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The Effect of Different Root Canal Irrigants When Activated with Endoactivator and Manual Dynamic Agitation on *Enterococcus Faecalis*- A Comparative in Vitro Evaluation

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Settings and Design:

- Tertiary care setting – Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research, Trivandrum.
- Biogenix Lab, Poojapura, Trivandrum.
- Jawaharlal Nehru Tropical *Botanical Garden* and Research Institute, Palode.

Keywords: E.faecalis, endo activator, manual dynamic agitation, aloe vera, Q Mix.

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The Effect of Different Root Canal Irrigants When Activated with Endoactivator and Manual Dynamic Agitation on *Enterococcus Faecalis*- A Comparative in Vitro Evaluation

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Methods and Material: Forty-two single-rooted, noncarious human premolar teeth having a single canal with similar sizes, and completed apices are selected. Pro Taper rotary files shape the root canals up to an F3 master apical file size. Aloe vera extract is taken and subjected to antimicrobial activity and Minimum inhibitory concentration tests.

To get pure colonies, a pure culture of E.faecalis (American Type Culture Collection[ATCC] 29221) is subcultured in Muller –Hilton Agar and incubated overnight at 37°C. The single colonies are picked up and transferred to 1ml of sterile MH broth and incubated at 37 °C to get the turbidity of 0.5 McFarland standard. The root canals are injected with an inoculum of E.faecalis using a sterile syringe. Sterile paper points are transported to 1 ml PBS in a test tube and vortexed. A BHI agar plate is swabbed with 50 µL of PBS to get individual colonies (colony count in CFU/mL). The specimens are then randomly divided into six groups with test solutions.

Based on the group, the irrigation is done with the appropriate test solution. All teeth are then flushed with 30 ml saline to prevent the carryover of the irrigants.

In each group, specimens will be subjected to CFU counting and then MTT ASSAY, which will determine the % of cell viability.

Statistical analysis used: The comparison of E.faecalis removal between two different irrigating protocols is carried out using an independent t-test. The comparison among the three different irrigating solutions is carried out by one-way ANOVA, and the Post hoc test is made use of for pairwise comparison.

Results: Among the three solutions, Sodium Hypochlorite displayed the best anti-microbial activity followed by Q mix and Aloe vera. Among the two irrigation activation techniques, Endo activator was the best in terms of removing *E. faecalis.* Manual dynamic agitation also showed a considerable amount of reduction in the bacterial count but was associated with operator fatigue.

Keywords: *E.faecalis, endo activator, manual dynamic agitation, aloe vera, Q Mix.*

Key Message: Sodium hypochlorite, in combination with Endo activator, was the most effective in removing *E. faecalis* from infected root canals followed by the combination of Q mix with Endo activator.

I. Introduction

A multitude of studies on humans as well as animals, have enlightened us about the fact that microorganisms play a pivotal role in causing and sustaining pulpal and periapical diseases. The flora that resides in the pulp space is involved in the development of periapical infections in teeth with caries extending into the pulp .1-3 Their removal from the root canal through various shaping methods, irrigation procedures, and, when needed intracanal medicaments, form the rationale of Endodontic treatment.[4] The bacteria, *Enterococcus faecalis* which forms a part of the normal microbial flora of the oral cavity has been associated with asymptomatic, persistent pulpal and periapical infections and failed root canal treatments.[5] Q mix, a root canal irrigant introduced in the market in 2012, is a combination of EDTA, chlorhexidine, and detergent. Using a single solution, which is a mixture of different components, not only saves time and adds simplicity to the procedure but also equips the clinician with beneficial effects of all the individual components.[6] Currently, many researches are being carried out to find herbal alternatives for pulp space disinfectants in Endodontics, owing to their efficiency, safety, and ease of accessibility. [7]

Adopting an appropriate method for activating an irrigating solution is equally important as selecting an ideal irrigant. The Endo Activator System is a sonically-driven system designed to safely activate various

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intracanal reagents and vigorously produce the hydrodynamic phenomenon. [8]

Machine-assisted agitations are effective in debridement. However, each of these methods need special gadgets. In 1980, Match proposed a simple technique for agitation by moving a well-fitted gutta-percha (GP) point inside a prepared root canal, which is now known as Manual Dynamic Agitation.

Studies have shown that gently moving a well-fitting gutta-percha master cone up and down in a short 2- to 3-mm stroke within a prepared canal can produce an effective hydrodynamic effect and significantly improve the displacement and exchange of any given reagent. [9]

Thus, developing a potent irrigant-irrigation activation regimen with maximum desirable properties and minimum adverse effects, that too effective against microbial species prevalent in secondary infections could be a boon to the endodontic fraternity.

II. Subjects and Methods

a) Aloe Vera Extract

Freshly collected healthy, mature leaves of Aloe vera are washed with clean water and longitudinally dissected. Using a sterile knife, the colorless, parenchymatous tissue (aloe gel) is scrapped out carefully, without the green fibers and processed in a blender. Cold percolation method extracts the fresh Aloe vera pulp using 70% ethanol for 72 hours. The extracts are then subjected to filtration using a double-layered muslin cloth. This filtered Aloe vera extract is used in the study.

b) Antibacterial Activity

i. *Agar- Well Diffusion Method*

Petri plates containing 20ml Muller Hinton Agar Medium are seeded with the bacterial culture of *Enterococcus faecalis* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm are bored using a well cutter, and different volumes of the sample such as 25µL, 50µL, 100µL are added. Following which, the plates are incubated at 37°C for 24 hours. The diameter of the inhibition zones around the well is measured to assay the antibacterial activity (NCCLS, 1993). Streptomycin acts as a positive control.

c) Determination of Minimal Inhibitory Concentration

Two-fold serial dilution methods helped determine the minimal inhibitory concentration (MIC) with *Enterococcus faecalis* as the indicator organism. The samples added in increasing concentrations of 50, 100, 200, 400, 800, and 1000 µL respectively were incubated overnight at 37˚C. Visual inspection immediately followed by optical density (OD) measurement at 620 nm made using a spectrophotometer measured the growth. At each dilution of the plant extract, growth inhibition for the test wells is determined by the formula:

$$\text{Percentage of inhibition} = (\text{OD of control} - \text{OD of test})/(\text{OD of control}) \times 100\%$$

Forty-two single-rooted, noncarious human mandibular premolar teeth with similar sizes and closed apices are selected. The root surfaces are mechanically debrided from the soft tissues and calculi with a periodontal scaler. Buccolingual and mesiodistal radiographs were taken from the specimens to evaluate their anatomy. Radiographs are taken to verify that the selected teeth are having only a single root canal. Distilled water at 4 °C is used to store the teeth until used. Specimens were then decoronated with a diamond disc using water as a coolant to obtain a standardized root length of 13 mm.

Type II GIC is used to seal the apices of all teeth. Pro Taper rotary files up to an F3 (size 30) master apical file size shaped the root canals, and 2 ml of 3% NaOCl solution is used to irrigate the root canals after each instrument. Subsequently, an autoclave at 121˚C and 15 lbs of pressure, is used to sterilize the samples for 15 minutes.

d) Treatment of Tooth Samples

A pure culture of *E.faecalis* (ATCC 29221) was subcultured in Muller–Hilton Agar and incubated at 37°C overnight to get pure colonies. The single colonies were picked up and transferred to 1ml of sterile MH broth and incubated at 37˚ C to get turbidity of 1.0 McFarland standard.

These colonies of *Enterococcus faecalis* inoculated the sterilized tooth samples. The now infected tooth samples are kept in Brain Heart Infusion broth and incubated for four weeks, with the media being replaced every 48hrs. After the period of incubation, the teeth are treated and categorized accordingly as A, B, C, D, E, and F.

GROUP A-3 ml of 3.0% Sodium hypochlorite for 1 minute using Manual Dynamic Agitation (MDA)

GROUP B-3 ml OF 3.0% Sodium hypochlorite for 1 minute using Endoactivator

GROUP C- 3 ml of Q Mix for 1 minute using MDA

GROUP D-3 ml of Q Mix for 1 minute using Endoactivator

GROUP E- 3 ml of Aloe Vera for 1 minute using MDA

GROUP F-3 ml of Aloe Vera for 1 minute using Endoactivator

The tooth samples were kept in a minimal amount of BHI overnight after treatment.

e) Determination of Colony Forming Units

The scraping from the cavity of each tooth mixed well in 1ml sterile PBS is used to determine the colony-forming units (CFUs) present. BHI agar plates

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swabbed with 10µl from each sample, were kept overnight at 37 ˚C. The control was an untreated tooth. After incubation, the colony-forming units (CFUs) observed were counted, and expressed as CFUs/ml.

f) Mtt Assay

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

The cavities of treated teeth samples were rinsed with sterilized PBS and was added with 10µl of reconstituted MTT and then incubated at 37°C in a humidified 5% CO2 incubator for 4 hours. After the removal of the supernatant and the addition of 100µl of MTT Solubilization Solution (DMSO) following the incubation period, and the cavities were mixed gently by pipetting up and down to solubilize the formazan crystals. A microplate reader at a wavelength of 570 nm measured the absorbance values.

The percentage of growth viability was calculated using the formula:

$$\%\ of\ Viability = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

III. Results and Statistical Analysis

a) Determination of Colony Forming Units

Pretreatment 41.2×10^4

Descriptive statistics for CFU

Table 1: Descriptive statistics for CFU based on group

	Group A	Group B	Group C	Group D	Group E	Group F
Mean	6.9	2.8	7.7	4.8	17.0	12.1
SD	1.1	0.3	1.5	1.0	0.6	0.6
Median	6.2	2.9	7.3	4.3	17.0	12.2
Mode	6.2	2.4	5.6	3.9	16.1	11.4
Minimum	5.9	2.4	5.6	3.9	16.1	11.4
Maximum	8.7	3.1	10.0	6.8	18.2	13.1

Table 2: Descriptive statistics for CFU based on solution

	3%NaOCL	Q Mix 2 IN 1	Ethanol extract of Aloe vera
Mean	4.8	6.3	14.5
SD	2.3	2.0	2.6
Median	4.5	6.2	14.6
Mode	6.2	3.9	11.4
Minimum	2.4	3.9	11.4
Maximum	8.7	10.0	18.2

Table 3: Comparison of CFU based on Solution

Solution	Mean	SD	N	F	Sig.	Scheffe Multiple Comparisons		
						Pair	F`	p
3% NaOCl (A)	4.8	2.3	14	71.97**	0.000	A & B	1.3	0.279
Q Mix 2 IN 1 (B)	6.3	2.0	14			A & C	61.7**	0.000
Ethanol extract of Aloe vera (C)	14.5	2.6	14			B & C	45**	0.000

**: - Significant at 0.01 level

Table 4: Descriptive statistics for CFU based on activation

	Manual dynamic agitation	Endoactivator
Mean	10.6	6.5
SD	4.8	4.1
Median	8.2	4.3
Mode	6.2	2.4
Minimum	5.6	2.4
Maximum	18.2	13.1

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Table 5: Comparison of CFU based on activation

Activation	Mean	SD	N	t	p
Manual dynamic agitation	10.6	4.8	21	2.89**	0.006
Endo activator	6.5	4.1	21		

**: - Significant at 0.01 level

b) Determination of % of Cell Viability (Mtt Assay)

Control- Absorbance 0.7992 Viability 100%

Descriptive statistics for % viability

Table 6: Descriptive statistics for % viability based on group

	Group A	Group B	Group C	Group D	Group E	Group F
Mean	48.0	20.5	52.3	30.8	63.5	60.3
SD	4.9	2.3	3.5	5.5	1.4	0.8
Median	48.4	20.1	52.1	30.2	63.2	60.2
Mode	41.8	18.5	48.3	24.8	62.1	59.1
Minimum	41.8	18.5	48.3	24.8	62.1	59.1
Maximum	54.3	25.3	59.1	39.3	65.5	61.4

Table 7: Descriptive statistics for % viability based on solution

	3%NaOCL	Q Mix 2 IN 1	Ethanol extract of Aloe vera
Mean	34.3	41.5	61.9
SD	14.8	12.0	2.0
Median	33.6	43.8	61.8
Mode	18.5	24.8	59.1
Minimum	18.5	24.8	59.1
Maximum	54.3	59.1	65.5

Table 8: Comparison of % viability based on Solution

Solution	Mean	SD	N	F	Sig.	Scheffe Multiple Comparisons		
						Pair	F`	p
3%NaOCl (A)	34.3	14.8	14	23.52**	0.000	A & B	1.5	0.234
Q Mix 2 IN 1 (B)	41.5	12.0	14			A & C	21.9**	0.000
Ethanol extract of Aloe vera (C)	61.9	2.0	14			B & C	11.9**	0.000

**: - Significant at 0.01 level

Table 9: Descriptive statistics for % viability based on activation

	Manual dynamic agitation	Endoactivator
Mean	54.6	37.2
SD	7.5	17.6
Median	53.3	30.2
Mode	41.8	18.5
Minimum	41.8	18.5
Maximum	65.5	61.4

Table 10: Comparison of % viability based on activation

Activation	Mean	SD	N	t	p
Manual dynamic agitation	54.6	7.5	21	4.18**	0.000
Endoactivator	37.2	17.6	21		

**: - Significant at 0.01 level

IV. Discussion

Colony-forming units and viability incidence were the two dependent variables that were used in this study to quantify the amount of reduction of *E.faecalis* from tooth samples after treating with different irrigants and their activation. Results of the study demonstrated that none of the irrigating solutions and their activation

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were able to completely remove *E.faecalis* from tooth samples.

But when different groups were compared, out of the three irrigants, 3% NaOCl was the most effective followed by Q mix and aloe vera in terms of mean reduction in the CFU and percentage of viable organisms. Out of the two activation methods, Endoactivator (EA) showed a greater reduction in both CFU and % viability when compared with Manual Dynamic Agitation (MDA). When the different irrigant-irrigation activation combination were compared the most effective combination was 3%NaOCl with EA(mean value of CFU-2.8×10^4 ,% Viability-20.5%) followed by Q Mix with EA(mean value of CFU-4.8×10^4,% Viability-30.8%), 3%NaOCl with MDA (mean value of CFU-6.9×10^4,% Viability-48%), Q mix with MDA(mean value of CFU-7.7×10^4 ,% Viability-52.3%), Aloe vera with EA(mean value of CFU-12.10×10^4 ,% Viability-60.3%) and Aloe vera with MDA(mean value of CFU-17×10^4 ,% Viability-63.5%) in terms of both CFU and % viability.

The results of the current study are in agreement with most of the previous studies, which evaluated NaOCl and Q Mix as irrigating agents. Aloe Vera being an organic irrigant, requires further investigation to prove its efficacy.

The main reason for choosing *Enterococcus faecalis* in this study despite being only occasionally found in cases of primary endodontic infections is that they are frequently isolated or detected where endodontic therapy has failed. *E.faecalis* can adhere to the root canal walls, accumulate, and form communities organized in biofilm, which helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms. In the current study, root canals were infected for four weeks to ensure the organization and maturation of the biofilm.[10]

One of the effective methods to eradicate *E.faecalis* is the use of various concentrations of sodium hypochlorite. Due to the various disadvantages of sodium hypochlorite like the unpleasant taste, toxicity, and potential weakening of the tooth structure by decreasing the hardness and structural integrity of the dentin within the root canal, finding an effective alternative has become imperative.[11]

In recent years, herbal products are widely investigated as root canal disinfectants in Endodontics because of their efficiency, safety, and accessibility. Bhardwaj et al. assessed the antibacterial activity of Aloe Vera gel as long as 1, 3, and 5 days.[12] Aloe Vera showed good antibacterial activity on the first day of incubation. They noted that Aloe Vera had 75 potentially active constituents such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids, which were possible reasons for its antimicrobial action.[7] In the present study, agar well diffusion method was used to study the antimicrobial activity of aloe vera against *E faecalis.*

The results of the current study showed that aloe vera had significantly lesser antimicrobial activity against *E.faecalis* when compared with 3% NaOCl and Q mix. Several factors could have contributed to this outcome. The first one is the time of contact of the solution with tooth surface wouldn't have been sufficient for Aloe vera to apply its inhibitory effect against *E.faecalis*. Second, tooth structures themselves might lessen the antibacterial effect of Aloe vera solution. Lawrence et al. stated that microbial toxicity of Aloe Vera is related to the site and number of hydroxyl groups in the phenol groups.[13] Hydroxyl groups are responsible for alkalinity and antibacterial action of calcium hydroxide. However, the dentin buffering action relatively neutralizes its effect. Therefore, this mechanism suppressed the antibacterial activity of Aloe Vera. Third, the gel-like consistency of Aloe Vera could cause a limited flow of the substance through the irregularities of the root canal system.

QMiX is a novel endodontic irrigant for smear layer removal with added antimicrobial agents. It contains EDTA, CHX, and a detergent. QMiX is a clear solution, ready to use with no chair-side mixing. In this current study, NaOCl and Qmix were not used in combination to avoid the formation of even a minute amount of the carcinogenic precipitate. Surface active agent lowers the surface tension of solution and increases their wettability and enables better penetration of an irrigant in the root canal. The potential benefit of bisbiguanide in this mixture is that it prevents the microbial colonization on the dentin surface. Calcium chelating agents can cause cell wall damage in gram-negative bacteria by chelating and removing divalent cations (Mg+2 and Ca+2) from the bacterial cell membranes and increasing its permeability.[14] After analyzing data from the current study, NaOCl had better activity against E.faecalis when compared to Q mix, and thus it would be more beneficial to use Q mix as a final rinse after NaOCl.

In a preliminary study, Gulabivala (2006) has shown that the EndoActivator removes simulated biofilms in extracted teeth. The action of the EndoActivator tip frequently produces a cloud of debris that can be observed within a fluid-filled pulp chamber. The primary function of the EndoActivator is to produce vigorous intra canal fluid agitation through acoustic streaming and cavitation. This hydrodynamic activation serves to improve the penetration, circulation, and flow of irrigant into the more inaccessible regions of the root canal system (Guerisolo[15] et al. 2002).

In the present study, manual dynamic agitation has not performed as effectively as sonic agitation. The reason behind this could be, the energy created by the push-pull motion of the GP point (3.3 Hz) is much lesser than sonic energy of 1-6 kHz, but manual dynamic

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agitation is a simple, cost-effective way of root canal agitation technique, which removes significantly more bacterial biofilm than syringe irrigation in the absence of any gadgets.[16]

According to Ying Liu et al. (2015) and Elakanti et al. (2015), Q mix had superior anti-microbial efficacy against *E.faecalis* when compared with NaOCl, which is in contrast to the results obtained in this current study which showed NaOCl to be much superior. [17,18] This difference could be because of the variation in contact time and quantity of the irrigating solution as well as the difference in the study models used.

At the same time, studies by Ordinola-Zapata r, et al. (2013) and Morgental et al. (2013) have reported inferior anti-biofilm efficacy of Q mix compared to various concentrations of NaOCl with EDTA combinations.

V. Summary and Conclusion

1. Sodium hypochlorite, in combination with Endo activator, was the most effective in removing *E. faecalis* from infected root canals followed by the combination of Q mix with Endo activator.
2. Among the three solutions, Sodium Hypochlorite displayed the best anti-microbial activity followed by Q mix and Aloe vera. Even though Aloe vera showed antimicrobial activity, its performance compared to the other two solutions was below par.
3. Among the two irrigation activation techniques, Endo activator was the best in terms of removing *E. faecalis.* Manual dynamic agitation also showed a considerable amount of reduction in the bacterial count but was associated with operator fatigue.

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