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Comprehensive assessment of antibacterial efficacies of biopure and niclor against planktonic and biofilm forms of enterococcus faecalis and candida albicans: an *in vitro* (original research) study

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Abstract

Aim: This study was undertaken to logically and intricately evaluate and compare antimicrobial effects of BioPure MTAD (MTAD) and Niclor 5 irrigating solutions as observed against planktonic and biofilm forms of E. faecalis and Candida albicans.

Materials & Methods: Total 45 recently extracted non carious permanent maxillary incisors teeth of both genders were studied. Biomechanical preparation of the intended canals was attempted precisely as mentioned in the standard literature and books by renowned researchers. Authors had intended to limit their biomechanical preparation up to 40 K sized file only. Group I (BioPure MTAD [MTAD]), group II (Niclor 5). The third group was kept as control group (sterile distilled water). The antimicrobial effect in each group was observed, documented, statistically analyzed and compared.

Statistical Analysis and Results: Statistical analysis was completed using statistical software Statistical Package for the Social Sciences version 21. The measured mean for group I, II and III was 25.57, 23.42, 26. 58 respectively. Standard deviation was noted least for group II (0.142) and maximum for group I (0.185). Standard error was noted minimum for group I (0.123) while it was maximum for group III (0.571). Candida albicans colony count was highest in group III and lowest in group II. Particularly it was 4.98±0.34 in group I, 2.12±0.67 in group II, 11.09± 2.43 in group III. E.

Conclusion: Niclor 5 exhibited to be remarkably efficient against colonies of planktonic and biofilm forms of E. faecalis and Candida albicans. Therefore Niclor 5 may be clinically employed as an effective root canal irrigant. In this study, the effectiveness of two different irrigating solutions was tested, wherein Niclor 5found to be more efficient in reducing bacterial count.

Keywords: niclor, antimicrobial, planktonic enterococcus faecalis, biopure, candida albicans

Introduction

As we all know that endodontic success depends on the elimination of microorganisms from the root canal system and prevents recolonization. To accomplish this desired result, a union of chemical and mechanical approaches is used. Due to complex canal anatomy microbe can linger even after rigorous disinfections regime, thus complete sterilization of the canals is not achieved [1, 3]. Enterococcus faecalis, a Gram-positive facultative anaerobe is found abundantly in untreated canals and failed root canals. Its capability in tolerating unfavorable environmental conditions results in high survival rates and made these bacteria to be more resistant to intracanal medications. Intracanal medicaments supplement this to eliminate the persistent bacteria and prevent their re-growth, thereby making root canal environment conducive for periapical tissue repair [4, 8]. Calcium hydroxide is one of the most commonly used intracanal medicaments having antibacterial activity on a wide range of microflora present in the root canal. The success of endodontic treatment depends mainly on elimination of infecting microorganisms. This is achieved through chemomechanical preparation of root canals and leaving antimicrobial dressings in the root canal between appointments. However, microorganisms might still survive these challenges. Because cleaning and shaping procedures alone do not reliably eliminate bacteria, it seems logical to medicate canals with an antimicrobial agent after canal preparation. Viable microorganisms remaining after root canal preparation and disinfection contribute significantly to failure in endodontic therapy. Intracanal medicaments are used against endodontic microbial flora. They are proven to be effective against microbes that survive instrumentation and irrigation [9, 14]. It is imperative to exterminate the intracanal microbes for the success of root canal treatment therefore interappointment medicaments is applied. It should provide disinfection, should be efficacious throughout the given period, and pervade through the dentinal tubules annihilating bacteria that maybe present, with little toxicity to the peri-radicular tissues. Enterococcus faecalis is the most persistent organism in the peri-radicular area. This indomitable property is due to its high alkali tolerance and its tubular invasion potentiality. Secondary intra-radicular infections are caused during treatment or between appointments or post-treatment. Candida albicans are found in such infection [15, 17]. They can infiltrate the pulp space even after the completion of root filling. This study was undertaken to logically and intricately evaluate and compare antimicrobial effects of BioPure MTAD (MTAD) and Niclor 5 irrigating solutions as observed against planktonic and biofilm forms of E. faecalis and Candida albicans.

Materials & Methods

For the execution of the study, authors had selected 45 freshly extracted non carious permanent maxillary incisors teeth with

the history of non-traumatic extraction. Each of the selected study samples was attained from both male and female subjects. For maintaining uniformity, authors had attempted to screen total 50 subjects. Out of these selected subjects 25 were male and 25 were female. Relative etiologies and method of the sample tooth extractions were also taken into considerations. Predominantly, teeth those removed because of orthodontic or periodontal reasons were included in the study. The study was discussed and approved by the ethical committee of the institute. In the beginning, authors have decided to section the sample teeth in their cervical region. This exercise was completed by sectioning them with diamond disc powered by high velocity air rotor headpiece. Accurate working length measurement for each selected root was completed by concerned radiographic analysis. Biomechanical preparation of the intended canals was attempted precisely as mentioned in the standard literature and books by renowned researchers. Authors had intended to limit their biomechanical preparation up to 40 K sized file only. Initially, we had taken care of profuse root canal irrigation by using plane distilled water. However in few instances, authors have also employed advanced root canal irrigating solutions also. Following biomechanical preparation of sample canals, the apical foramen was sealed by using self-curing acrylic resins. The processed roots canals were then attempted to completely sterilize following biomechanical preparation. The microbial suspension of mixed culture of the tested microorganisms was inserted into prepared root canals. All proposed test samples were incubated for 72 hours. Authors have additionally separated the sample teeth randomly into 3 groups of 15 each. This segregation was solely based on irrigating solutions employed. Group I samples were subjected to BioPure MTAD (MTAD), group II samples were subjected to Niclor 5 (5% NaOCl) solution and group III samples were subjected sterile distilled water. BioPure MTAD (Mixture of doxycycline, citric acid, and Tween 80) has been suggested as a final rinse because of its antimicrobial properties and its ability to remove the smear layer. BioPure MTAD (MTAD) is less cytotoxic than 5.25% NaOCl. Whereas the Niclor 5 (Ogna Laboratori Farmaceutici, Muggiò, MB, Italy) is actually aqueous solution of 5% NaOCl. At first, microbiological samples were imported from root canal spaces which were further verified. In such microbial suspensions, the total number of Colony Forming Units (CFU) of studied bacterium was evaluated by routine microscopy. To fasten the process and make it more comprehensible, operators have sectioned each root across their root length so as to make them more accessible. Such exercises have entirely opened the canal spaces and the canal was completely visible with no interference. All such processed and cut sections were immersed into prepared bacteriological solutions for minimum 72 hours. Authors have also made sure to manage the relative quantity of root canal irrigating suspensions. This was done comprehensively in each studied sample group. It was kept maintained at 5 ml with immersion time of 5 minutes. Results thus obtained were subjected to statistical analysis wherein P value less than 0.05 was considered significant.

Statistical Analysis & Results

Comprehensive statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0 for windows (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL) statistical analysis software. Mean and standard deviation were determined for intended parameters. The resulting data was subjected to suitable statistical tests to obtain p values, mean, standard deviation, chi- square test, standard error and 95% CI. Response evaluation and analysis exhibits some very intricate inferences. These inferences were shown to have clinical explicabilities too. Here in this study, two different root canal irrigating solution were used. They were BioPure MTAD (MTAD) and Niclor 5 while Sterile distilled water was kept as control [standard]. Irrigation exercise was completed legitimately in group I, II and III samples. Table 1 shows types of irrigating solutions used in study and group wise allocation of the samples. There were 15 teeth in each group. Table 2 shows about evaluation of Mean values with Standard Deviation & Standard Error at 95% Coefficient of interval. The measured mean for group I, II and III was 25.57, 23.42, 26.58 respectively. Standard deviation was noted least for group II (0.142) and maximum for group I (0.185). Standard error was noted minimum for group I (0.123) while it was maximum for group III (0.571). Candida albicans colony count was highest in group III and lowest in group II. Particularly it was 4.98±0.34 in group I, 2.12±0.67 in group II, 11.09± 2.43 in group III. Planktonic and biofilm forms of E. faecalis count were found maximum in group III and minimum in group II. Efficiently it was 4.13± 0.32 in group I, 2.98± 0.32 in group II, 10.32± 2.87 in group III (Table 3 & Graph 1). The difference was significant (P< 0.05). The overall results undoubtedly pointed out that NAC had maximum antibacterial activity as compared with other studied irrigating solutions including distilled water.

Table 1: Types of irrigating solutions used in study: Sample Grouping and assortments

Groups	Group I	Group II	Group III
Solutions	BioPure MTAD (MTAD)	Niclor 5 (5% NaOCl)	Sterile distilled water [Control]
No. of teeth	15	15	15

Table 2: Evaluation of Mean values (M) with Standard Deviation (SD) & Standard Error (SE) at 95% Coefficient of interval

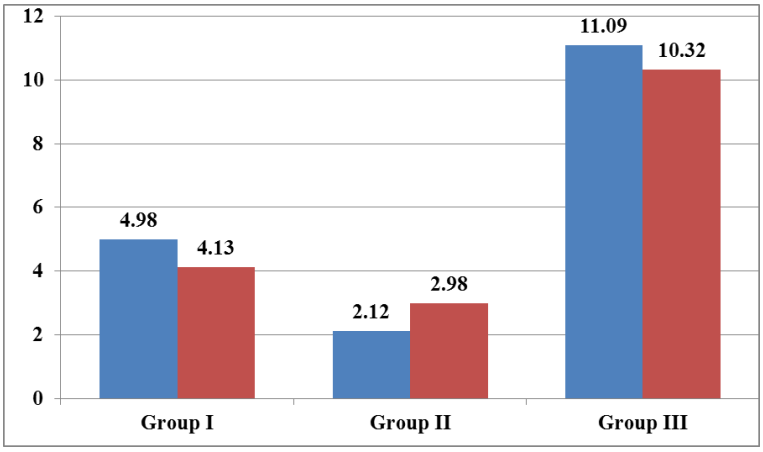
Group	n	Mean	SD	SE	95% CI for Mean	Level of Significance (p value)
Group I	15	25.57	0.185	0.123	18.341	0.006*
Group II	15	23.42	0.142	0.209	21.499	0.090
Group III	15	26.58	0.169	0.571	23.655	0.080

*p<0.05 significant

Table 3: Bacterial colony counts Group wise

Microbial Genus	Group I	Group II	Group III	P value
Candida albicans	4.98±0.34	2.12±0.67	11.09± 2.43	0.01*
E. Faecalis	4.13± 0.32	2.98± 0.32	10.32± 2.87	0.01*

p<0.05 significant



Graph 1: Microbial colony counts Group wise

Discussion

The fundamental goal of root canal treatment is to eliminate bacteria from the root canal and prevent infections. Because of the complex anatomy of root canal systems biomechanical preparation procedures do not completely eliminate them. Enterococcus faecalis and Streptococcus mutans are the most commonly isolated microorganisms from infected root canals. E. faecalis is associated with persistent apical infection of root canals [18, 22]. Endodontic infections are considered polymicrobial and more than 150 bacterial species are usually found in combinations of 3 to 6 species in each canal. Also, microorganisms, such as yeasts may be commonly found in root canals with pulp necrosis. Some of the pioneer researchers studied the prevalence of Candida genus in different sites of the oral cavity. They had verified that 55% of the root canals presented these microorganisms. Other clinicians analyzed the microbiota from the root canals of teeth with pulp necrosis and showed that in 15.3% of the cases Candida albicans was identified [23, 26]. Enterococcus faecalis is also frequently isolated from root canals in cases of pulp infection and also recalcitrant infections after endodontic treatment. Eradication of bacteria from the root canal system is important in ensuring the long-term success of root canal therapy. The microorganisms are the primary etiological agent in endodontic infections, and failure to eradicate them affects the outcome of endodontic therapy. Studies have shown that the bacterial flora in endodontic infections is polymicrobial, with a predominance of anaerobic species [27, 29]. Enterococcus faecalis is part of the human normal flora and an important pathogen in opportunistic infections in humans. E. faecalis is rarely present in primary apical periodontitis, but it is the dominant microorganism in root canal-treated teeth presenting with posttreatment apical periodontitis. Eradication of E. faecalis from the root canal remains a challenge, since it is resistant to a variety of antimicrobial agents. Studies have also shown that E. faecalis may be one of the reasons of failure of endodontic treatments [30, 34]. These results were quite comparable with our study's results. The control and suppression of E. faecalis in these dental procedures are of primarily importance in decreasing the penetration of bacteria inside the dentinal tubules and also limiting the formation of any relationship with other microorganisms, as in virulence factors, environment, and the biofilms [35].

Conclusion

Niclor 5 exhibited to be remarkably efficient against planktonic and biofilm forms (colonies) of E. faecalis and Candida albicans. Therefore, Niclor 5 may be clinically employed as an effective root canal irrigant. In this study, the effectiveness of two different irrigating solutions was tested, wherein Niclor 5 found to be more efficient in reducing microbial count. Nevertheless, future research is required to be conducted using larger sample size and widened study objectives. Our study results must be considered as suggestive for presuming prognosis for similar clinical conditions. However, we expect some other large scale studies to be performed that might further establish certain standard and concrete guidelines in these perspectives.

References

1. Henriques LC, de Brito LC, Tavares WL, Teles RP, Vieira LQ, Teles FR. *et al*. Microbial ecosystem analysis in root canal infections refractory to endodontic treatment. J Endod. 2016; 42(8):1239-1245.
2. Kim SY, Shin Y, Lee CY, Jung IY. *In vivo* quantitative evaluation of live and dead bacteria in root canal infection by using propidium monoazide with real-time PCR. J Endod. 2013; 39(11):1359-1363.
3. Aw V. Discuss the role of microorganisms in the aetiology and pathogenesis of periapical disease. Aust Endod J. 2016; 42(2):53-59.
4. Al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML. *et al*. The antimicrobial effect of various endodontic sealers. Oral Surg Oral Med Oral Pathol. 1990; 70(6):784-790.
5. Brito PR, Souza LC, Machado de Oliveira JC, Alves FR, DeDeus G, Lopes HP. *et al*. Comparison of the effectiveness of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: an *in vitro* study. J Endod. 2009; 35(10):1422-1427.
6. Desai P, Himel V. Comparative safety of various intracanal irrigation systems. J Endod. 2009; 35(4):545-549.
7. Howard RK, Kirkpatrick TC, Rutledge RE, Yaccino JM. Comparison of debris removal with three different irrigation techniques. J Endod. 2011; 37(9):1301-1305.
8. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006; 32(2):93-98.
9. Subapriya R, Nagini S. Medicinal properties of neem leaves: a review. Curr Med Chem Anticancer Agents. 2005; 5(2):149-156.

10. Dutta A, Kundabala M. Antimicrobial efficacy of endodontic irrigants from Azadirachta indica: an *in vitro* study. Acta Odontol Scand. 2013; 71(6):1594-1598.
11. Dutta A, Kundabala M. Comparative anti-microbial efficacy of Azadirachta indica irrigant with standard endodontic irrigants: a preliminary study. J Conserv Dent. 2014; 17(2):133-137.
12. Owolabi LL, Gbotolorun SC, Akpantah AO, Ekong MO, Eluwa MA, Ekanem TB. *et al*. Effect of methanolic extract of Neem leaf (Azadirachta indica) on ovarian histology and hormonal milleu. Nig Q J Hosp Med. 2008; 18(4):194-197.
13. Nayak A, Nayak RN, Soumya B, Bhat K, Kudalkar M. Evaluation of antibacterial and anticandidial efficacy of aqueous and alcoholic extract of neem (Azadirachta indica): an *in vitro* study. Int J Res Ayurveda Pharm, 2011; 2:230-235.
14. Rôças IN, Siqueira JF Jr. Root canal microbiota of teeth with chronic apical periodontitis. J Clin Microbiol. 2008; 46(11):3599-3606.
15. Sjögren 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; 30(5):297-306.
16. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol. 1994; 78(4):522-530.
17. Chávez De Paz LE, Dahlén G, Molander A, Möller A, Bergenholtz G. Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. Int Endod J. 2003; 36(7):500-508.
18. Zehnder M. Root canal irrigants. J Endod. 2006; 32(5):389-398.
19. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J. 2001; 34(6):424-428.
20. Venkateshbabu N, Anand S, Abarajithan M, Sheriff SO, Jacob PS, Sonia N. *et al*. Natural therapeutic options in endodontics – a review. Open Dent J. 2016; 10:214-226.
21. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). Curr Sci. 2002; 82(11):1336-1345.
22. Dubey S, Chaodary M, Gupta P. Comparative study of the antimicrobial efficiency of neem leaf extract, sodium hypochlorite and biopure MTAD – an *in vitro* study. Indian J Dent Adv. 2012; 4(1):740-743.
23. Zoletti GO, Pereira EM, Schuenck RP, Teixeira LM, Siqueira JF Jr, dos Santos KR. *et al*. Characterization of virulence factors and clonal diversity of Enterococcus faecalis isolates from treated dental root canals. Res Microbiol. 2011; 162(2):151-158.
24. Ferrer-Luque CM, Bejarano I, Ruiz-Linares M, Baca P. Reduction in Enteroccocus faecalis counts – a comparison between rotary and reciprocating systems. Int Endod J. 2014; 47(4):380-386.
25. Dakin HD. On the use of certain antiseptic substances in the treatment of infected wounds. Br Med J. 1915; 2(2852):318-320.
26. 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; 92(4):446-450.
27. Busslinger A, Sener B, Barbakow F. Effects of sodium hypochlorite on nickel-titanium Lightspeed instruments. Int Endod J. 1998; 31(4):290-294.
28. Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod. 2000; 26(12):751-755.
29. Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of Azadirachta indica, Mimusops elengi, Tinospora cardifolia, Ocimum sanctum and 2% chlorhexidine gluconate on common endodontic pathogens: an *in vitro* study. Eur J Dent. 2014; 8(2):172-177.
30. Mistry KS, Sanghvi Z, Parmar G, Shah S, Pushpalatha K. Antibacterial efficacy of Azadirachta indica, Mimusops elengi and 2% CHX on multispecies dentinal biofilm. J Conserv Dent. 2015; 18(6):461-466.
31. Mustafa M. Antibacterial Efficacy of Neem (Azadirachta indica) Extract against Enterococcus faecalis: An *in vitro* Study. J Contemp Dent Pract. 2016; 17(10):791-794.
32. Halkai KR, Mudda JA, Shivanna V, Rathod V, Halkai R. Antibacterial efficacy of biosynthesized silver nanoparticles against Enterococcus faecalis Biofilm: An *in vitro* study. Contemp Clin Dent, 2018; 9:237-41.
33. Sridevi A, Sindhu J, Naveen DN, Nirupama DN, Nainan MT. Antibacterial effect of fluorinated graphene and zinc oxide nanoparticles incorporated in zinc oxide-based sealers on Enterococcus faecalis (*in vitro* study). Saudi J Oral Sci, 2019; 6:81-7.
34. Rodrigues CT, de Andrade FB, de Vasconcelos LRSM. Antibacterial properties of silver nanoparticles as a root canal irrigant against Enterococcus faecalis biofilm and infected dentinal tubules. Int Endod J. 2018; 51(8):901-911.
35. Kim SW, Shin DH. Antibacterial effect of urushiol on E. faecalis as a root canal irrigant. Restor Dent Endod. 2017; 42(1):54-59.
36. Ravinanthanan M, Hegde MN, Shetty VA, Kumari S. Antimicrobial assay of combination surfactant irrigant regimen on vancomycin-resistant *Enterococcus faecalis*. An *in vitro* direct contact test. Dent Res J (Isfahan). 2018; 15(6):397-403.
37. Tandon B, Uppin VM, Hogade SA, Chaudhry S, Bansal S, Bansal S. *et al*. Antibacterial activity of four root canal sealers against Enterococcus faecalis at 1st, 3rd, 5th, and 7th day duration: A *In vitro* study. Indian J Dent Sci, 2017; 9:98-104.