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

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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.
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-apical area. 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. 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 one of the main cause of dental caries. 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. Prevalence of* Enterococcus faecalis* in primary endodontic infection is 40 % and persistent Endodontic infection is 24 to 77 %. Calcium hydroxide was introduced in 1920 and is the most commonly used intracanal medicament. It has a various biological properties like antimicrobial activity, tissue dissolving ability, inhibition of tooth resorption and induction of repair by hard tissue formation. However, calcium hydroxide fails to effectively eliminate enterococci from root canals and its long-term use as intracanal medicament tends to reduce dentinal strength. 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. When used as an intracanal medicament, chlorhexidine was more effective than calcium hydroxide against* Enterococcus faecalis* infection in dentinal tubules. 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. 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.

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*, *Emblica officinalis*, *Moringa pterygosperma* and *Glycyrrhiza glabra*. It has been reported to possess antibacterial, anti-inflammatory and wound healing properties. It is said to be helpful in treating Gram - positive as well as Gram-negative infections.

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**

a. Calcium hydroxide.

b. Chlorhexidine digluconate (0.2 % gel) (CHX).

c. Septilin.

**Micro-Organisms**

a. *E. faecalis*.

b. *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 % H 2 and 10 % CO 2 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.

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.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Groups</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecalis</em></td>
<td>CHX</td>
<td>29.5000</td>
<td>5.7735</td>
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<td></td>
<td>Calcium Hydroxide</td>
<td>26.0000</td>
<td>0.0000</td>
<td></td>
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<tr>
<td></td>
<td>Septilin</td>
<td>20.0000</td>
<td>8.1650</td>
<td></td>
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<td><em>S. mutans</em></td>
<td>CHX</td>
<td>28.5000</td>
<td>1.0000</td>
<td></td>
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<tr>
<td></td>
<td>Calcium Hydroxide</td>
<td>24.2500</td>
<td>9.5743</td>
<td></td>
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<td></td>
<td>Septilin</td>
<td>19.5000</td>
<td>1.2909</td>
<td>0.000</td>
</tr>
</tbody>
</table>

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

Table 2. Comparison of the Antimicrobial Activity (Mean Diameter of Zone of Inhibition) between Different Groups

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
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<tr>
<td></td>
<td>CHX</td>
<td>Septilin</td>
<td>9.000000</td>
<td>.000</td>
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<tr>
<td></td>
<td>Calcium Hydroxide</td>
<td>CHX</td>
<td>-4.500000</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Calcium Hydroxide</td>
<td>Septilin</td>
<td>5.000000</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Septilin</td>
<td>Calcium Hydroxide</td>
<td>-9.500000</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Calcium Hydroxide</td>
<td>CHX</td>
<td>-5.000000</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Calcium Hydroxide</td>
<td>Septilin</td>
<td>4.250000</td>
<td>.001</td>
</tr>
<tr>
<td><em>S. mutans</em></td>
<td>CHX</td>
<td>Calcium Hydroxide</td>
<td>9.000000</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>CHX</td>
<td>Septilin</td>
<td>-4.250000</td>
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</tbody>
</table>

Table 2. Comparison of the Antimicrobial Activity (Mean Diameter of Zone of Inhibition) between Different Groups

DISCUSSION

This study evaluated the antibacterial 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. To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytometer 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., Gomes et al., and V Ballal et al.

The antimicrobial action of Septilin might be due to synergistic action of ingredients. Emblica officinalis was active against *Escherichia coli*, Klebsiella pneumoniae, *Klebsiella ozaena*, Proteus mirabilis, Serratia marcesens, 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. Antibacterial and antiviral properties of Moringa pterygosperma inhibit the growth of gram-positive and gram-negative bacteria such as *E. coli*, *Styphi* and *S. paratyphi*. 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. stating 2 % chlorhexidine and Septilin demonstrated significant inhibition against *E. faecalis* followed Byaloeveera, Propolis and Calcium Hydroxide. Moreover, Septilin has a dark brownish colour which has high chances of causing tooth discoloration 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

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