



## **Antimicrobial Efficacy of Calcium Hydroxide and Triple Antibiotic Paste Combination on *E. faecalis* Biofilm- An *in vitro* Study**

**Azima Hanin S. M.<sup>1</sup>, Anjaneyulu K.<sup>2\*</sup> and Muralidharan N. P.<sup>3</sup>**

<sup>1</sup>Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, India.

<sup>2</sup>Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, India.

<sup>3</sup>Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University Chennai-600077, India.

### **Authors' contributions**

This work was carried out in collaboration among all authors. Author AHSM performed the study and statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AK designed the study and managed the analyses of the study. Author MNP assisted in microbiological evaluation. All authors read and approved the final manuscript.

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### **ABSTRACT**

Calcium hydroxide (CH) is an intracanal medicament that has been widely used in endodontics, which can eliminate bacteria because of its high alkalinity. However, *E. faecalis* is resistant to CH. Triple antibiotic paste (TAP) is a mixture of ciprofloxacin, minocycline, and metronidazole, and is highly effective against *E. faecalis*. Hence the main aim of this study was to find the antimicrobial efficacy of CH and TAP combination against *E. faecalis*. The study was done by agar diffusion method, three wells were punched in Tryptone soya agar and filled with CH, TAP, and the combination of both. The zone of inhibition values was recorded and subjected to statistical analysis using SPSS. One way ANOVA and Post Hoc tests were used to compare the means. The combination of CH with TAP was found to be significantly better than CH and TAP used alone ( $p$ -

\*Corresponding author: E-mail: [Kanjaneyulu.sdc@saveetha.com](mailto:Kanjaneyulu.sdc@saveetha.com);

value <0.05). Within the limits of this study, it can be concluded that TAP is more efficient when compared with CH. The combination of CH and TAP proved to be more effective when compared to the two when used alone.

**Keywords:** Calcium hydroxide; triple antibiotic paste; *E. faecalis*; intracanal medicaments; root canal Medicaments.

## 1. INTRODUCTION

Complete elimination of microorganisms and removal of pulp tissue from the root canal system is of primary importance during endodontic therapy [1]. The success of root canal treatment mainly depends on mechanical preparation, microbial control, and complete filling of the root canal system [2]. Microorganisms may survive during endodontic procedures due to anatomical structural complexities in root canal systems which cannot be accessed by instrumentation and irrigants [3]. To ensure elimination of root canal bacteria, effective antimicrobial agents like Calcium Hydroxide and Triple Antibiotic Pastes are used for a predetermined time for predictable eradication of the remaining bacteria [4]. Calcium Hydroxide is a white odourless powder with low solubility in water and a high alkaline pH value (about 12.5-12.8). Hydroxyl ions released during the ionic dissociation of Calcium Hydroxide are responsible for the high alkaline property. The usage of calcium hydroxide is not confined to dentistry alone and has varied industrial uses such as food processing, production, construction and sewage treatments [5].

Infection in the root canal system is polymicrobial and is dominated by anaerobes [6]. Microorganisms such as streptococci and enterococci are capable of surviving different conditions of filled root canals [7]. Various literature reveals that a Gram positive facultative anaerobe *E. faecalis* is the most frequently isolated microorganism in failed root canal treatment teeth [8,9]. This is because of its unique capability to proficiently invade dentinal tubules and surviving extreme levels of basic Ph and limited nutrition [10]. The placement of intracanal medicaments helps in the elimination of the surviving microorganism and promotes the success of the treatment [11].

Calcium hydroxide (CH) is an intracanal medicament that has been widely used in endodontics, which has the ability to eliminate bacteria due to its high alkalinity. The high pH level of CH changes the lipopolysaccharide component in the cell wall of gram-negative

bacteria which inactivates membrane transportation and eventually leads to cell death [12]. However, *E. faecalis* is resistant to CH because of its ability to penetrate deeper into the dentinal tubules, where the pH of the medicament is neutralized by the buffering action of dentin [13,14]. In addition, CH reduces the flexural strength of dentin, and its efficacy in eliminating the bacteria, even after prolonged contact with root canal walls, has been questioned [15].

Triple antibiotic paste (TAP) is a mixture of three antibiotics namely; ciprofloxacin metronidazole, and minocycline. It is used as an intracanal medicament for the disinfection of the root canals of immature necrotic teeth during regenerative procedures [16]. The rationale of using TAP in this study is the effectiveness of this mixture against the common endodontic pathogens which has been proved in several studies [17-19]. A recent *in vitro* study by Adl et al, reveals that TAP is highly effective against *E. faecalis* in comparison to CH [20]. Another report on teeth contaminated with *E. faecalis*, reveals that TAP can reduce the colony count of the bacteria more effectively than CH [21]. However, no studies exist on the combined antimicrobial efficacy of TAP and CH against *E. faecalis*.

Hence the main aim of this study is to find the antimicrobial efficacy of Calcium Hydroxide and Triple Antibiotic Paste combination against *E. faecalis*.

## 2. MATERIALS AND METHODS

This study was conducted at the Department of Conservative and Endodontics in Collaboration with the Department of Microbiology at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India.

### 2.1 Sample Preparation

The test microorganism used in this study was the pure culture of *E. faecalis*. Twenty-four-hour colonies of a pure culture of *E. faecalis* were grown on tryptone soy agar and incubated for 24

h at 37°C. The plates were checked for purity before the introduction of the study groups. Intracanal medicaments were prepared for three groups: Group I (N=5): Calcium Hydroxide powder + Saline, Group II (N=5): Triple Antibiotic Paste and Group III (N=5): Calcium Hydroxide powder + Triple Antibiotic Paste.

The intracanal medicaments were tested using agar diffusion method to study their antimicrobial efficacy. 3 uniform wells with 4mm depth were punched at equidistant points in the agar plate with the use of a sterile copper coil. The wells were filled with the three study groups immediately. Five such samples were made and inhibition zones around medications were measured after the incubation of the plates at 37°C for 24 h. This method proves only the in-vitro elimination of the bacteria, not the in vivo one which was not reproduced or mimicked here.

## 2.2 Data Collection and Statistical Analysis

Zones of inhibition of the three groups in five samples were measured in millimeters and recorded in Microsoft Excel 2010. The collected data was exported to Statistical Package for Social Science (SPSS) and subjected to statistical analysis. Descriptive statistics were applied to the data to find the average zone of inhibition values for the three groups and was represented using bar graphs. ANOVA test and Post Hoc statistical tests were used to analyze the significance between the three different groups of intracanal medicaments. P-value of less than 0.05 was considered to be statistically significant.

## 3. RESULTS AND DISCUSSION

From this study, it was evident that the maximum zone of inhibition with a mean of 47.3 mm was seen in Calcium Hydroxide + Triple Antibiotic Paste combination which was followed by 42.6 mm in Triple Antibiotic Paste (Fig. 1). Both Triple Antibiotic Paste and the combination of Calcium Hydroxide with Triple Antibiotic Paste were found to be significantly better than calcium hydroxide used alone (p value= 0.001<0.05). The combination of Calcium Hydroxide with Triple Antibiotic Paste was also found to be significantly better than Triple Antibiotic Paste when used alone. (p value= 0.01<0.05). The combination of Calcium Hydroxide and Triple Antibiotic Paste

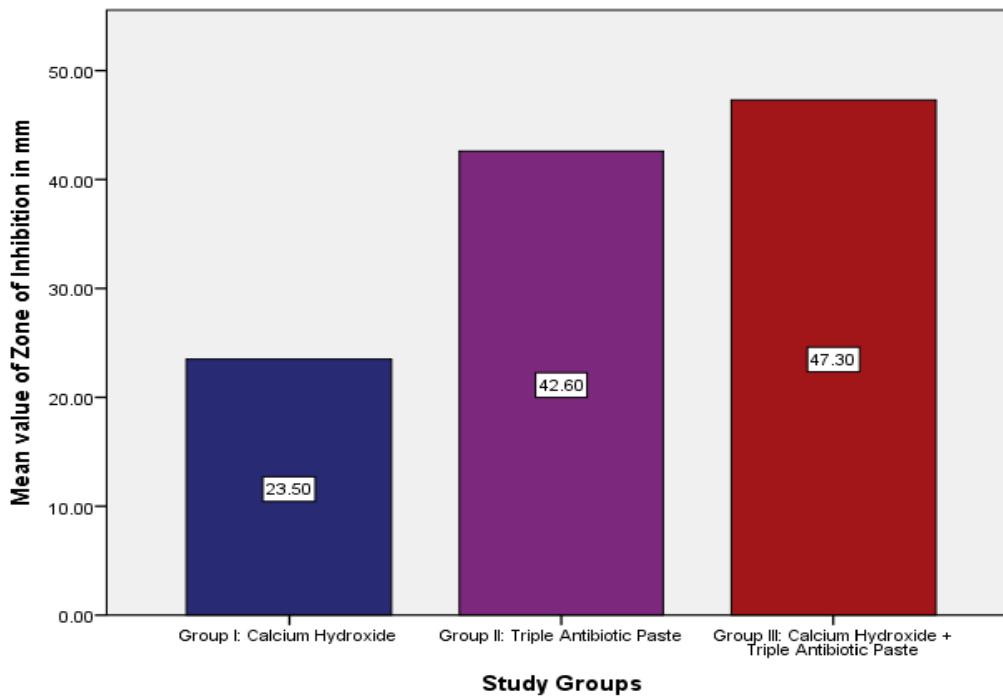
proved to be significantly better than when used alone as Calcium Hydroxide and Triple Antibiotic Paste (p value= 0.001<0.05) (Table 3).

In this 20th century, the world is witnessing many revolutions in medical care and treatments. Considering the shortcomings of Calcium hydroxide on *E. faecalis*, combining it with an effective intracanal medicament like Triple Antibiotic Paste would be beneficial. Hence this study was done to analyze the antimicrobial efficacy of the combination.

Various literature reports have been proposed for the evaluation of different intracanal medicaments against the resistant microflora in the root canal system [22,23]. *E. faecalis* was selected for this study as its most frequently associated with failed root canal treated teeth in addition to the fact that Calcium Hydroxide is less effective against it [24].

Because of the complexity of the root canal infection by *E. faecalis*, a combination of antibiotics is required to address the diverse flora encountered in root canal infections. The Triple Antibiotic Paste is a mixture of three antibiotics namely metronidazole, ciprofloxacin, and minocycline. Findings from the present study depict that Triple Antibiotic Paste is more effective against *E. faecalis* when compared to Calcium Hydroxide. This finding corroborates with findings of a recent study by Madhubala et al., in which Triple Antibiotic Paste had higher antibacterial effects than Calcium Hydroxide on *E. faecalis* [21] (Fig. 1).

In the present study, it was observed that the bactericidal effect of Calcium Hydroxide and TAP when used alone against *E. faecalis* was significantly lower when compared to the combination of Calcium Hydroxide and Triple Antibiotic Paste (Table 3). This observation may be due to the remarkable efficacy of Triple Antibiotic Paste against *E. faecalis* enhances Calcium Hydroxide efficacy against it. Although the results of this study confirmed the bactericidal efficacy of the combination of Calcium Hydroxide + Triple Antibiotic Paste against *E. faecalis*, more clinical studies in a similar subject is necessary to illustrate its antimicrobial efficacy as an intracanal medicament, especially in non-surgical retreatment of failures in endodontic therapy.



**Fig. 1.** This Bar graph represents the mean value of zones of inhibition of the three groups. The X-axis represents the three different study groups and Y-axis represents the zone of inhibition against *E. faecalis* in millimeter (mm). Blue color represents Group I (CH), purple color represents Group II (TAP) and the red color represents Group III (CH + TAP combination). From the graph, it was evident that the maximum zone of inhibition with a mean of 47.3 mm was seen in Group III (CH+ TAP combination) which was followed by 42.6 mm in Group II (TAP)

**Table 1.** Table representing the descriptive statistics of the zone of inhibition for the three study groups. From the table, it is evident that the combination of Ca(OH)<sub>2</sub> with Triple Antibiotic paste shows a maximum zone of inhibition when compared to the other groups

	N	Mean	Std. deviation	Std. error	95% Confidence interval for mean	
					Lower bound	Upper bound
Group I- Calcium Hydroxide	5	23.500	2.5000	1.1180	20.396	26.604
Group II- Triple Antibiotic PAste	5	42.600	2.8151	1.2590	39.105	46.095
Group III-Calcium Hydroxide + Triple Antibiotic Paste	5	47.300	1.3038	.5831	45.681	48.919
Total	15	37.800	10.8641	2.8051	31.784	43.816

**Table 2.** The table depicts the results of the one-way ANOVA test between the three groups. It was observed from the graph that there is a statistically significant difference between the three groups. (p value= 0.001<0.05)

	Sum of squares	df	Mean square	F	Sig.
Between Groups	1588.900	2	794.450	150.132	.000
Within Groups	63.500	12	5.292		
Total	1652.400	14			

**Table 3. Table depicting the results from the Post Hoc test done between the three groups to compare their significance. Both TAP and the combination of CH + TAP were found to be significantly better than CH used alone (p value= 0.001<0.05). The combination of CH + TAP was also found to be significantly better than TAP when used alone. (p value= 0.01<0.05). The combination of CH + TAP proved to be significantly better than when used alone as Calcium Hydroxide and TAP (p value= 0.001<0.05)**

(I) Groups	(J) Groups	Mean difference (I-J)	Std. error	Sig.
Group I- Calcium Hydroxide (CH)	Group II- Triple Antibiotic Paste (TAP)	-19.1000	1.4549	.000
	Group III-Calcium Hydroxide + Triple Antibiotic Paste (CH+TAP)	-23.8000	1.4549	.000
Group II- Triple Antibiotic Paste (TAP)	Group I- Calcium Hydroxide (CH)	19.1000	1.4549	.000
	Group III-Calcium Hydroxide + Triple Antibiotic Paste (CH+TAP)	-4.7000	1.4549	.018
Group III-Calcium Hydroxide + Triple Antibiotic Paste (CH+TAP)	Group I- Calcium Hydroxide (CH)	23.8000	1.4549	.000
	Group II- Triple Antibiotic Paste (TAP)	4.7000	1.4549	.018

\* The mean difference is significant at the 0.05 level

**4. CONCLUSION**

Within the limits of this study, it can be concluded that Triple Antibiotic Paste is more efficient when compared to Calcium Hydroxide against *E. faecalis*. The combination of Calcium Hydroxide and Triple Antibiotic Paste proved to be more effective when compared to the two when used alone. The study was limited to an in vitro environment. Further research is necessary to understand the effects of the combination of TAP and CH in an in vitro environment.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

Before carrying out the present study, ethical approval was obtained from the institutional ethics committee.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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