ORIGINAL RESEARCH PAPER

Dental Science

KEY WORDS: Enterococcus Faecalis, Intracanal Medicament, Nisin, Omeprazole.

COMPARATIVE EVALUATION 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-AN INVITRO STUDY

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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: In this study 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. In group IV the maximum diameter of 22 mm is obtained . 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.

INTRODUCTION :

Enterococcus faecalis is the one the important causative organism of root canal treatment failures. *E faecalis* is the most common facultative anaerobic bacteria isolated from both secondary and persistent root canal infections. As per the studies conducted by Love **1**, Stuart et al **2**, and Sirén et al **3** , apart from the contributing factors, such as complex root canal anatomy and ineffective chemo mechanical instrumentation, *E faecalis* possesses certain virulence factors (lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid), invades and adheres to the dentinal tubules with a depth of penetration ranging from 500 to 1000 micrometer and has the ability to survive in harsh environmental conditions due to its potential to transform into the viable but non-cultivable state.

Now-a-days has calcium hydroxide [Ca(OH)2] used an intra-canal medicament, most commonly an effective antibacterial action against most endodontic micro-flora. But *E faecalis* can withstand the antimicrobial activity of Ca(OH)2 due to its PPI action and its potential to withstand high alkalinity. The pH in the canal reaches neutral levels (in the presence of *E faecalis*) on the use of Ca(OH)2, leading to bacterial growth and survival in the root canal. 3

So the search for an effective intracanal medicament aims to achieve superior disinfection of the root canal system, long-term clinical success of endodontic therapy and this led to the recent advances in the development of various materials such as Propolis, bioactive glass, ozonated water, corticosteroids, grape seed extract, Nisin, and PPIs. **4**

Nisin commonly used as a food preservative to preserve meat and dairy products, is only very recently recommended for use as an intracanal medicament.. It is a naturally occurring antimicrobial cationic peptide, produced by Streptococcus lactis Chemically, it is a polycyclic antimi crobial peptide with 34 amino acid residues which includes uncommon amino acids, such as lanthionine, methyllanthionine, didehydroalanine, and didehydroa minobutyric acid. It has antimicrobial activity against a wide range of Gram-positive bacteria and their spores, even against drug-resistant *E faecalis* isolates. **5-8**

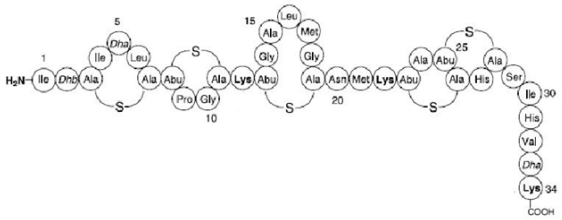

Fig- Chemical structure of Nisin

Proton pump inhibitors are a group of drugs with the mechanism of action of pronounced and long-lasting reduction of gastric acid secretion, most commonly used for the treatment of peptic ulcer **9**. The PPIs not only reduce acid secretion but also increase the sensitivity to antimicrobials, maintaining the alkaline pH.

Hence this study aimed at evaluating antimicrobial efficacy of calcium hydroxide as an intracanal medicaments and combination of nisin and proton pump inhibitor against *E faecalis*

In our study we selected Omeprazole as it is comparatively cheaper than Pantoprazole or Lansoprazole.

MATERIALS AND METHODS

- **Bacterial Strain used in Our 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**
- 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,

AGARWELL DIFFUSION ASSAY:

Using the well diffusion susceptibility test, the antibacterial efficacy was detected by challenging bacterial isolates with antibacterial agents on the wells that were created on the surface of an agar plate seeded with a lawn culture of E. faecalis for 24 hours.

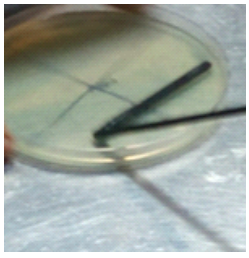

FIG A

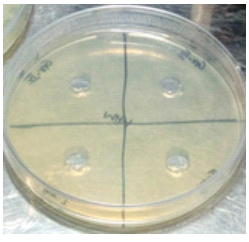

FIG B

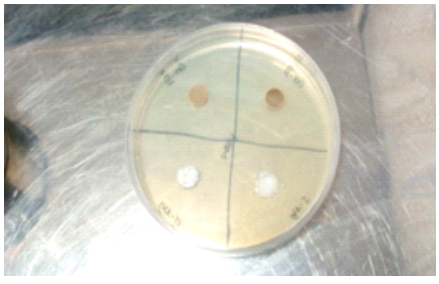

FIG C

FIG D

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

MINIMUM INHIBITORY CONCENTRATION (MIC)

- To determine the MIC value of the test solutions, Micro broth dilution assay was done as per CLSI Institute guidelines. 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. The assay was performed in triplicates for all the test solutions. The MIC was the lowest concentration of the test solution that completely inhibited the growth of *E faecalis.*

RESULTS

AGARWELL 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 20 mm, group I is 20mm and group III is 21mm. Group I is positive control group with which other groups are compared.

FIG E: group IV showed the maximum zone of inhibition

MINIMUM INHIBITORY CONCENTRATION

- The MIC values of the experimental groups against planktonic cells of *E faecalis* ATCC 29212 according *to* this study. There is no statistically significant differences between the experimental groups.

Test Solution	Group I	Group II	Group III	Group IV	Growth Control
E faecalis	0.45%	0.2 mg/ml	0.45%+0.03mg/ml	≤0.01%	Normal

STATISTICAL ANALYSIS

- Statistical analysis was done using Kruskal–Wallis Test and chi-square test. There is statistically significant difference when the probability value was $p < 0.05\%$.

STATISTICAL ANALYSIS TABLE FOR AGAR DIFFUSION TEST

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	0.368	0.368

STATISTICAL ANALYSIS TABLE FOR MIC VALUES

Chi-square	**0.600**
Degree of freedom	3
P-value	0.896

There is no statistically significant difference between the experimental groups

DISCUSSION

In this study the result has shown that the experimental groups exhibited antimicrobial action against *E faecalis*. Among the four groups, Group- IV (calcium hydroxide and omeprazole) showed the superior antimicrobial activity compared with Ca(OH)2.

Omeprazole is the first PPI developed and is weak base, highly lipophilic, and easily crosses the cell membrane. It has been used to eradicate Helicobacter pylori which is implicated as the main causative agent of peptic ulcer by affecting its proton pump. The first attempt to eradicate *E faecalis* using calcium hydroxide with omeprazole is by Wagner et al. In their study they used the combination of omeprazole, a PPI, with Ca(OH)2 as an intracanal medicament which had shown an increased antimicrobial efficacy against *E faecalis* producing superior healing of periapical lesions.**10**

In group I, 29% concentration of Ca(OH)2 showed 20mm zone of inhibition. The antibacterial effect of calcium hydroxide is related to the release and diffusion of hydroxyl radicals and the velocity of its release depends on the vehicle with which it is manipulated. The reduced antibacterial efficacy of Ca(OH)2 against E. faecalis in our study may be due to the difference in the methodology when compared with other experimental studies.

The antimicrobial activity of calcium hydroxide is due to its alkaline pH. The antibacterial effect of calcium hydroxide is related to the release and diffusion of hydroxyl ions. The ion release is retarded with a viscous vehicle and is accelerated with an aqueous vehicle.

When nisin is combined with calcium hydroxide,the zone of inhibition showed 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. Experiments conducted by Severina et al **7** proved that nisin is less toxic, odourless, colorless, tasteless, and has low drug resistance rates compared with other similar antimicrobial peptides.

According to Jack et al**11,** Nisin exhibits its antibacterial effect by the following mechanisms: it acts by inserting into the bacterial plasma membrane and triggering the activity of bacterial murein hydrolases, resulting in damage or degradation of the peptidogly-cans and lysis of cells. Du Plessis et al **12** reported that it is due to interaction with the phospholipids membrane of the target bacterial cell causing autolysis and irreparable damage to plasma membrane. Crandal et al **13** showed that it disrupts the cellular mechanism, inducing leakage of small intracellular contents from the cell.

IN OUR STUDY THERE ARE SOME LIMITATIONS LIKE -

- 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 the reduced diffusion ability of the experimental stock solution when the agar diffusion assay is used for testing.

Further studies are needed to evaluate the chemical interaction between Omeprazole and Ca (OH)2 and its antibacterial efficacy against *E faecalis* at various concentrations and also in vivo conditions.

CONCLUSION

The results of our present study is concluded as: The antimicrobial efficacy of omeprazole (PPI) combined with calcium hydroxide showed the maximum zone of inhibition and according to this study , this combination showed the most potent intracanal medicament .

REFERENCES:

1. Love RM.Enterococcus faecalis—a mechanism for its role in endodontic failure. Int Endod J 2001 Jul; 34(5):399-405.
2. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis—its role in root canal treatment failure and current concepts in retreatment. J Endod 2006 Feb; 32(2):93-98.
3. Sirén EK, Haapasalo MP, Waltimo TM, Ørstavik D. In vitro antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on Enterococcusfaecalis. Eur J Oral Sci 2004 Aug; 112(4):326-331.
4. Tong Z, Ling J, Lin Z, Li X, Mu Y. The effect of MTADN on 10 Enterococcus faecalis isolates and bioflm: an in vitro study.J Endod 2013 May; 39(5):674-678.
5. Hemadri M, Thakur S, Sajjan G. Nisin vs. calcium hydroxide—antimicrobial effcacy on Enterococcus faecalis—an in-vitro study. Int J Contemp Dent 2011 Jun;2(3):55-61.
6. Chinni SK, Veni AB, Srinivasan MR, Rajamani I. An in vitro investigation of a newer intracanal medicament Nisin on Enterococcus faecalis in comparison with chlorhexidine andcalcium hydroxide. J Int Clin Dent Res Organ 2011 Jan; 3(1):21-24.
7. Severina E, Severin A, Tomasz A. Antibacterial effcacy of nisin against multidrug-resistant Gram-positive pathogens.J Antimicrob Chemother 1998 Mar; 41(3):341-347.
8. Tong Z, Zhou L, Li J, Jiang W, Ma L, Ni L. In vitro evaluation of the antibacterial activities of MTAD in combination with nisin against Enterococcus faecalis. J Endod 2011Aug;37(8):1116-1120
9. SHANDS. Drugs and therapy bulletin. Vol. 15(1). Gainesville (FL): Shands at the University of Florida; 2001.
10. Wagner C, Barth VC Jr, de Oliveira SD, Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an in vivostudy. J Endod 2011 Sep; 37(9):1253-1257.
11. Jack FW, Tagg JR, and Ray B. Bacteriocins of gram positive bacteria. Microbiol Rev 1995 Jun; 59(2):171-200.
12. Du Plessis DJ, Nouwen N, Driessen AJ. The Sec translocase. Biochem Biophys Acta 2011 Mar; 1808(3):851-865.
13. Crandal AD, Montville T. Nisin resistance in Listeria monocytogenes ATCC 700302 is a complex phenotype. Appl Environ Microbiol 1998 Jan;64(1):231-237