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Evaluation and Comparison of Efficacy of Combinations of Calcium Hydroxide – 2% Chlorhexidine Gluconate and Calcium Hydroxide – Propolis Against *E Faecalis*: An Invitro Study

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ABSTRACT

Background: An efficient antimicrobial protocol is a must for successful endodontic treatment. Various root canal medicaments have been used in this regard to ensure minimum microbial count in the root canals.

Aim: The present study was conducted in order to investigate the antimicrobial activity of 2 combinations of root canal medicaments, calcium hydroxide – 2% chlorhexidine gluconate and calcium hydroxide – propolis against Enterococcus faecalis. A third medicament carbopol served as negative control.

Methods: 3 blood agar plates were used to make lawn culture of the inoculae of these organisms. Wells were made within the prepared lawn culture plates using a sterile punch. The prepared wells in the plates were filled with two combination medicaments under test and carbopol(negative control) respectively. The medicament filled agar culture plates were incubated overnight at 37°C. Zone of inhibition test was used to measure the antimicrobial activity of the medicaments under test. The zones of inhibition were measured after 24 hr and 48 hr.

Statistical analysis: Mann Whitney U and Wilcoxon Signed Ranks Test

Result and Conclusion: The present study suggested that calcium hydroxide – propolis is most effective against E. faecalis compared with calcium hydroxide – 2% chlorhexidine gluconate.

Keywords: Calcium hydroxide - propolis, chlorhexidine gluconate, Enterococcus faecalis, root canal infection.

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INTRODUCTION

An effective control of root canal infection is must for a successful endodontic treatment of teeth with apical periodontitis.[1-2] Though a diminution in the number of microorganisms can be achieved to a large extent by proper biomechanical preparation absolute eradication is almost impossible.[3] Consequently an efficient antimicrobial treatment procedure becomes a must in order to reduce the bacterial affront to as minimum as possible.[4-5] Enterococcus faecalis forms a small proportion of normal oral flora and is commonly detected in root canals of infected and untreated teeth. It plays a chief role in endodontic infections and is known to be one of the most persistent species isolated from root canals of teeth with chronic apical periodontitis. [6]

Various studies *have* been conducted in order to study the activity of different intracanal medicaments against E. faecalis. By far, calcium hydroxide has been considered a benchmark and compared with other medicaments. [7-9] Studies reveal that, calcium hydroxide is unable to eliminate the entire gamut of micro-organisms and also needs a protracted time for its antimicrobial action, which limits its use as a root canal medicament.[10-12] Studies have reported that E. Faecalis is sensitive to antimicrobial action of Chlorhexidine gluconate.[13-14] Studies have shown that the antimicrobial effect of Chlorhexidine gluconate was equal to that of the conventional irrigants and medicaments. [15-16] It has got substantivity property which may prevent colonisation of microorganisms on to the dentinal surface.[12,16]

Propolis is a flavanoid-rich resinous substance created by honeybees. It has been used in various studies for its antimicrobial and healing properities.[17] A range of studies conducted mainly on animals and to a few on humans, have explored the use of propolis in different dental fields. [18-19] Lately, a study to assess pulpal response to propolis done in rats, proposed that the flavonoids present in propolis cause delay in pulpitis due to their ability to cause of transforming growth factor (TGF-β1) production and also synthesis of collagen by dental pulp cells. [20] In the current scenario, sodium hypochlorite and calcium hydroxide have been routinely used as irrigant and intracanal medication respectively for root canal disinfection. Unfortunately, as substantiated by numerous studies, E. faecalis microorganisms still persist despite the use of the above antimicrobial agents. [21-22] This further permits colonisation of bacteria at the apical and periapical areas and worsens the prognosis of treatment due to delay or stoppage of healing. [23]

The focus of recent studies has been towards inventing alternative protocols for root canal disinfection, to eliminate even such resistant microorganisms more swiftly. Escalating evidence obtained in the recent past is that it is highly difficult to attain sterility of the infected root canal by currently treatment means. [3,24] The objective of the present study was to determine the antibacterial effect of combination of Calcium hydroxide – 2% Chlorhexidine gluconate and Calcium hydroxide - propolis on E. faecalis, using microbiologic dilution and agar diffusion tests (ADT).

MATERIALS AND METHODS

Calcium hydroxide – (Merck India Ltd., Mumbai, India)

2% Chlorhexidine gluconate – (Dentochlor Ammdent)

Propolis – (Probee propolis)

Division of the experimental groups:

The agar plates containing the combination of medicaments were divided as follows:

• Group 1 (n = 10): $Ca(OH)_2$ + 2% CHX

• Group 2 (n = 10): $Ca(OH)_2$ + Propolis

Medicaments with antimicrobial activity were prepared in 1:1, (volume–volume relationship). Agar diffusion test was performed to check the antimicrobial activity of each group. The zone of inhibition for most of the antimicrobial agents is determined by the concentration of minimal inhibition (CIM) of the antibiotics.

The test of the CIM of an antimicrobial agent is the minimal concentration of the antimicrobial agent that disables the multiplication and production of a

visible growth of a bacterial strain given in the system of test.

Agar diffusion test

The present study used E. faecalis ATCC No. 29212 (NCIM Pune India) strains cultured blood agar and incubated in aerobic conditions. Sterile petri dishes were used to prepare the agar plates and placed overnight at 37°C to ensure sterility. Viable inoculae of the E.faecalis strains were prepared by diluting in sterile saline. McFarland's turbidity standard tube No.0.5 was used to evaluate the turbidity of the prepared solution. Lawn culture of the inoculae was done using sterile cotton swabs on prepared sterile blood agar plates. A sterile punch was used to make wells of 4 mm depth and 6 mm diameter in agar plates. A total of 15 wells in three blood agar plates for E. Faecalis were prepared (1 plate = 5 wells).

The wells in the first two plates were filled with the test gels: Calcium hydroxide - 2% Chlorhexidine gluconate (KMC Pharmacy, Manipal, India), Calcium hydroxide – propolis wells in the third plate contained carbopol (BF Goodrich, Belgium) which served as the negative control. The blood agar plates were then incubated at 37°C for 24 hr and 48hr following which the zone of inhibition was measured with a plastic ruler and documented for each material. Figure 1 and 2 illustrate the zone of inhibitions after 24hrs of incubation and Fig. 3 and 4 show the zones produced after 48 hrs of incubation. Data were analyzed using statistical software (SPSS v.10.5, IBM, Chicago, IL.). The p value of <0.05 was considered significant. An average of the results (mean – SD) for each parameter at 24th and 48th hours was taken. Mann Whitney U test and Wilcoxon Signed Ranks Test were used to analyse the differences in mean values on inter and intra group comparison.

RESULT

The inter group comparison showed significant difference between Group I and Group II ($P < 0.05$) with the later being more effective against E. faecalis after 24hrs an 48hrs duration [Table 1]. In addition, a considerable difference was also established on intragroup comparison of samples collected after 24hrs and 48hrs of application of the medicaments ($P < 0.05$) [Table 2].

DISCUSSION

The ultimate aim of endodontic treatment of teeth pulp and periapical pathology is to eradicate the microorganisms from the root canal system. Thoough various chemomechanical procedures are performed during the treatment, the microorganisms contained by the dentinal tubules may stay uninfluenced. E. Fecalis is commonly encountered in the infected root canals and has always posed a challenge for endodontic treatment. Large quantitites of E. faecalis microorganisms have been located in failed root canal treated teeth. [23,25] Hence, E. Fecalis was chosen for the present study. The culture medium used in the present study was blood agar, as it is easily available and routinely used E. faecalis culture. To determine the antimicrobial efficacy of the medicaments under study, agar diffusion test was used. Agar diffusion test is a commonly used and accepted method for testing antimicrobial activity of medicaments. In addition, the test is easy to perform and reproduce and also is relatively inexpensive. Nevertheless, some factors like pH of the substrate, incubation period, toxicity and drug diffusion capacity might have an impact on the antimicrobial activity of the test materials in the plates.[12]

The study evaluated the efficacy of Calcium hydroxide because it has been most widely used as an intracanal medicament. The high alkaline pH associated with discharge of hydroxyl ions in an aqueous milieu, confers the most applauded antimicrobial property to Calcium hydroxide. It has also been known to be efficient in eradicating most of the endodontic pathogens with few exceptions like Enterococcus faecalis and Candida albicans.[4,21] Chlorhexidine gluconate on the other hand possesses an antimicrobial property due to the ability of its positively charged molecules to adsorb onto the surface of the dentin and prevent microbial colonization for a protracted period of time. Chlorhexidine has been proved to be effective against Candida albicans and Enterococcus faecalis in earlier studies. [12,22,26]

Propolis, is a brown resin substance accumulated by bees from plants. It is also referred to as bee glue or bee propolis. It is used to reinforce the combs and to

Table 1: Inter-group comparison of Enterococcus Faecalis at 24 hrs and 48 hrs.

		N	Mean	Std. Deviation	Z-value	p-value
24 hrs	Group I	10	11.20	0.78	-3.877	0.000
	Group II	10	7.20	1.03		
48 hrs	Group I	10	12.40	0.51	-3.877	0.000
	Group II	10	9.00	0.94		

Table 2: Intra-group comparison of Enterococcus Faecalis at 24 hrs and 48 hrs.

		Mean	N	Std. Deviation	Z-value	p-value
Group I	24 hrs	11.20	10	0.78	9.00	0.000
	48 hrs	12.40	10	0.51		
Group II	24 hrs	7.20	10	1.03	5.51	0.000
	48 hrs	9.00	10	0.94		

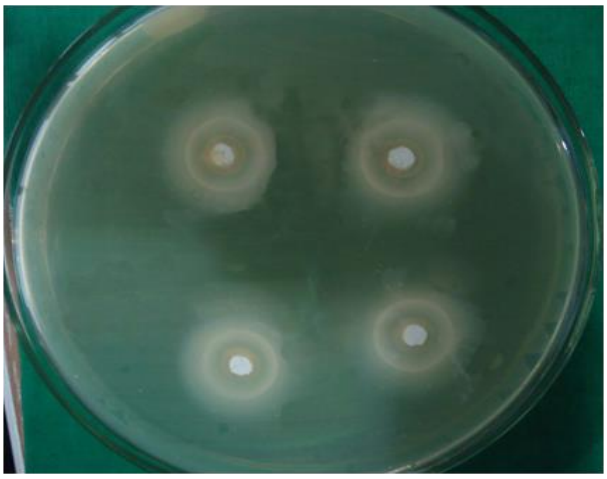

Fig 1: Blood agar plate showing Inhibition zone of Group 1 after 24hrs

keep the hive environment aseptic. [17] It is a potent antimicrobial, antioxidant, and anti-inflammatory agent. Along with a range of aromatic compounds, phenols and flavonoids form the major chemical composition of propolis. Flavonoids are famed as plant composites with antibacterial, antifungal, antiviral, anti-inflammatory and antioxidant properties. [27-28]

Fig 2: Blood agar plate showing Inhibition zone of Group 2 after 24hrs.

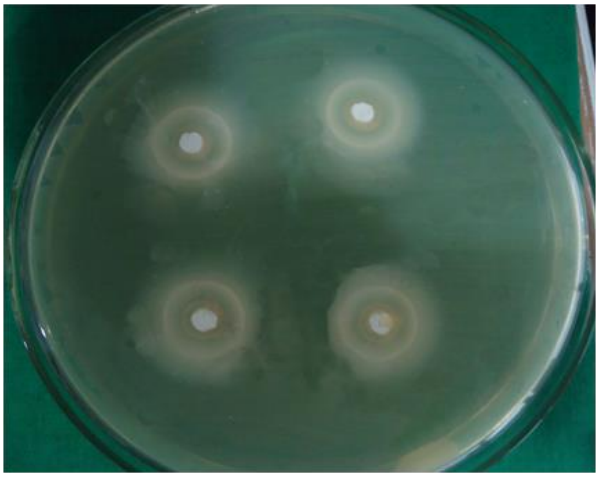

Fig 3: Blood agar plate showing Inhibition zone of Group 1 after 48hrs.

Fig 4: Blood agar plate showing Inhibition zone of Group 2 after 48hrs

To our knowledge this is the first study comparing mixture of calcium hydroxide and 2% chlorhexidine gluconate with calcium hydroxide and propolis. However calcium hydroxide and propolis has shown better antimicrobial efficacy than calcium hydroxide and 2%chlorhexidine gluconate. More advanced clinical and laboratory research should be carried out in order to validate the findings of the present study and also to evaluate the beneficial effects of propolis as intracanal medicament or as any other endodontic material.

In conclusion, the results of the present study show the additive benefits achieved by combining calcium hydroxide with propolis in the treatment of E. Faecalis.

CONCLUSION

The present study suggests that calcium hydroxide-propolis combination as an intracanal medicament has substantial antimicrobial effect against Enterococcus faecalis. It is more effective than calcium hydroxide and 2%chlorhexidine gluconate at 24 hrs and 48 hrs.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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