J. Int Oral Health 2011

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.

Madhu Pujar* Chetan Patil† and Ajay Kadam‡

*M.D.S, Professor and Head, †M.D.S, Asst Professor, ‡Post Graduate Student, Department of conservative dentistry and endodontics, MMNGH institute of dental sciences and research centre, Belgaum, Karnataka.
Contact: ajjukaddy@gmail.com.

Abstract:

Objective: To evaluate antimicrobial efficacy of Triphala, Green tea polyphenols (GTP) and 3% of sodium hypochlorite against *E. faecalis* biofilm formed on tooth substrate. Method: Human extracted teeth were biomechanically prepared, vertically sectioned and placed in wells containing *E. faecalis* to form a biofilm. After 2 weeks all groups were treated for 10 minutes with test solutions (Triphala, GTP, 3% of sodium hypochlorite and saline) and were analysed quantitatively. Results: Quantitative analysis showed complete inhibition of bacterial growth with 3% of sodium hypochlorite. Groups treated with Triphala, GTP and saline showed 2.3±0.59 × 104 CFU/ml, 3.8±0.79 × 104 CFU/ml and 9.90±0.52 bacterial growth respectively. Conclusion: Sodium hypochlorite has shown maximum antibacterial activity against *E. faecalis* biofilm formed in tooth substrate. Triphala and GTP have shown significantly better antibacterial activity. Herbal alternatives can be used as root canal irrigants, considering the undesirable effects of sodium hypochlorite.

P- ISSN
0976 – 7428

E- ISSN
0976 – 1799

Journal of International Oral Health

Conservative & Endodontics

Original Research

Received: Dec, 2010
Accepted: Feb, 2011

Bibliographic listing:
EBSCO Publishing
Database, Index Copernicus, Genamics Journalseek Database, Proquest, Open J Gate.

Key words: Polyphenols, Sodium hypochlorite, biofilm, Enterococcus faecalis.

Introduction:

Primary root canal infection is caused by microorganisms colonizing the necrotic pulp tissue.[1] Primary endodontic infections, which are polymicrobial in nature and dominated by gram-negative anaerobic rods, the microorganisms involved in secondary infections are composed of one or a few bacterial species. *E. faecalis* is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora[2-5]. Moreover it is found as major component of the flora of endodontically treated teeth with chronic apical periodontitis[5,6]

E. faecalis can survive in harsh conditions such as well instrumented and obturated root canals alone with scanty available nutrients also[2,7]. Its mode of growth is through biofilm formation[1]. Biofilms consist of one or more communities of microorganisms, embedded in a glycocalyx, that are attached to a solid surface. The reason for the existence of a biofilm is that it allows microorganisms to stick to and to multiply on surfaces. Biofilms facilitate processing and uptake of nutrients, as well as the development of an appropriate physicochemical environment[8].

Total cleaning of the root canal system using mechanical instrumentation is ineffective due to extremely complex root canal anatomy. Irrigants serve several purposes, including tissue solvent, disinfection, flushing of gross debris and lubrication. Proper irrigation of the root canal system during endodontic treatment is essential for successful treatment[9-11].

Sodium hypochlorite (NaOCl) has been widely used as an irrigant since its introduction in endodontics by Walker in 1936[12]. Sodium hypochlorite solutions require careful handling and several factors are associated with its safety concerns[13]. The main disadvantages of sodium hypochlorite are unpleasant taste, high toxixity, corrosive to instruments[14], inability to remove smear layer[15] and reduction in elastic modulus and flexural strength of dentin[16].

Due to delirious effects of synthetic drugs on teeth, it has prompted to look for herbal drugs which have no delirious effects as such. Triphala (Zandu pharmaceuticals) is an Indian ayurvedic herbal formulation consisting of dried and powdered fruits of three medicinal plants *Terminalia bellerica*, *Terminalia chebula*, and *Emblica officinalis*[17]. It has a potential of antibacterial activity against enteric pathogens[18], and also have anti-inflammatory activity[19].

Green tea polyphenols(GTPs, Ambe Phytoextracts) is the traditional and most widely consumed beverage of China and Japan, obtained from the young shoots of tea plant *Camellia sinensis*[20]. The cathechins and the flavins are considered as microbiologically active ingredients[21].

The purpose of this in vitro study was to evaluate the antimicrobial efficacy of Triphala (Zandu pharmaceuticals), Green tea polyphenols,(Ambe Phytoextracts) and 3% sodium hypochlorite against E.faecalis biofilm formed on tooth substrate of extracted human teeth.

Materials and Methods:

A pure culture of E. faecalis was grown on brain heart infusion (BHI, Himedia, Mumbai, India), inoculated into brain heart infusion broth (Himedia, Mumbai, India) incubated at 37°C overnight (fig.2). Triphala and GTP powders were made into a solution by dissolving them in 10% dimethyl sulfoxide (DMSO) (S.D. Fine Chem Pvt Ltd, Mumbai, India).

Biofilm Formation on Tooth Substrate:

Single-rooted human mandibular premolars with fully formed apices were used in this study. The teeth were cleaned of superficial

debris, calculus, and tissue tags and stored in normal saline. The tooth specimens were sectioned below the cementoenamel junction with a diamond disc to obtain a standardized tooth length of 8 mm for uniform specimen. The root canals were then instrumented using the step-back technique with hand instruments and the canals were enlarged to an apical size 40 k-file. Two milliliters of 3% NaOCl was used between each instrument during the cleaning and shaping procedure. All the teeth were then vertically sectioned along the midsagittal plane into two halves. The concave tooth surface was minimally grounded to achieve a flat surface to enable placement in the tissue culture wells, exposing the root canal surface to *E. faecalis* to form a biofilm.

The sectioned samples were then divided into four experimental groups. Each group consisted of 10 samples each and assigned as, group 1 - Triphala, group 2 - GTPs, group 3 - 3% sodium hypochlorite, and group 4 - normal saline (control). The samples were placed in the wells of culture plates. Wells containing tooth samples were inoculated with 2 ml of bacterial solution and incubated at 37°C for 2 weeks.

At the end of the second week, all groups were treated for 10 minutes as follows: group 1, immersed in 3 mL of Triphala (60 mg/mL in 10% DMSO); group 2, immersed in 3 mL of GTP (60 mg/mL in 10% DMSO); group 3, immersed in 3 mL of 3% NaOCl; and group 4: immersed in 3 mL sterile saline. Then, the biofilm on the root canal portion was scraped and inoculated on BHI plates and incubated for 24 hours at 37°C. The quantitative analysis was performed by serial dilution method for all the groups (figs.3,4,5,6).

Statistical Analysis:

Statistical analysis was performed by using one-way analysis of variance and compared by the Post hoc Tukey test. The criterion for statistical significance was defined as $p < 0.05$.

Results:

Table 1: bacterial count from each sample (bacterial count in CFU/ml)

TRIPHALA	GREEN TEA	SODIUM HYPOCHLORIDE	SALINE
1.5×104	5×104	0	1.05×105
2×104	4×104	0	1×105
1.5×104	3×104	0	1×105
2×104	3×104	0	8.5×104
2×104	4×104	0	1×105
2.5×104	5×104	0	1×105
3×104	4×104	0	1×105
3×104	4×104	0	1×105
2.5×104	3×104	0	1×105
3×104	3×104	0	1×105

Table 2: Mean and standard deviation of each group (bacterial count in CFU/ml)

	TRIPHALA	GREEN TEA	SODIUM HYPOCHLORIDE	SALINE
MEAN*	2.30×104	3.80×104	0.00	9.90×105
S.D.*	±0.59×104	±0.79×104	0.00	±0.52×105

***comparison by post hoc Tukey test and data analyzed by ANOVA (p<0.05)**

Figure1: Test solutions: Triphala and GTP

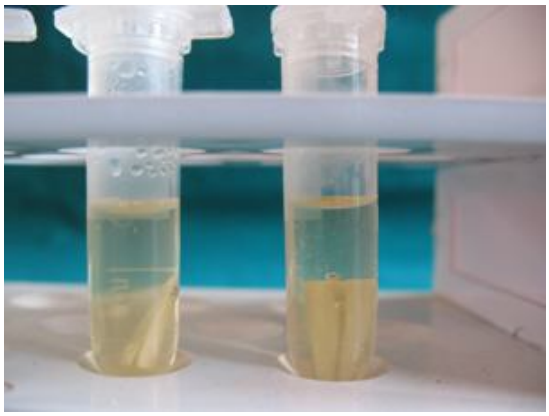

Figure2: Extracted teeth in culture wells containing *E. faecalis*

Figure3: Bacterial growth on samples treated with Triphala

Figure4: Bacterial growth on samples treated with GTP

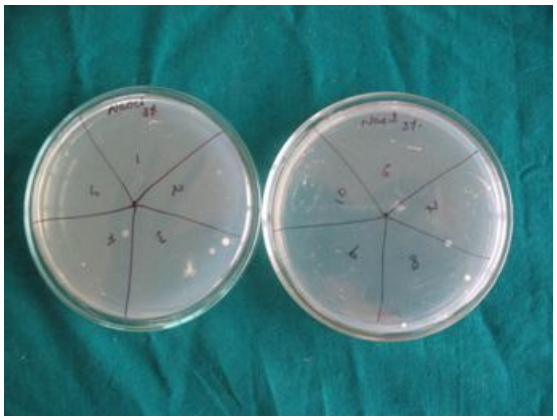

Figure5: Bacterial growth on samples treated with NaOCl

Figure6: Bacterial growth on samples treated with Saline

Table 1 shows bacterial growth in all the groups. The bacterial count was maximum in the group treated with normal saline where as NaOCl group showed no growth.

Table 2 shows the mean and standard deviation of all the groups treated on a 2-week biofilm. NaOCl showed the maximum zone of inhibition. In quantative analysis, samples treated with Triphala and green tea showed, 2.3±0.59 × 104 CFU/ml and 3.8±0.79 × 104 CFU/ml (mean± standard deviation), respectively. The groups statistical differed from each other ($p<0.05$)

Discussion:

E. faecalis is the most common *Enterococcus species* recovered from root filled teeth with chronic apical periodontitis[2,4,22]. The resistance of these microorganisms is increased by formation of biofilm. It has shown that the antibiotic resistance is thousand times higher when there is a biofilm formation as compared to that of planktonic cells[8,23].

The ability of *E. faecalis* to form calcified biofilm on root canal dentin may be a factor that contributes to their persistence after endodontic treatment. the biofilm-forming capacity and its structural organization are influenced by the chemical nature of the substrate[8]. Biofilm experiments conducted on polycarbonate or glass substrate will not provide a true indication of the bacteria-substrate interaction[24]. Hence, *E. faecalis* biofilm was formed on a tooth substrate in this study. All the groups were tested in direct contact with the biofilm formed on tooth substrate.

A recent study reported that NaOCl was capable of eradicating *E.faecalis* biofilm after 1 minute at a concentration of 0.00625%[25] that was grown in the Calgary biofilm forming device. But the same concentration may not be effective on biofilm formed on tooth substrate.

DMSO was used as a solvent for Triphala and GTP. DMSO is a clean, safe, highly polar, aprotic solvent that helps in bringing out the pure properties of all the components of the herb being dissolved[26].

Triphala showed more potency on E. faecalis biofilm. This may be attributed to its formulation, which contains three different medicinal plants in equal proportions. Tannic acid represents the major constituent of the ripe fruit of T .chebula, T.belerica and E. officinalis. Earlier studies reported that tannic acid is bacteriostatic or bactericidal to some gram positive and gram negative pathogens[27].

3% NaOCl was best among all the groups. It exhibited excellent antibacterial activity in 2-week biofilm. But NaOCl is very caustic and nonspecific agent, and also has deleterious effects on dentine like reduction of the elastic modulus and the flexural strength[12,14,28].

Triphala and GTPs are proven to be safe, containing active constituents that have beneficial physiologic effect apart from its curative property such as antioxidant, anti-inflammatory, and radical scavenging activity and may have an added advantage over the traditional root canal irrigants[19,29].

The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity, and lack of microbial resistance reported so far[30].

Conclusion:

Within the limitations of this study, 3% sodium hypochlorite showed maximum antibacterial activity against 2-week *E. faecalis* biofilm formed on tooth substrate. Triphala and GTPs showed significantly better antibacterial activity against 2-week biofilm. The use of herbal alternatives as a root canal irrigant might prove to be advantageous considering the several undesirable characteristics of NaOCl. Further research is needed to conclusively recommend herbal solutions as a root canal irrigant.

Acknowledgement:
I will be thankful to Dr. Kishor Bhat, department of microbiology, Maratha Mandals institute of dental sciences and research centre.

References:

1. Tronstad L, Sunde PT. The evolving new understanding of endodontic infections. Endod Topics 2003; 6: 57-77
2. 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: 93-8.
3. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Path Oral Radiol Endo. 1998; 85: 86-93.
4. Pinheiro ET, Gomes BPFA, Ferraz CC, Ferraz CCR, Sousa ELR, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J. 2003; 36: 1-11.
5. Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J. 2001; 34: 429-34.
6. Fouad AF, Zerella J, Barry J, Spangberg LS. Molecular detection of Enterococcus species in root canals of therapy-resistant endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005; 99: 112-8.
7. Love RM. Enterococcus faecalis-a mechanism for its role in endodontic failure. Int Endod J. 2001; 34: 399-405.
8. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontology 2000. 2002; 28: 12-55.
9. Becker TD, Woollard GW. Endodontic irrigation. Gen Dent. 2001; 44: 272-6.
10. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endod Topics. 2005; 10: 77-102.
11. Zehnder M. Root Canal Irrigants. J Endod. 2006; 32: 389-98.
12. Siqueira JF, 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; 30: 279-82.
13. Clarkson RM, Moule AJ. Sodium hypochlorite and its use as an endodontic irrigant. Aus Dent J. 1998; 43: 4.
14. Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J. 2008; 58: 329-41.
15. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod. 1975; 1: 238-42.
16. Sim TPC, Knowles JC, Ng Y-L, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int Endod J. 2001; 34: 120-32.
17. Jagetia GC, Baliga MS, Malagi KJ, Sethukumar KM. The evaluation of the radioprotective effect of Triphala (an Ayurvedic rejuvenating drug) in the mice exposed to radiation. Phytomedicine. 2002; 9: 99-108.
18. Tambekar DH, Dahikar SB, Lahare MD. Antibacterial potentials of some herbal preparations available in India. Res J Med Medicinal Sci. 2009; 4: 224-7
19. Sumantran VN *et al*. Hyaluronidase and collagenase inhibitory activities of the herbal formulation Triphala guggulu. J Biosci. 2007; 32: 755-61.
20. Hamilton-Miller JM. Anti-cariogenic properties of tea (Camellia sinensis). J Med Microbiol. 2001; 50: 299-302.
21. Smullen J, Koutsou GA, Foster HA, Zumbé A, Storey DM. The antibacterial activity of plant extracts containing polyphenols

against Streptococcus mutans. Caries Res. 2007; 41: 342-9.
22. de Paz LC. Redefining the persistent infection in root canals: possible role of biofilm communities. J Endod. 2007; 33: 652-62.
23. Mah TFC, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001; 9: 34-9.
24. Carlos, Blitzkow G, Poli JA, de Araujo CR. A model system to study antimicrobial strategies in endodontic biofilms. J Appl Oral Sci. 2009; 17: 87-91.
25. Arias-Moliz MT, Ferrer-Luque CM, Espigares-Garcia M, et al. Enterococcus faecalis biofilms eradication by root canal irrigants. J Endod 2009; 35: 711-4.
26. de la Torre JC. Biological actions and medical applications of dimethyl sulfoxide. Ann NY Acad Sci. 1983; 411: 1-403.
27. Jagadish L, Anand Kumar VK, Kaviyarasan V. Effect of Triphala on dental bio-film. Indian J Sci Technol. 2009; 2: 30-3.
28. Grigoratos D, Knowles J, Ng Y-L, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. Int Endod J 2001; 34: 113-9.
29. Naik GH, Priyadarsini KI, Bhagirathi RG, Mishra B, Mishra KP, Banavalikar MM, Hari M. *In vitro* antioxidant studies and free radical reactions of triphala, an ayurvedic formulation and its constituents. Phyto Res. 2005; 19: 582-6.
30. Abascal K, Yarnell E. Herbs and drug resistance. Part 1-clinical implications of research on microbial resistance to antibiotics. Altern Complementary Therapies. 2002; 8: 237-41.

Source of Support: Nil

Conflict of Interest: No Financial Conflict