

“Comparative Evaluation of the Antibacterial Activity of Calcium Hydroxide with Different Vehicles Against Microorganisms Which Are Commonly Found In Infected Root Canals: In Vitro Study”

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Abstract: Calcium hydroxide has been included in several materials and antimicrobial formulations that are used in several treatment modalities in endodontics, such as inter-appointment intracanal medicaments. The purpose of this article was to review the antimicrobial properties of calcium hydroxide in combination with solution, anaesthetic nilgiri oil and camphorated monochlorophenol in endodontics. Calcium hydroxide has a high pH (approximately 12.5-12.8) and is classified chemically as a strong base. Calcium hydroxide has a wide range of antimicrobial activity against common endodontic pathogens. Calcium hydroxide is also a valuable anti-endotoxin agent.

Date of Submission: 02-07-2019

Date of acceptance: 17-07-2019

I. Introduction

The success of endodontic therapy depends mainly upon instrumentation, irrigation, thorough cleaning and shaping of the root canal for elimination of responsible microbial flora from the root canal. In 1920, Hermann introduced calcium hydroxide to dentistry as a pulp-capping material but today it is used widely in the field of endodontics.

Calcium hydroxide is a white odourless powder with the chemical formula $\text{Ca}(\text{OH})_2$ & classified as a strong base in contact with aqueous fluids (its pH is about 12.5 - 12.8), and dissociate into calcium and hydroxyl ions.

It is a basic compound, as such it is mildly irritating to vital pulp tissue. It has bacteriostatic properties which mean it keeps bacteria from actively spreading. Both of these qualities make it a good lining material for restoration in close proximity to pulp. Its bactericidal or bacteriostatic action is related to its dissociation into calcium and hydroxyl ions thereby creating alkaline environment in its vicinity, not allowing the growth of acidophilic microorganisms.

In the field of endodontics application of calcium hydroxide is facilitated by mixing it with various vehicles such as glycerin, anaesthetic solution, nilgiri oil and camphorated monochlorophenol. To find out the effect of vehicle on the action of calcium hydroxide numerous studies have been carried out.

II. Materials And Methods

For evaluation five microorganisms commonly found in infected root canal were selected in study namely; *Psuedomonas*, *E.coli*, *C.albicans*, *Streptococcus*, *Staphylococcus*. The study was conducted in Department of Microbiology, Rohilkhand Medical College and Hospital, Bareilly.

Calcium Hydroxide powder was pre-weighed (1g) and stored in autoclaved vials. Calcium Hydroxide paste preparations were divided into four groups and tested for efficacy.

- 22 Group: -
- Group I – Calcium Hydroxide and Glycerine
- Group II – Calcium Hydroxide and anaesthetic solution
- Group III – Calcium Hydroxide and nilgiri oil
- Group IV – Calcium Hydroxide and camphorated monochlorophenol

Paste was prepared using the ratio 1:1 (1g powder with 1 cubic centimeter [cc] of appropriate liquid). Sterile instruments were used for each preparation.

A volume of 0.01 ml of the suspension of culture of test organism was spread across the plate using sterile cotton swab. Then it was filled with the four different preparation of Calcium Hydroxide and culture plates were then incubated. The plates of aerobic organisms were incubated aerobically at 37⁰ C and the zone of

inhibition measured after 24, 48, 72 hours accordingly. The antibacterial sensitivity pattern represented as the zone of inhibition at its maximum diameter was measured around each well using a caliper and results observed were tabulated.

III. Results

In the current study

Group I (Ca(OH)₂&Glycerine) showed positive zone of inhibition and antibacterial activity against all the microorganisms tested. Calcium hydroxide glycerine paste (Group I) produced zone of inhibition that was significantly larger as compared to other groups.

Group II (Ca(OH)₂&Anaesthetic Agent) also showed positive zone of inhibition and antibacterial activity.

Group III (Ca(OH)₂ and Nilgiri Oil) showed the zone of inhibition that was significantly smaller than other groups tested.

Group IV (Ca(OH)₂ and CMCP) exhibited relatively high inhibition zone in all the groups.

Micro Organisms	Day of Incubation	Zone of Inhibition (mms)			
		GROUP - 1 Ca(OH) ₂ with Glycerine	GROUP - 2 Ca(OH) ₂ with Anaesthetic solution	GROUP - 3 Ca(OH) ₂ with Nilgiri Oil	GROUP - 4 Ca(OH) ₂ with Camphorated monochloro phenol
Streptococci	Day 1	8	6	5	8
	Day 2	8	6	5	7
	Day 3	8	6	5	7
Staphylococci	Day 1	6	5	3	3
	Day 2	6	5	3	3.5
	Day 3	6	5	3	3.5
Pseudomonas	Day 1	6	7	5	7
	Day 2	8	9	6	7
	Day 3	8	9	6	7
E. coli	Day 1	6	5	3	5
	Day 2	6	5.5	3	6
	Day 3	6	5	3	7
C. Albicans	Day 1	8	7	4	9.5
	Day 2	8	7	4	5
	Day 3	6	5	3	7

IV. Discussion

Calcium Hydroxide is a versatile endodontic medicament whose clinical applications are very wide. Calcium hydroxide is the recommended intracanal medication for the treatment of apical periodontitis. Its antimicrobial mechanism of action is influenced by its speed of dissociation into calcium ions and hydroxyl ions, in a high pH environment which inhibits enzymatic activities that are essential to microbial life, i.e. metabolism, growth and cellular division. The antimicrobial potential of calcium hydroxide pastes with different vehicles was evaluated over periods of 24, 48 and 72hrs.

In the present study, **group I (Ca(OH)₂&Glycerine)** showed positive zone of inhibition and antibacterial activity against all the microorganisms tested. Glycerine possess significant antibacterial activity and they can enhance the antibacterial activity of calcium hydroxide by acting synergistically with it.⁵⁰ A calcium hydroxide glycerine paste was found to have superior density of fill in the root canals of teeth. Glycerine compares favorably with other groups but it is found to be more toxic and cannot be administered parentally. It has been found to produce local irritation when applied topically on the mucous membrane and exposed tissues.⁵¹

Group II (Ca(OH)₂&Anaesthetic Agent) also showed positive zone of inhibition and antibacterial activity. Although dental local anaesthetic solutions have an acidic pH (between 4 and 5), they provide an adequate vehicle because Ca(OH)₂ is a strong base, which is affected minimally by acid. When Ca(OH)₂ prepared with local anaesthetic agent, anaesthetic agent acts as a vehicle only. It does not possess any antibacterial property.

Group III (Ca(OH)₂ and Nilgiri Oil) showed the zone of inhibition that was significantly smaller than other groups tested. Studies have reported that the antimicrobial activity of Eucalyptus essential oil only in pure concentration on Pseudomonas aeruginosa and Escherichia coli as well as on Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis and Staphylococcus aureus.⁵⁵ Considering the antimicrobial potential of eucalyptus essential oil against resistant microorganisms, it is expected that its association with Calcium hydroxide contributes to the control of endodontic infections. However, more

laboratorial steps are to be conducted in order to confirm the potential for constituting it in intra canal medicaments and to consider the clinical applicability of these associations.

Group IV (Ca(OH)₂ and CMCP) exhibited relatively high inhibition zone in all the groups. The results are consistent with the previous studies. The maximum size of zone was attained in first 48hrs, which remain unchanged in the subsequent observation period. This indicates that activity was predominant only in first 48hrs. Therefore, to obtain a sustain and long-term effect it has to be frequently replenished.

V. Conclusion

Calcium Hydroxide has a great value in endodontics. Vehicles like CMCP, glycerine, Anaesthetic agent and nilgiri oil constitute non setting calcium hydroxide paste for intracanal application. Use of vehicles judiciously can influence the desired therapeutic effect of the paste.

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Dr. Sansrati. “Comparative Evaluation of the Antibacterial Activity of Calcium Hydroxide with Different Vehicles Against microorganisms Which Are Commonly Found In Infected Root Canals: In Vitro Study.” *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, vol. 18, no. 7, 2019, pp 32-34.