Comparative evaluation of the antimicrobial efficacy of calcium hydroxide- chlorhexidine combination with the addition of Curcuma longa or retinoic acid – an in vitro study

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Abstract

Background: The aim of the study was to evaluate and compare the antimicrobial efficacy of three medicament combinations namely, calcium hydroxide (CH) powder and 2 percent chlorhexidine (CHX) liquid (Group 1); CH, CHX and Curcuma longa (Turmeric powder [TP]) (Group 2); and CH, CHX and Retinoic acid (vitamin A [VA]) (Group 3). Materials and methods: Agar diffusion test was used to compare the antimicrobial efficacy of medicament combinations against Enterococcus faecalis after 24 hrs of incubation at 37°C, by measuring the zones of inhibition. Statistical analysis was performed using one way ANOVA and Tukey-Kramer multiple comparison tests. Results: Group 3 (CH+CHX+VA) had significantly (P<0.01) higher zone of inhibition than Group 1 (CA+CHX) with least being Group 2 (CA+CHX+TP). Conclusion: Vitamin A can be used in combination with CA and CH as an intracanal medicament.

Key words: Calcium hydroxide, chlorhexidine, Retinoic acid, Curcuma longa

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Introduction

Complete eradication of microorganisms is a significant factor in determining the success of a root canal treatment. Biomechanical preparation contributes a major part in attaining this goal, but it is inadequate to obtain the root canal system completely devoid of bacteria. Hence, there is a need of intra canal medicaments to further decrease the bacterial count.1

Among various intra canal medicaments, calcium hydroxide (CH) is most commonly employed in root canal therapy which exhibits antibacterial activity due to its high alkaline nature. But, it is a proved fact that Enterococcus faecalis (E. faecalis) is resistant to calcium hydroxide medicament.2

Hence, the need of the hour is to develop a new intracanal medicament or use drug combinations to obtain root canal system which is devoid of bacteria. As a result, research progressed towards the use of calcium hydroxide and chlorhexidine combinations which was proven to show a synergistic effect against E. faecalis than when calcium hydroxide was solely used.3,4

But the above-mentioned advantage is accompanied by a disadvantage too, that is, chlorhexidine in the

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presence of alkaline environment liberates excess reactive oxygen species (ROS), which are not only detrimental to bacteria, but also to host periradicular tissues. This disadvantage can be defeated by counteracting excess ROS by an antioxidant.

Antioxidants used in previous studies not only neutralized ROS but also improved the antibacterial effect of calcium hydroxide and chlorhexidine when used as a combination. In the present study, calcium hydroxide and chlorhexidine combination is mixed with two antioxidants, namely turmeric powder and vitamin A powder.

India is a land of herbal wealth and has been using herbs in the treatment of various medical diseases. The roots are now extending even into dentistry, where herbs are being used for various purposes. The inclination towards their usage is due to the fact that they have fewer side effects, are economical and are renewable sources. *Curcuma longa*, which is commonly referred as turmeric, is known for its anti-inflammatory, anti-oxidant, anti-microbial, and antifungal properties. The antioxidant property of *Curcumum* is due to the presence polyphenol components. Vitamin A, a chemically retinoic acid, is known for its antioxidant property. The property of this fat-soluble vitamin to neutralize ROS is being used even in the treatment of cancers.

No study in the literature has been undertaken with the above-mentioned antioxidants to evaluate and compare the antimicrobial efficacy when mixed with calcium hydroxide and chlorhexidine combination against *E. faecalis*. Therefore the aim of this study was to evaluate the antimicrobial efficacy of three drug combinations, namely calcium hydroxide (CH) powder and 2 percent chlorhexidine liquid (CHX) (G1), CH, CHX and *Curcuma longa* (TP) (G2) and CH, CHX and retinoic acid (RT) vitamin A (G3).

**Materials and methods**

*Preparation of medicaments:* Figure 1 shows the materials used for the study.

- Group 2 was the combination of 32 mg of calcium hydroxide, 2 percent 0.5 ml chlorhexidine liquid and 32 mg of turmeric extract (CH+CHX+TP). The turmeric powder was prepared from its rhizomes which were initially washed with distilled water and then dried. Irregular large pieces of the same were then made and they were further dried till moisture free. Once dry, these irregular large sized pieces were ground to form a fine powder.
- Group 3 was the combination of 32 mg of calcium hydroxide, 2 percent 0.5ml chlorhexidine and 32 mg of vitamin A powder (CH+CHX+VA). Vitamin A powder was prepared by grinding chewable vitamin A tablets 50,000 IU (Piramal Enterprises Limited) after removing the superficial coating.

**Agar diffusion test (ADT):** Agar was evenly distributed over the surfaces of the petri dishes to a thickness of 5mm. A loopful of bacterial colonies from stock blood agar plate for *Enterococcus faecalis* was mixed with 1 ml dialyzed Brain Heart Infusion Broth (equivalent to 0.5 McFarland Unit) and was poured and spread with sterile swabs on the prepared blood agar plates. Standard wells with a diameter of 5.0 mm were punched into the agar with the blunt end of a Pasteur pipette. A total of 10 agar plates with three wells in each were used. Three wells in each agar plate represented three groups. Prepared medicament combinations were introduced aseptically in to the wells as shown in the Figure 2. Agar plates were incubated at 37 °C for 24 hours. The diameters of the inhibition zones around the materials were measured in millimetres using Vernier calliper after...
24 hours. Figure 3 shows the inhibitory zones of three groups after 24 hours of incubation.

**Statistical analysis:** The results were analyzed statistically using one way ANOVA and Tukey-Kramer multiple comparison tests.

![Figure 2: Intra canal medicaments placed three in wells](image)

![Figure 3: Inhibition zones of the three groups](image)

**Results**

As shown in the Table 1 there was overall significant difference in the mean scores among the three groups (p < 0.001). Group 3 (CA+2%CH+Vit A) had significantly higher zone of inhibition than group 1 (CA+2%CH) with least being group 2 (CA+2%CH+TURMERIC). The results are diagrammatically represented in Graph 1.

![Graph 1: Comparison of mean zone of inhibition values](image)

**Table 1:** Comparison of mean zone of inhibition values were compared using ANOVA with post-hoc Tukey – Kramer test

<table>
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<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
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**Discussion**

*Enterococcus faecalis*, which is a Gram positive facultative anaerobic bacterium, was selected to check antimicrobial efficacy as it has been known to be one among the most commonly isolated microorganisms from endodontically failed cases. High alkaline pH of Calcium hydroxide is responsible for destruction of bacterial cell membrane. Chlorhexidine which is known for its substantivity exhibits antibacterial property by interacting with negatively charged molecules of bacterial cell membrane. When above medicaments are used in combination, there is increase in the release of ROS by chlorhexidine in an alkaline environment. ROS in lesser quantities has shown to be bactericidal. However, when released in higher quantities, ROS exhibits harmful effects to host cells by its oxidative property which leads to damage of DNA, amino acids in proteins and cofactors of various enzymes. These ROS behaves as a double edge sword which are not only responsible for antibacterial effect, but also destruction of periradicular tissues when equilibrium is disturbed.

Hence, antioxidants are added to neutralize excess ROS so that its antibacterial effect is utilized without harming the host tissues. Turmeric and vitamin A are used with CA, 2 percent CH combination as antioxidants to neutralize the excess ROS. But addition of antioxidants may increase, decrease, or may not affect the primary goal of the combinations that is eradicating bacterial colonies. Hence in this present study CA, 2 percent CH and vitamin A and CA, 2 percent CH and turmeric combination were compared against CA and 2 percent CH control.

Antioxidants are becoming popular in dentistry. In a study conducted by Mageshwaran, it was proved that addition of lycopene and proanthocyanidin to
CA and CH combination not only decreased ROS formation, but also exhibited higher antibacterial effect than control group containing CA and CH. An added advantage of addition of antioxidants is that it increases the fracture resistance of radicular dentine which was proved in a study conducted by Madhusudhana, where fracture resistance of lycopene added group was significantly higher than the group containing CA and CH only.

Agar diffusion test (ADT) was selected to check the antimicrobial efficacy of the medicament combination. ADT has certain drawbacks because the results obtained depends on various factors like state of the medicament (liquid or gel), molecular size, solubility and diffusion ability of the medicament, agar viscosity, storage conditions of the agar plates and incubation time.

Results indicated that vitamin A (RT) group exhibited higher zones of inhibition which was significantly higher when compared to control and turmeric groups. RT is a subclass of a family of lipid-soluble compounds referred to as retinoic acids. These consist of four isoprenoid units joined in a head to tail fashion. Enhanced antimicrobial activity of RT group might be due to unaltered basic pH of CH even when combined with vitamin A and secondly might be due inherent bactericidal properties of vitamin A. However, further research is needed to know about this enhanced efficacy. Turmeric powder (Curcumin longa) chemically a phenolic compound apart from antibacterial property also has antioxidant, anti-inflammatory, antimicrobial, antispasmodic, anticancer, and many other properties. But turmeric, a natural antibacterial agent, when combined with CH and CHX exhibited lesser zone of inhibition than control group. This can be explained as:

- Curcumin is stable at a pH of 6.5 but highly unstable at neutral-basic pH condition;
- Possible chemical interactions between three agents may have reduced the effective antibacterial agents leading lesser zone of inhibition; and
- Lastly it can be explained as alkaline pH of Calcium hydroxide which is responsible for antibacterial effect may be altered by acidic pH of turmeric extract.

**Conclusion**

Within the limitations of the study it can be concluded that:

- Addition of vitamin A enhanced the antibacterial property of calcium hydroxide-chlorhexidine combination.
- Addition of turmeric powder had a negative impact on the antibacterial property of calcium hydroxide-chlorhexidine combination.
- Therefore, vitamin A gives a new ray of hope as intracanal medicament which can be used in the clinical scenario after more in-vitro and in-vivo evaluations.

**References**

7. Mageshwaran T, Ebenezar AR, Madhanamadhubala M, Kavitha S, Mahalaxmi S. Counteraction of reactive oxygen species and determination of antibacterial efficacy of proanthocyanidin and lycopene when mixed with calcium hydroxide and chlorhexidine mixture.


