

**To Evaluate the Effectiveness of Omeprazole Associated With Calcium Hydroxide and Erythromycin as an Intra Canal Medicament - An Invivo Study on Rats**

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**Abstract**

**Background :** Proton pump inhibitors are used in combination with calcium hydroxide as an intracanal medicament for elimination of enterococcus faecalis. The aim of this invivo study on wistner rats was to evaluate the efficacy of association of omeprazole with calcium hydroxide and erythromycin as intra canal medicament in rats induced with periapical lesions.

**Materials and Methods :** Periapical lesions were induced on incisor teeth of 30 male Wistner rats. After 28 days, root canal of each tooth was prepared, filled with intra canal medicaments and divided into three groups. Group 1 - Calcium hydroxide + PEG, Group 2 - Calcium Hydroxide + Omeprazole + PEG, Group 3 - Calcium Hydroxide + Omeprazole + Erythromycin + PEG, and access cavity was sealed with cavit for 15days.

Microbiological samples were taken in 2 periods. S<sub>1</sub>- after 28 days of access cavity preparation, S<sub>2</sub> -15 days after placement of intracanal medicaments.

**Results:** Microbiological Analysis revealed significant decrease of colony forming units from S<sub>1</sub> to S<sub>2</sub> time periods in all the 3 groups.

**Conclusion:** Results showed that association of Omeprazole with Calcium Hydroxide and Erythromycin favoured superior Antibacterial Efficacy against Enterococcus faecalis in comparison with Calcium hydroxide and Calcium hydroxide used in combination with Omeprazole.

**Keywords:** Calcium hydroxide, omeprazole, erythromycin, proton pump inhibitors.

## Introduction

The complete debridement and disinfection of the root canal system is essential for good endodontic prognosis. Although instrumentation of root canal is the primary means of canal debridement, irrigants and intracanal medicaments are critical adjuncts. Despite proper cleaning and shaping procedures complete bacterial elimination is difficult to achieve due to anatomic complexities and persistence of micro organisms in the apical third of the root canal.[1] Various methods are been tried during chemo mechanical preparation of root canal as irrigants and after preparation as intra canal medicaments for complete bacterial elimination from the root canals. Despite adequate cleaning and shaping procedures enterococcus faecalis is the most common microorganism commonly seen in persistent periradicular infections.[2] Calcium hydroxide is the gold standard of intracanal medicament used in endodontic practice earlier, but the efficiency of the medicament is reduced due to dentine buffering capacity,[3,4] moreover the presence of inherent proton pump in the cell membrane of E faecalis maintains its own pH and remains unaffected by the external alkaline environment of calcium hydroxide.[5] To overcome this proton pump inhibitors were used in combination with calcium hydroxide and has shown positive results against E faecalis.[6] Engstrom has done susceptibility tests and [7] suggested Erythromycin or Chloromphenicol for local administration against intra canal Enterococci. Considering the mechanisms of resistance showed by E.faecalis species and ability of E faecalis to express proton pump linked to cell membrane and efficacy of erythromycin and calcium hydroxide against enterococcus faecalis [7] aim of the present study was to verify whether association of proton pump inhibitor omeprazole with erythromycin could increase the effectiveness of calcium

hydroxide as intracanal medicament against enterococcus faecalis.

## Materials And Methods

30 Male wistner rats (Fig 1) were used and experimental protocols were approved by local Animal Ethic committee. On day 1 each rat was anesthetized by an intra peritoneal injection of xylazine (10 mg/kg) combined with ketamine (100 mg/kg) (Fig 2). Pulp exposure was performed on incisor tooth by using ½ no. round bur to depth of bur diameter (Fig 3) followed by instrumentation of canal with #10 no. k file endodontic instrument under irrigation with saline solution. Exposed pulps were left open to oral environment to allow formation of periapical lesions for 28 days.[10]



Fig 1- Male wistner rats



Fig 2 – Intra peritoneal injection of local anesthesia



Fig 3 – Access cavity preparation

### Intracanal Medicaments

Pastes were prepared at moment of application in glass slab by using a stainless steel spatula with Calcium hydroxide (Prime Dental Products Pvt Ltd, Bhiwandi, India), Omeprazole (Sigma Aldrich; Mumbai, Maharashtra, India) and Erythromycin (Mehta API Pvt Ltd, India) with polyethylene glycol 400 (PEG 400; Acacia life science private limited - Hyderabad) as vehicle. Intracanal dressings were prepared according to following experimental groups. Group 1(n=10) - Calcium hydroxide + PEG, Group 2(n=10) - Calcium Hydroxide + Omeprazole + PEG, Group 3 (n=10) - Calcium Hydroxide + Erythromycin + Omeprazole + PEG.

### Procedure For Root Canal Preparation

On day 28, rats were reanesthetised and root canals were instrumented to 25 no. k file (Mani Inc, Japan) with NaOCl (1%) (Vishal Dental Pvt Ltd, India) as irrigating solution followed by EDTA 17% (Prevest Dental Products, India) for 3 min and final wash with saline solution. Subsequently canals were dried with #20no. sterilized paper points and filled with intra canal medications according to respective groups and sealed with cavit.

### Microbiological Analysis

Microbiological samples were collected by using #20 sterilized saline solution at 2 different periods. S1- day 28 before endodontic treatment (Fig 4), S2-15 days after placement of intracanal medication. In all cases absorbent paper points were aseptically transferred to 1.5ml microcentrifuge tubes (aliquots) containing brain heart infusion broth. Then solution is agitated by vortexing to suspend attached bacteria into solution.

To estimate the number of colony forming units per ml, serial decimal dilutions were prepared. Aliquots of 100 $\mu$ l of each dilution and original suspension were spread onto surface of blood agar and incubated aerobically at 37<sup>o</sup> c for 24hrs.



Fig 4 – Collection of microbial sample

### Statistical Analysis

Result were analysed as mean  $\pm$  standard error of mean of 10 animals in each experimental group. Data were subjected to analysis of variance, followed by Newman Keuls post hoc test.

### Results

#### Microbiological Analysis

After placement of intra canal medicaments the results are as (Table 1)

Group 1 - There was a mean reduction of 53% in colony forming units of E.faecalis

Group 2 - There was a mean reduction of 77% in colony forming units of E.faecalis.

Group 3 - There was a mean reduction of 98% in colony forming units of E.faecalis.

**Table 1** – Implicates the colony forming units after treatment with Ca(OH)<sub>2</sub>, Ca(OH)<sub>2</sub>+Erythromycin, Ca(OH)<sub>2</sub>+Erythromycin+Omeprazole.

Groups	S1 Sample( CFU/ml) Average mean value of 10 rats	S 2 sample (CFU/ml) Average mean value of 10 rats
Calcium hydroxide	50621±1653	23446±1230
Calcium hydroxide erythromycin	49876±1254	11879±852
Calcium hydroxide erythromycin, omeprazole	49346±1127	624±68

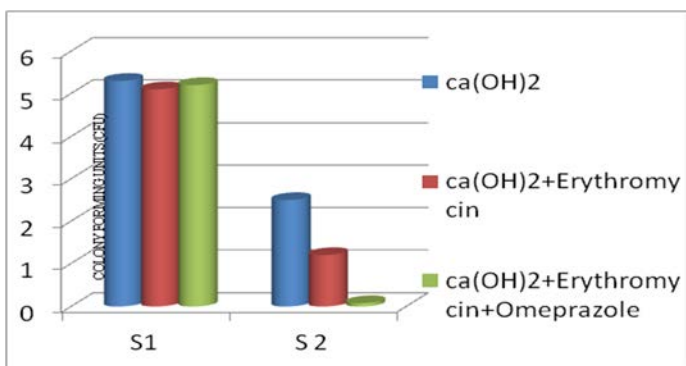
Comparison of effects of Ca(OH)<sub>2</sub>, Ca(OH)<sub>2</sub>+Erythromycin, Ca(OH)<sub>2</sub>+Erythromycin+Omeprazole on enterococcus faecalis. (Graph 1)

**Graph 1** - Comparison of effects of Ca(OH)<sub>2</sub>, Ca(OH)<sub>2</sub>+Erythromycin, Ca(OH)<sub>2</sub>+Erythromycin+Omeprazole on enterococcus faecalis.

S<sub>1</sub> Sample- Before applying intracanal medicament.

S<sub>2</sub> Sample- After application of intra canal medicaments

**(Mean Bacterial Reduction )**



**Discussion**

Intracanal medicaments by virtue of their contact time in the root canal can effectively eradicate microorganisms from the root canal. Calcium hydroxide due to its high pH of around 12.5 is one of the most commonly used intra

canal medicament but however in the most peripheral layer of dentin of the root the pH is reduced to 7.4 due to the buffering effect of dentin.[4] The formation of endodontic biofilms commonly seen in endodontic infections prevents the action of calcium hydroxide to its full extent.[10]

There is an ongoing debate in literature regarding the pros and cons for local antibiotics in treatment of oral infections in combination with other intra canal medicaments.[11] Main advantage of local antibiotics compared with systemic use is that systemic consequences and complications are prevented and that substantially higher concentration can be used.[12] Some disadvantages connected to local antibiotic delivering periodontal pocket can be easily overcome in root canal. Exposure to surrounding periapical vital tissue through apex is small and need for carrier system is not critical when root canal can be used as vehicle itself. Concentration can be optimized so that even microbial concentration can be used. The latter is important because there is no host defense system present and killing effect is necessary for complete elimination if inhibitory concentrations are used which is achieved with systemic use of antibiotics. This may lead the bacteria in root canal to become dormant. This condition is related to that of biofilm with poor penetration of drug and low susceptibility of bacteria caused by low metabolic activity.

Erythromycin had superior performance over tetracycline, especially in cases of pure cultures of Enterococci.[8] Enterococcus faecalis is a gram positive facultative anaerobe most commonly seen in persistent periradicular infections and retreatment cases.[5,13] The presence of proton pump in cell membrane of E faecalis imparts resistance to calcium hydroxide. Wagner et al [6] conducted a study on wistner rats by supplementing calcium hydroxide with omeprazole for healing of

periapical lesion and found out superior results. The proton pump inhibitors also exert anti inflammatory and pro reparative effects which enhance the healing of the periapical area.[14-16] The results of our study showed superior antibacterial properties with calcium hydroxide mixed with omeprazole and calcium hydroxide mixed with omeprazole and erythromycin groups. These results were similar to study conducted by Wagner et al and Molander A et al.[6,8]

In present study we decided to investigate effects of associating Omeprazole with calcium hydroxide and erythromycin in rat model of periapical lesions. Usefulness of rat models to emulate human endodontic lesions has been well established.[18] Most studies have used this model to evaluate effect of systemic medications on endodontic lesions.[19] However use of rat model to analyze intracanal dressings has not been fully characterized before. Furthermore association of Omeprazole with calcium hydroxide and erythromycin has never been tested in endodontics. Therefore this study was conducted considering previous evidence on relevance of proton pump for survival of bacteria resistant to endodontic treatment [2] as well as in current therapy of gastric ulcers associated with H pylorie infection in which proton pump inhibitors not only reduces the acid secretion but also increases bacterial sensitivity to antibiotics.[20]

The antimicrobial effect of  $\text{Ca}(\text{OH})_2$  is related to release and diffusion of hydroxyl radicals [21,22] and velocity of release depends on vehicle in which it is used for manipulation.[23] When  $\text{Ca}(\text{OH})_2$  is prepared in more viscous vehicle, PEG 400, as presented in our study ion release is slower than that observed when aqueous vehicle is used.[24] Interestingly according to the previous literature studies[6] demonstrated clear anti-inflammatory, proreparative effects for PPI's such as Omeprazole that indicates more effective healing of periapical tissue in

(Calcium hydroxide + Erythromycin + Omeprazole) group. Thus erythromycin mixed with calcium hydroxide and omeprazole seems to be valuable option in battle against Monoinfections of Enterococci. But further research directed towards more effective treatment strategies against Enterococci infected teeth is needed.[8] Therefore our results for new intra canal medicament are promising for endodontic clinical sitting.

### Conclusion

Our data showed association of Omeprazole with  $\text{Ca}(\text{OH})_2$  and erythromycin favoured superior anti bacterial efficacy against E.faecalis in comparison with other medicaments ( $\text{Ca}(\text{OH})_2$  alone, and  $\text{Ca}(\text{OH})_2$  + Omeprazole).

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