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Original Research Article

Comparative evaluation of antibacterial efficacy of ayurvedic and homeopathic drugs against *E. fecalis* with different concentrations of sodium hypochlorite

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Abstract: One of the main goals of endodontic treatment is root canal disinfection and to prevent subsequent chances of reinfection. Adjuvant to instrumentation, root canal irrigants are required to eliminate the bacteria found on the root canal walls and lateral canals within the dentinal tubules. The aim is to measure and compare the antibacterial efficacy of ayurvedic and homeopathic drugs against E. Faecalis with different concentrations of sodium hypochlorite. Twenty agar plates were prepared by using Brain Heart Infusion (BHI) in 90mm diameter Petri dishes inoculated with *Enterococcus faecalis*-American Type Culture Collection (ATCC) 29212. 10 ml of each test solution 5.25 % sodium hypochlorite, 3%sodium hypochlorite, triphala, myrestica sebifera solutions respectively and normal saline was used as control in one of the well. Plates were incubated aerobically at 37˚C. Results were expressed as per the terms of the diameter of the inhibition zone. Results suggested a statistically significant difference in the zones of inhibition between five irrigating solutions ($p < 0.001$). Under the circumstances of this *in vitro* study, Triphala was more effective on cultures of *E. faecalis* compared to 3%NaOCl and Myrestica Sebifera.
Keywords: Antibacterial Sensitivity, Disinfection, Endodontic treatment, Irrigation

INTRODUCTION:

One of the main goals of endodontic treatment is root canal disinfection and to prevent subsequent chances of reinfection. Adjuvant to instrumentation, root canal irrigants are required to eliminate the bacteria found on the root canal walls and lateral canals within the dentinal tubules. Irrigating the root canals with antimicrobial solutions helps to decrease or completely eliminate microorganisms from the root canal system [1]. Incomplete elimination of microorganisms from the root canals may lead to the persistence or survival of the microorganisms in the complex root canal system leading to the failure of endodontic treatment. An ideal irrigant destroys bacteria, dissolves necrotic debris, lubricates the root canal and removes the smear layer without irritating healthy tissues [1, 5].

At present, Naocl is the most common root canal irrigant. It is a strong proteolytic substance and provides sufficient antimicrobial effect [5-9]. However, adverse effects of NaOCl have been reported including unpleasant odor and taste, toxicity, possible paresthesia of the mandibular nerve, allergy and an increase in coronal micro leakage of adhesive restorations [7-9]. Occasionally isolated organism from primary endodontic infections is Enterococcus faecalis (E.Faecalis) and also it is the frequently isolated organism from treatment failure cases. Because of its adhesion to dentin and penetration into the dentinal tubules and resistance to the antimicrobial effects of calcium hydroxide (CH), elimination of this microorganism is very difficult, if not impossible [10]. A remedy of great antiseptic powers, prepared from trituration of red, acrid, very poisonous gum, obtained by incising the bark of Myristica sebifera tree. Boericke describes the drug, for the treatment of inflammation of skin, cellular tissue and periosteum. Traumatic infections, Parotitis. Fistulas, Carbuncles. *Specific action in panaritium*. Coppery taste and burning in throat. Tongue white and cracked. Phlegmonous inflammations. Hastens suppuration and shortens its duration. Often does away with use of the knife. Inflammation of middle ear, suppurative stage, Fistula

Triphala [three (tri) fruits (phala)] is a plant-derived composition developed in India; the powder is a combination of three dried plants naming *Terminaliabellerica*, *Terminalia-chebula* and *Emblicaofficinalis* with tannic acid being its principal constituent. It has been used in Indian traditional medicine for treatment of headaches, constipation and hepatic disorders. Initial studies have shown

bacteriostatic or bactericidal effect of tannic acid on gram-positive and gram-negative pathogens [16]. Compared to commonly used root canal irrigants, it is safe and is composed of compounds with proper physiologic effects in addition to its anti-oxidative and anti-inflammatory properties. The most important advantages of Triphala include easy access, low cost, long-term substantively, less toxicity and absence of microbial resistance. The present experimental study was designed to evaluate and compare the antibacterial activity of Triphala, myrestica sebifera, 3% and 5.25% concentrations of NaOCl against *E. faecalis*.

MATERIALS AND METHODS:

The present in-vitro study was conducted at Department of Conservative Dentistry and Endodontics and Department of Microbiology, sharad pawar dental college Sawangi in 2016. Twenty agar plates were prepared by using Brain Heart Infusion (BHI) in 90mm diameter Petri dishes. These petri dishes were incubated at 37 ˚C for overnight before use to verify that they had remained sterile. A suspension of pure culture of E. faecalis –American type culture collection (ATCC) 29212 was prepared by adding colonies from pure culture of E.Faecalis to freshly prepared BHI broth and incubated for overnight. To adjust the turbidity of broth 0.5 McFarland standard was used as a reference. The antimicrobial sensitivity was performed by agar well diffusion method. Twenty BHI agar plates with single well having the diameter of 6 mm and dept 4mm were prepared .These plates were inoculated with BHI Broth culture of E. faecalis with sterile cotton swabs to provide an even lawn culture . 10 ml of each test solution 5.25 % sodium hypochlorite, 3%sodium hypochlorite, triphala, myrestica sebifera solutions respectively and normal saline was used as control in one of the well. BHI agar plates were marked on the bottom of the plate to identify the irrigating solution. Twenty BHI agar plates were incubated at 37˚C for 24hrs. After 24hrs, microbial zones of inhibition were measured across the diameter in millimeters (mm) with a pair of Vernier Calipers.

Statistical Analysis:

Data was entered in Microsoft Excel and analyzed using SPSS 18.0. Data was described using mean (standard deviation) and median and their 95% Confidence Intervals. 95% Confidence Intervals were obtained by using bootstrapping technique as sample size was very small. As mean and median values were almost similar, parametric test was used. One-way ANOVA was used to test null hypothesis that whether mean inhibition zones of antibiotics differed significantly. Tukey's *post-hoc* test was used to find out which pairs differed significantly from one another contributing to significance of ANOVA. P-value <0.05 was used as level of significance.

RESULTS:

In the present study, to evaluate the antimicrobial efficacy of antibiotics as root canal irrigating solutions, four irrigating solutions were evaluated. *Enterococcus faecalis* was inoculated on agar and the irrigating solutions were pipetted. After incubation, inhibition zones were measured. Table 1 depicts the readings of inhibition zones for different antibiotics tested in the present study.

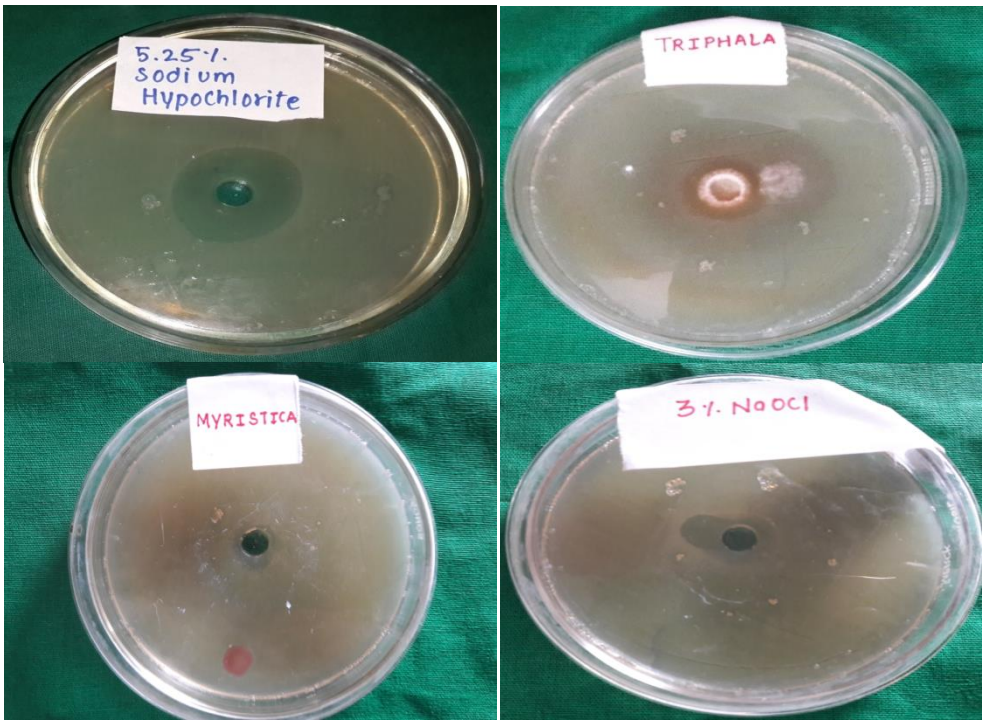

Table 1: Inhibition zones (in mm) for different irrigating solutions

Irrigating solutions	3% Sodium hypochlorite	*Triphala*	5% Sodium hypochlorite	MS
Inhibition zones in mm	13.0	16.0	30.0	5.0
	14.0	18.0	24.0	6.0
	15.0	21.0	25.0	6.0
	15.0	18.0	25.0	7.0
	13.0	17.0	25.0	5.0

Table 2 depicts the descriptive statistics of the above readings. Among the four irrigating solutions, 5% Sodium hypochlorite had larger mean inhibition zones 25.8 mm (24.4-28.0) followed by Triphala 18.0 (16.6-19.6) followed by 3% Sodium hypochlorite 14.0 (13.2-14.8) respectively. Myrestica sebifera had least inhibition zone among all the irrigating solutions tested. The median values do not differ significantly from mean values and hence parametric tests can be used. 95% confidence intervals were generated by bootstrapping technique since sample size was very small.

Table 2: Mean and median inhibition zones for all the solutions along with their 95% CI

Irrigating solutions	Mean	Standard deviation	95% Confidence interval	Median	95% Confidence interval
3% Sodium hypochlorite	14.0	1.0	13.2-14.8	14.0	13.0-15.0
Triphala	18.0	1.8	16.6-19.6	18.0	16.0-21.0
5% Sodium hypochlorite	25.8	2.4	24.4-28.0	25.0	24.0-30.0
MS	7.8	2.8	5.8-9.8	7.0	5.0-12.0

Null hypothesis that there is no difference between the mean inhibition zones of different irrigating solutions was tested by one-way ANOVA. P-value was less than 0.001 signifying that inhibition zones of one or more antibiotics are different (Table 3).

To identify the irrigating solutions pairs whose inhibition zones differ significantly, Tukey's HSD post-hoc test was used. Table 4 summarizes post-hoc pair wise comparison. $C_{critical}$ for p=0.01, k=6, v=24 is 5.3738 and $Q_{critical}$ for p=0.05, k=6, v=24 is 4.3729.

Table 3: Tukey's HSD test

Irrigating solution pair	**Tukey HSD Q statistic**	**Tukey HSD p-value**	**Tukey HSD Inference**
3% NaOCl Vs Triphala	2.7802	0.390943	Insignificant
3% NaOCl Vs 5% NaOCl	8.2016	0.001005	p<0.01
3% NaOCl Vs MS	4.3093	0.055085	significant
Triphala Vs 5% NaOCl	5.4214	0.009233	p<0.01
Triphala Vs MS	7.0895	0.001005	p<0.01
5% NaOCl vs. MS	12.5109	0.001005	p<0.01

Inhibition zone of 5% Sodium hypochlorite was found to be significantly larger than that of 3% Sodium hypochlorite, *Triphala* and MS (p<0.01). But inhibition zone of 3% Sodium hypochlorite was not significantly different than that of Triphala and MS (p>0.05).

DISCUSSION

This experimental study compared the antibacterial properties of an herbal endodontic irrigation (Triphala) and homeopathic drug myrestica sebiferia with different concentrations of NaOCl on *E. faecalis* and showed that the antimicrobial properties of Triphala and 3% and 5% NaOCl were comparable. The principal aim of endodontic treatment is to prevent or eliminate microbial contamination of the root canal system [1, 2], and the main reason for the majority of treatment failures is persistence of infections within these spaces. Although mechanical instrumentation and use of irrigation solutions with strong antimicrobial properties eliminate the majority of intracanal microorganisms, it has been demonstrated that it is not possible to completely eliminate microorganisms [6, 7, 10]. On the other hand, some microorganisms are resistant to antimicrobial agents used within the root canal [4]. *E. faecalis* is a gram-positive facultative anaerobic microorganism, which has been isolated from almost 38% of teeth with failed endodontic treatment [17]. It is resistant to CH which is the most commonly used intracanal antimicrobial agent [4, 10]. This microorganism has been used in a large number of studies for the evaluation of antimicrobial properties

due to its role in retreatment failure. *E. faecalis* can survive even in obturated canals without support from other microorganisms or with very small amounts of nutrients [17].

NaOCl is currently the most commonly used intracanal irrigation solution at various concentrations [9, 11]. It has a broad antimicrobial activity against endodontic microorganisms and biofilms, including difficult-to-eliminate species like *Enterococci*, *Acitinomycetes* and *Candida albicans* [5, 18]. It is demonstrated that 2.5% NaOCl can reduce the intracanal bacteria by 90% [19]. In another study 5.25% NaOCl displayed the most efficient antibacterial action and had significantly greater substantively at different time intervals [20]. Studies evaluating cytotoxicity of NaOCl have shown higher cytotoxicity and caustic effects of 5.25% NaOCl compared to its 3% and 1% concentrations on healthy tissues [9, 11]. In many countries concerns about the chemical and toxic effects of the solution have resulted in the use of 0.5 and 1% concentrations of NaOCl as an intracanal irrigation solution instead of 5.25% concentration [6, 8, 21].

In recent years, there has been an increased tendency to use plant-derived alternative irrigation solutions with pharmaceutical properties. Previous studies regarding the comparison of antimicrobial activities of Triphala and NaOCl have used 0.5 and 1% concentrations of NaOCl [11, 14]. In the present study concentrations of 3% and 5% NaOCl were used because studies have shown no significant differences in the antimicrobial activity between 1 and 5% NaOCl solutions [11, 22].

Myristica sebifera mother tincture has no direct anti-microbial action E.Faecalis microbe. Homeopathic drugs have no direct antibacterial efficacy against E.Faecalis organism, however further trials need to be conducted. The results of the present study showed higher antimicrobial activity of Triphala compared to 3% NaOCl and that it can be used as an appropriate irrigation solution in Endodontics given the advantages of natural medications and the disadvantages of NaOCl. This claim needs more investigation. Another difference between this study and previous studies is the fact that previous studies have not used the biofilms of *E. faecalis*. Biofilms are more resistant to antibacterial agents compared to planktonic bacteria [17]. Therefore, conducting similar studies with biofilms *of E. faecalis* is suggested in order to compare the antimicrobial activity of Triphala with lower concentrations of NaOCl solution.

CONCLUSION

Under the circumstances of this *in vitro* study, Triphala was more effective on cultures of *E. faecalis* compared to 3%NaOCl and Myrestica Sebifera.

REFERENCES

1. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endodontic topics. 2005 Mar 1; 10(1):77-102.
2. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. European Journal of Oral Sciences. 1981 Aug 1; 89(4):321-8.
3. 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. International endodontic journal. 1997 Sep 1; 30(5):297-306.
4. Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. International endodontic journal. 2003 Jan 1; 36(1):1-1.
5. Rahimi S, Janani M, Lotfi M, Shahi S, Aghbali A, Pakdel MV, Milani AS, Ghasemi N. A review of antibacterial agents in endodontic treatment. Iranian endodontic journal. 2014 Jun 29; 9(3):161-8.
6. Barbosa SV, Safavi KE, Spångberg LS. Influence of sodium hypochlorite on the permeability and structure of cervical human dentine. International Endodontic Journal. 1994 Nov 1; 27(6):309-12.
7. Gernhardt CR, Eppendorf K, Kozlowski A, Brandt M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. International Endodontic Journal. 2004 Apr 1; 37(4):272-80.
8. Serper A, Özbek M, Çalt S. Accidental sodium hypochlorite-induced skin injury during endodontic treatment. Journal of endodontics. 2004 Mar 31; 30(3):180-1.
9. Ehrich DG, Brian JD, Walker WA. Sodium hypochlorite accident: inadvertent injection into the maxillary sinus. Journal of endodontics. 1993 Apr 1; 19(4):180-2.
10. Kayaoglu G, Ørstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Critical Reviews in Oral Biology & Medicine. 2004 Sep; 15(5):308-20.
11. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate: an in vitro study. Journal of endodontics. 2010 Jan 31; 36(1):83-6.
12. de Almeida Gomes BP, Vianna ME, Matsumoto CU, Zaia AA, Ferraz CC, de Souza Filho FJ. Disinfection of gutta-percha cones with chlorhexidine and sodium hypochlorite. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2005 Oct 31; 100(4):512-7.
13. Park JB, Park NH. Effect of chlorhexidine on the in vitro and in vivo herpes simplex virus infection.

Oral surgery, oral medicine, oral pathology. 1989 Feb 1; 67(2):149-53.
14. Pujar M, Patil C, Kadam A. Comparison of antimicrobial efficacy of Triphala,(GTP) Green tea polyphenols and 3% of sodium hypochlorite on Enterococcus faecalis biofilms formed on tooth substrate: in vitro. Journal of International Oral Health. 2011 Apr 1; 3(2).
15. Jagadish L, Kumar VA, Kaviyarasan V. Effect of Triphala on dental bio-film. Indian Journal of Science and Technology. 2009 Jan 1; 2(1):30-3.
16. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation triphala and its constituents. International Journal of Pharmacognosy. 1997 Jan 1; 35(5):313-7.
17. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1998 Jan 1; 85(1):86-93.
18. Siqueira JF, Machado AG, Silveira RM, Lopes HP, Uzeda MD. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. International Endodontic Journal. 1997 Jul 1; 30(4):279-82.
19. Abbaszadegan A, Khayat A, Motamedifar M. Comparison of antimicrobial efficacy of IKI and NaOCl irrigants in infected root canals: An in vivo study. Iranian endodontic journal. 2010 Oct 18; 5(3):101-6.
20. Sharifian MR, Shokouhinejad N, Aligholi M, Emaneini M, Alizadeh J. Antibacterial substantivity of Carvacrol and sodium hypochlorite in infected bovine root dentin. Iranian endodontic journal. 2009 Oct 27; 4(2):45-8.
21. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, Drucker DB. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. International Endodontic Journal. 2004 Jul 1; 37(7):438-46.
22. Pataky L, Iványi I, Grigár Á, Fazekas Á. Antimicrobial efficacy of various root canal preparation techniques: an in vitro comparative study. Journal of endodontics. 2002 Aug 31; 28(8):603-5.