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Research Article

COMPARING THE ANTIBACTERIAL EFFICACY OF INTRACANAL MEDICAMENTS IN COMBINATION WITH CLOVE OIL AGAINST *ENTEROCOCCUS FAECALIS*

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ABSTRACT

Objective: To demonstrate whether clove oil enhances the antibacterial effect of intracanal medicaments.

Methods: Materials used include clove oil, *Enterococcus faecalis* microorganism, intracanal medicaments (metronidazole, ciprofloxacin, clindamycin, and calcium hydroxide), agar plates, motor, pestle, weighing machine, propylene glycol, glass slab, scale. Agar well diffusion method is employed to study the antibacterial effects of intra canal medicaments with and without clove oil against *E. faecalis.*

Results: From the obtained results it can be concluded that intra canal medicaments combined with clove oil showed greater antibacterial effects against the microorganism, *E. faecalis*, than those intracanal medicaments without clove oil.

Conclusion: The ultimate goals of intracanal medicaments are complete elimination of bacteria, their by-products and pulpal remnants from infected root canals and the complete seal of disinfected root canals. Clove oil can be attributed to its antimicrobial, antifungal, antiseptic, antiviral, aphrodisiac, and stimulating properties. Eugenol in clove essential oil has an inhibitory effect on various bacteria and should be used in minimum inhibitory concentration for safe and effective results. It is used to treat patients with pain from a dry socket, as well as used in a number of temporary restorative materials. Intracanal medicaments when combined with other substances may produce additive or synergistic effects. In this study, the synergistic effect of clove oil when mixed with other intracanal medicaments is tested and found to be more effective.

Keywords: Intracanal medicaments, Eugenol, Clove, Intra canal infection.

INTRODUCTION

The main purpose of endodontic therapy is to clean and shape the root canal by means of instruments and after chemical irrigation intra canal medicaments are used, to eliminate the microorganisms, when the pulp is necrosed [1-3]. After disinfection, the canal is sealed with a root filling, and one of main property of intra canal medicament is to have antimicrobial activity [4]. These include the elimination or reduction of microorganisms, rendering canal contents inert, prevention of post-treatment pain, and to enhance anaesthesia.

Calcium hydroxide has been used commonly as an intracanal medicament. This is mainly due to its high alkaline pH and antibacterial action. Metronidazole is a nitroimidazole compound that exhibits broad spectrum of activity against protozoa and anaerobic bacteria. Ciprofloxacin is a synthetic floroquinolone with rapid bactericidal action [5]. Clindamycin has been found to be effective against various endodontic pathogens. Triple antibiotic paste (TAP), a mixture of metronidazole, ciprofloxacin, and minocycline, is the widely used intracanal medicament in endodontic regeneration. However, crown discoloration has been associated with TAP. Therefore, recent studies have suggested substituting minocycline with other antibiotic [6].

A modified triple antibiotic composed of metronidazole, ciprofloxacin, and clindamycin was successfully used as an intracanal medicament to disinfect necrotic immature teeth during an endodontic regeneration procedure [7]. In this study, the antibacterial effect of TAP without clove oil (Group I), TAP in combination with clove oil (Group II), calcium hydroxide paste without clove oil (Group III) and calcium hydroxide paste in combination with clove oil (Group IV) is studied against *Enterococcus faecalis.*

E. faecalis is Gram-positive cocci that occur singly in pairs or in short chains. It is a facultative anaerobe present in a small proportion of the flora of untreated canal as a part of the polymicrobial flora. It is a predominant bacteria implicated in root canal failures and persistent infections [8-10]. *E. faecalis* is known to colonize dentinal tubules, isthmus, rami, lateral and accessory canals. It remains to be the most frequently identified species in canals of root-filled teeth with periapical lesions. The root canal is hardly a nutrient-rich medium, but *E. faecalis* may survive on serum components from the dentinal fluid [11].

Bacteria may survive after intracanal medication for several reasons. Bacterial strains present in the root canal infection may be intrinsically resistant to the medicament, bacterial cells may be enclosed within anatomical variations inaccessible to the medicament, the medicament may be neutralized by tissue components and by bacterial cells or products, losing its antibacterial effects, medicaments may remain in the root canal system for insufficient time to reach and kill bacterial cells and bacteria may also alter their pattern of gene expression after changes in the environmental conditions. This alteration may allow them to survive in unfavorable environments [12].

Eugenol is the most important medicament seen in a dental office. Besides its pure form as sedative dressing and obtundent, it is used in varieties of zinc oxide eugenol cement formulations and liquid of endodontic sealers. It is also known as allyl guaiacol or eugenic acid, an odoriferous principle, the active ingredient of oil of cloves, comprising 70-80% of its bulk [13]. *Syzygium aromaticum* commonly known as Clove belongs to the family *Myrtacea*. Oil of cloves, also known as clove oil, is an essential oil from the clove plant, *Syzygium aromaticum*. It is a natural analgesic and antiseptic used primarily in dentistry for its main ingredient eugenol [14]. The germicidal properties of the oil make it very effective for relieving dental pain, toothache, sore gums, and mouth ulcers. As a result, clove oil is added to numerous dental products and medications, including mouthwash and toothpaste and filling material as a temporary alternative to a root canal. Eugenol is

also said to have several disadvantages. Sometimes the free eugenol in endodontic sealers can cause certain toxicity. Eugenol is also a well-known allergen; it may also act as a tissue irritant and cause a burning sensation. The intracanal medicaments are generally removed after 5-7 days. Combining it with clove oil enhances its efficacy so that it can be removed earlier. The mean minimum inhibitory concentration of clove oil was found to be 0.62±0.45 [15]. Today, there is a renewed interest in the uses of traditional plant sources as medicine. This revival of interest is mainly due to the widespread belief that 'green medicine' is safe and more dependable than the costly synthetic drugs. This study was done to evaluate the antimicrobial activity of the few endodontic sealers in combination with clove oil. Hence, this study was done to evaluate the antibacterial activity of clove oil with intracanal medicaments and bring about its use in the field of dentistry.

METHODS

Preparation of test microorganism

E. faecalis MTCC was added to the nutrient broth which was incubated at 37°C for 24 hrs. It was then sub-cultured onto a nutrient agar plate and incubated at 37°C for 24 hrs and maintained in the Department of Microbiology, Saveetha Dental College. The inoculum for the antimicrobial activity was prepared by adjusting the density of an organism to approximately 10^8 colony forming units/ml with the help of 0.5 McFurland opacity standards [16].

Methodology

A TAP was prepared by mixing 250 mg ciprofloxacin, 250 mg metronidazole, and 150 mg clindamycin. It was prepared by removing the coating and crushing of antibiotic ciprofloxacin, metronidazole, and clindamycin tablets separately using a mortar and pestle. The crushed powder was passed through a fine sieve to remove heavy filler particles and obtain a fine powder. The ciprofloxacin, metronidazole, and clindamycin powders thus obtained were weighed separately, and this TA mixture was dispensed and mixed with one drop of propylene glycol to get a thick paste-like consistency. A total of 100 mg of CH powder was dispensed and mixed with one drop of propylene glycol on a clean and dry glass slab to prepare a thick-pastelike consistency [17].

Screening of antibacterial activity (agar well diffusion method)

Four petri dishes were prepared and a lawn culture of the test organisms from the bacterial suspension made matching 0.5 McFarland's standard was made on the Muller Hinton agar (MHA-Hi media M1084) media using sterile cotton swab and the plates were dried for 15 minutes. Five wells were made in the agar with a metal tube to receive the testing material. The TAP without and with clove oil was placed in the agar well in respective plates. In the other, paste of calcium hydroxide with and without clove oil was placed in the well in two different plates. The plates were incubated at 37°C overnight and the zone of inhibition of growth around the wells containing the medicaments was measured in millimetres. All the tests were done 5 times to minimize the test errors.

RESULT

The antibacterial activity of the medicaments with and without clove oil was screened by agar well diffusion method and the zone of inhibition was measured (Figs. 1 and 2). Group II was found to be more effective with a zone of inhibition of 23 mm, 22 mm, 24 mm, 26 mm, and 25 mm. Group IV was also found to be more effective against *E. faecalis* than Group III with a zone of inhibition of 32 mm, 37 mm, 34 mm, 32 mm, and 29 mm (Table 1).

DISCUSSION

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. Plant essential oils and extracts have been used in preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used traditionally to improve the quality of health care. Essential oils are potential sources

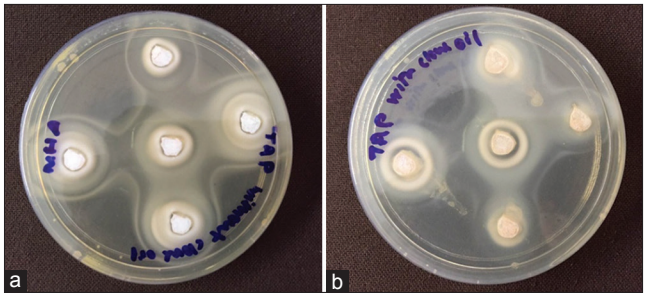

Fig. 1: Inhibition zones promoted by triple antibiotic paste without clove oil (a) and combined with clove oil (b) against *Enterococcus faecalis*

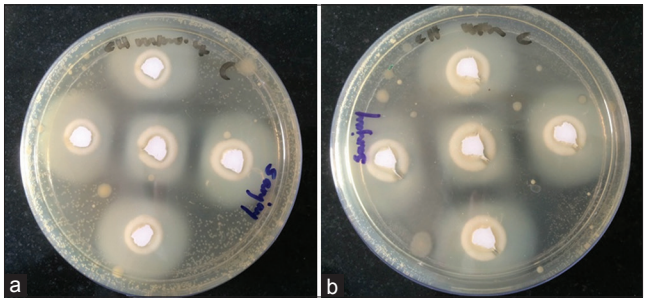

Fig. 2: Inhibition zones promoted by calcium hydroxide paste without clove oil (c) and combined with clove oil (d) against *Enterococcus faecalis*

Table 1: *In vitro* evaluation of the antimicrobial activity of intracanal medicaments without clove oil and in combination with clove oil against *E. faecalis*

Intra canal medicaments	Zone of inhibition in millimeter					Mean (mm)
Group I	19	18	23	26	20	21.2
Group II	23	22	24	26	25	24.2
Group III	30	27	26	25	23	26.2
Group IV	32	37	34	32	29	39.2

E. faecalis: Enterococcus faecalis

of novel antimicrobial compounds against bacterial pathogens. Cloves are used in Indian Ayurvedic medicine, Chinese medicine and western herbalism and in dentistry. The essential oil is used in aromatherapy. Topical application over the abdomen/stomach is said to warm the digestive tract. It also relieves toothache. Herbal medicines like clove are abundantly available, easily accessible, economically feasible, and culturally acceptable and may possess minimal side effects and can be recommended for long term uses.

E. faecalis is the most commonly implicated microorganism in asymptomatic persistent endodontic infections. It is one of the most resistant species usually found in root canal infections. The present study was to evaluate the antibacterial effect of clove oil combined with the intracanal medicaments. The results from this study show that clove oil has enhanced antibacterial effect when combined with the other intracanal medicaments against *E. faecalis*.

CONCLUSION

Systematic study and exploration will help in developing indigenous and economic dental materials and medicaments which are phytotherapeutics. Hence, this study concludes that apart from traditional use of clove, antibacterial effects of essential oils like clove oil also can provide an effective intracanal antiseptic medicament against oral pathogens.

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