Original Article

An *in vitro* comparison of calcium ions release and diffusion ability of calcium hydroxide-based intracanal medicament in combination with three different vehicles like propolis, chitosan, and propylene glycol

Pinku T. Thomas, Jyoti Sumi Issac, Parvathy Girija, Lekshmi S. Chandran, D. S. Arjun, A. J. Siddik

Department of Pediatric and Preventive Dentistry, Azeezia College of Dental Sciences and Research, Kollam, Kerala, India

Abstract

Context: Calcium hydroxide, which is an intracanal medicament, is widely used in endodontics. Improvements can be made to its effectiveness, as calcium hydroxide is dependent on the vehicle.

Aim: The study aims to compare and evaluate the release and diffusion ability of calcium hydroxide when mixed with – propolis, chitosan, and propylene glycol.

Methods: For this study, 33 single-rooted extracted premolar teeth have been decoronated. After the working length and enlargement of the canals had been established, different preparations of calcium hydroxide with vehicles such as propolis, chitosan, and propylene glycol were loaded into the canals. Atomic absorption spectrophotometry was used to analyze the release of calcium ions in three groups, while a digital pH meter was used to determine an acid change.

Results: Atomic absorption spectrophotometry showed sustained releases of calcium ions and the digital pH meter showed increased diffusion capacity in the propylene glycol paste group in comparison to the other two groups.

Conclusion: Propylene glycol vehicle made it easier to enter calcium hydroxide into the dentinal tubules.

Keywords: Atomic absorption spectrophotometry; calcium hydroxide; chitosan; pH; propolis; propylene glycol

INTRODUCTION

The removal of debris may not always be complete with mechanical instrumentation during endodontic treatment due to morphological and physical barriers.[1] To disinfect and promote periapical healing, calcium hydroxide has been given as the gold standard intracanal medicinal product since 1920.[2]

The ability of calcium hydroxide to dissociate directly with Ca^{2+} and OH-ions results in a higher pH locally.[3] The concentration and velocity of ionic liberation are directly linked to the type of vehicle in various *in vitro* studies.[4] In terms of chemical classification, vehicles can be divided into three categories; viscous, hydrosoluble, and oily. The use of an aqueous vehicle enhances solubility, leading to rapid phagocytosis by macrophages, but the canal needs to be cleaned multiple times before that effect is achieved. Although viscous vehicles such as propylene glycol are water soluble, they release calcium and hydroxyl

Address for correspondence:
Dr. Pinku T. Thomas,
Seonpurathil, Near Indian Oil Petrol Pump, Chekkalamukku, Sreekariyam, Thiruvananthapuram - 695 017, Kerala, India.
E-mail: pinkuthomas92@gmail.com

Date of submission : 02.11.2023
Review completed : 29.11.2023
Date of acceptance : 05.12.2023
Published : 08.02.2024

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/jcde
	DOI: 10.4103/JCDE.JCDE_258_23

How to cite this article: Thomas PT, Issac JS, Girija P, Chandran LS, Arjun DS, Siddik AJ. An *in vitro* comparison of calcium ions release and diffusion ability of calcium hydroxide-based intracanal medicament in combination with three different vehicles like propolis, chitosan, and propylene glycol. J Conserv Dent Endod 2024;27:190-4.

ions more slowly for a longer period due to their high molecular weight.[5] Research has been conducted on medically potential products such as propolis and chitosan in combination with calcium hydroxide due to the global trend in natural products. Honeybee produces a natural resinous mixture called propolis that contains highly active bioflavonoids and has antimicrobial, antioxidant, and anti-inflammatory properties.[6]

Chitosan is a polysaccharide that also naturally occurs and is obtained through partial deacetylation of chitin. When used as a vehicle for a longer duration, Ca^{2+} ions are released in a controlled manner from $Ca(OH)_2$.[7] Consequently, it is important to note that the combination of certain vehicles with calcium hydroxide can affect its therapeutic effects.

The present study aims

- To determine the efficacy of propolis, chitosan, and propylene glycol as vehicles when mixed with calcium hydroxide by determining their calcium ion release and diffusion ability.

Objective

- To evaluate the calcium ion release and diffusion ability of calcium hydroxide with propylene glycol
- To evaluate the calcium ion release and diffusion ability of calcium hydroxide with propolis and chitosan
- To compare the calcium ion release and diffusion ability of calcium hydroxide with propylene glycol, propolis, and chitosan.

METHODS

This study has been conducted at the Department of Pediatric and Preventive Dentistry, Azeezia College of Dental Sciences, Kollam, in cooperation with the Cashew Export Promotion Council of India (CEPCI) Laboratory and Research Centre. Before the initiation of this study, ethical approval has been obtained from Azeezia's Ethics Committee AEC/REV/2019/40. The study is *in vitro* and does not include any contact with human subjects; AEC has decided to provide a waiver of informed consent.

The sample size is calculated using the formula:

$$n = \frac{(Z1\text{-}\alpha/2 + Z1\text{-}\beta)^2\ ([S1]^2 + [S2]^2)}{(X_1 - X_2)^2}$$

where $X_1 = 9.70$, $X_2 = 9.16$, $S1 = 0.45$, $S2 = 0.3$

$= 11$ (each group)

Three groups are present, therefore, the total sample $= 11 \times 3 = 33$.

Thirty-three single-rooted premolar teeth, therapeutically extracted for orthodontic purposes, were selected to be studied in the experimental study.

Specimen preparation

Before the experiment was carried out, the teeth were cleaned with ultrasonics and preserved in a container of saline solution after the extraction. The teeth were decoronated and the length of each tooth was codified to a maximum of 12 mm [Figure 1]. The root canals have been widened to the size of 20 K file and tissue from the pulp has taken off using a broach. Working length was determined by adding 10 K files until they were visible on the apical foramen.

Canal preparation

The root canal has been treated with a filing technique up to a file size of 45 K for each tooth. For each file, 2 mL of 2.5% sodium hypochlorite using a 27-cm beveled needle was passively irrigated into the canal. The final infusion was to flush 2 mL of 17% ethylenediaminetetraacetic acid solution with 5 mL of distilled water for 1 min to remove any precipitates and store them safely in a sealed saline bottle. Based on the combination of $Ca(OH)_2$ with various vehicles such as propolis (Super Bee Natural Bee Propolis 30 mL), chitosan (Bangalore Fine Chem 50 g), and propylene glycol (Pioneer Propylene Glycol, 99.9%, 250 mL), calcium hydroxide (Prevest DenPro Calcium Hydroxide, Extra Fine Powder) has been divided into three groups. The teeth were divided into three groups and each group consisted of 11 teeth ($n = 11$).

Preparation of calcium hydroxide pastes

- Group 1: $Ca(OH)_2$ propolis paste, which is made from 1 g of $Ca(OH)_2$ powder with 2 mL of propolis
- Group 2: $Ca(OH)_2$ chitosan paste. To make a gel base, dissolve chitosan in 2 g of chitosan in 2 mL of acetic acid (Spectrum Reagents and Chemicals). 1 g of $Ca(OH)_2$

Figure 1: Preparation of tooth sample

powder in 100 mL of deionized water was added and mixed into the formed base
- Group 3: $Ca(OH)_2$ propylene glycol paste, which is made by adding 1 g of $Ca(OH)_2$ powder with 2 mL of propylene glycol.

A Lentulo spiral spreader was used to pack the accurately measured amounts of different compositions as soon as the canals were dried with paper points. Glass ionomer cement was used to seal the orifices of each tooth. To avoid moisture contamination, petroleum jelly has been applied over the cement. To allow only the apical third of roots to be exposed to deionized water, each tooth was encased in a glass vial measuring 3 cm and held by molding wax that contained 3 mL of dissolved water [Figure 2]. To avoid exposure to moisture, vials have been closed tightly by modeling wax and cork.

pH measurement

A digital pH meter of Orion 3 Star pH Benchtop at 24-h, 7-day, 15-day, and 30-day intervals has been used to determine changes for various formulations. The medium has been homogenized for 10 s before each reading by shaking the vial. pH electrode has been immersed fully into the deionized water in each vial. To remove any residues of Ca $(OH)_2$ between each measurement, the pH electrode has been rinsed thoroughly with deionized water and dried on absorbent paper.

Measurement of calcium ion concentration

After establishing a baseline reading, an atomic absorption spectrometer (Perkin Elmer India Pvt. Ltd., Model: Pinnacle 900 H) with a calcium-specific hollow cathode lamp was used to determine the mean concentration of the Ca^{2+} ions released. The 3 mL solution of each vial was withdrawn and replaced with fresh distilled water after every periodical measurement.

Figure 2: Each tooth from three groups with accurately measured quantities of different formulations was suspended in glass vials, such that only the apical third of the roots were immersed in deionized water

Statistical analysis

For the analysis of data, SPSS 26.0 (SPSS Inc., Chicago, IL, USA) statistical package was used and $P < 0.05$ was set as the level of significance. Descriptive statistics were compiled, and using the Shapiro–Wilkinson test, the normality of the data was assessed. Using the one-way ANOVA test, inferential statistics were used to determine the difference between the groups. In the group, a comparison was performed by repeated measures of the ANOVA test in combination with the *post hoc* test (Bonferroni test).

RESULTS

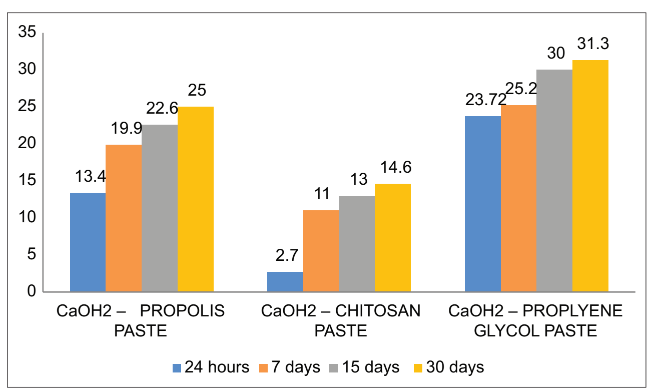
The release of ions was statistically significant during the intergroup comparison. The test shows a significant difference present at all the pair time intervals ($P < 0.05$) and the difference from 24 h to 30 days was 7.6 ± 0.98. Group 3 showed the highest release of calcium ions, while Group 2 showed the lowest. Propylene glycol was used as a vehicle in Group 3 to compare its proven efficacy with vehicles such as propolis and chitosan. Maximum diffusion was found in Group 3 with a sustained release of OH^- ions.

At 24 h, 7 days, 15 days, and 30 days, Table 1 shows the mean values of calcium ion release of three groups in deionized water. Graph 1 explains the summary of all three pastes and their intervals regarding the calcium ion release.

Table 1: The Mean values of calcium ion release of the entire three groups in deionized water at different time intervals

Groups	Group-1 $CaOH_2$ – Propolis paste	Group-2 $CaOH_2$ – Chitosan paste	Group-3 $CaOH_2$ – Propylene glycol paste
24 h	13.4±1.84[a]	2.7±0.34[a]	23.7±1.56[a]
7 days	19.9±1.13[b]	11±1.56[b]	25.2±1.34[b]
15 days	22.6±1.22[c]	13±1.12[c]	30±1.77[c]
30 days	25±1.87[d]	14.6±1.23[d]	31.3±1.54[c]
P (ANOVA)	0.0001*	0.0001*	0.0001*
Difference	12±0.29	11.9±0.89	7.6±0.98

Dissimilar superscripts denote statistical significance ($P<0.05$)

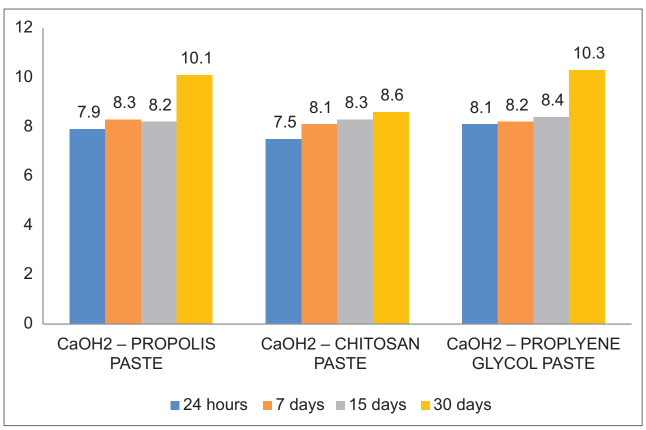


Graph 1: explains the summary of all three pastes and its intervals regarding the calcium ion release

Table 2 shows the respective mean pH of all three groups at different time intervals in deionized water. Graph 2 explains a summary of all three pastes and their intervals regarding the pH change.

DISCUSSION

According to Wang and Hume, the prime determinant of dentine permeation is molecular size, in which smaller molecules more readily diffuse.[8] After biomechanical preparations, some microorganisms are susceptible to antibiotic treatment and may survive in the root canal.[9] Estrela *et al*. conducted a study based on scientific evidence highlighting that Herman first proposed that calcium hydroxide be used as an intracanal medicament in treating dental pulp.[10] In clinical situations treated with this medicament, which is due to its antimicrobial effect, induction of hard-tissue formation, and ability to support regeneration, healing has been observed.[11] About 80.8% and 73.8% of success rates have been reported when calcium hydroxide used is for endodontic treatment in teeth with periapical lesions.[12] It may be more easily resorbed into apical tissues when calcium hydroxide powder is mixed with an appropriate vehicle.[13] Significant pH values were observed even in the calcium hydroxide–water combination.[14]



Graph 2: Explains the summary of all three pastes and its intervals regarding the pH change

Table 2: The mean pH of all three groups in deionized at different time intervals

Groups	$CaOH_2$ – Propolis paste	$CaOH_2$ – Chitosan paste	$CaOH_2$ – Propylene glycol paste
24 h	7.9±0.23[a]	7.5±0.29	8.1±0.32[a]
7 days	8.3±0.16[a]	8.1±0.19	8.2±0.34[a]
15 days	8.2±0.76[a]	8.3±0.69	8.4±0.43[a]
30 days	10.1±0.88[b]	8.6±0.62	10.3±0.68[b]
P (ANOVA)	0.0001*	0.992	0.0001*
Difference	2.2±0.65	1.1±0.33	2.2±0.36

Dissimilar superscripts denote statistical significance ($P<0.05$)

In a study carried out by Chandak *et al*., it has been established that the ion diffusion and pH of calcium hydroxide are larger in herbal pastes such as aloe vera.[15] Compared to aqueous or viscous vehicles, paste with oily vehicles has significantly larger mean inhibition zones.[16] Fava and Saunders highlighted that the physical and chemical characteristics of the compound can be affected by the vehicle which is composed of calcium hydroxide.[17]

Three experimental pastes with propolis, propylene glycol, and chitosan have been used to evaluate calcium ion release and diffusion ability of hydroxyl ions in this study.

In 1962, laws proposed that propylene glycol could be used as a vehicle in endodontics because of its good handling properties to the paste, and fibroblasts were shown to have a reduced impact.[18] Its alkalinizing properties can give an immediate and rapid increase in 24 h, followed by progressive increases of 15–30 days.[19] The possible use of propylene glycol in the delivery of intracanal medicament products has been proposed by Cruz *et al*., who noted that a rapid demonstration was made for the administration of dye through the root canal system.[20] The thickness of propolis promotes a diffusion that also has a superior push-out bond strength to 2% chlorhexidine gel, at the apical third.[21]

There are some limitations to propolis, such as changes in temperature, which can alter the form and color of the tooth.[22] Another natural, unbranched homopolymer derived from chitin is chitosan. The growth of *Enterococcus faecalis* and *Candida albicans* may be inhibited by combining calcium hydroxide with chitosan.[23] Alireza *et al*. noted that when it is mixed with calcium hydroxide the ions are released slowly.[24]

For determining the release of calcium ions, a number of techniques including atomic absorption spectrometry, ultraviolet spectrophotometer, and flame photometry have been used. In 1995, Simon *et al*. used Orion's research microprocessor ion analyzer 901 to measure the concentration of calcium ions.[25] According to Robertson and Marshall, the measurement of calcium ion release using an atomic absorption spectrophotometer was the most accurate method compared to other methods due to the potential advantages of being highly specific for calcium and minimal preparation required for the sample.[26] Earlier Wang *et al*.,[8] used a dual chamber system to measure diffusion ability. Grover *et al*.,[3] Montero *et al*.[13] observed pH changes by using a pH meter.

This study tried to estimate diffusion ability using a digital pH meter and quantitatively measuring calcium ion release from atomic absorption spectrophotometry since it is simple, rapid, and cost-effective.

Thus, the intercomparison among the three groups showed that Group 3 reported the highest amount of calcium release, followed by Group 1, and the least release of calcium ions was associated with Group 2. The diffusion of hydroxyl ions was similar concerning Group 1 and Group 3 where the latter group was marginally higher than Group 3. The ability to regulate and sustain Ca^{2+} and OH^{-} ion release is made possible by propylene glycol. This prevents an increase of pH above the critical optimum level needed for tissue regeneration and repair.

The difference between calcium hydroxide propolis paste and calcium hydroxide propylene glycol paste showed the largest variation over time intervals, followed by negligible differences for calcium hydroxide chitosan paste.

Limitations of the study

Since the study was conducted with a small sample size, future trials on a larger sample will make it possible to determine the benefits and advantages in a more precise way. Further, evaluation of different clinical conditions, and chemical reactions of the materials used, is required.

CONCLUSION

Within the limitations of the study:

- The highest release of calcium ions from the group calcium hydroxide propylene glycol paste group was found, followed by calcium hydroxide propolis paste, and the lowest with calcium hydroxide chitosan paste
- In the case of calcium hydroxide propolis paste and calcium hydroxide propylene glycol paste, the diffusion of hydroxyl ions was similar, where the latter group was slightly higher than the calcium hydroxide propolis paste.

Therefore, the study showed that the calcium hydroxide propylene glycol paste group promotes healthy releases of calcium and the ability to absorb hydroxyl ions from dentinal tubules. Because of enhanced clinical properties, and a reduced number of appointments, calcium hydroxide propylene glycol vehicle combination can be used as an effective intracanal medicament.

Acknowledgement

I thank all the co-authors for supporting me in conducting the study. I also thank all the staff at the CEPCI Laboratory and Research Institute, Kollam for the technical assistance.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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