the root canals of deciduous teeth are similar to those in the root canals of permanent teeth.6,7

Though sodium hypochlorite (NaOCl) is a commonly used root canal irrigant, it has an unpleasant odor and taste; it does not consistently disinfect the root canal system8 and is toxic when extruded into the periradicular tissues.9 Because of these limitations, a search for better root canal irrigant continues.

Torbinejad in 2003 introduced a new irrigant, Biopure MTAD (Tulsa Dentsply, Tulsa, OK) a mixture of Doxycycline, citric acid, and Tween-80, which is capable of safely removing the smear layer10 and eliminating E. faecalis.11
Propolis, a resinous bee-hive product, is a potent antimicrobial, antioxidant and anti-inflammatory agent. The pharmacologically active constituents in propolis are flavonoids, phenolics and aromatics. Propolis has been used in dentistry as pulp capping agent, as storage media for avulsed teeth, for prevention of caries and dentine hypersensitivity. But its use as a root canal irrigant has not been evaluated yet.

So the present study sought to compare the antimicrobial efficacy of 3% NaOCl, MTAD and propolis against *E. faecalis*.

**MATERIAL AND METHODS**

The methodology used was adapted from the research study protocol of Shabahang and Torbinejad with some modifications. Fifty-five extracted single rooted human permanent teeth were collected and stored in 10% formalin. Each tooth was radiographed with digital radiography to confirm the presence of patent canal. The teeth were decoronated and instrumented 0.5 mm short of apex up to size 40 k-type file maintaining 10 mm of working length. One ml of NaOCl was used for cleaning and shaping. One ml of 17% of EDTA was used to remove the smear layer. Later all the roots were autoclaved. Five autoclaved samples were transferred to sterile broth to serve as negative control.

A 24 hours culture of *E. faecalis* (ATCC 4082) was grown in BHI broth with concentration of 1×10⁸ cells/ml. Each root canal was inoculated with 1 µl of *E. faecalis* suspension using a sterile 1mL tuberculin syringe and incubated at 37°C for 48 hours. Five infected samples were kept as positive control. The infection of the specimen was confirmed by sampling the culture on McConkey’s agar. Growth was determined by visualization of individual white pin point colonies on agar plates. The rest of the contaminated samples were then divided into 3 experimental groups according to the irrigant used.

*Group I:* 15 samples were irrigated with 2 ml of 3% NaOCl and kept in the canal for 5 minutes.

*Group II:* 15 samples were irrigated with 2 ml of Biopure MTAD (Tulsa Dentsply Tulsa, OK) and kept in the canal for 5 minutes. (MTAD prepared according to manufacturer’s instructions).

*Group III:* 15 samples were irrigated with 2 ml of Propolis and kept in the canal for 5 minutes (Flow chart 1).

All the samples were then washed with distilled water to prevent potential carry-over of the irrigants, transferred into another tubes containing 2 ml of BHI broth and aseptically cultured in an incubator at 37°C for 96 hours. After 96 hours, two observers blinded as to the irrigant examined the tubes for the presence of turbidity. The numbers of positive and negative samples were recorded.

The chi-square test was used for statistical analysis to evaluate the differences between the groups.

**RESULTS**

All of the positive control samples caused turbidity in the tubes, whereas none of the negative controls showed turbidity. Data obtained for each irrigation regimen are presented in Table 1. Eight out of fifteen samples treated with 3% NaOCl remained contaminated as evidenced by turbidity of the BHI broth after 96 hours. While all the 15 samples which received irrigation with Propolis showed presence of turbidity, none of the samples treated with MTAD showed turbidity. There was statistical significant difference between samples treated with 3% NaOCl, MTAD and Propolis (p < 0.05).

**Table 1:** Comparision of antimicrobial effect of 3% NaOCl, MTAD and propolis

| Groups     | Sample size | Growth | No growth |
|------------|-------------|--------|-----------|
| Negative control | 5           | 0      | 5         |
| Positive control  | 5           | 5      | 0         |
| 3% NaOCl     | 15          | 8      | 7         |
| MTAD         | 15          | 0      | 15        |
| Propolis     | 15          | 15     | 0         |

**DISCUSSION**

One of the main aims of root canal treatment is to eliminate the bacteria, their byproducts and the substrate from the root canal system. The use of irrigation solution in this process is essential to ensure bacterial elimination and eradication of organic tissue remnants. Maximum antibacterial effect, maximum tissue dissolving effect on the necrotic tissues and the least toxic effect on the peripheral tissues are some important features of an ideal root canal irrigant. The complex morphology and the irregularity of
Comparative Evaluation of Antimicrobial Efficacy of MTAD, 3% NaOCl and Propolis Against E. faecalis

International Journal of Clinical Pediatric Dentistry, January-April 2010; 3(1): 21-25

Flow chart 1: Distribution of samples among the treatment groups

- 55 single rooted extracted human teeth
- Decoronation, instrumentation and sterilization
  - Negative control n = 5
  - Canal infected with E. faecalis for 48 hours
  - Positive control n = 5
- 5 minutes exposure to 3% NaOCl n = 15
- 5 minutes exposure to MTAD n = 15
- 5 minutes exposure to Propolis n = 15

the root canals of primary teeth negatively affect the success of chemo-mechanical endodontic treatment. Sodium hypochlorite is, till date, the most commonly employed root canal irrigant. The antimicrobial activity of NaOCl is by the release of hypochlorous acid (HOCl) and its oxidative action on sulfhydryl groups of bacterial enzymes thereby disrupting the metabolism of the microorganism. Although it is an effective antibacterial agent, NaOCl is toxic when extruded to the periradicular tissues. In root canal treatment of primary teeth, NaOCl can damage permanent tooth follicles, peripheral tissues and oral mucosa.

Therefore, research for new irrigants continues. MTAD, after its introduction in 2003 was subjected to various test procedures to evaluate its efficacy and was compared with various commonly used irrigants. The superior antimicrobial activity of MTAD over 3% NaOCl seen in this study are in agreement with the findings of Shabahang and Torabinejad’s study.

In present study the smear layer was removed before contaminating the teeth with E. faecalis to allow penetration of bacteria into the tubules. The lack of turbidity of the BHI by negative control group demonstrated that the sterilization procedure utilized was effective. The results from the samples in the positive group confirmed the presence of E. faecalis within the root canal systems. The roots were immersed in culture media for 96 hours so that if bacteria were present in the dentinal tubules, their movement into the main canal would result in turbidity of the media. The samples treated with MTAD showed absence of turbidity proving efficacy of MTAD deep into the canals. This effect is related to presence of detergent in it.

The organism E. faecalis was selected in this study because it is most commonly isolated in endodontic retreatment of apical periodontitis and has been identified to be resistant to currently used chemicals such as sodium hypochlorite, potassium iodide or calcium hydroxide and has been found to survive as a monoinfection in root canals.

Previous in vitro studies have shown a high level of susceptibility of E. faecalis to MTAD, even when this solution was diluted 200 times, whereas NaOCl loses its antibacterial activity against the same isolate beyond 32 times dilution. MTAD removes the smear layer with significantly less erosion of the dentinal tubules compared to EDTA. Also when MTAD was evaluated for biocompatibility, it was found to be less cytotoxic than Eugenol, 3% H₂O₂, Ca (OH)₂ paste, 5.25% NaOCl, 0.12% Chlorhexidine gluconate, and EDTA and more cytotoxic than 2.63%, 1.31% and 0.66% NaOCl. The antimicrobial efficacy of MTAD is because of anticollegenase activity of Doxycycline, its low pH, ability to be released gradually over time, and its action is facilitated...
by citric acid which removes the organic and inorganic substances. Tween-80 reduces surface tension on the dentinal tubules and allows deeper penetration of Doxycycline into the tubules.

One of the significant features of MTAD is its capacity to kill *E. faecalis* after a mere exposure of 5 minutes making it useful in the clinical situation. However this effect was not seen with NaOCl. Newberry et al showed that MTAD inhibited most strains of *E. faecalis* growth when diluted 1:8192 times and killed most strains of *E. faecalis* when diluted 1:512 times. Thus MTAD has properties of an ideal root canal irrigant.

The results of the present study are in accordance with the findings of Portenier et al, Ghoddusi et al, and Davis et al, while studies done by Dunavant et al, Baumgartner et al, and Krause et al are in disagreement with these results. The disparity in the results may be caused by differences in methodology and variance in strains tested. Failure to follow manufacturer’s recommendations may affect the results of the study. Torabinejad’s group recommended the use of 1.3% NaOCl for 15 to 20 minutes before the final rinse with MTAD. Some of these studies did not use NaOCl at all or used it for a shorter period than recommended. Use of MTAD in primary teeth is however limited due to chance of discoloration of permanent tooth buds present below the primary teeth. However its use in permanent teeth in younger age may not be controversial.

Natural products have been used in dental and medical practices for thousands of years and have become even more popular today. Propolis has gained increased interest due to its antimicrobial activity against a wide range of pathogenic organisms. Propolis is collected from various plant sources by honeybees and its chemical composition varies due to climate, season and location. The main chemical classes present in propolis are flavonoids, phenolics and aromatic compounds. Flavonoids are well known compounds that have antioxidants, antimicrobial, antifungal, antiviral and anti-inflammatory properties. Brazilian propolis is characterized by a very low concentration of flavonoids and esters of phenolic acids, but it has a high concentration of dihydrocinnamic acid, prenylated acetophenones and specific terpenoids, which have antimicrobial activity.

Though in the present study Propolis found to be less efficacious than NaOCl and MTAD, Al-Qathami and Al-Madi found that propolis was as equally effective as NaOCl when used as an antimicrobial irrigant on extracted human teeth. Koo et al tested the antimicrobial efficacy of EEP, using agar diffusion method and observed that it significantly inhibited all of the microorganisms tested, including facultative and strict anaerobic species. Oncag et al observed that Propolis has good antibacterial activity against *E. faecalis* in the root canal of extracted teeth suggesting that it can be used as an alternative intracanal medicament. Gafar et al used intracanal dressings of EEP in infected teeth and showed that it gave better results than camphorated paramonochlorophenol. When utilized as a pulp-capping agent, EEP induced hard tissue bridge formations in the same way as those induced by calcium hydroxide. Al Shaker et al using pulp and periodontal fibroblast cell culture observed that propolis exerts minimal toxicity on either type of cell.

Propolis has shown biocompatibility and proven its antibacterial efficacy in the pulp – periapical infections. It is important to remember that the flavonoid composition of propolis is qualitatively and quantitatively different depending on the region where propolis is collected. So to include in the arsenal of intracanal substances further research such as *in vivo* and *in vitro* studies are required for better understanding of action of propolis as an intracanal irrigant.

To conclude, in the present study biopure MTAD has been shown to be a better irrigant when compared to 3% NaOCl and propolis. Further *in vitro* and *in vivo* studies are essential to validate the use of propolis as an irrigant against *E. faecalis*.

REFERENCES

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965 Sep;20:340-349.
2. Ingle JI. Root canal obturation. J Am Dent Assoc 1956 Jul;53(1):47-55.
3. Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997 Sep;30(5):297-306.
4. Rocas IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004 May;30(5):315-320.
5. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990 Aug;6(4):142-149.
6. Marsh SJ, Largent MD. A bacteriological study of the pulp canals of infected primary molars. J Dent Child 1967 Nov;34(6):460-470.
7. Tomic-Karovic K, Jelinek E. Comparative study of the bacterial flora in the surroundings the root canals and sockets of deciduous molars. Int Dent J 1971 Sep;21(3):375-388.
Comparative Evaluation of Antimicrobial Efficacy of MTAD, 3% NaOCl and Propolis Against E Faecalis

8. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981 Aug;89(4):321-328.

9. Chang YC, Huang FM, Tai KW, Chou MY. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001 Oct;92(4):446-450.

10. Torabinejad M, Khademii AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S. A new solution for the removal of the smear layer. J Endod 2003 Mar;29(3):170-175.

11. Torabinejad M, Shabahang S, Apresco R, Kettering JD. The antimicrobial effect of MTAD: an in vitro investigation. J Endod 2003 Jun;29(6):400-403.

12. Sabir A, Tabbu CR, Agustiono P, Sosroseno W. Histological analysis of rat dental pulp tissue capped with propolis. J Oral Sci 2005 Sep;47(3):135-138.

13. Martin MP, Pileggi R. A quantitative analysis of Propolis: A promising new storage media following avulsion. Dent Traumatol 2004 Apr;20(2):85-89.

14. Duarte S, Rosalen PL, Hayacibara MF, Cory JA, Bowen WH, Marquis RE, Rehder VL, Sartoratto A, Ikigaki M, Koo H. The influence of novel propions on mutans streptococci biofilms and caries development in rats. Arch Oral Biol 2006 Jan;51(1):15-22.

15. Mahmoud AS, Almas K, Dahlan AA. The effect of propolis on dentinal hypersensitivity and level of satisfaction among patients from a university hospital Riyadh, Saudi Arabia. Indian J Dent Res 1999 Oct-Dec;10(4):130-137.

16. Shabahang S, Pourseimail M, Torabinejad M. In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. J Endod 2003 Jul;29(7):450-452.

17. Siqueira JF Jr, Lima KC, Magalhaes FA, Lopes HP, de Uzeda M. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod 1999 May;25(5):322-335.

18. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod 1998 Jul;24(7):472-476.

19. Türkün M, Gökay N, Özdemir N. Comparative investigation of the toxic and necrotic tissue dissolving effects of different endodontic irrigants. J Dent Faculty Istanbul Univ 1998;32:87-94.

20. Thomas AM, Chandra S, Pandey RK. Elimination of infection in pulpectomized deciduous teeth: short-term study using iodoform paste. J Endod 1994 May;20(5):233-235.

21. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. Int Endod J 1997 Jul;30(4):279-282.

22. Molander A, Reit C, Dahlen G. The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. Endod Dent Traumatol 1999 Oct;15(5):205-209.

23. Estrela C, Pimenta FC, Ito IY, Bammann LL. Antimicrobial evaluation of calcium hydroxide in infected dentinal tubules. J Endod 1999 Jun;25(6):351-353.

24. Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J 2003 Jan;36(1):1-11.

25. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-Tetrazolium method. J Endod 2003 Oct;29(10):654-657.

26. Shabahang S, Torabinejad M. Effect of MTAD on Enterococcus faecalis-contaminated root canals of extracted human teeth. J Endod 2003 Sep;29(9):576-579.

27. Newberry BM, Shabahang S, Johnson N, Apresco RM, Torabinejad M. The antimicrobial effect of Biopure MTAD on eight strains of Enterococcus faecalis: an in vitro investigation. J Endod 2007 Nov;33(11):1352-1354.

28. Portenier I, Waltimo T, Orstavik D, Haapasalo M. Killing of Enterococcus faecalis by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentine powder or BSA. J Endod 2006 Feb;32(2):138-141.

29. Ghodduisi J, Rohani A, Rashid T, Ghaziani P, Akbari M. An evaluation of microbial leakage after using MTAD as a final irrigation. J Endod 2007 Feb;33(2):173-176.

30. Davis JM, Maki J, Bahcall JK. An in vitro comparison of the antimicrobial effects of various endodontic medicaments on Enterococcus faecalis. J Endod 2007 May;33(5):567-569.

31. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against Enterococcus faecalis Biofilms. J Endod 2006 Jun;32(6):527-531.

32. Johal S, Baumgartner JC, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/BioPure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. J Endod 2007 Jan;33(1):48-51.

33. Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on Enterococcus faecalis. J Endod 2007 Jan;33(1):28-30.

34. Santos FA, Bastos EM, Maia AB, Uzeda M, Carvalho MA, Farias LM, Moreira ES. Brazilian propolis: physicochemical properties, plant origin and antibacterial activity on periodontopathogens. Phytother Res 2003 Mar;17(3):285-289.

35. Almas K, Mahmoud A, Dahlan AA. A comparative study of Propolis and saline application on human dentin: A SEM study. Indian J Dent Res 2001 Jan-Mar;12(1):21-27.

36. Al-Qathami H, Al-Madi E. Comparison of sodium hypochlorite, propolis and saline as root canal irrigants: a pilot study. Saudi Dent J 2003 May-Aug;15(2):100-103.

37. Koo H, Gomes BP, Rosalen PL, Ambrosano GM, Park YK, Cory JA. In vitro antimicrobial activity of Propolis and Arnica Montana against oral pathogens. Arch Oral Biol 2000 Feb;45(2):141-148.

38. Oncag O, Cogulu D, Uzel A, Sorkun K. Efficacy of propolis as an intracanal medicament against Enterococcus faecalis. Gen Dent 2005 Sep-Oct;54(5):319-322.

39. Gafar M, Sacalas E, David E, David N. The treatment of simple pulp gangrene with the epitherapeutic agent “Propolis” [Tratamentul gangrenei pulpare simple cu produsul apiterapic “Propolis”]. Rev Chir Oncol Radiol O R L Oftalmol Stomatol Ser Stomatol 1986 Apr-Jun;33(2):115-117. (Rom).

40. Bretz WA, Chiego DJ, Marcucci MC, Cunha I, Custodio A, Schneider LG. Preliminary report on the effects of propolis on wound healing in the dental pulp. Z Naturforsch C 1998 Nov-Dec;53(11-12):1045-1048.

41. Al-Shaher A, Wallace J, Agarwal S, Bretz WA, Baugh D. Effect of propolis on human fibroblasts from the pulp and periodontal ligament. J Endod 2004 May;30(5):359-361.