Efficacy of turmeric extract as an intracanal medicament in deciduous teeth against *Enterococcus faecalis*: An in vitro study

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ABSTRACT

The purpose of this study was to evaluate, in vitro, the antimicrobial efficacy of turmeric extract as an intracanal medicament in deciduous teeth against *Enterococcus faecalis*. Sixty single canalled deciduous teeth were made into standardized segments & infected with *Enterococcus faecalis*. They were treated with a paste made of either turmeric extract, calcium hydroxide and saline for one week. Dentinal shavings were collected from within the canal, suspended in solution & spread on MRS broth. Colony forming units (CFU) were enumerated using a digital colony counter. The pH of the medicaments used was measured with the help of pH meter. The results were analysed statistically by ANOVA & TUKEY’s test. The results of both types of evaluation showed, Untreated Group 3 (mean 82.70 ± 11.79) was significantly different from Group 1 (mean 41.80 ± 7.93), Group 2 (mean 41.05 ± 7.68), indicating that Group 1 and Group 2 were effective against the species *E. faecalis*. The experimental result obtained were analysed using ANOVA test of significance p<0.05. The analysis showed that the result obtained is significant. The present study concludes that turmeric extract i.e Curcumin is effective against *E. faecalis*. Calcium hydroxide and Curcumin showed better antibacterial effect. There is 50% reduction in colony count was observed when treated with Curcumin. Curcumin is effective against *E. faecalis*. Hence there is possibility of use of Ca(OH)₂ which can be substituted with Curcumin as an intracanal medicament.

Introduction

The main goal of endodontic treatment depends on identifying and eliminating the causative factors in the development of apical periodontitis so that optimal healing can be achieved. Since microorganisms play a vital role in the development and perpetuation of pulpal and periradicular diseases (Leung, 1980) and are the major causative factors associated with endodontic treatment failures (Pinheiro et al., 2003), endodontic research assumes special importance in finding methods and materials to predictably eradicate root canal infection. Therefore, the reduction and elimination of bacteria and their by-products should be given the utmost importance towards achieving a successful endodontic therapy.
Studies have revealed that the prevalence of *E. faecalis* in previously root-filled teeth is up to 33%, holding it responsible for a majority of endodontic failures.

Re-treatment requires the use of suitable intra-canal medicaments that will eliminate these bacteria, prevent their inter-appointment proliferation, act as a barrier against their ingress and cut off their nutrient supply. Though calcium hydroxide has been the most effective intracanal medicament against a variety of microorganisms of the root canal flora, it has clearly been demonstrated to be ineffective against *E. faecalis* (Orstavik and Haapasalo, 1990; Leung, 1980; Donatus *et al.*, 1875).

Curcumin is the principle curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). Turmeric is used extensively in foods for its flavour and color, particularly as an anti-inflammatory and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic (Kawamori *et al.*, 1999). Turmeric can also be applied topically in poultices to relieve pain and inflammation (Siqueira and Lopes, 1999). It possess good antioxidant (Bystrom and Sundquist, 1983), hepatoprotective (Subasree *et al.*, 2014), antimicrobial (Distel *et al.*, 2002) and anti cancer activity (Kontakiots and Nakou, 1995; PDR for herbal medicines, 2000).

The purpose of this study was to evaluate, in vitro the efficacy of turmeric extract as an intracanal medicament in deciduous teeth against *Enterococcus faecalis*.

**Materials and Methods**

The study was conducted in Department of Pedodontics, Bharati Vidyapeeth Dental College and Hospital in collaboration with the Dept. of Microbial Biotechnology, Rajiv Gandhi Institute of IT & Biotechnology, B.V.D.U., Pune. The organism selected in the present investigation were *E. faecalis* (NCIM 5025), as it has been the most prevalent bacteria in the failed root canal system.

**Preparation of samples**

- Sixty single canalled deciduous teeth which were extracted for therapeutic reasons were stored in saline till use for the study after disinfecting with 5.25% NaOCl to remove surface soft tissue & organic debris.
- The teeth were sectioned horizontally into coronal, middle and apical section using a carbide disc in a straight hand piece.
- The 5mm middle segment was taken and the root canal of each specimen was enlarged with No.10 round bur to standardize the internal diameter of canal.

**Smear layer removal from the samples**

- The smear layer was removed by placing it in a 17%. EDTA followed by 5.25% NaOCl for 5 minutes. Each segment was then placed in a sterile tray.

**Sterilization of samples**

- Samples were sterilized by autoclaving at 121°C, 15 psi for 30 minutes.

**Microorganism**

- Standard strain of *E. faecalis* (NCIM 5025) from National Collection of
Industrial Microorganisms, Pune was used in this study.

**Preparation of the inoculums**

- Single colony of *E. faecalis* from MRS agar plate was inoculated into MRS broth. The broth was then incubated at 37°C for 48 hours to obtain bacterial suspension.

**Inoculation of the segments**

- The segments were placed in MRS broth containing the culture of *E. faecalis* and incubated for 5 days at 37°C to infect the dentinal tubules.
- After 5 days the segments were removed from the broth with a sterile forceps and rinsed with sterile water and blotted dry with sterile gauze.
- The 60 specimens were randomly divided into 3 groups of 20 specimens each and kept upright in petridishes which contained nutrient agar by pressing the segment into the media as follows.

Group 1: A paste made from Turmeric extract (curcumin with propylene glycol)
Group 2: A paste comprising of calcium hydroxide & saline.
Group 3: Control group in which no medicament is placed.

- The medicaments were mixed on a sterile glass slab. The glass slab was sterilized by passing over a Bunsen flame 3 times and the cement spatula by passing in a Bunsen flame for 1 minute.
- The powder was dispensed with the cement spatula and the liquid medicament with a sterile syringe and needle.
- The powder and liquid was mixed to a creamy consistency. The creamy medicament was then taken in a lentulospiral and applied in to canal space and the entire space was filled.
- The pH of the medicaments immediately on mixing was measured with help of a pH strips.
- The medicaments were placed in the canal space of each segment and these were placed back in nutrient agar and kept for incubation at 37°C and 100% humidity for 1 week.
- At the end of one week, the segments were removed from the Petri dishes and the paste was removed using 2ml of sterile water irrigation and dried with gauze and paper points.
- For testing the bacterial survival, dentinal shavings within the canal were collected using round burs of increasing diameter. They were collected in 2ml of Eppendorff tube.
- These were suspended in 1ml solution of MRS broth. The MRS suspensions were further diluted using double dilution method.
- In order to determine CFU, 0.1ml of suspension from each tube was spread on MRS plates. The plates were incubated at 37°C for 48 hrs. C.F.U was enumerated using a digital colony counter.

The obtained results were compared using ANOVA and TUKEY’S test to detect the statistical difference among and between groups.

**Results and Discussion**

The total number of CFU count of *E. faecalis* is shown in Table 1. Untreated Group 3 (mean 82.70 ± 11.79) (Fig.3) was significantly different from Group 1 (mean 41.80 ± 7.93) (Fig.1), Group 2 (mean
41.05 ± 7.68) (Fig.2), indicating that Group 1 and Group 2 were effective against the species *E. faecalis* (Graph 1).

The experimental result obtained were analysed using ANOVA test of significance p<0.05. The analysis showed that the result obtained is significant. This is shown in Table 2. The pH of medicaments is shown in Table 3.

*E. faecalis*, which is an opportunistic, facultative anaerobe, was chosen as the test organism because it is well recognised as a pathogen associated with persistent apical periodontitis in endodontically treated teeth and is highly prevalent in failed root filled teeth (Kiso *et al.*, 1983; Siqueira and Rocas, 2005; Schafer and Bossmann, 1999).

- It is non-fastidious and easy to culture (Haapasalo and Orstavik, 1987).
- It has been used successfully in previous studies (Haukvik *et al.*, 2005).
- It has been previously shown to infect dentinal tubules rapidly and persist within it as a mono infection for up to 10 days without any nutrition (Orstavik and Haapasalo, 1990).
- It has been clearly shown to be resistant to calcium hydroxide therapy (Bystrom and Sundquist, 1983). Studies have shown that *E. faecalis* will get killed only at a pH greater than 10-11 due to an inbuilt proton pump which enables it to survive in such alkaline environments.

Calcium hydroxide, discovered by Hermann in 1920, has been advocated and used as an intra-canal medicament since ages. Its antimicrobial properties have been attributed to its:
- High pH (11–12.5)
- Its dissociation into the highly interactive and lethal hydroxyl ions which kill bacterial cells by damaging the cytoplasmic membrane, protein denaturation and damaging the DNA (Donatus *et al.*, 1990).
- Its ability to absorb carbon dioxide, which deprives capnophillic bacteria, which mainly rely on it for their nutrition from thriving (Donatus *et al.*, 1990).
- Its physical presence, which prevents the ingress of bacteria either coronally or apically.
- It enhances the tissue dissolution action of sodium hypochlorite (Mortellini *et al.*, 2000).

In the present study Ca(OH)$_2$ + saline was ineffective in eliminating 100% *E. faecalis* from the root canals. This is in agreement with previous studies (Lenet *et al.*, 2000; Haukvik *et al.*, 2009; http://www.freepatentsonline.com/EP1792581A1.html) done but contradicts the finding of others (Sundqvist *et al.*, 1998; Siqueira, 2001; Apisariyakul *et al.*, 1995). This can be attributed to presence of inherent proton pump in *E. faecalis* and also to the buffering nature of root dentin which prevents attaining a high alkalinity necessary to kill *E. faecalis*. The proton pump provides a means of maintaining pH homeostasis. This is accomplished by pumping protons into the cell, which lowers the internal pH. It has been reported that by inhibiting the proton pump the susceptibility of *E. faecalis* to medicaments at high pH increases. Evans *et al.*, blocked the proton pump and showed a 20 fold to 70 fold reduction in survival at high pH. The proton pump of *E. faecalis* appears to function until it is overwhelmed at pH values of 11.5 and greater (Mchugh, 2004).

Bystrom and Sundquist, (1983) reported that a pH decrease from 12.5 to 11.5 of calcium hydroxide resulted in a high recovery of *E. faecalis* in test tube conditions even after 24 hour, as saturated calcium hydroxide killed this microorganism in a few minutes.
Curcumin is the principle curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). Turmeric is used extensively in foods for its flavour and color, as well as having a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory (Chandra and Gupta, 1972) and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic (Kawamori et al., 1999). Turmeric can also be applied topically in poultices to relieve pain and inflammation (Siqueira and Lopes, 1999).

Turmeric can be used in following ways offer relief from dental problems (Bystrom and Sundqvist, 1981)

- Rinsing the mouth with turmeric water (boil 5 g of turmeric powder, two cloves, and two dried leaves of guava in 200 g water) gives instant relief.
- Massaging the aching teeth with roasted, ground turmeric eliminates pain and swelling.
- Applying the powder of burnt turmeric pieces and bishop’s weed seed on teeth and cleaning them makes the gums and teeth strong.
- Applying a paste made from 1 tsp of turmeric with ½ tsp of salt and ½ tsp of mustard oil provides relief from gingivitis and periodontitis. Rub the teeth and gums with this paste twice daily.

Pit and fissure sealant

It has been found that tinted pit and fissure sealant is useful for applying to tooth surfaces for the prevention or reduction of dental caries. This sealant can be produced from a composition comprising a polymerizable resin system containing acrylic monomer and at least one colorant selected from the group consisting of Annatto extract, turmeric extract, and β-Apo-8-Carotenal (Prasanna et al., 2011).

Dental-plaque detection system

Caries or periodontal diseases are thought to be infectious diseases caused by bacteria present in dental plaques and it is known that the removal of dental plaques is highly important for the health of oral cavities. However, a dental plaque is not easy to identify by the naked eye and it is difficult to confirm its attachment site and extent precisely. Accordingly, dental plaque is generally stained with dental-plaque disclosing agents, which contain dyes, to reveal their locations in order to uncover the attached dental plaques. The dental plaque detection system includes a dental-plaque disclosing agent, which contains yellow pigment derived from either of beni-koji, turmeric extracts, and curcumin; and a light-emitting apparatus, which outputs light having a wavelength within a range of 250 to 500 nm to an object in the oral cavity where the dental-plaque staining agent is attached. A yellow pigment of beni-koji and turmeric are known as staining agents also used for other purposes (Lynne et al., 2003).

Haukyik et al studied the phototoxic effects of curcumin against gram positive bacteria like E. faecalis, streptococcus intermedius and gram negative bacteria E. coli were investigated in aqueous preparations (Sukawat and Srisuwan, 2002).
Prasanna et al., (2011) conducted an in vitro study to evaluate the antimicrobial efficacy of curcumin against E. faecalis considering sodium hypochlorite (3%) as reference for comparison. The result of his study revealed that curcumin had significant antibacterial activity against E. faecalis he concluded that the antibacterial activity of curcumin was similar to sodium hypochlorite and thus herbal medicine can be used in endodontics for root canal failure (Vahdaty et al., 1993).

The present study compared the antibacterial activity of the predominant active component of turmeric i.e., curcumin, against E. faecalis, the most resistant bacteria in root canal treatment. This appears to be the first report on the endodontic applications of curcumin in deciduous teeth. This material has shown antibacterial, antifungal and antiviral activity in previous studies (Mchugh et al., 2004; Schafer and Bossmann, 1999).

The results of this study showed that Ca(OH)$_2$ and Curcumin achieved 50% killing of bacteria. The ability of Ca(OH)$_2$ to eliminate E. faecalis is in accordance with other reports (Haapasalo and Orstavik, 1987; Bystrom and Sundquist, 1983). We speculate that Turmeric (curcumin) is able to eliminate the matrix and the bacteria which warrant further investigation. Microbial communities in vivo are quite resistance to and difficult to eradicate with antimicrobials owing to the fact that the microorganisms to be targeted are organized in structures attached to each other and/or the root canal walls often involving a multitude of species known as microbial biofilms. The testing of antimicrobial agents against bacterial biofilms is yet to be standardized and no in vitro method accurately reflects the conditions under which microorganisms grow in vivo. So, caution should be exercised while extrapolating these results to the clinical scenario.

A recent report suggested that Curcumin in aqueous preparation exhibits phototoxic effect against gram positive and gram negative bacteria (Sukawat and Srisuwan, 2002). This opens up avenues for further research on the use of turmeric in photodynamic therapy of root canal systems.

The widespread use of calcium hydroxide is, to a large degree, based on its long lasting alkalinity and blocking of nutrient diffusion to residual bacteria. The antibacterial effect of curcumin i.e. turmeric and calcium hydroxide may move to be of benefit in the treatment of certain types of persistent infections in primary and particularly in retreatment cases where E. faecalis is the most common isolate.
Table 1: Showing the total number of CFU counts of *E. faecalis*

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</table>

GROUP 1: A paste made from Ca(OH)2 + Saline
GROUP 2: Curcumin + Propylene glycol
GROUP 3: Control (untreated)
Table 2: Comparison of CaOH$_2$, Turmeric and untreated with respect to CFU

<table>
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<th>Number of cases</th>
<th>CFU (Mean ± SD)</th>
<th>p-value</th>
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<td>Turmeric</td>
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<td>Untreated</td>
<td>20</td>
<td>82.70 ± 11.79</td>
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Table 3: Showing the pH of medicaments

<table>
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<th>MEDICAMENTS</th>
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<tr>
<td>Curcumin + propylene glycol</td>
<td>6</td>
</tr>
</tbody>
</table>

References


Lenet, B.J., Komorowski, R., Wu, X.Y., Huang, J., Lawrence, H.P.,


