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EVALUATION OF CALCIUM AND HYDROXYL ION RELEASE WHEN CALCIUM HYDROXIDE IS COMBINED WITH GRAPHENE OXIDE AS A VECHICLE -AN IN- VITRO STUDY

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ABSTRACT

Background: Calcium hydroxide is widely used as an intracanal medicament because its dissociation releases calcium and hydroxyl ions that contribute to antimicrobial activity and hard-tissue formation. Graphene oxide has been proposed as a vehicle, but its influence on ion release requires evaluation. Aim: To compare the in vitro release of calcium and hydroxyl ions from calcium hydroxide mixed with distilled water and calcium hydroxide mixed with graphene oxide. Materials and Methods: Calcium hydroxide preparations were divided into two groups (n = 5): calcium hydroxide with distilled water and calcium hydroxide with graphene oxide solution. The preparations were placed in polyethylene tubes and immersed individually in 20 mL of deionized water. Calcium ion release was quantified by the o-cresolphthalein complexone colorimetric method at 570 nm at predetermined intervals from 5 minutes to 7 days. Hydroxyl ion release was calculated from the measured calcium ion release, and the groups were compared statistically. Results: Calcium hydroxide mixed with distilled water demonstrated greater calcium and hydroxyl ion release than the graphene oxide-containing preparation throughout the observation period. Significant between-group differences were observed at 20 minutes and on days 6 and 7. The graphene oxide group showed a gradual increase in ion release but remained lower than the distilled-water group. Conclusion: Under the conditions of this study, distilled water promoted greater release of calcium and hydroxyl ions from calcium hydroxide than graphene oxide when used as a vehicle.

KEYWORDS: Calcium hydroxide; graphene oxide; intracanal medicament; calcium ion release; hydroxyl ion release; endodontics.

INTRODUCTION

Endodontic treatment is directed toward the prevention and control of pulpal and periradicular infections. The outcome of the endodontic therapy depends on reduction or elimination of microorganisms. Complete chemo-mechanical preparation may be considered an essential step in root canal disinfection. However, total elimination of bacteria from the root canal is difficult to accomplish.[1,2] Hence, by remaining in the root canal in between appointments, intracanal medicaments help to eliminate surviving bacteria.

Since its introduction by Hermann in 1920, calcium hydroxide has been widely used in endodontics. It is a strong alkaline substance, which has a pH of approximately 12.5. In an aqueous solution, calcium hydroxide dissociates into calcium and hydroxyl ions. Various biological properties have been attributed to this substance, such as antimicrobial activity,[3] inhibition of tooth resorption[4] and induction of repair by hard tissue formation.[5] Because of such effects, calcium hydroxide has been recommended for use in several clinical situations.[6,7]

Most of the endodontic pathogens are unable to survive in the highly alkaline environment provided by calcium hydroxide.-[7] Antimicrobial activity of calcium hydroxide is related to the release of hydroxyl ions in an aqueous environment. Hydroxyl ions are highly oxidant free radicals that show extreme reactivity with several biomolecules. [8]

Graphene oxide is a two-dimensional allotrope of carbon, with a honey comb lattice. The mechanism of graphene oxide has been demonstrated to be depended on purity, size of nanosheets, concentration of Graphene oxide and exposure time. Aqueous dispersion of Graphene oxide were found to be stable in the pH range of 4-12, with excellent dispersibility in the range of 7-11 .

The aim of our study was to compare the in vitro release of calcium and hydroxyl ions from the two different calcium hydroxide preparations used in endodontic treatment

AIM

To compare and evaluate the. calcium ion and hydroxyl ion release in calcium hydroxide based intracanal medicaments when combined with graphene oxide. as a vechicle

OBJECTIVE

To evaluate the calcium and hydroxyl ion release when calcium hydroxide is combined with graphene oxide as a vehicle

STUDY DESIGN

Study design: in vitro experimental design
Type of study: in vitro study
Sample size: 5 in each group

MATERIALS AND METHOD

Graphene oxide 10mg/ml dispersion in water is purchased from modern scientific solution, Aluva
Calcium hydroxide powder from prevest denpro
Distilled water
Deionized water
Lentulospirals
Temporary sealer
Polyethelene tubes
Colorimeter

SAMPLING PROCEDURE

PREPARATION OF SAMPLES

Calcium hydroxide-based substances will be divided into two groups:

Group A: Calcium hydroxide powder with distilled water
Group B: Calcium hydroxide powder with Graphene oxide solution

PREPARATION OF MEDICAMENTS:

Commercially available calcium hydroxide powder (prevest denpro) is weighed in a digital weighing scale with an accuracy of +/- 0.001gm accuracy, and mixed with distilled water to make a 10 mg / ml solution. Graphene oxide 10mg/ml dispersion in water (Modern scientific solution, Aluva) is mixed with Calcium Hydroxide (10mg/ml) in a 1:1 concentration.

SAMPLE PREPARATION:

Ten-millimetre polyethylene cylindrical tubes is filled with the respective materials of each group. Before this, one end of all the tubes is sealed with a 1-mm layer of temporary sealer. A lentulospiral was used to introduce carefully the calcium hydroxide pastes into the tubes through the opening, avoiding bubble formation. The tubes was then immersed in separate test tubes, each with 20 mL of deionized water which was used as the extraction solution. The extraction solutions were maintained at room temperature and without agitation. For each group, five samples were analyzed.

Calcium quantification

Calcium ion liberation was measured by a colorimetric method (o-cresolphthalein complex one calcium reaction). For each group, samples were analyzed after 0, 5, 10, 20 and 40 minutes; and after 1, 2, 6, 24 and 48 hours till 1 week.At each interval, 200 µL of solution was retrieved from the respective extraction solutions. For calcium quantification, 0.5 mL of buffered solution and 0.5 mL of color reagent was added. The cresolphthalein–calcium complex formation were followed by colorimetric method at 570 nm, which is directly proportional to calcium concentration. The linearity of the method was verified through calcium standards up to 25 mg/dL.

Analysis of liberated hydroxyl Ions

From the calculations of the calcium ions liberated, it will also be possible to determine the amount of hydroxyl ions liberated. The molecular weight of 2 mol hydroxyl ion is 34, and the molecular weight of 1 mol calcium ion is 40.08, and therefore the molecular weight of the complete molecule is 74.08. The percent of the two in the total weight can be simply calculated to be 45.89% and 54.11%, respectively. Therefore, in 1 mol of calcium hydroxide, there are 45.89% hydroxyl ions and 54.11% calcium ions. After determining the quantity of liberated calcium ions, the quantity of hydroxyl ions can be calculated as they are in direct proportion.

PLAN OF ANALYSIS

Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference post hoc tests with PASW Statistics version 18 to compare the statistical difference

RESULTS:

Calcium ion release

The measurement of calcium release over 1 week was compared for calcium hydroxide powder with distilled water and calcium hydroxide powder with graphene oxide . The delivery of calcium ion whereas calcium hydroxide powder with distilled water showed greater amounts of release in a short period, and a time-dependent increase of delivery was observed. Calcium hydroxide powder with distilled water released more calcium ions than other groups in the first hour. Calcium hydroxide powder with distilled water showed a significant increase after 2 hours, reaching a maximum level after 24 hours and 1 week, respectively.

While calcium hydroxide with Graphene oxide showed release of calcium ions less than that of calcium hydroxide with distilled water, where the values increased gradually and reached a maximum value by one week, but comparably less than that of calcium hydroxide and Distilled water.

Hydroxyl ion release

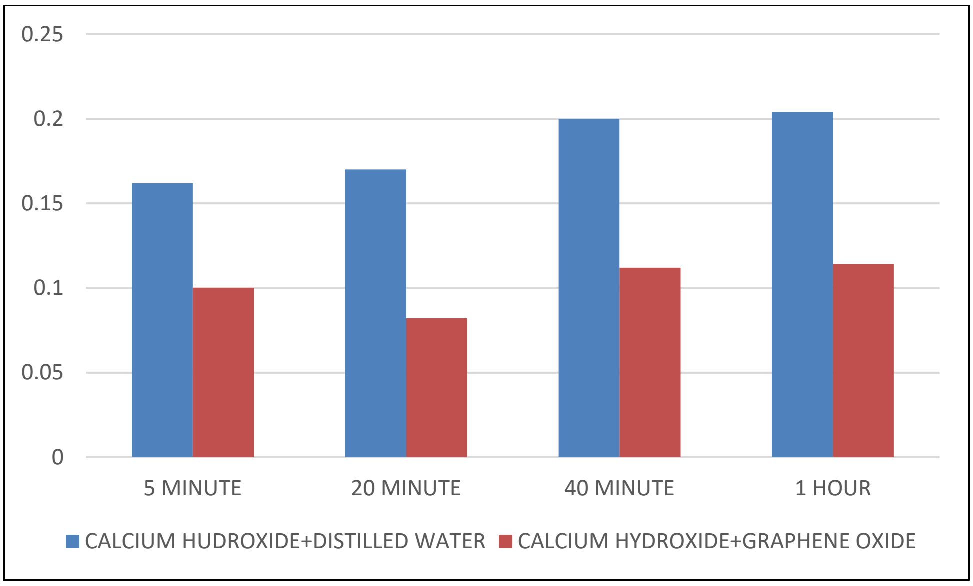
Hydroxyl ion release was calculated from the calcium ion release which was measured. The calculated percent of liberation of hydroxyl ions was thus 84.80% of the liberated calcium ions. The order of release of hydroxyl ions was calcium hydroxide powder with distilled water where it was the highest, followed by and calcium hydroxide graphene oxide combination.

T-Test

Group Statistics					
	Group	**N**	**Mean**	**Std. Deviation**	**P VALUE**
5 Minute	Calcium Hudroxide+Distilled Water	5	.1620	.01643	.139
	Calcium Hydroxide+Graphene Oxide	5	.1000	.05701	
20 Minute	Calcium Hudroxide+Distilled Water	5	.1700	.01225	.002
	Calcium Hydroxide+Graphene Oxide	5	.0820	.07791	

40 Minute	Calcium Hudroxide+Distilled Water	5	.2000	.00707	.081
	Calcium Hydroxide+Graphene Oxide	5	.1120	.03962	
1 Hour	Calcium Hudroxide+Distilled Water	5	.2040	.00548	.076
	Calcium Hydroxide+Graphene Oxide	5	.1140	.03782	

The Table Shows The Comparison Between The Groups At Different Time Periods. The Comparison At 20 Minutes Was Found To Be Statistically Signficant.

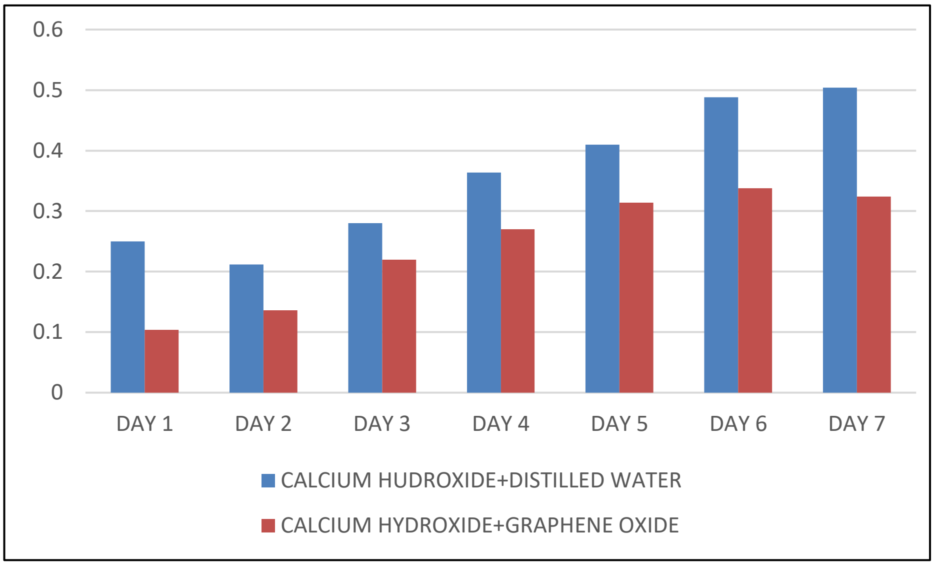


T-Test

Group Statistics					
	Group	**N**	**Mean**	**Std. Deviation**	**p Value**
Day1	Calcium Hudroxide+Distilled Water	5	.2500	.02236	.227
	Calcium Hydroxide+Graphene Oxide	5	.1040	.05595	
Day2	Calcium Hudroxide+Distilled Water	5	.2120	.03421	.286
	Calcium Hydroxide+Graphene Oxide	5	.1360	.07021	
Day3	Calcium Hudroxide+Distilled Water	5	.2800	.08216	.801
	Calcium Hydroxide+Graphene Oxide	5	.2200	.07649	
Day4	Calcium Hudroxide+Distilled Water	5	.3640	.06877	.842
	Calcium Hydroxide+Graphene Oxide	5	.2700	.07211	
Day5	Calcium Hudroxide+Distilled Water	5	.4100	.06856	.411
	Calcium Hydroxide+Graphene Oxide	5	.3140	.07925	
Day6	Calcium Hudroxide+Distilled Water	5	.4880	.04087	.014
	Calcium Hydroxide+Graphene Oxide	5	.3380	.08556	

Day7	Calcium Hudroxide+Distilled Water	5	.5040	.03050	.007
	Calcium Hydroxide+Graphene Oxide	5	.3240	.10407	

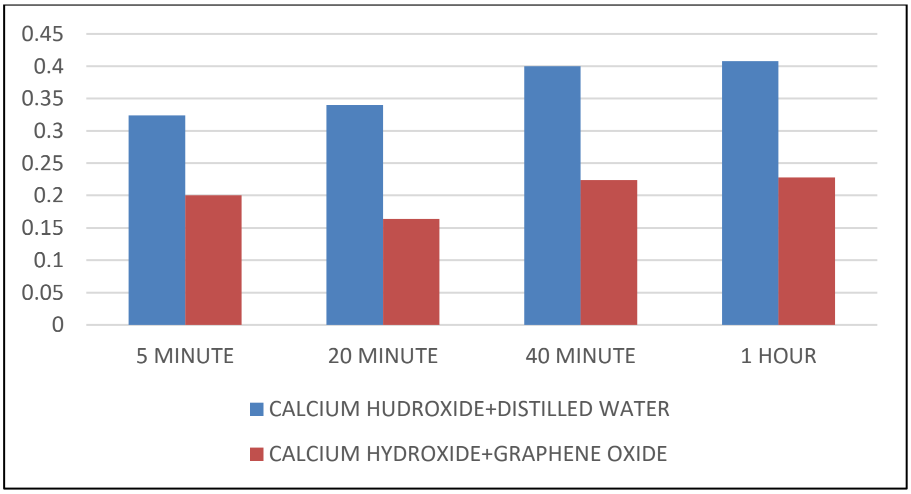
The Table Shows The Comparison Between The Groups In Day 1-7. The Comparison At Day 6 And Day 7 Was Found To Be Statistically Signficant.



Analysis Of Hydroxyl Ions
T-Test

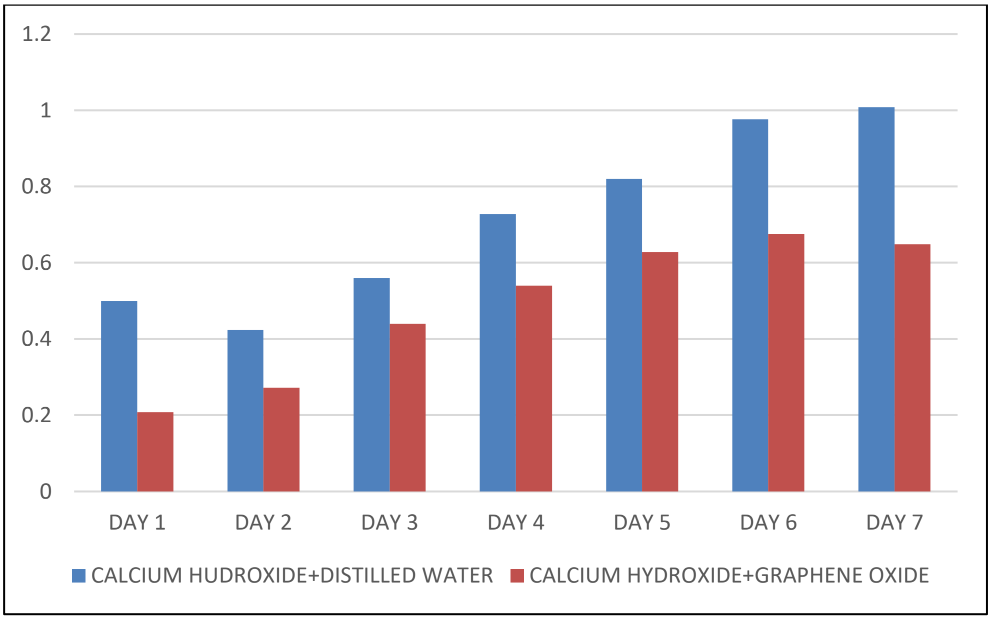
Group Statistics					
	Group	**n**	**Mean**	**Std. Deviation**	**p Value**
5 Minute	Calcium Hydroxide+Distilled Water	5	.3240	.03286	.139
	Calcium Hydroxide+Graphene Oxide	5	.2000	.11402	
20 Minute	Calcium Hydroxide+Distilled Water	5	.3400	.02449	.002
	Calcium Hydroxide+Graphene Oxide	5	.1640	.15582	
40 Minute	Calcium Hydroxide+Distilled Water	5	.4000	.01414	.081
	Calcium Hydroxide+Graphene Oxide	5	.2240	.07925	
1 Hour	Calcium Hydroxide+Distilled Water	5	.4080	.01095	.076
	Calcium Hydroxide+Graphene Oxide	5	.2280	.07563	

The Table Shows The Comparison Between The Groups At Different Time Periods. The Comparison At 20 Minutes Was Found To Be Statistically Signficant.



Group Statistics					
	Group	**n**	**Mean**	**Std. Deviation**	**p Value**
Day1	Calcium Hydroxide+Distilled	5	.5000	.04472	.227

	Water				
	Calcium Hydroxide+Graphene Oxide	5	.2080	.11189	
Day2	Calcium Hydroxide+Distilled Water	5	.4240	.06841	.286
	Calcium Hydroxide+Graphene Oxide	5	.2720	.14043	
Day3	Calcium Hydroxide+Distilled Water	5	.5600	.16432	.801
	Calcium Hydroxide+Graphene Oxide	5	.4400	.15297	
Day4	Calcium Hydroxide+Distilled Water	5	.7280	.13755	.842
	Calcium Hydroxide+Graphene Oxide	5	.5400	.14422	
Day5	Calcium Hydroxide+Distilled Water	5	.8200	.13711	.411
	Calcium Hydroxide+Graphene Oxide	5	.6280	.15849	
Day6	Calcium Hydroxide+Distilled Water	5	.9760	.08173	.014
	Calcium Hydroxide+Graphene Oxide	5	.6760	.17111	
Day7	Calcium Hydroxide+Distilled Water	5	1.0080	.06099	.007
	Calcium Hydroxide+Graphene Oxide	5	.6480	.20813	



DISCUSSION

The therapeutic effect of calcium hydroxide is due to its ability to break down into calcium and hydroxyl ions. Hydroxyl ions show an affinity to various biologically active substances.[9] Their antimicrobial activity is important since microbes cause the majority of endodontic diseases. The antibacterial activity of hydroxyl ion is related to the formation of a potent alkaline medium leading to the destruction of lipids, the main component of bacterial cell membrane and causing structural damage to bacterial proteins and nucleic acids.[10]

Calcium hydroxide, apart from its bacterial enzymatic inhibition which represents an important antibacterial property, has the capability of activating tissue enzymes which favour tissue restoration through mineralization.[11]

Calcium hydroxide in direct contact with conjunctive tissue is the origin to a zone of necrosis, altering the physio-chemical state of intercellular substance which, through rupture of glycoprotein, seems to determine protein denaturation.[11] The formation of mineralized tissue after contact of calcium hydroxide with conjunctive tissue has been observed from about the 7th day to the 10th day.[11,12]

Many substances have been added to the calcium hydroxide powder to improve its properties such as the antibacterial action, radiopacity, flow, and consistency. The ideal vehicle should allow a gradual and slow release of calcium and hydroxide ions. The vehicles suggested can be classified as aqueous and oily. Safavi et al. studied the effect of mixing vehicle on dissociation of calcium hydroxide in solution. They measured the conductivity values for saturated solutions of calcium hydroxide in water and in pure glycerine or propylene glycol. They found that the use of non-aqueous mixing vehicles may impede the effectiveness of calcium hydroxide as a root canal dressing.[17] In this study, we have used both aqueous and oily vehicles, along with a new type which was calcium hydroxide gutta-percha points.
The diffusion of hydroxyl ions through dentin from different calcium hydroxide medicaments was determined by Sevimay et al.[19] They found that non-setting calcium hydroxide based materials have an effective release of hydroxyl ions compared with calcium hydroxide plus points. The results of present study are comparable to those observed in previous studies. hydroxyl ion and calcium ion release observed in this study, the materials used can be ranked in the following order: 1) calcium hydroxide with sterile water 2) calcium hydroxide with Graphene oxide as a vehicle.

CONCLUSION

Calcium hydroxide with sterile water showed a significant increase in hydroxyl ion and calcium ion release and this increase was superior to that shown by calcium hydroxide with graphene oxide.

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Conflict of Interest: None declared

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