

A Comparative Evaluation of Antimicrobial Activity of Septilin, Calcium Hydroxide and 2 % Chlorhexidine as an Intracanal Medicament - An In Vitro Study

Akshay Khandelwal¹, Ajitha Palanivelu²

¹Department of Conservative and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute Of Medical and Technical Sciences Chennai, Tamil Nadu, India. ²Department of Conservative and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences Chennai, Tamil Nadu, India.

ABSTRACT

BACKGROUND

One of the most important objectives of root canal treatment is elimination of microorganisms in the root canals. The use of intra-canal medicament may be helpful in eliminating remaining bacteria that survived inside the root canals after complete chemo-mechanical preparation. This study aims to evaluate and compare the antimicrobial activity of Septilin, chlorhexidine and calcium hydroxide against *Enterococcus faecalis* and *Streptococcus mutans*. Intra-canal medicament helps in eliminating remaining bacteria that survived inside root canals after complete chemo-mechanical preparation.

METHODS

The well variant of agar diffusion test using brain heart infusion agar was used for evaluating the antimicrobial activity of the intra-canal medicaments. McFarland 0.5 turbidity standard was taken as reference to adjust the turbidity of bacterial suspensions. The wells were prepared and filled with of Septilin, chlorhexidine and calcium hydroxide. This was done for both the test organisms and plates were incubated in an incubator for 24 hrs. at 37 °C. After incubation, antimicrobial effectiveness was determined using digital caliper (mm) by measuring zone of inhibition.

RESULTS

The mean zone of inhibition for chlorhexidine, calcium hydroxide, and Septilin were 29.50 ± 0.58 , 25.00 ± 0.00 , 20.00 ± 0.82 for *E. faecalis* and 28.50 ± 1.00 , 24.25 ± 0.96 , 19.50 ± 1.29 for *S. mutans* respectively.

CONCLUSIONS

Septilin showed significant inhibition against *E. faecalis* and *S. mutans* but its antibacterial activity is less compared to that of calcium hydroxide and chlorhexidine. Moreover, the dark colour of the material might cause tooth discoloration.

KEY WORDS

Septilin, Antimicrobial Efficacy, Intracanal Medicaments, *Enterococcus faecalis*, *Streptococcus mutans*.

Corresponding Author:

Dr. Ajitha Palanivelu.

Department of Conservative and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, 162, Poonamallee High Road, Chennai - 600077, Tamil Nadu, India., E-mail: ajitharijesh@gmail.com

DOI: 10.14260/jemds/2020/581

How to Cite This Article:

Khandelwal A, Palanivelu A. A comparative evaluation of antimicrobial activity of septilin, calcium hydroxide and 2% chlorhexidine as an intracanal medicament- an in vitro study. J Evolution Med Dent Sci 2020;9(37):2670-2673, DOI: 10.14260/jemds/2020/581

Submission 28-05-2020,

Peer Review 28-07-2020,

Acceptance 04-08-2020,

Published 14-09-2020.

Copyright © 2020 JEMDS. This is an open access article distributed under Creative Commons Attribution License [Attribution 4.0 International (CC BY 4.0)]

BACKGROUND

The most important objective of root canal treatment is elimination of microorganisms in the root canals. One of the major factors associated with endodontic failure is persistence of microbial infection in root canal system and peri-radicular area.¹ The chemo-mechanical preparation of root canals reduces the bacterial count to a good extent but in case of severely infected root canal system with pain and exudate an intra-canal medicament with antibacterial action is required to maximize the disinfection.^{2,3} Intra - canal medicament helps in eliminating remaining bacteria that survived inside root canals after complete chemo-mechanical preparation.

Streptococcus mutans is a facultative anaerobe, gram - positive coccus commonly found in the oral cavity and is a one of the main cause of dental caries.⁽⁴⁾ Facultative anaerobic bacteria *Enterococcus faecalis* is considered to be the most resistant species in oral cavity, and a possible cause of root canal treatment failure.⁵ Prevalence of *Enterococcus faecalis* in primary endodontic infection is 40 % and persistent Endodontic infection is 24 to 77 %.⁶

Calcium hydroxide was introduced in 1920⁷ and is the most commonly used intracanal medicament. It has a various biological properties like antimicrobial activity,⁸ tissue dissolving ability,⁹ inhibition of tooth resorption¹⁰ and induction of repair by hard tissue formation.¹¹ However, calcium hydroxide fails to effectively eliminate enterococci from root canals¹² and its long-term use as intracanal medicament tends to reduce dentinal strength.¹³ The antimicrobial effect of Chlorhexidine is related to the cationic molecule binding to negatively charged bacterial cell walls, thereby altering the cell's osmotic equilibrium.¹⁴ When used as an intracanal medicament, chlorhexidine was more effective than calcium hydroxide against *Enterococcus faecalis* infection in dentinal tubules.¹⁵ Although synthetic chemical medicaments such as calcium hydroxide and chlorhexidine are commonly used, development of antibiotic-resistant strains and the side effects associated with it have directed the researchers to look for the use of herbal alternatives for root canal disinfection.¹⁶ Compared to conventional drugs herbal alternatives are easily available, cost effective, have better shelf life, less toxic and lack of microbial resistance reported so far.¹⁷

Septilin is a herbal preparation by The Himalaya Drug Company, Bangalore, India. It contains powders of *Balsamodendron mukul* and shankha bhasma, and extracts of Maharasnadi kwath, *Tinospora cordifolia*, *Rubia cordifolia*, *Emblia officinalis*, *Moringa pterygosperma* and *Glycyrrhiza glabra*.¹⁸ It has been reported to possess antibacterial,¹⁹ anti-inflammatory,²⁰ and wound healing properties.²¹ It is said to be helpful in treating Gram - positive as well as Gram-negative infections.^{22,23}

This study aims to evaluate and compare the antimicrobial effect of Septilin, chlorhexidine and calcium hydroxide against *Streptococcus mutans* and *Enterococcus faecalis*.

METHODS

This is an in vitro study carried out in the microbiology department of our institution. Ethical approval was obtained

from the Institutional Review, [IHEC Ref. No. - IHEC/SDC-ENDO-1712/20/156].

Medicaments Included

- Calcium hydroxide.
- Chlorhexidine digluconate (0.2 % gel) (CHX).
- Septilin.

Micro-Organisms

- E. faecalis*.
- S. mutans*.

Extract Preparation

Septilin (net weight: 452 mg; batch number - E 281004; manufacture date: 10 / 08 / 2017; expiry date: 30 / 04 / 2020) in tablet form was crushed by means of a sonicator then diluted in 35 mL of distilled water. The sample was incubated on a shaker for 1 hour at ambient temperature. The sample was then centrifuged at 40,000 rpm for 10 mins. After that it was filtered using 0.50 nm sterilized filters and stored in 1 mL aliquots at - 80 degree Celsius.

Experimental Procedure

Micro-organisms were maintained in brain heart infusion (BHI) broth. Turbidity of the inoculum, prepared in BHI, was adjusted to the turbidity of a 0.5 McFarland Standard (1.5 x 10⁸ bacteria / mL).

The agar diffusion test was used. Petri plates containing BHI agar supplemented with hemin and menadione were inoculated with the micro - organisms to be tested using sterile cotton - tipped applicators that were brushed across the medium. Four wells of 5 mm depth and 6 mm diameter were punched in each agar plate and filled with the medicaments to be tested. The bacteria agar plates were placed into anaerobic jars. Anaerobic conditions were produced by the evacuation-replacement procedure, in which the air in the jar is removed using a vacuum pump and replaced with a mixture containing 10 % H₂ and 10 % CO₂ in nitrogen. The jars were incubated at 37 ° C for 1 day. Afterward, the diameters of the zones of bacterial inhibition were measured and recorded for each material tested.

Statistics

Data was entered in Microsoft Excel spread sheet and analysed using SPSS software (version 19). Descriptive statistics were used. The data were statistically analysed with one-way analysis of variance followed by Tukey multiple comparison means to check the difference in bacterial inhibition between groups (p < 0.01). For significance level, a p value of < 0.05 was considered statistically significant.

RESULTS

The means of the zones of bacterial inhibition for each medicament for the two bacterial strains are presented in

Table 1. Chlorhexidine showed large zones of inhibition against *E. faecalis* and *S. mutans* with mean diameter 29.50 and 28.50 mm respectively. Calcium hydroxide showed zone of inhibition with mean diameter of 25.00 and 24.25 mm for *E. faecalis* and *S. mutans*. Septilin showed zone of inhibition with mean diameter of 20.00 and 19.50 mm for *E. faecalis* and *S. mutans*. Chlorhexidine showed the most antibacterial activity followed by calcium hydroxide followed by Septilin.

The mean difference in the diameter of zone of inhibition between the groups is presented in Table 2, comparing the antibacterial activity of the respective groups. There was a significant difference between all the three groups for both the bacterial strain.

Bacteria	Groups	Mean	Std. Deviation	P-Value
<i>E. faecalis</i>	CHX	29.5000	.57735	0.000
	Calcium Hydroxide	25.0000	.00000	
	Septilin	20.0000	.81650	
<i>S. mutans</i>	CHX	28.50000	1.00000	0.000
	Calcium Hydroxide	24.2500	.95743	
	Septilin	19.5000	1.29099	

Table 1. Mean Diameters of Zones of Inhibition (mm)

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Sig.
<i>E. faecalis</i>	CHX	Calcium Hydroxide	4.50000*	.000
		Septilin	9.50000*	.000
	Calcium Hydroxide	CHX	-4.50000*	.000
		Septilin	5.00000*	.000
	Septilin	CHX	-9.50000*	.000
		Calcium Hydroxide	-5.00000*	.000
<i>S. mutans</i>	CHX	Calcium Hydroxide	4.25000*	.001
		Septilin	9.00000*	.000
	Calcium Hydroxide	CHX	-4.25000*	.001
		Septilin	4.75000*	.000
	Septilin	CHX	-9.00000*	.000
		Calcium Hydroxide	-4.75000*	.000

Table 2. Comparison of the Antimicrobial Activity (Mean Diameter of Zone of Inhibition) between Different Groups
 *. The mean difference is significant at the 0.05 level

DISCUSSION

This study evaluated the antimicrobial activity of Septilin against *Enterococcus faecalis* and *Streptococcus mutans* and compared it to that of chlorhexidine and calcium hydroxide. Agar well diffusion method was used of the variety of laboratory methods available to evaluate the antimicrobial activity in vitro as it provides direct estimation of antimicrobial activity against a specific microorganism and has added advantages of simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease of results interpretation. Even though there are new technologies in the field of microbiology, agar diffusion is still one of the preliminary tests to assess the antimicrobial activity of a material.²⁴⁻²⁵ To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are recommended, which provide information on the nature of the inhibitory effect whether bactericidal or bacteriostatic, time-dependent or concentration-dependent.

Chlorhexidine showed maximum antibacterial activity followed by calcium hydroxide followed by Septilin. Chlorhexidine showed more antibacterial activity than calcium hydroxide, this result is similar to previous studies done by Krithikadatta et al.²⁶ Gomes et al.¹⁵ and V Ballal et al.²⁷

The antimicrobial action of Septilin might be due to synergistic action of ingredients. *Embllica officinalis* was active against *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella ozaena*, *Proteus mirabilis*, *Serratia marcescens*, *Salmonella paratyphi A* and *B*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. *Rubia cordifolia* was found to be significantly active against *B. subtilis* and *S. aureus* compared with penicillin G and streptomycin.²⁸ Antibacterial and antiviral properties of *Moringa pterygosperma* inhibit the growth of gram-positive and gram-negative bacteria such as *E. coli*, *S.typhi* and *S. paratyphi*.²⁹ although Septilin showed significant zone of inhibition its anti-microbial activity was less compared to calcium hydroxide and chlorhexidine. This result contradicts the results of study done by Priya S et al.³⁰ stating 2 % chlorhexidine and Septilin demonstrated significant inhibition against *E. faecalis* followed Byaloevera, Proplolis and Calcium Hydroxide. Moreover, Septilin has a dark brownish colour which has high chances of causing tooth discolouration if used as an intracanal medicament.

CONCLUSIONS

Septilin showed significant inhibition against *E. faecalis* and *S. mutans* but its antibacterial activity is less compared to that of calcium hydroxide and chlorhexidine. Moreover, dark colour of the material might cause tooth discoloration.

Financial or Other Competing Interests: None.

REFERENCES

- [1] Nair PNR, Sjögren U, Key G, et al. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy- resistant periapical lesions: a long-term light and electron microscopic follow-up study. *J Endod* 1990;16(12):580-8.
- [2] Lee Y, Han SH, Hong SH, et al. Antimicrobial efficacy of a polymeric chlorhexidine release device using in vitro model of *Enterococcus faecalis* dentinal tubule infection. *J Endod* 2008;34(7):855-8.
- [3] Spangberg LSW. Intracanal medication. In: Ingle JJ, Bakland LK, eds. *Endodontics*. 4th edn. Baltimore: Williams & Wilkins 1994:627-40.
- [4] Loesche WJ. Microbiology of dental decay and periodontal disease. Chap- 99. In: Baron S, ed. *Medical microbiology*. 4th edn. Galveston (TX): University of Texas Medical Branch at Galveston 1996.
- [5] Gomes BPFA, Montagner F, Berber VB, et al. Antimicrobial action of intracanal medicaments on external root surface. *J Dent* 2009;37(1):76-81.
- [6] Stuart CH, Schwartz SA, Beeson TJ, et al. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J Endod* 2006;32(2):93-8.
- [7] Hermann BW. Calciumhydroxyd als mittel zum behandein und fullen von zahnwurzelkanalen. Univ Wurzburg Med Dissertation 1920.
- [8] Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the

- treatment of infected root canals. *Endod Dent Traumatol* 1985;1(5):170-5.
- [9] Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. *J Endod* 1988;14(3):125-7.
- [10] Tronstad L. Root resorption--etiology, terminology and clinical manifestations. *Endod Dent Traumatol* 1988;4(6):241-52.
- [11] Foreman PC, Barnes F. A review of calcium hydroxide. *Int Endod J* 1990;23(6):283-97.
- [12] Gomes BP, Drucker DB, Lilley JD. Clinical significance of root canal microflora. *J Dent* 1996;24(1-2):47-55.
- [13] Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol* 2002;18(3):134-7.
- [14] Delany GM, Patterson SS, Miller CH, et al. The effect of chlorhexidine gluconate irrigation on the canal flora of freshly extracted necrotic teeth. *Oral Surg Oral Med Oral Pathol* 1982;53(5):518-23.
- [15] Gomes BPFA, Souza SFC, Ferraz CCR, et al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine in vitro. *Int Endod J* 2003;36(4):267-75.
- [16] Newell CA, Anderson LA, Phillipson JD. *Herbal medicines: a guide for health care professional*. London: Pharmaceutical Press 1996.
- [17] Pujar M, Makandar SD. Herbal usage in endodontics - a review. *Int Journal of Contemporary Dentistry* 2011;2(1):34-7.
- [18] Daswani, BR, Yegnanarayan R. Immunodulatory activity of Septilin, a polyherbal preparation. *Phytother Res* 2002;16(2):162-5.
- [19] Ross DG. The anti-infective and anti-bacterial efficacy of Septilin. *Probe* 1984;23(2):84-7.
- [20] Kumar PV, Kuttan G, Luttan R. Immunomodulatory activity of Septilin. *Probe* 1993;33:1-5.
- [21] Udapa AL, Rao SG, Kulkarni DR. Wound healing profile of Septilin. *Indian J Physiol Pharmacol* 1989;33(1):39-42.
- [22] Gadekar HA, Vijay A, Komawar JV, et al. Septilin in acute tonsillitis in children below 12 years of age. *Probe* 1986;25:164-5.
- [23] Sharma SK, Agarwal HC, Pal D, et al. Septilin in infective dermatoses. *Probe* 1986;25:156-61.
- [24] Gomes BPFA, Pedroso JA, Jacinto RC, et al. In vitro evaluation of the antimicrobial activity of five root canal sealers. *Braz Dent J* 2004;15(1):30-5.
- [25] Gomes BPFA, Vianna ME, Sena NT, et al. In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medicament. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;102(4):544-50.
- [26] Krithikadatta J, Indria 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(12):1473-6.
- [27] Ballal V, Kundabala M, Acharya S, et al. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. *Aust Dent J* 2007;52(2):118-21.
- [28] Basu S, Ghosh A, Hazra B. Evaluation of the antibacterial activity of *Ventilago madraspatana* Gaetrn., *Rubia cordifolia* Linn. and *Lantana camara* Linn.: isolation of emodin and physcion as active antibacterial agents. *Phytotherapy Research* 2005;19(10):888-94.
- [29] Saeed S, Tariq P. Antibacterial activities of *Emblica officinalis* and *coriandrum sativum* against gram negative urinary pathogens. *Pak J Pharma Sci* 2007;20(1):32-5.
- [30] Priya S, Vundavalli RM, Reddy VKK, et al. Dentinal tubule disinfection with 2% chlorhexidine gel, aloe vera, propolis, Septilin and calcium hydroxide. *Int J Med Res Rev* 2016;4(6):950-5.