AJDS LAZHAR Al-Azhar Journal of Dental Science Print ISSN 1110-6751 | online ISSN 2682 - 3314
Vol. 27- No. 1- 147:152- January 2024 https://ajdsm.journals.ekb.eg
Orthodontic & Pediatric Dentistry Issue (Orthodontics and Pediatric Dentistry)

COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFICACY OF ALOE VERA, 3% NANO SILVER SOLUTION, AND 2% CHLORHEXIDINE GLUCONATE ON *ENTEROCOCCUS FAECALIS*: AN IN VITRO STUDY

Mohammed Elsayed Tolba Rezk [1*], Ebrahim Farouk Barakat [2], Alaa Nabil Abbass[3]

ABSTRACT

Objectives: The present study aims to evaluate and compare the antimicrobial efficacy of irrigant solutions with different natures against *E. faecalis*. **Subjects and methods**: A total of 48 contaminated roots were randomly divided into four groups (n = 12) according to the irrigation regimen used as follow: Group I: A 80% Aloe Vera extract irrigant solution. Group II: A 3% Nanosilver irrigant solution. Group III: A 2% Chlorhexidine irrigant solution. Group IV: A 1 % Sodium hypochlorite irrigant solution. The microbial counts were assessed by CFU/ml. **Results:** a significant reduction in the CFU count results of *E. faecalis* after irrigation in all studied groups. NaOCl had the highest antimicrobial effectiveness against *E. faecalis* and showed significant differences compared with CHX, *Aloe Vera*, and AgNPs solutions. **Conclusion**: NaOCl had the strongest and most significant effects of the studied irrigants against E faecalis, followed by CHX, AgNPs, and Aloe Vera.

KEYWORDS: Enterococcus faecalis, irrigant, NaOCl, Aloe Vera, Nano Silver, Chlorhexidine

INTRODUCTION

Primary dentition is important as it guides the eruption of permanent dentition and contributes to jaw development. Premature loss of primary teeth leads to the disturbance of permanent teeth eruption. Active carious lesions of the occlusal surfaces of the primary teeth are always associated with the presence of microbial biofilm. Once dental caries progress deeper, bacteria that are located at the advanced frontline of the biofilms are directly involved in inducing damage and consequential pulp tissue infection (1).

Success in endodontic treatment was originally based on the triad of debridement, thorough disinfection, and obturation of the root canal system, with each and every aspect equally important. The debridement and disinfection procedures require the removal of the irritants from the canal and periapical tissues. Therefore, to achieve a favorable endodontic treatment outcome, it is crucial to remove inflamed or necrotic pulpal tissue and to eliminate or at least reduce the number of microorganisms which is the main etiological factor for pulp space infection (2).

1. Masters Candidate, Dentist at the Egyptian Ministry of Health
2. Associate Professor and head of Pedodontics and Oral Health Department, Faculty of Dental Medicine (Boys, Cairo), Al-Azhar University
3. Lecturer of Pedodontics and Oral health Department, Faculty of Dentistry (Boys, Cairo), Al-Azhar University

• ***Corresponding author*:** ahmedmohammed27877@gmail.com

DOI: *10.21608/AJDSM.2022.157712.1358*

The most up-to-date techniques for a bacterial reduction in endodontic therapies include mechanical instrumentation to clean and expand the root canal space, chemical disinfection by irrigation and intracanal medication, referred to as an antimicrobial dressing, and mechanical disinfection by irrigation. However, the root canal system has a complex anatomical structure, and even with rotary files, root canal instrumentation often only exposes 60% of the root canal wall surface[3]. Therefore, the use of irrigants in combination with mechanical instrumentation is essential for loosening and helping to remove debris and bacteria. However, sodium hypochlorite (NaOCl) is considered the widely used endodontic irrigant solution but it has numerous disadvantages such as bad taste, toxicity, and risk of tissue destruction and denatures, as well as the inability to eliminate all the microorganisms present in infectious canals. Chlorhexidine (CHX) gluconate can be applied clinically as an antimicrobial agent alone or in combination with NaOCl during all phases of the root canal preparation[4].

The use of herbal products such as *Aloe Vera extracts* as root canal disinfectant irrigants have been widely investigated in endodontics because of their efficiency, safety, anti-inflammatory properties, as well as their stimulating dental pulp cell proliferation, differentiation, and extracellular matrix mineralization. Also, silver nanoparticles (Ag-NPs) were used as irrigant solution because of their antimicrobial effects, biocompatibility, and effective surface area [2]. However, the endodontic infections have a polymicrobial nature but it was found that *Enterococcus faecalis* (*E. faecalis*) is one important microorganism to be controlled as it observed in 22% and 32% of necrotic deciduous teeth and it is more prevalent in secondary infection than in primary endodontic infections. Additionally, it has been considered as the most antibiotic-resistant and the most common species recovered from teeth with failed endodontic treatment [4]. However, no irrigant solution appears to be ideal as a disinfectant solution against *E. faecalis*. Therefore, the present study aims to evaluate and compare the antimicrobial efficacy of irrigant solutions with different natures against *E. faecalis*.

SUBJECTS AND METHODS

Study Design: An in vitro experimental comparative study.

Study Setting: The freshly extracted human primary teeth (serially extracted for orthodontic purposes) were collected from the out-patient clinic of the Pediatric Dentistry and Public Health Department, Faculty of Dentistry, Al-Azhar University.

Sample Size:

Based on the previous study by Moradi et al[2], a power calculation of sample size indicated that a minimum of 12 teeth per group were required to detect a significant difference between groups. The effect size (dz=4.148) and the required sample size were calculated for a 95% confidence interval and power of (0.80).

Sample Grouping:

A total of 48 contaminated roots were randomly divided into four groups (n = 12) according to the irrigation regimen used as follow: Group I: A 80% Aloe Vera extract irrigant solution. Group II: A 3% Nanosilver irrigant solution. Group III: A 2% Chlorhexidine irrigant solution. Group IV: A 1% Sodium hypochlorite irrigant solution.

Ethical Consideration:

This work was approved by the Ethical Committee of the Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo), with the permission number EC Ref. No. (569/3433).

Eligibility criteria for teeth selection: [2,5]

Inclusion criteria: All teeth should have at least two-thirds of root length. The roots have no

sign of perforation in the root wall or furcation area. The roots did not have macroscopic cracks..The roots did not have internal resorption.

Exclusion criteria: Teeth of root with external resorption more than one-third. The roots with macroscopic root cracks, or fractures. The roots with signs of perforation in the root wall or furcation area.

Teeth cleaning, disinfection, and storage: The collected teeth were cleaned with a prophylaxis brush so that deposits and soft tissue residues were removed. Then, the extracted teeth were disinfected by soaking in 2.5% NaOCl for 24 hours to remove any remaining residual loose tissue and debris from the root surface [6]. To prevent dehydration, the teeth were stored sterile in normal saline at room temperature [2].

Intervention:

1. **Tooth preparation:**

The root apices were sealed with Cyanoacrylate adhesives Z105899 (Sigma-Aldrich, USA) (to prevent bacterial leakage) and then the roots were mounted in self-cure acrylic resin Acrostone cold cure (Acrostone, Egypt) blocks for ease of instrumentation. All teeth were decoronated to assimilate the conditions of the teeth at the cement-enamel junction (CEJ) with the use of a long cylindrical carbide bur and low-speed handpiece (W&H company, Austria) under excessive water irrigation. K-file file #15 (Mani company, Japan) was inserted in the root canal of each tooth and moved in the apical direction until the tip of the file was observed through the root apex. A 1 mm less than the distance passed by the K- file was considered the working length of each tooth. Then, each root was individually shaped, using K-files #15 to #30 by the use of the step-back technique. During cleaning and shaping, 2 ml sterile distilled water was used after each instrument size. Finally, the root canals were flushed with 5 mL of distilled water to remove any debris. All roots were then sterilized in a steam autoclave (steam autoclave) for 15 minutes under 15 psi pressure at 121°C [2,5].

Microbiology culturing procedures:

The bacterial strains used in this study are a known resistant strain of E. faecalis strain (ATCC19433). To create a standard infection in all samples, the primary culture was raised by inoculating E. faecalis in the brain heart infusion (BHI) broth after incubation at 37°C for 24 hours. Then, the root canals were cautiously inoculated via injection of the bacterial suspension using a sterile 20 µL insulin syringe of the freshly prepared suspension of the organisms with a concentration of 1 McFarland (CFU/ml 3 × 108). This procedure was repeated every 72 h for 60 days, always using 24 hours cultures adjusted to tube 1 of the MacFarland turbidity standard. The roots were incubated and maintained in a humid environment at 37°C between each repetition cycle. All the root samples were handled with sterile gloves and sterile tweezers to prevent contamination [2, 5, 7].

Baseline CFU counting

The microbial counts were expressed as CFU/ml of the sample by using an automated colony counter[2,5,7].

Data management and analysis:

The collected data during the study were tabulated and statistically analyzed using SPSS version 22. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Numerical data were described as mean and standard deviation. F-test (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups, followed by Post-hoc comparisons between groups using Bonferroni corrections. A t-test was used for normally distributed quantitative variables, to compare between two groups. The level of significance was set at $P<0.05$.

RESULTS

E. faecalis count (CFU/ml) among groups at before irrigation: There was a statistically no significant difference in E. faecalis count (CFU/ml) at the baseline between groups (P= 0.45301). The involved root canals showed a statically significant reduction in E. faecalis (CFU/ml) count after irrigation with Aloe Vera from (4.55X105±0.221) to (3.98X105±0.131) respectively. The involved root canals showed a statically significant reduction in E. faecalis (CFU/ml) count after irrigation with AgNPs solution from (4.65 X105±0.135) to (3.15 X105±0.077) respectively. The involved root canals showed a statically significant reduction in E. faecalis (CFU/ml) count after irrigation with CHX solution from (4.59 X105 ± 0.189) to (2.45 X105±0.285) respectively. The involved root canals showed a statically significant reduction in E. faecalis (CFU/ml) count after irrigation with NaOCL solution from (4.67 X105 ± 0.171) to (2.11 X105±0.096) respectively. Table (1)

E. faecalis count (CFU/ml) among groups after irrigation: There was a statistically significant difference in E. faecalis count (CFU/ml) between the involved root canals of the different tested groups after the use of irrigation with a P-value of (P<0.00001) as indicated by the One-way ANOVA test. The higher (mean ± SD) of E. faecalis (CFU/ml) count of (3.98X105 ±0.131) was recorded with the Aloe Vera (group I), followed AgNPs (group II) with E. faecalis (CFU/ml) count of (3.15X105 ±0.077), and CHX (group III) with E. faecalis (CFU/ml) count of (2.45 X105 ±0.285). While NaOCL (group IV) showed a lower (mean ± SD) E. faecalis (CFU/ml) count of (2.11X105 ±0.096). Table (1)

TABLE (1) E. *faecalis* count (CFU/ml) among groups at before and after irrigation

	Aloe Vera	AgNPs	CHX	NaOCl	p-value
Before irrigation	4.55 $X10^5$ ±0.221	4.65 $X10^5$ ±0.135	4.59 $X10^5$ ±0.189	4.67 $X10^5$ ±0.171	0.45301
After irrigation	3.98$X10^5$ ±0.131	3.15$X10^5$ ±0.077	2.45 $X10^5$ ±0.285	2.11$X10^5$ ±0.096	<0.00001*
P0	<0.0001*	<0.0001*	<0.0001*	<0.0001*	

**; The result is significant at p < 0.05.*

DISCUSSION

It is well established that the most probable cause of the failure in root canal treatments is the presence of oral bacterial flora in the apical portion of the root canal [5,7]. The results of the present study revealed that Aloe Vera extract has a significant antimicrobial effect (P<0.00001) against E. faecalis (CFU/ml) count after irrigation with a reduction in bacterial count from (4.55X105±0.221) to (3.98X105±0.131) after irrigation. This could be attributed to the potentially active constituents in the Aloe Vera extracts such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids which were possible reasons for its antimicrobial action [8]. This results in agreement with the results of Sureshchnadra et al., [9] who found that the Aloe Vera extract is effective against E. faecalis in agar medium. Additionally, Lawrence et al, [10] claimed that the location and quantity of hydroxyl groups in the phenol groups are connected to the microbiological toxicity of aloe vera.

The results of this study showed that AgNPs have a significant antimicrobial effect (P<0.00001) against E. faecalis (CFU/ml) count after irrigation with a reduction in bacterial count from (4.65 $X10^5$±0.135) to (3.15 $X10^5$±0.077) after irrigation. This could be

explained as silver and silver nanoparticles in an aqueous solution releasing silver ion, these ions can interact with the thiol groups of many vital enzymes and inactivate them, and these ions are biologically active and actually mediate the bactericidal effect. Additionally, it is thought that the nanoparticles' large surface areas provide them more strength to penetrate bacteria[2,11]. On the cell surface, there were "pits" that had formed, and nanoparticles had accumulated there[12]. These results agreed with Moradi and Haghgoo[2] results, who reported that AgNPs have a significant antimicrobial effect against E. faecalis in vitro. Additionally, the results of Bhandi et al.,[13] revealed that AgNPs as irrigant solution has significant antimicrobial against E. faecalis in vitro, and they found that; decreasing the size of silver particles to the nanoscale increases their antimicrobial effects and effective surface area.

Furthermore, the results of the current study exhibited that CHX has a significant antimicrobial effect ($P<0.00001$) against E. faecalis (CFU/ml) count after irrigation with a reduction in bacterial count from ($4.59 X10^5 \pm 0.189$) to ($2.45 X10^5 \pm 0.285$) after irrigation. This could be related to the bactericidal effect of CHX and its ability to precipitancy or coagulate the cellular cytoplasm, which is attributable to cross-linking proteins at high concentrations.[14,15] These results agreed with the results of Williamson et al,[16] and Endo et al,[17].

Additionally, the results of the present study showed that NaOCl has a significant antimicrobial effect ($P<0.00001$) against E. faecalis (CFU/ml) count after irrigation with a reduction in bacterial count from ($4.67 X10^5 \pm 0.171$) to ($2.11 X10^5 \pm 0.096$) after irrigation. This is because when hypochlorous acid in NaOCl solution, comes in connection with organic tissue it acts as a solvent that releases chlorine and combines with the protein amino group to form chloramines. These results agreed with Moradi and Haghgoo [2] results and Senthil et al., [14] reported that NaOCl kills bacteria very rapidly even at low concentrations. The results of the present study also showed that NaOCl had the highest antimicrobial effectiveness against E. faecalis and showed significant differences compared with CHX, Aloe Vera, and AgNPs solutions ($P < 0.001$). This could be attributed to the hypochlorous acid in NaOCl solution coming in connection with organic tissue it acts as a solvent that releases chlorine and combines with the protein amino group to form chloramines [13, 14].

The results of the current study show that Aloe Vera has a far less inhibitory effect on E. faecalis than the antimicrobial effect of NaOCl, CHX, and AgNPs solutions. Lawrence et al, [15] stated that microbial toxicity of Aloe Vera is related to the site and number of hydroxyl groups in the phenol groups. Calcium hydroxide has hydroxyl groups that give it its alkalinity and antibacterial properties, however the buffering action of dentin largely negates these effects.

CONCLUSION

The use of Aloe Vera extracts, AgNPs, CHX, and NaOCl as an irrigant solution has a significant antimicrobial effect against E. faecalis. NaOCl had the strongest and most significant effects of the studied irrigants against E faecalis, followed by CHX, AgNPs, and Aloe Vera.

REFERENCES

1. Mitrakul K, Vongsawan K, Watcharakirin W, Khererat P. Quantitative Analysis of Lactobacillus and Enterococcus faecalis between Irreversible Pulpitis and Pulp Necrosis in Primary Teeth. Dent Res Oral Health. 2019; 2: 17-31.
2. Moradi F, Haghgoo R. Evaluation of antimicrobial efficacy of nanosilver solution, sodium hypochlorite and normal saline in root canal irrigation of primary teeth. Contemp Clin Dent. 2018;9: 227-32.
3. Hussein HH, Abood FM, Alhela AG. Some virulence factors of Enterococcus faecalis isolated from root of the anal infections combined with effect of some irrigation solution against E. Faecalis. Sys Rev Pharm. 2020; 11: 742-48.

4. Lemos SS, Cesar DE, Procópio SW, Machado FC, Ribeiro LC, Ribeiro RA. Qualitative and quantitative molecular analysis of bacteria in root canals of primary teeth with pulp necrosis. Braz Oral Res. 2020;34: 93-97.
5. Sahebi S, Khosravifar N, SedighShamsi M, Motamedifar M. Comparison of the antibacterial effect of sodium hypochlorite and Aloe Vera solutions as root canal irrigants in human extracted teeth contaminated with Enterococcus Faecalis. J Dent Shiraz Univ Med Sci, March 2014; 15: 39-43
6. Rezende G, Daniel A. Decurcio DA, Estrela C. Costa L. Antibacterial action of intracanal medicaments on infected dentin of deciduous and permanent teeth. Dental Press Endod. 2011; 1:34-39.
7. Eggmann F, Vokac Y, Eick S, Neuhaus KW. Sonic irrigant activation for root canal disinfection: power modes matter. BMC Oral Health. 2020; 20:3-9.
8. Karkare S R. Comparative evaluation of antimicrobial activity of hydroalcoholic extract of Aloe vera, garlic, and 5% sodium hypochlorite as root canal irrigants against Enterococcus faecalis: An in vitro study. J Indian Soci Pedodontics and Preventive Dent. 2015; 33: 274-81.
9. Sureshchandra B, Kumar AJ. Antibacterial efficacy of aloe vera extract on resistant antimicrobial strains in endodontics. Endodontology 2011; 23: 56-60.
10. Lawrence R, Tripathi P, Jeyakumar E. Isolation, Purification and Evaluation of Antibacterial Agents from Aloe vera. Braz J Microbiol 2009; 40: 906-915.
11. Razumova S, Brago A, Serebrov D, Barakat H, Kozlova Y, Howijieh A, et al. The Application of Nano Silver Argitos as a Final Root Canal Irrigation for the Treatment of Pulpitis and Apical Periodontitis. In Vitro Study. Nanomaterials 2022; 12: 248-54.
12. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007; 3:95–101.
13. Bhandi S, Mehta D, Mashyakhy M, Chohan H, Testarelli L, Thomas J, et al. Antimicrobial efficacy of silver nanoparticles as root canal irrigant's: A Systematic Review. J Clin Med. 2021; 10:1152-8.
14. Senthil B, Gurunathan D, Vasantharajan M, Lakshmi T. Primary tooth root canal irrigants - A review. Int J of Pure Appl Math 2018;120: 565-89.
15. Lawrence R, Tripathi P, Jeyakumar E. Isolation, Purification and Evaluation of Antibacterial Agents from Aloe vera. Braz J Microbiol 2009; 40: 906-915.
16. Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of Enterococcus faecalis. J Endod 2009; 35: 95-7.
17. Endo MS, Martinho FC, Zaia AA, Ferraz CC, Almeida JF, Gomes BP. Quantification of cultivable bacteria and endotoxin in post-treatment apical periodontitis before and after chemo-mechanical preparation. Eur J Clin Microbiol Infect Dis 2012; 31: 2575-83.