Original Article

In vitro evaluation of antibacterial efficacy of calcium hydroxide in different vehicles

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Abstract

Aim: This study was aimed to evaluate the antibacterial effect of calcium hydroxide (CH) in different vehicles in an *in vitro* model. **Materials and Methods:** Calcium hydroxide paste prepared with two conventionally used vehicles namely, camphorated monochlophenol, distilled water and propylene glycol. The antibacterial activity of these paste were tested against five micro-organisms that can commonly occur in the infected root canals. **Results:** The results of this study indicate that a paste of CH made with propylene glycol exerts significant antibacterial action. Hence, it can be recommended for use as an intracanal medicament in preference to a paste prepared with a tissue toxic phenolic compound like camphorated monochlorophenol.

Key words: *Antibacterial efficacy, calcium hydroxide, camphorated monochlorophenol, propylene glycol*

INTRODUCTION

The successful endodontic therapy depends upon instrumentation, irrigation, thorough cleaning and shaping of the root canal for elimination of responsible microbial flora from the root canal.[1-4]

Calcium hydroxide (CH) is known to dentistry for its application since its introduction by Herman in 1930. In present day, it has become more popular as intra canal medicament. 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 addition to this, it also inhibits the enzymatic activity that is essential for microbial growth.[5]

To facilitate its application in the field of endodontics, it is generally mixed with vehicles such as camphorated monochlorophenol, distilled water, glycerin, or propylene glycol. Various studies are carried out to identify or to understand the most suitable vehicle and also to find out the effect of vehicle on the action of the CH as an intracanal medicament.[3,6-9] CH when mixed with camphorated monochlorophenol showed dual effect that is its high pH has toxic action on bacteria and tissue mineralization due to activation of hydrolytic enzyme alkaline phosphatase. CH when mixed with propylene glycol exhibits dual effect and no tissue irritating effect and being alcoholic in nature, it remains in paste form for a longer time.[4,6-9]

However, there are very few studies related to propylene glycol that serves as better vehicle when mixed with CH to form a paste for the purpose of

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intracanal medicament. Hence, the present study was done to evaluate the antibacterial effect of CH when mixed with distilled water, camphorated monochlorophenol or propylene glycol.

MATERIALS AND METHODS

Five microorganisms, which are commonly found in infected root canal were selected in the study for evaluation namely; *Hemophilus streptococcus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeuorginosa* and *Candida albicans*. The study was conducted in Department of Microbiology, Grand Medical College, Sir. J.J. Group of Hospital Mumbai.

Calcium hydroxide powder was preweighed (1 g) and stored in autoclaved vials. Calcium hydroxide paste preparations were divided into four groups and tested for efficacy.

- Group I-CH and distilled water.
- Group II-CH and propylene glycol 90%.
- Group III-CH and propylene glycol 100%.
- Group IV-CH and camphorated monochlorophenol.

The ratio used in this study to prepare paste was 1:1 (1 g CH powder with 1 cubic centimeter [cc] of appropriate liquid). Sterile instrument were used for each preparation.

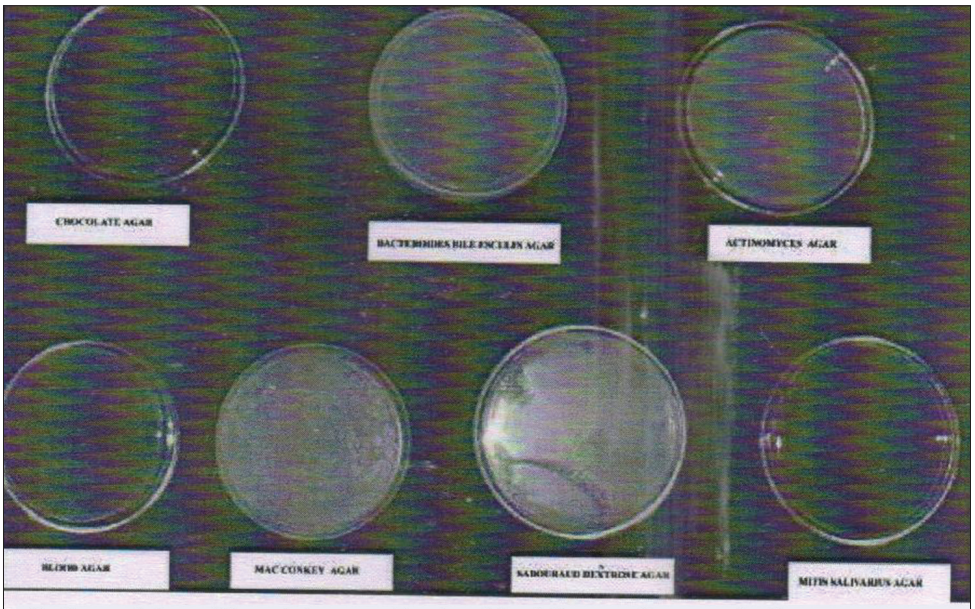
A volume of 0.01 ml of the suspension of culture of test organism was spread across the plate using sterile cotton swab [Figure 1]. Then, it was filled with the four different preparation of CH and the culture plates were then incubated [Figure 2]. The plates of aerobic organisms were incubated aerobically at 37°C and the zone of inhibition measured after 24, 48, and 72 h 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 [Figure 3].

RESULTS

In the present study, the result showed that CH distilled water paste (Group I) produced zone of inhibition that was significantly smaller than other groups, only *C. albicans* exhibited a large zone of inhibition. It requires more incubation period. It is a well-established fact that CH has antibacterial property. CH paste prepared with distilled water does not possess antibacterial property.

Group II further divided into two subgroups. Group IIA, where 1 g of CH powder mixed with 1 cc of 100% of propylene glycol. Group IIB, where 1 g of CH powder mixed with 1 cc of 90% propylene glycol.

It was observed in the present study that among all the CH paste, only CH propylene glycol paste remain in the paste form for the longer period of time, which indicated good handling qualities and exerts significant antibacterial action. The average diameter of zone of inhibition for



Culture plates - Chocolate agar, Bacteroides bile esculin agar, Actinomyces agar, Blood agar, Mac Conkey's agar, Sabourauds dextrose agar, Mitis Salivarious agar

Figure 1: Culture plates for incubations

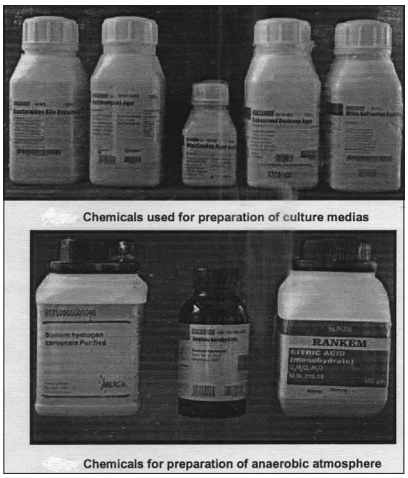


Figure 2: Chemicals used for culturing

Group II (CH + 90% propylene glycol) and Group III were similar (CH + 100% propylene glycol). Group III showed maximum zone of inhibition on culture plates.

Different preparation of CH used in this study produced some degree of inhibition of growth of the test organisms including enterococci. The average diameter of the zones of inhibition for aerobic organisms is shown in Table 1. The inhibition of growth was exhibited as clear zone, around the well. It had the appearance of a fresh agar surface and was smooth and clear.

Among the various preparation of CH, CH camphorated monochlorophenol combination demonstrated the highest degree of growth inhibition; all organisms exhibited high sensitivity to the paste.

The average diameter of the zone of inhibition for both experimental group CH with 90% propylene glycol and CH with 100% propylene glycol combinations were equal. In case of CH and distilled water paste, which acts as a control group, only *C. albicans* exhibited a large zone of inhibition.

There was distinct growth inhibition observed with enterococcus on 3 days of incubation with CH camphorated monochlorophenol paste. *C. albicans* showed no growth on the first day of incubation in all specimens. It required more incubation period.

DISCUSSION

It is a well-known fact that success in endodontic therapy depends upon adhering strictly to various norms laid down namely diagnostic phase, preparatory phase, and phase of obturation.[10] CH is effective in eliminating micro-organisms from the root canal

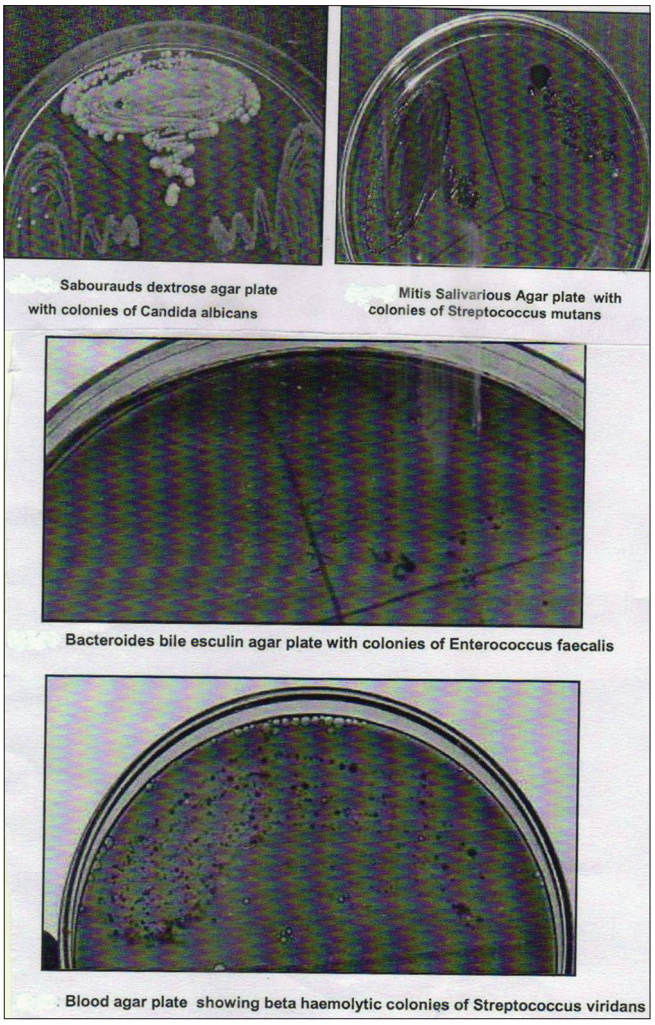


Figure 3: Cultured plates after incubation

space.[11] CH has bactericidal effect and its capacity to neutralize the bacterial endotoxins.[12]

In the present study, Group I (control) showed zone of inhibition that was significantly smaller than other groups. Only *C. albicans* exhibited a large zone of inhibition. It requires more incubation period. It is a well-established fact that CH has antibacterial property. When CH paste prepared with distilled water, water acts as a vehicle only. It does not possess antibacterial property.[7,9,13]

Paul in 1997 evaluated the antibacterial efficacy of CH with different vehicles such as distilled water, camphorated monochloro phenol (CMCP), and propylene glycol. The results showed that CH paste with CMCP, and propylene glycol was more effective as compared to the CH distilled water paste as seen in our study.[14]

Antony in 1997 studied the antibacterial property of CH with four different vehicles namely distilled water,

Table 1: Antibacterial activity of calcium hydroxide with various vehicles

Organism (fungal and aerobic)	Zone of inhibition (in mm)				
	Days of incubation	CH+ distilled water	CH+ 90% PG	CH+ 100% PG	CH+ CMCP
Candida albicans	1st (no growth) candida required more incubation period				
	2nd	22	24	24	34
	3rd	22	25	24	36
Hemophilus streptococcus	1st	15	18	18	30
	2nd	16	19	19	32
	3rd	16	19	19	32
Enterococcus faecalis	1st	R	R	R	16
	2nd	R	R	R	16
	3rd	R	R	R	16
Staphylococcus aureus	1st	13	13	14	30
	2nd	13	14	14	30
	3rd	13.5	16	16	32
Pseudomonas aeroginosa	1st	12	12	14	26
	2nd	12	12	14	29
	3rd	12	12	14	29

CH = Calcium hydroxide, CMCP = Camphorated monochlorophenol

glycerin, CMCP, and propylene glycol. The result of the study showed that all the different preparation of CH paste with glycerin, CMCP, and propylene glycol was more effective in controlling the micro-organisms as compared to CH distilled water paste.[6]

Study done by Paul showed that the paste of the CH made with propylene glycol exert significant antibacterial action and it can be considered for use as an intracanal medicament in preference to the paste prepared with tissue irritating phenolic compound like CMCP.[14]

Propylene glycol which was suggested by Laws in 1962 has been evaluated for its possible use as a vehicle in the field of endodontics.[8] It permits the release of calcium and hydroxyl ions essential for the therapeutic action. It has been found to be antibacterial, nonirritating to the periapical tissue. Both 90% and 100% propylene glycol CH paste showed antibacterial properties, which might be due to the slow diffusion of calcium and hydroxyl ions as compared to the paste prepared with distilled water. The addition of 10% water was done in Group IIB with a view of facilitating immediate ionization of the CH which is necessary for its action. The addition of water did not facilitate the enhancement of antibacterial action.

In Group III, although maximum zone of inhibition was observed, but its clinical use if objectionable due to tissue irritating property of 100% propylene glycol. Its clinical use as objectionable due to its tissue irritating property. The size of the zone of bacterial inhibition does not necessarily reflect the strength of antibacterial agent. The zone size may be influenced by the molecular size of the chemical and its diffusion. An agent that diffuses more easily will exhibit a large zone and CMCP has been shown to diffuse readily through blood agar medium. Since disinfection of the root canal is established by comprehensive effect of biomechanical Preparation, irrigation and intra canal medicament. The loss of long-term activity can be attributed to the insoluble weak salt formed in combination with the hydroxyl ions dissociation, thus affecting the biological activity of CH.[15]

Calcium hydroxide camphorated monochlorophenol paste has a tendency to become sticky and set fast. Besides, CMCP in volatile liquid and can be lost from the paste rapidly. The toxicity of phenol, camphorated phenol and camphorate monochlorophenol was evaluated by Saekont which confirmed the cytotoxicity of these antiseptics. They are irritant to the periapical tissue.[16] Even CMCP alone or with CH is effective more than other two pastes, but it is not preferred as it causes irritation to the periapical tissue.

CONCLUSIONS

The routine use of propylene glycol in place of camphorated monochlorophenol can be recommended as vehicle for CH in view of its efficacy and better handling properties of the resulting paste. The diffusion of CH propylene glycol paste along with tissue irritation and its efficacy *in vivo* require further investigation.

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