

## COMPARISON OF ANTIMICROBIAL EFFICACY OF CALCIUM HYDROXIDE AND 2% CHLORHEXIDINE AS MEDICAMENTS

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### ABSTRACT

#### INTRODUCTION:

The use of intracanal medicaments help in elimination of bacteria that remain even after cleaning and shaping. This study is done to compare the antimicrobial efficacy of 2% chlorhexidine gel and calcium hydroxide as an intracanal medicament against *E. faecalis*.

#### MATERIAL & METHODS:

Sixty extracted single-rooted human teeth were prepared with standard method. After contaminating the canals with *E. Faecalis* and incubated for seven days, the samples were divided into four groups ( $n=15$ ). Normal saline was used as control group. The teeth in each group were treated with normal saline, calcium hydroxide and 2% chlorhexidine gel. Microbial samples were obtained from the dentinal shavings of root and colony forming units (CFU) of *E. faecalis* were recorded after 24hrs, 72hrs and 7 days.

#### RESULTS:

To determine the significance of the differences between the different groups, ANOVA (Analysis of variance) and Tukeys Honestly significant Difference (HSD) Post hoc test were performed. Mean CFU of calcium hydroxide group and 2% chlorhexidine group was statistically significantly different from CFU of all other groups ( $p<0.01$ ).

#### CONCLUSION:

Mean CFU of calcium hydroxide was found to increase at 24 hrs and decreases at 72 hrs and 7 days. The antimicrobial efficacy 2% chlorhexidine gel was better than calcium hydroxide paste.

**KEYWORDS:** Calcium hydroxide, Chlorhexidine, Colony forming units, *E. faecalis*, Intracanal Medicaments

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## INTRODUCTION

Microorganisms are the major causative agents in the development of pulpal and periapical inflammation. Without microbial involvement in pulp and associated periapical tissues, there would be no need for endodontic therapy. Complete disinfection in root canal system is difficult because of anatomic complexities and diversity of root canals, as well as the subsequent limitations in access, by instruments and irrigants<sup>1</sup>.

Study has shown that instrumentation and antibacterial irrigation renders 50% to 70% of infected canals free of microorganisms while the remaining canals contain vital bacteria which are thought to be source of persistent endodontic infection. *E. faecalis* is the most commonly isolated species in persistent root canal infections. It has been detected in 77% of failed endodontic cases<sup>2</sup>. Therefore, the use of intracanal medicaments help in elimination of bacteria that remain even after cleaning and shaping<sup>3</sup>.

Different types of intracanal medicaments have been used to disinfect the root canals. Among them, calcium hydroxide is one of the most widely used intracanal medicament because of its well documented antibacterial activity against most of the strains identified in root canal infections. Since, the pH of calcium hydroxide is about 12.5, several bacterial species commonly found in infected root canals are eliminated after a short period when in direct contact with this substance.

Apart from calcium hydroxide, 2% Chlorhexidine gluconate gel has also been used as intracanal medicaments as it has broad spectrum antimicrobial activity affecting both gram positive and gram negative bacteria. It possesses properties that include extended residual activity and relative absence of toxicity<sup>4</sup>. The rationale of this in-vitro study is to compare the antimicrobial efficacy of 2% Chlorhexidine gel and Calcium hydroxide as intracanal medicaments against *E. faecalis*.

## MATERIAL & METHODS:

### Preparation of the Samples

This study was conducted in the Department of Conservative dentistry and endodontics, Universal College of Medical Sciences, College of Dental Surgery, Bhairahawa, Nepal for 3 months period in 2015. 45 freshly extracted anterior human teeth with straight single canal and mature apex were collected from the department of Oral and Maxillofacial surgery. Teeth with immature root apices, fracture or craze lines, restoration, hypo calcification, or hypoplasia, presence of multiple or lateral canals, calcification, more than 2 roots/canals and curved root with more than 30 degree curvature of root were excluded from the study. Teeth were decoronated using a

diamond disc (Axis Products) at low speed to standardized root length of 18 mm  $\pm$  2mm. Root canal treatment was done. Then, the teeth were individually placed in bottles containing 2ml of Brain heart infusion medium and autoclaved at 121°C, 20 lbs for 15 mins. They were then kept in an incubator at 37 °C for 24 hr to check the efficiency of the sterilization treatment. Sterilization of teeth was confirmed by gram staining the BHI broth and pour plating the broth.

### Bacterial culture

Isolated 24 hr colony of pure culture of *E. faecalis* was grown in blood agar and suspended in brain heart infusion broth. Turbidity of BHI broth was verified by using the MacFarland Turbidity Scale and adjusted to 0.5, corresponding to  $1.5 \times 10^8$  organisms CFU per milliliter. All the microbiological experiments were conducted under aseptic conditions in a laminar flow hood to avoid contamination by outside organisms.

### Contamination of the samples

Tubes containing each specimen were opened. Sterile pipettes were used to remove 2ml of sterile BHI and were replaced with 2ml of bacterial inoculums. Pure culture of *E. faecalis* (ATCC 29212) was used as a test organism. At the end of each experimental run, bacterial viability and purity of cultures was confirmed by gram staining, Catalase production, colony morphology on BHI agar bacterial viability and purity of cultures was confirmed by gram staining, Catalase production, colony morphology on BHI agar. At the end of 7 days, the blocks were irrigated with sterile saline to remove the incubation broth. Canals were dried with sterile paper points (Dentsply, Maillefer, Switzerland).

### Antibacterial Assessment

The blocks were randomly assigned to the following groups divided into 4 groups (n=15) group 1- Normal Saline (negative control), group 2- Calcium hydroxide (Ecodont, India), and group 3- 2% Chlorhexidine gel (Conceptsis, Ultradent, USA). Calcium hydroxide was mixed with sterile saline in the ratio of 1:1 (wt/vol) to obtain paste like consistency and the mixture was placed into root canal with a lentulo spiral. 2% Chlorhexidine gel was placed into root canal with a lentulo spiral. The medicaments were placed inside the canals. A sterile cotton pellet was placed in the canal orifice and sealed with tin foil. Blocks were incubated in an anaerobic environment for 37 °C in incubator (Naran Scientific works, India). After the loading of various medicaments, all groups were subdivided into three subgroups of 5 samples and incubated for different experimental time periods of 24 hrs, 72 hrs and 7 days.

Harvesting of the dentin was carried out with round carbide bur of diameter 1mm. The dentinal shavings obtained were collected in each sterile petridish and then transferred into 1ml of sterile BHI broth and incubated in an anaerobic environment at 37°C for 24 hrs. After 24 hrs, the contents of each tube was serially diluted, 100ul of the broth in 300ul of sterile saline for 8 times. 100 µl of the dilution was then plated on nutrient agar plate with L shaped rod and incubated for 24hrs. Colonies were counted using colony counter. The same procedures were repeated at time intervals of 72hrs and 7 days in each five samples respectively. The data was entered manually on Microsoft excel (MS Office Excel 2000; Microsoft Corporation, Redmond, WA, USA), checked for possible data entry errors. Frequencies and percentages were taken out for categorical variables. The data were analyzed using SPSS version 21.0 (IBM Corp. Armonk, NY: IBM Corp). For inferential statistics One way ANOVA followed by post hoc Tukey (HSD) was used for multiple comparisons between different groups. The level of significance was established as  $P < 0.01$ .

## RESULTS

Table 1 shows the mean  $\pm$  SD CFU count for the study groups at different time intervals. On comparison of the means at particular time showed statistically significant difference among the groups ( $p < 0.01$ ). Table 1 also shows pair wise comparison using Tukey's test of groups at 24hrs, 72hrs and 7 days. Mean CFU of control group and calcium hydroxide group was statistically significantly different from mean CFU of all other groups ( $p < 0.01$ ). Mean CFU of control group and chlorhexidine group was statistically significantly different from mean CFU of all other groups ( $p < 0.01$ ). Mean CFU of calcium hydroxide group and 2% chlorhexidine group was statistically significantly different from CFU of all other groups ( $p < 0.01$ ).

GROUP	24hrs	72hrs	7 DAYS
Normal saline (NS)	450	186.6 $\pm$ 55.1	175.8 $\pm$ 26.8
Calcium hydroxide (CH)	113.2 $\pm$ 32.6	90.00 $\pm$ 26.3	68.6 $\pm$ 17.8
2% Chlorhexidine gel (CHX)	8.6 $\pm$ 10.1	13.2 $\pm$ 13.06	14.6 $\pm$ 17.2
F value	36.77	23.6	58.00
P value	0.01	0.01	0.01
Significant pairs in Post Hoc Tukey test	NS vs CH; NS vs CHX; CH vs NS; CH vs CHX; CHX vs NS; CHX vs CH	NS vs CH; NS vs CHX; CH vs NS; CH vs CHX; CHX vs NS; CHX vs CH	NS vs CH; NS vs CHX; CH vs NS; CH vs CHX; CHX vs NS; CHX vs CH

Table 1. Descriptive and Comparative Analysis showing Mean  $\pm$  Standard Deviation of CFU of groups at different time intervals of 24hrs, 72hrs and 7 days (one week).

## DISCUSSION

The aim of this study is to compare the antimicrobial efficacy of chlorhexidine gel and calcium hydroxide as intracanal medicaments against *E. faecalis*. The calcium hydroxide group was used to compare with newer intracanal medicaments because it is widely used in Endodontics. In calcium hydroxide, the mean CFU was found highest at 24hrs and least at 7 days. Even though calcium hydroxide does have some antibacterial action, under the experimental run, it was not able to eliminate sufficient cells of *E. faecalis* at any time. This is in accordance with the findings of other studies. This is in accordance with the findings of other reports.<sup>6,7,8</sup> The antimicrobial efficacy of calcium hydroxide is lower in comparison to chlorhexidine gel due to the buffering of the alkalinity of calcium hydroxide by dentin and dentin components, low diffusibility of hydroxyl ions in dentinal tubules and colonization of *E. faecalis* within dentinal tubules forming dense biofilm. Calcium hydroxide have shown lower efficacy than chlorhexidine gel in all time intervals.

The present study revealed that highest antibacterial activity was observed with 2% chlorhexidine gel in comparison to other groups in all the time intervals (Figure 1). The antimicrobial efficacy of 2% chlorhexidine gel may be due to the high concentration, lethal bactericidal mode of action and enhanced diffusion into dentinal tubules as chlorhexidine gel has low contact angle with dentin and thus penetrates the dentinal tubules effectively at faster rate.<sup>9,10</sup> 2% Chlorhexidine gel was not able to eliminate *E. faecalis* completely as it may be due to the inhibitory effect of dentin, other molecules as serum albumin and collagen as well as killed microbes present in the root canal which can alter the efficacy of intracanal medicaments.

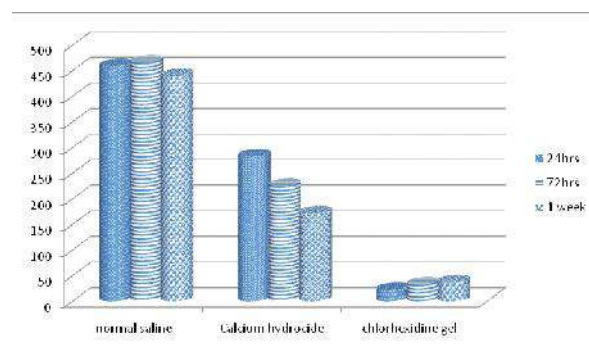


Figure 1. Mean CFU at 24hrs, 72hrs and 7 days (1 week)

There are various limitations in the study. Even though, to minimize the biases only single rooted canal of similar dimensions of the similar age group patients are included in

the study, each tooth in oral cavity has certain variation in anatomy. It is expected to get this variation in this study too. Every effort was made but this in vitro could not simulate the intraoral environment inside the infected root canal. It was not possible to standardize certain variables such as the quantity of dentinal shavings analyzed per sample, amount of time required for drilling a punch hole, the amount of heat generated during the procedure.

## CONCLUSION

Although, the present study has shown 2% chlorhexidine gel to be the more effective antibacterial agent than calcium hydroxide at all-time intervals. Under the limitations of this study, it can be concluded that calcium hydroxide and chlorhexidine gel have antimicrobial efficacy against *E. faecalis*.

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