

Antibacterial Efficacy of the Grape Seed Extract as an Irrigant for Root Canal Preparation

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ABSTRACT

Objective: The purpose of this research was to compare relative effectiveness of sodium hypochlorite 5.25% (NaOCl), 2% chlorhexidine gel (CHX) and 6.5 % grape seed extract (GSE) against *Enterococcus faecalis* using instrument Reciproc R25 in root canal preparation.

Methods: Forty-five mesiobuccal root canals from extracted human maxillary molars were collected and infected with *Enterococcus faecalis*. The samples were divided into five groups according to the different types of irrigants: saline (positive control) (n=5); in the other groups were used 10 root canals for each group: NaOCl+EDTA; CHX gel+EDTA; GSE solution+EDTA; GSE gel+EDTA. All the groups were prepared with reciprocating instruments Reciproc R25. Bacterial reduction was measured by two-way ANOVA ($P<0.001$) followed by Tukey HSD post-hoc tests, from the counting of colony forming units (CFUs) from samples collected before instrumentation and after. The significance level established at 5% ($P<0.05$).

Results: The group prepared with the NaOCl resulted in highest antimicrobial capacity among of all ($P>0.05$), followed by CHX and GSE gel ($P<0.05$). Control and GSE solution showed similar results ($P<0.05$) and resulted in the lowest percentage of the reduction of the microorganism into the root canals.

Conclusion: NaOCl had the higher elimination capacity of *Enterococcus faecalis* than GSE and CHX.

Keywords: Chlorhexidine, enterococcus faecalis, grape seed extract, reciprocating, sodium hypochlorite

HIGHLIGHTS

- This study evaluated the antibacterial efficacy of the 6.5% grape seed extract (GSE) gel and solution for root canal preparation compared to 2% CHX gel and 5.25% NaOCl.
- 6.5% GSE gel showed antibacterial efficacy similar 2% CHX gel.
- Irrigation with 5.25% NaOCl was significantly more effective than 2% CHX gel and 6.5% GSE gel in bacterial reduction into the root canal.
- Control and GSE solution showed no statistically significant difference between them.

INTRODUCTION

During root canal preparation is important the association between endodontic instruments and root canal irrigants, because this combination reduces intra-canal bacterial population (1, 2). Preliminary studies showed that the exclusive use of instruments without the use of an irrigant with antimicrobial ability did not provide adequate cleanliness of the root canals (3), and it has been necessary the use of the irrigant solutions with potential for disinfection during the preparation (2, 4, 5).

Enterococcus faecalis plays a fundamental role in the aetiology of periapical pathologies, with the ability to survive into root canal as sole microorganism (2), it can withstand in an environment with a high pH value and intense salt concentration (6), besides being resistant to the calcium hydroxide (7). This microorganism is commonly associated with endodontic failures (2, 7).

Sodium hypochlorite (NaOCl) has been widely applied as an irrigant, due to its antimicrobial and dissolution effectiveness (4, 8). Nevertheless, it may cause cytotoxic activity, in cases of NaOCl extrusion beyond the apex during root canal therapy (5,8,9). Besides, NaOCl may affect the mechanical properties of dentine (10-12).

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Investigators have been researching other possible irrigants. Chlorhexidine digluconate (CHX) has been used because of its antimicrobial activity (13) and substantivity (14, 15). One of the advantages of CHX is that it does not interfere with the integrity of the dentine wall (10) and exhibits low cytotoxicity (13). It is beneficial to teeth restored with resin-based materials.

Some studies have shown that GSE provided greater stability for the interface of resin-based restorations (11, 16, 17). This fact, favours the process of dental structure remineralization (17, 18), and there are still reports of antimicrobial activity (11, 18, 19). Studies showed good antimicrobial activity when associated with manual instrumentation of root canals (11). The results of some studies indicate GSE as valid option for irrigation of the root canals (11, 12, 17-21).

On the other hand, GSE gel had not been tested, as well as its association with the reciprocating system for root canal preparation. This study compared antibacterial efficacy of different root canal irrigants associated with EDTA against *Enterococcus faecalis*.

MATERIALS AND METHODS

Root preparation

This study was approved by the Research Ethics Committee of the Local University, presenting with statement number 570.397. Forty-five mesiobuccal roots of maxillary molars were collected, from Teeth Biobank of the University of Passo Fundo. Teeth without fracture, or calcifications and no previous root canal treatment were selected and confirmed by visual and radiographic analysis. The dental crowns were cut to determine a root length of 13 mm. The apical patency was determined by inserting a size 08 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until the tip was visible at the apical foramen. Only narrow canals with initial apical diameters no larger than a size 10 K-file (Dentsply Maillefer) were included. The working length was determined 1 mm short, and the samples instrumented with files #10 and #15. The samples were washed with 17% EDTA (Sigma-Aldrich, St. Louis, MO, USA) for 3 min through passive ultrasonic irrigation (PUI) and 5 mL of distilled water to remove the smear layer from the samples.

Subsequently, the cyanoacrylate (Loctite, Itapeva, São Paulo, Brazil) was applied to the external surface of the roots and the root apex was sealed with a resin composite (3M ESPE; St. Paul, MN, USA). At the end of this stage, 45 microtubes (Odeme, Joaçaba, Santa Catarina, Brazil) were filled with Zetalabor (Zhermack Spa, Badia Polenesia, Rivogo, Italy) laboratory silica, and the roots being accommodated with their cervical portion upwards in the silicone. All samples were autoclaved (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) for a period of 30 minutes at 120 °C. At the end of the sterilization, the roots were placed in a shelf for microtubes to initiate the contamination.

Bacteria preparation and contamination

The bacteria culture and inoculum were prepared according to preceding study (22). The reference strain was *Enterococcus faecalis* (American Type Collection 19433), prepared in

the laboratory of Microbiology of the Institute of Biological Sciences of the local University. The bacterium was cultivated in aerobiosis on Brain Heart Infusion (BHI) agar broth for 24 hours at 37°C in microbiological incubators. In each one of the specimens, 100 µL of the culture of *Enterococcus faecalis* were introduced within the canal. The culture of *Enterococcus faecalis* remained for 30 days in order to promote bacterial growing, renewing the BHI broth every 48 h. The procedures were executed in a laminar flow hood under aseptic conditions. An aliquot of BHI from a casually sample to confirm the presence of a single microorganism was performed by Gram staining and cultured on blood agar followed by catalase and esculin tests.

Division of experimental groups

Forty-five samples, after contamination, were randomly divided into 5 groups as follows:

G1-(n=5): NaCl: saline (Basso, Caxias do Sul, RS, Brazil).

G2-(n=10): 5.25% NaOCl (Natupharma, Passo Fundo, RS, Brazil)+EDTA.

G3-(n=10): 2% CHX gel (Chlorhexidine gel 2%-Natupharma, Passo Fundo, RS, Brazil)+EDTA.

G4-(n=10): 6.5% GSE solution (6.5% Grape Seed Extract-Mega-Natural, Madera, CA, USA)+EDTA.

G5-(n=10): 6.5% GSE (Grape Seed Extract-Mega-Natural)+EDTA.

The GSE powder is produced by Hot Water Extraction, and its molecular weight is 590.581 g/mol, according to the manufacturer. The extract was dissolved in distilled water and the pH of the slightly acidic solutions was adjusted to 7.2 using NaOH.

One operator performed all root canal instrumentation. The root canals were prepared with the Reciproc R25 instrument (tip size 25, 0.08 taper) (VDW GMBH, Munich, Germany), operated in a reciprocating motion. The file was passively inserted within the canal in an in-and-out pecking motion. After these three motions inside the canal, when more pressure was needed to make the instrument advance within the canal, the file was withdrawn and cleaned. In the next step, the file was reused in the same manner along the middle third followed by irrigation with 2 mL of sodium chloride. This protocol was repeated until the R25 instrument reached the working length. After that, the file was used to the full working length with a brushing motion against the walls of the root canal and a final flush with 2 mL of sodium chloride was performed. The file was activated in a reciprocating movement for an electric engine Reciproc® Silver (VDW GMBH) using the settings predefined by the manufacturer. Patency of the apical foramen with small hand files was checked after each instrument.

The root canal was irrigated with sterile disposable syringe and 30-gauge cannula (Navi-Tip, Ultradent, South Jordan, UT, United States) using 5 mL each. A total of 10 mL was used per canal in all groups, and the time was no more than 4 min. After the root canal preparations, final irrigation was performed

with 1 mL 17% EDTA for 1 min and 1 mL of saline, respectively; except in the control group.

When CHX gel and GSE gel were used, during the preparation, the specimens were filled and then removed with 10 mL of saline.

Microbiological analysis

Microbiological analysis were developed in two stages (after and before root canal preparation): the first sample (F1) was collected immediately after contamination with *Enterococcus faecalis* and second sample (F2) before instrumentation procedures. This collection was performed with the saline-filled canal. A sterile K-file #15 (Dentsply Maillefer) promoting agitation of the saline for 30 s, and after that, a sterile absorbent paper point #15 was placed within the canal and agitated in acircumferential manner to ensure contact with the walls for 30 s in the F1. Within this context, the same procedure was done to collect samples F2 where the absorbent paper point used was the size 25.

After that, the points were transferred to a microtube containing 1000 ml of saline. This solution was homogenized, and serial dilutions were made up to 10⁻². An amount of 75 µL of the solution and its dilutions were used for sowing. The Drop technique was performed, where 5 drops of 15 µL of each concentration were plated onto Petri dishes containing PCA (Plate Count Agar) and incubated for 48 hours. After the incubation period, the CFUs (colony-forming units) were counted to quantify the initial contamination. The efficiency of decontamination protocols was calculated through the percentage reduction of the colony units before and after instrumentation with the different irrigants. The number of CFUs were measured on the plates, three times by two different trained observers and the mean values were used.

Statistical analysis

The counts of CFUs were converted into Log₁₀, to normalize the data, and to perform statistical analysis. The test used to confirm the normal distribution of bacterial reduction of the data was Kolmogorov-Smirnov test. Data were evaluated by two-way ANOVA followed by Tukey HSD post-hoc tests. The data were analyzed using BioEstat 5.0 (Fundation Mamirauá, Belém, PA, Brazil) with statistical significance level was established at P<0.05.

RESULTS

The medium values and their respective standard deviations of the antibacterial efficacy of root canal irrigants are shown in Table 1. Mean values of bacterial counts [colony-forming units (CFUs) and in log numbers (Log₁₀)] and reduction percentage (%) before and after root canal preparation with different irrigants associated with EDTA, as well as the statistical analysis are reported in Table 1. No tested irrigant was able to promote complete disinfection of the samples. The antimicrobial effectiveness of NaOCl was higher than that of CHX (P<0.05). The samples that used CHX and GSE gel had similar results, but with lower mean bacterial reduction than NaOCl (P<0.05). There was no statistical difference in relation to the control group and GSE solution (P>0.05).

DISCUSSION

In this study, the antibacterial efficacy of the GSE were evaluated as a root canal irrigant because of its desirable properties reported in the literature (11, 12, 17-21). The available irrigants for root canal preparation have some limitations (11). NaOCl is cytotoxic and can cause collagen degradation (4, 5, 8, 11), and CHX has the inability to dissolve organic matter (8, 13).

The use of reciprocating and rotary instruments for root preparation root canal has improved the quality of the instrumentation considerably. It is possible to make it with small changes in the internal anatomy of root canal, with a reduction of the apical foraminal transposition, less dentin wear and short working time due to the simplification of the technique (1, 3, 23).

The antimicrobial effectiveness of NaOCl was higher than that of CHX, and similar results were found in other studies (6, 7, 24). Dornelles-Morgental et al. (25) evaluated NaOCl 2.5%, CHX 2% and peracetic acid 1%, and found that NaOCl was the most potent antimicrobial agent similar to results in this study. The action of NaOCl on bacteria is mainly due to its high pH (4, 7, 8). This action occurs on the cytoplasmic membrane of the bacteria, promoting biosynthetic alterations, causing their denaturation (4, 8).

Preliminary results using GSE showed good bacterial efficacy besides preserving mechanical properties of dentine, at concentrations tested to promote dentine collagen crosslinking (17, 20, 21). Still, it was able to inhibit the growth of different strains associated with dental caries, as *Streptococcus* spp. (18),

TABLE 1. Mean values of bacterial counts [colony-forming units (CFUs) and in log numbers (Log₁₀)] and reduction percentage (%) in the root canals before and after chemomechanical instrumentation using different preparation techniques and irrigation regimens

Groups	Before root canal instrumentation		After root canal instrumentation		% Reduction (Means±SD)	Tukey test
	Initial CFUs (Means±SD)	Initial Log ₁₀ (Means±SD)	Final CFUs (Means±SD)	Final Log ₁₀ (Means±SD)		
NaCl	50033.33±44170.09	4.49±0.57	3893.33±3097.32	3.47±0.38	72.60%±8.39%	C
NaOCl+EDTA	39752.59±52031.46	4.26±0.58	32.592±38.94	1.36±0.34	99.69%±0.40%	A
CHX+EDTA	211140.95±303251.22	4.63±1.07	554.29±550.01	2.37±0.76	92.05%±19.02%	B
GSE Gel+EDTA	30118.52±15763.45	4.42±0.26	4111.11±2785.09	3.51±0.34	85.65%±8.92%	B
GSE Solution+EDTA	44044.45±41590.84	4.33±0.66	3219.05±5173.19	3.05±0.76	76.39%±25.60%	C

The data are showed as meas±standard deviation of the samples evaluated from the groups. The values of P are meaningful using the analysis of variance two-way ANOVA P<0.001 e F=6.0427 where different letters represent in the table significant statistical differences in the post hoc test (Tukey test). NaCl: Saline solution, NaOCl: Sodium hypochlorite, CHX: Chlorhexidine gel, and, GSE: Grape seed extract

and had better interface performance of resin-based restorations (11, 16, 17). Because of GSE has in its composition Proanthocyanidins (17), which have the characteristic of crosslinking (17, 26, 27). When associated with manual instrumentation of straight root canals showed good antimicrobial activity (11).

Because of these characteristics, GSE was tested at 6.5% concentration, as an irrigant for root canal preparation, in gel or solution base, compared to NaOCl and CHX. Comparing GSE in gel and solution, better result was found for GSE in gel-base. However, both GSE gel and solution base had less reduction percentage of UFCs than NaOCl. Furthermore, GSE gel results were statistically similar to CHX; these findings seem to be promising.

In spite of these results, there was no statistical difference in relation to the control group and GSE solution ($P > 0.05$). Although, the similar design, GSE showed better result when associated with manual instrumentation of straight root canal (11). In this study, the authors used mesiobuccal root canals, instrumentation for Reciproc, and no activation. Root canal anatomy as well as no activation can be responsible for discrepancies between studies (28, 29).

No root canal irrigant used during preparation completely decontaminated the root canal after preparation, similar to a previous study (11). This findings, suggest the importance of the association for the different protocols, and the instrumentation and irrigation are important parts of root canal treatment (28).

Despite that CHX having less antimicrobial activity than NaOCl, had a greater ability to reduce *Enterococcus faecalis* when compared to GSE solution and control. Its antimicrobial potential is due to having a positive charge and this charge interacts with the cell wall of negatively charged bacteria, interfering with the osmotic balance of bacteria, leading to bacterial death (13, 14), in addition to its substantivity (14, 15), being released slowly with residual activity inside the root canal. However, it has the disadvantage of having the inability to remove organic tissues (4, 13). Previous studies have shown that CHX associated with EDTA does not negatively affect the resistance of adhesive materials (10) or mechanical properties of dentine (11, 12).

As natural extracts have previous results that report the time-dependent action (17, 27), it is necessary to examine the concentration and the ideal time for an effective antimicrobial action for clinical use in dentistry. Recent study found favourable results for GSE regarding its antimicrobial efficacy (30).

Further studies in larger concentrations of GSE should be performed to verify the feasibility of using as an irrigant for root canal preparation, since it has low cytotoxicity (11, 17) and can strengthen the remaining dental structure by improving in the mechanical properties of dentine (12, 20).

CONCLUSION

With the limitations of this study, it can be concluded that NaOCl has the best elimination capacity of *Enterococcus faecalis* from the root canals, compared to CHX and the GSE gel.

Disclosures

Conflict of interest: No conflict of interest.

Ethics Committee Approval: The Research Ethics Committee of the Local University, presenting with statement number 570.397, approved this study.

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