ORIGINAL ARTICLE

AN INVITRO COMPARATIVE STUDY OF THE ANTIBACTERIAL EFFICACY OF CALCIUM HYDROXIDE AS AN INTRACANAL MEDICAMENT AND COMBINATION OF CALCIUM HYDROXIDE WITH FOOD PRESERVATIVES AND PROTON PUMP INHIBITOR AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Aim: To evaluate the antimicrobial effcacy of nisin and calcium hydroxide with and without omeprazole against Enterococcus faecalis.

Materials and methods: The antibacterial effect of the following experimental groups as intracanal medicaments (Group I- $Ca(OH)_2$, Group II-$Ca(OH)_2$ + Nisin, Group III - $Ca(OH)_2$ + Nisin + PPI, Group IV-$Ca(OH)_2$ + PPI) was evaluated using the agar diffusion test for a time period of 24 hours.

The minimum inhibitory concentration (MIC) against E. Faecalis were also determined. Statistical analysis was performed using Kruskal-Wallis Test and chi-square test.

Results: The antibacterial efficacy is detected by the formation of the zone of inhibition around the wells inoculated with the experimental groups. Groups I, II, III and IV showed inhibitory zones. The maximum diameter of 22 mm is obtained with group IV. The MIC values for the experimental groups I, II, III, and IV were 0.45%, 0.2mg/ml, 0.45%+0.03mg/ml, ≤0.01% respectively.

Conclusion: The antimicrobial efficacy of omeprazole (PPI) combined with calcium hydroxide shows the maximum zone of inhibition and according to this study; this combination shows the most potent intracanal medicaments.

KEY WORDS

Enterococcus faecalis, Intracanal medicament, Nisin, Omeprazole.

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INTRODUCTION

Enterococcus faecalis is the most common facultative anaerobic bacteria isolated from both secondary and persistent root canal infections which possesses extraordinary ability to withstand adverse conditions and live inside dentinal tubules. Stuart et al have shown that apart from the contributing factors, such as complex root canal anatomy and ineffective chemomechanical instrumentation, E. faecalis possesses certain virulence factors which invades and adheres to the dentinal tubules with a depth of penetration ranging from 500 to 1000 µm, and also survive in harsh environ-mental conditions as it has the potential to transform into the viable but noncultivable state.[1]

Calcium hydroxide, commonly used as an intracanal medicament, has an effective antibacterial action against most endodontic microflora. But E. faecalis is resistant to the antimicrobial activity of $Ca(OH)_2$ due to its PPI action and its potential to withstand high alkalinity. On the use of $Ca(OH)_2$ the pH in the canal reaches neutral levels in the presence of E. faecalis leading to bacterial growth and survival in the root canal.[2]

Recent advances in the development of various materials such as Propolis, bioactive glass, ozonated water, corticosteroids, grape seed extract, Nisin, and PPIs in order to achieve superior disinfection of the root canal system and for long-term clinical success of endodontic therapy.[3]

Nisin which is a chemical commonly used as a food preservative, nowadays it is used as an intracanal medicament. Nisin is a naturally occurring antimicrobial cationic peptide, produced by Streptococcus lactis subspecies lactis found in the year 1928. It is a polycyclic antimicrobial peptide with 34 amino acid residues which includes uncommon amino acids, such as lanthionine, methyllanthionine, didehydroalanine, and didehydroaminobutyric acid. It has antimicrobial activity against a wide range of Gram-positive bacteria and their spores, even against drug-resistant E. faecalis isolates.[4]

Proton pump inhibitors are a group of drugs with which have a long-lasting reduction of gastric acid secretion, mostly used for the treatment of peptic ulcer.

By maintaining the alkaline pH, PPIs not only reduce acid secretion but also increase the sensitivity to antimicrobials.[6,7]

This study aimed to find out the potent intracanal medicaments that is very effective against Enterococcus faecalis and objectives of the study are to evaluate the antimicrobial efficacy of calcium hydroxide as an intracanal medicaments and combination of calcium hydroxide with food preservatives and proton pump inhibitor against Enterococcus faecalis and also to find out the best intracanal medicaments which have a low market price value against Enterococcus faecalis.

MATERIALS AND METHODS

Bacterial Strain used in the Study

Enterococcus faecalis ATCC 29212 (American Type Culture Collection) was maintained in the Microbiology laboratory of our institution and was revived in Brain Heart Infusion Broth and stored at 4°C.

Preparation of the Stock Solutions (FIG A-G)

- Group I-Ca(OH)2 : Calcium hydroxide was prepared in sterile distilled water at a concentration of 29%.
- Group II – Ca(OH)2 + Nisin : Calcium Hydroxide and Nisin dissolved in sterile injectable water at a concentration of 10 mg/mL.
- Group III - Ca(OH)2 + Nisin + PPI : Calcium Hydroxide and Nisin and omeprazole (20 mg) were dissolved in 10 ml of sterile injectable water
- Group IV- Ca(OH)2 + PPI : Calcium Hydroxide and omeprazole (20 mg) were dissolved in 10 ml of sterile injectable water

Preparation of culture media

The culture media reagents were bought from the market and mixed with distilled water and autoclaved. After autoclaving the solutions were kept inside the laminates for cooling . When the solutions cooled down the pH were tested by using parchment paper. The solutions were all alkaline in nature.

AGAR DIFFUSION ASSAY

According to the CLSI guidelines, agar diffusion assay was done. The antibacterial efficacy was detected using the well diffusion susceptibility test, by creating wells on the surface of an agar plate seeded with a lawn culture of E. faecalis and the solutions that were prepared for each group were poured into the wells respectively and incubated for 24 hours.


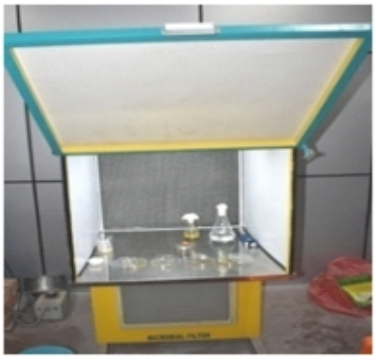


FIG A: the reagents were autoclaved;FIG B: after autoclaving the solutions were kept inside the laminated for cooling ; FIG C: the pH of the solutions were tested by using parchment paper

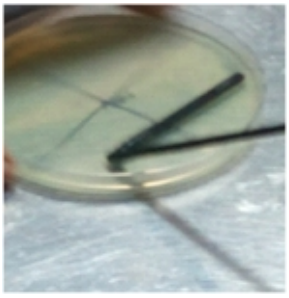
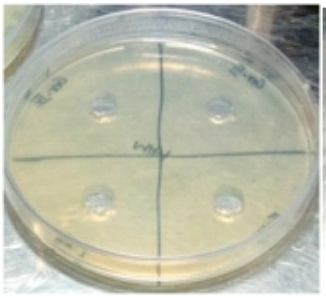
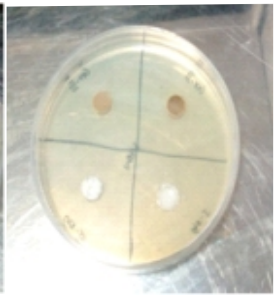


FIG D **FIG E** **FIG F** **FIG G**

FIG D: Lawn culture was done ; FIG E: Four well were made ; FIG F: Four test solutions are given in four wells respectively according to groups ; FIG G: The plate was placed in the incubator

Minimum Inhibitory Concentration

● As per CLSI Institute guidelines[7], Microbroth dilution assay was done to determine the MIC value of the test solutions. The analysis was performed using doubling dilutions of the test solutions. The test solutions were double serially diluted from wells 1 to 11 of each row. The last well of each row served as the culture control where no test solution was added. For all the test solutions the assay was performed in triplicates. The Minimum Inhibitory concentration was the lowest concentration of the test solution which had completely inhibited the growth of E. faecalis .

RESULTS

Agar Well Diffusion Assay

The antibacterial efficacy is detected by the formation of the zone of inhibition around the wells inoculated with the experimental groups. Groups I, II, III and IV showed inhibitory zones. The maximum diameter of 22 mm is obtained with group IV. The inhibitory zones of group II is 20mm, group I is 20mm and group III is 21mm. Group I is positive control group with which other groups are compared. **(FIG -H, Table 1)**

FIG H: group IV showed the maximum zone of inhibition

Minimum Inhibitory Concentration

The MIC values of the experimental groups against E. faecalis according to this study showed no statistically significant differences between the experimental groups.

TABLE 1

TEST SOLUTION	GROUP I	GROUP II	GROUP III	GROUP IV	GROWTH CONTROL
E.faecalis	0.41%	0.1 mg/ml	0.41%,0.03mg/ml	≤0.01%	Normal

STATISTICAL ANALYSIS

● Statistical analysis was done using Kruskal-Wallis Test and chi-square test. There was statistically significant difference when the probability value was $p < 0.05\%$.(Table 2, 3)

Statistical analysis table for Agar Diffusion Test

TABLE 2

Groups	Group I	Group II	Group III	Group IV
Chi-Square	2.000	2.000	2.000	2.000
Degree of freedom	2	2	2	2
p-value	1.000	1.000	0.364	0.364

Statistical analysis table for MIC values

TABLE 3

Chi-square	0.600
Degree of freedom	3
P-value	0.869

There is no statistically significant difference between the experimental groups

DISCUSSION

The result has shown that the experimental groups exhibited antimicrobial action against E. faecalis. In this study, group IV (calcium hydroxide and omeprazole) showed the superior antimicrobial activity compared with $Ca(OH)_2$. Omeprazole is the first PPI to be develope as it is a weak base, highly lipophilic, and easily crosses the cell membrane . Many reasons are proposed for the resistance of E. Faecalis with calcium hydroxide but the presence of PPI helps to maintain cytoplasmic pH. [8,9]

Omeprazole has been used to eradicate helicobacter pylori which is implicaed as the main causative agent of peptic ulcer by affecting its proton pump. In dental literature, Wagner et al did the first attempt to eradicate E. faecalis using calcium hydroxide in combination with omeprazole. Wagner et al in his study used the combination of omeprazole, a PPI, with $Ca(OH)_2$ as an intracanal medicament which has shown an increased antimicrobial efficacy against E. faecalis which showed superior healing of periapical lesions. [9,10]

In group I, 29% concentration of $Ca(OH)_2$ showed 20 mm zone of inhibition. The antibacterial effect of calcium hydroxide is related to the release and diffusion of hydroxyl radicals. When compared with other experimental studies, $Ca(OH)_2$ showed reduced antibacterial efficacy against E. Faecalis in this study may be due to the difference in the methodology The antimicrobial activity of calcium hydroxide is attributed to its alkaline pH. [11,12,13]

When nisin is combined with calcium hydroxide, the zone of inhibition shows 20 mm. The results indicated that nisin (in combination with calcium hydroxide) improves the antimicrobial action of calcium hydroxide against pathogenic bacteria. Nisin has a potent antimicrobial activity against a wide range of gram-positive microorganisms. Severina et al proved that nisin is less toxic, odorless, colorless, tasteless, and has low drug resistance rates compared with other similar antimicrobial peptides. Nisin showed its antibacterial effect by the following mechanisms. According to Jack et al[14], nisin acts by inserting into the bacterial plasma membrane and triggering the activity of bacterial murein hydrolases, which results in degradation of the peptidoglycans and lysis of cells and Du Plessis[15] et al reported that it is due to interaction with the phospholipid membrane of the target bacterial cell causing autolysis and irreparable damage to plasma membrane. Crandal et al showed that by disrupting the cellular mechanism, nisin induced leakage of small intracellular contents from the cell.[16]

The drawbacks of this study include the following:

- The antimicrobial efficacy of the experimental groups is not tested on root canal biofilm samples;
- The antimicrobial efficacy of omeprazole may be inhibited by the agglomerate formation when combined with calcium hydroxide and when the agar diffusion assay is used for testing the experimental solution showed the reduced diffusion ability.

However, it remains clear that further studies are required to evaluate the chemical interaction between Omeprazole and $Ca(OH)_2$ and its antibacterial efficacy against E. faecalis at various concentrations.

CONCLUSION

The result of the present study concluded that :

The antimicrobial efficacy of omeprazole (PPI) combined with calcium hydroxide shows the maximum zone of inhibition and according to this study, this combination shows the most potent intracanal medicaments .

- Omeprazole has also a low market price value than other PPI like pantoprazole ,lansoprazole .

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