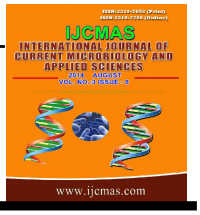
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 17-25**
http://www.ijcmas.com



Original Research Article

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

Jay Chamele* and Chetan Bhat

Department of Pedodontics and Preventive Dentistry, Maitri Dental College, Durg - 491001, India
**Corresponding author*

A B S T R A C T

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.

Keywords

Calcium hydroxide; Turmeric extract (Curcumin); CFU; *Enterococcus faecalis*;

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 (Kontakiotis 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 121^0C, 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 37^0C 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 37^0C 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 37^0C 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 Eppendroff 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 37^0c for 48 hrs. C.F.U was enumerated using a digital colony counter.

The obtained results were compared using ANOVA and TUKEYS 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.freepatents online.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). The active constituents of turmeric are the flavonoid curcumin (diferuloylmethane) and various volatile oils, including tumerone, atlantone, and zingiberone. Other constituents include sugars, proteins, and resins (Mchugh *et al.,* 2004). It possess good antioxidant (Haukvik *et al.,* 2009), hepatoprotective (Subasree *et al.,* 2014), antimicrobial (Distel, 2002) and anticancer activity (Kontakiotis and Nakou, 1995; PDR for herbal medicines, 2000).

Dental Application of Turmeric

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.

Graph.1 Comparison of Mean CFU /mg among three different groups

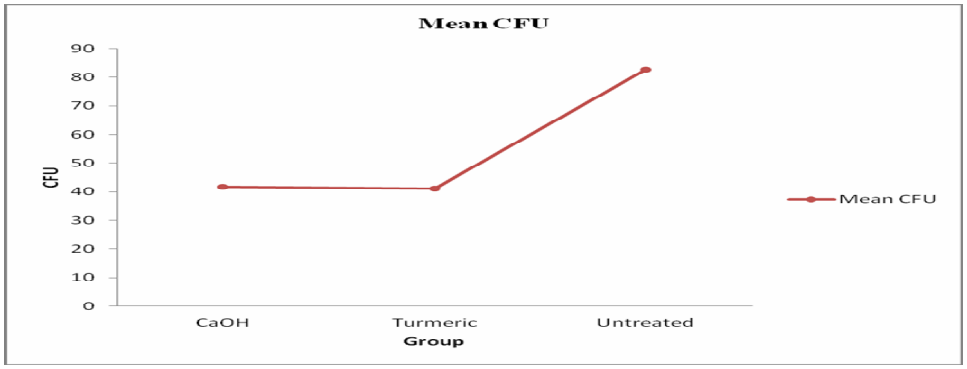


Table.1 Showing the total number of CFU counts of *E. faecalis*

GROUP 1		**GROUP 2**		**GROUP 3**	
TOOTH NO	CFU COUNT	TOOTH NO	CFU COUNT	TOOTH NO	CFU COUNT
1	37	1	39	1	78
2	38	2	36	2	80
3	34	3	32	3	69
4	40	4	41	4	82
5	39	5	37	5	72
6	45	6	47	6	91
7	56	7	55	7	103
8	40	8	38	8	88
9	67	9	62	9	112
10	45	10	50	10	99
11	41	11	39	11	80
12	40	12	38	12	78
13	40	13	37	13	72
14	33	14	33	14	65
15	42	15	44	15	86
16	44	16	41	16	82
17	47	17	46	17	90
18	36	18	37	18	75
19	38	19	36	19	78
20	34	20	33	20	74

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

	Number of cases	CFU (Mean ± SD)	p-value
$CaOH_2$	20	41.80 ± 7.93	< 0.001
Turmeric	20	41.05 ± 7.68	
Untreated	20	82.70 ± 11.79	

Table.3 Showing the pH of medicaments

MEDICAMENTS	pH
Calcium hydroxide + saline	12
Curcumin + propylene glycol	6

References

Apisariyakul, A., Vanittanakom, N., Buddhasukh, D. (1995). Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). *J. Ethnopharmacol.*, 49: 163–169.

Bystrom, A., Sundquist, G. 1983. Bacteriologic evaluation of the effect of 0.5% NaOCl in endodontics therapy. *Oral Surg.*, 55: 307.

Bystrom, A., Sundqvist, G. (1981). Bacteriological evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand. J. Dent. Res.*, 89: 321–328.

Chandra, D., Gupta, S. (1972). Anti-inflammatory and anti-arthritic activity of volatile oil of *Curcuma longa* (Haldi). *Indian J. Med. Res.*, 60: 138–142.

Distel, J., Hatton, J., Gillespie, M. (2002). Biofilm formation in medicated root canals. *J. Endod.*, 28: 689–693.

Donatus, I.A., Sardjoko, Vermeulen, N.P. (1990). Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. *Biochem. Pharmacol.*, 39: 1869–1875.

Haapasalo, M., Orstavik, D. (1987). *In vitro* infection and disinfection of dentinal tubules. *J Dent Res.*, 66(8): 1375–1379.

Haukvik, T., Bruzell, E., Kristensen, S., Tonnesen, H.H., (2009). Photo killing of bacteria by curcumin in different aqueous preparations. Studies on curcumin and curcuminoids XXXVII. *Pharmazie,* 64(10): 666–673.

http://www.freepatents online.com /EP1792581A1.html.

Kawamori, T., Lubet, R., Steele, V.E., et al. (1999) Chemopreventative effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Res.*, 59: 597–601.

Kiso, Y., Suzuki, Y., Watanabe, N., et al. (1983). Antihepatotoxic principles of *Curcuma longa* rhizomes. *Planta Med.*, 49: 185–187.

Kontakiotis, E., Nakou, M., (1995). In vitro study of the indirect action of calcium hydroxide on the anaerobic flora of the root canal. *Int. Endod. J.*, 28(6) :285–289.

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

Friedman, S. (2000). Antimicrobial substantivity of bovine root dentin exposed to different chlorhexidine delivery vehicles. *J. Endodon.,* 26: 652–655.

Leung A. (1980). Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. New York, NY: John Wiley, pp. 313–314

Lynne, R.E., Liewehr, F.R., West, L.A., Patton, W.R., Buxton, T.B., McPherson, J.C. (2003). *In vitro* antimicrobial activity of various medication preparations on *E. faecalis* in root canal dentin. *J. Endod.,* 29: 187–190.

Mchugh, C., Zhang, P., Michalek, S., Eleazer, P. (2004). pH required to kill *Enteroccoccus faecalis in vitro*. *J. endod.,* 30: 218–219.

Mortellini, R., Foresti, R., Bassi, R., Green, C.J. (2000). Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. *Free Radic. Biol. Med.,* 28: 1303–1312.

Orstavik, D., Haapasalo, M. (1990). Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod. Dent. Traumatol.,* 6: 142–149.

PDR for herbal medicines, 2nd Ed., (2000). Medical Economics Company, Montvale, NJ, p. 776.

Pinheiro, E., Gomes, B., Ferraz, C., Sousa, E., Txeira, F., Souza-Filho, F. (2003). Microorganisms from device to disinfect dentinal tubules. *J. Endodontics,* 29: 416–418.

Prasanna, N., Chandana, S., Chandragiri, V. (2011). Analysis of antibacterial activity of curcumin against *Enterococcus fecalis*. *Int. j. curr. res. rev.,* 3(4): 68–77.

Schafer, E., Bossmann, K. (1999). Antimicrobial effect of camphorated chloroxylenol (ED 84) in the treatment of infected root canals. *J Endod.,* 25: 547–551.

Siqueira, J.F. Jr, Lopes, H.P. (1999). Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int. Endod. J.,* 32: 361–369.

Siqueira, J.F. Jr. (2001). Aetiology of root canal treatment failure: why well-treated teeth can fail (Literature review). *Int. Endod. J.,* 34: 1–10.

Siqueira, J.J., Rocas, I. (2005). Exploiting molecular methods to explore endodontic infections: part 2-redefining the endodontic microbiota. *J. Endod.,* 31: 488–498.

Subasree, S., Karthikeyan, M., Sripradha. S., Niha, N. (2014). Effects of turmeric on oral health: an overview. *Int. J. Pharm. Sci. Health Care*, 2(4): 6–14. (http://www.freepatentsonline.com/4261879.html. Last access on 2008 Feb 20).

Sukawat, C., Srisuwan, T. (2002). A comparison of the antimicrobial efficacy of three calcium hydroxide formulations on human dentin infected with *Enterococcus faecalis*. *J. Endod.,* 28: 102–104.

Sundqvist, G., Figdor, D., Sjogren, U., (1998). Microbial analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg.,* 85: 86–93.

Vahdaty, A., Pitt Ford, T.R., Wilson, R.F. (1993). Efficacy of chlorhexidine in disinfecting dentinal tubules *in vitro*. *Endod. Dent. Traumatol.,* 9: 243–248.