

An *in-vitro* evaluation of antibacterial activity of curcumin against common endodontic bacteria

Praveenkumar S. Mandroli and Kishor Bhat

¹Department of Pedodontics & Preventive Dentistry NGH Institute of Dental Sciences and Research centre, R.S. No 47 A/2, Bauxite road, Belgaum-590010, Karnataka state, India.

²Department of Molecular and Microbiology NGH Institute of Dental Sciences and Research centre, R.S. No 47 A/2, Bauxite road, Belgaum-590010, Karnataka state, India.

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ABSTRACT

Aim of the study was to investigate the anti-bacterial potential of curcumin, against standard strains of common endodontic bacteria. The bacterial strains of *Streptococcus mutans* (ATCC 35668), *Actinomyces viscosus* (ATCC 10048), *Lactobacillus casei* (ATCC 334), *Porphyromonas gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611), *Enterococcus faecalis* (ATCC 29212) from the stock were revived by plating on blood agar medium. Isolated colonies were transferred to sterile Brain Heart Infusion (BHI) broth and once again incubated overnight. The growth concentration was adjusted to 5×10^5 organisms / ml by using 0.5 McFarland's turbidity standard. MIC was determined, by serial broth dilution of curcumin to 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2, 1 μ g/ml. respectively. The tubes were then incubated for 24 hours at 37° C. The last tube with clear supernatant was considered to be without any growth and taken as MIC value. Mean MIC values of curcumin were as follows: *S. mutans* (333.33 μ g/ml), *A. viscosus* (167.67 μ g/ml), *L. casei* (125 μ g/ml), *P. gingivalis* (125 μ g/ml), and *P. intermedia* (208.33 μ g/ml). There was no action against *E. faecalis*. Thus, we can conclude that curcumin has the potential to be developed into medicament for the treatment of various endodontic diseases.

INTRODUCTION

Traditional medicine is known to be fertile ground for the source of modern medicines (Corson, Crews, 2007). One medicine in that category is turmeric (*Curcuma longa*) and belongs to the ginger (*Zingiberaceae*) family. Turmeric is extensively used as a spice, food preservative and coloring material in India, China and South East Asia. Since the time of Ayurveda (1900 BC) numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders.² Components of turmeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin. Curcumin, a yellow colored phenolic pigment, is the most important fraction which is responsible for the biological

activities of turmeric (Chattopadhyay *et al.*, 2004). Curcumin was first isolated in 1815 by Vogel and Pelletier. Its chemical structure was determined in 1910 by J. Milobedzka and V. Lampe (Aggarwal *et al.*, 2004). Extensive research on curcumin have demonstrated a wide spectrum of therapeutic effects such as anti-inflammatory, antibacterial, antiviral, antifungal, anti-diabetic, anti-coagulant, hepato-protective, anti-ulcer, hypo-tensive and hypo-cholesteremic (Chattopadhyay *et al.*, 2004 and Kohli *et al.*, 2005). How a single agent could exhibit all these effects is an enigma under intense scrutiny (Alpers, 2008). Curcumin possesses antibacterial property against a number of Gram positive and Gram-negative bacteria (Negi, 1999). Also, its anti-inflammatory properties are well documented (Kohli *et al.*, 2005 and Jurenka, 2009). Action of curcumin has never been tested against endodontic bacteria. Keeping in view the important role of curcumin as an anti-inflammatory and anti-bacterial agent, the present in-vitro study was intended to investigate the anti-bacterial potential of curcumin against standard strains of common endodontic bacteria.

* Corresponding Author

E mail: pedopravin@rediffmail.com; drkgbhat@yahoo.com

Phone no : + 919449230407; Fax no : +91 08312479323

MATERIALS AND METHODS

The study was conducted at Department of Molecular and Microbiology, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Centre, Belgaum (India). The test agent in this study was 95% curcumin (Himedia laboratories Pvt Ltd, Mumbai, India). The test organisms were as follows: a) Bacteria of deep carious lesions (Love, 2009 and Roy, 2009) namely *Streptococcus mutans* (ATCC 35668), *Actinomyces viscosus* (ATCC 10048), *Lactobacillus casei* (ATCC 334) and b) Bacteria of infected root canals (Da Silva *et al.*, 2006 and Tavares *et al.*, 2011). namely *Porphyromonas gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611), *Enterococcus Faecalis* (ATCC 29212).

Bacterial strains and maintenance procedure

The respective bacterial strains from the stock were revived by plating on blood agar medium. After overnight incubation at 37^o C, isolated colonies were selected and the identities of the organisms were confirmed. Isolated colonies were transferred to sterile BHI broth and once again incubated overnight. The growth concentration was adjusted to 5 X 10⁵ organisms / ml by using 0.5 McFarland's turbidity standard.

Determination of Minimum inhibitory concentration

The procedure was carried out for all the test organisms (*Streptococcus mutans*, *Actinomyces viscosus*, *Lactobacillus casei*, *Porphyromonas gingivalis*, *Prevotella. intermedia*, *Enterococcus faecalis*) and each test was carried out in triplicate.

Stock solution of the test agent (curcumin) was made up in DMSO (dimethylsulfoxide; Merck, Germany) to ensure complete solubilization. 200 µL of the BHI broth was added in each of ten MIC tubes per bacterial strain. In the first MIC tube containing 200 µL broth, 200 µL of stock was added. After mixing well, 200 µL was transferred to the second MIC tube. This was continued till the last (10th) tube. From the last tube 200 µL of the final solution was discarded. By following this serial dilution, the concentrations of the aqueous extract achieved was the following – 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2, 1 µg /ml. respectively.

To each of the ten such prepared MIC tubes with varying concentrations, 200 µL of the earlier prepared strain of organism was added such that the final volume per tube was 400 µL. The tubes were then incubated for 24 hours at 37^o C.

After the incubation, the MIC values were determined by visual inspection of the tubes. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value. Turbidity in the MIC tube indicated growth of the bacteria implying that the bacteria are resistant to curcumin.

RESULTS

All the tests were performed in triplicate. The mean MIC values are summarized in Table 1.

Table 1: MIC of curcumin against test organisms.

Organism	Mean MIC (µg /ml)
<i>Streptococcus mutans</i>	333.33
<i>Lactobacillus casei</i>	125
<i>Actinomyces viscosus</i>	167.67
<i>Prevotella intermedia</i>	208.33
<i>Porphyromona gingivalis</i>	125
<i>Enterococcus faecalis</i>	--

DISCUSSION

The present study investigated antibacterial activity of curcumin against standard strains of most prevalent organisms of deep carious lesions namely *Streptococcus mutans*, *Lactobacillus casei*, *Actinomyces viscosus* and most prevalent strains of root canal bacteria namely *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Enterococcus faecalis*. The results indicated that curcumin has antibacterial activity against all the test organisms except *E. faecalis*.

Curcumin has been shown to kill several pathogenic Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus* that cause infections such as skin diseases, pneumonia, meningitis and urinary tract infections in human beings (Negi, 1999). It has been suggested that curcumin inhibits bacterial cell division, by perturbing the cytokinetic Z-ring through a direct interaction with FtsZ. (Rai *et al.*, 2008)

Most pathoses of dental pulp and peri-radicular tissues are either directly or indirectly related to microorganisms. Dental caries is the most common source of bacteria and their by-products affecting the pulp. Dental caries and pulp inflammation beneath it are clearly microbial in origin. The pulpal injury beneath restorations is also microbial (Holland, 2008). The antibacterial action demonstrated by curcumin is encouraging.

Research has shown curcumin to be a highly pleiotropic molecule capable of interacting with numerous molecular targets involved in inflammation. Curcumin has been shown to regulate numerous transcription factors, cytokines, protein kinases, adhesion molecules, redox status and enzymes that have been linked to inflammation (Aggarwal and Harikumar, 2009). Curcumin's potent anti-inflammatory properties have lead to active research on its use for a variety of inflammatory conditions, including postoperative inflammation, arthritis, uveitis, inflammatory pseudotumors, dyspepsia, irritable bowel syndrome, inflammatory bowel disease, pancreatitis, and *Helicobacter pylori* infection. Most studies are promising and further exploration of curcumin's therapeutic value for inflammatory conditions is warranted (Jurenka, 2009).

Clinical trials with human participants and studies in various animal models show that curcumin can be used safely in a wide range of concentrations without toxicity (Yeon *et al.*, 2010). An agent which possesses both anti-bacterial and anti-inflammatory actions can be considered as ideal for developing into medicaments with a range of possible applications in endodontic procedures.

Lack of antibacterial action against *E. faecalis*, which is the most resistant bacterial species to chemo-mechanical preparation and using disinfecting irrigants and antibacterial dressings (Portenier *et al.*, 2003) can be considered a limitation of curcumin. However, curcumin has also been shown to potentiate the antimicrobial action of cefexime, cephalexin, vancomycin and tetracycline (Moghaddam *et al.*, 2009) suggesting a possible utilization in combination with other medicaments. Further studies with more number of root canal organisms can be considered to validate the findings of the present study.

CONCLUSION

Possession of useful properties, pharmacological safety and negligible cost make curcumin an attractive agent to explore further for its potential therapeutic applications in various endodontic procedures.

REFERENCES

- Corson TW, Crews CM. Molecular understanding and modern application of traditional medicines: triumphs and trials. *Cell*, 2007;130:769-74.
- Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: Biological actions and medicinal applications. *Current Science*, 2004;87:44-53.
- Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. *Trends in Pharmacological Sciences*, 2008;30:85-94.
- Kohli K, Ali J, Ansari MJ, Raheman Z. Curcumin: A natural anti-inflammatory agent. *Indian J Pharmacol*, 2005;37:141-47.
- Alpers DH. The potential use of curcumin in management of chronic disease: too good to be true? *Curr. Opin. Gastroenterol*, 2008; 24: 173-75.
- Negi PS, Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. *J Agric Food Chem*, 1999; 47: 4297-300.
- Julie Jurenka, MT. Anti-inflammatory Properties of Curcumin, a Major Constituent of *Curcuma longa*: A Review of Preclinical and Clinical Research. *Altern Med Rev*, 2009; 14: 141-53.

Love RM. 2009. Microbiology of caries and dentinal tubule infection. In:Fouad AF, ed. *Endodontic Microbiology*. Iowa: Wiley-Blackwell. pp 28.

Roy RR. Changing concepts in caries microbiology. *Am J Dent* 2009; 22: 304-10.

Da Silva LAB, Nelson-Filho P, Faria G, De Souza-Gugelmin MCM, Ito IY. Bacterial Profile in Primary Teeth with Necrotic Pulp and Periapical Lesions. *Braz Dent J*, 2006;17:144-48.

Tavares WLF, Neves de Brito LC, Teles RP, et al. Microbiota of deciduous endodontic infections analysed by MDA and Checkerboard DNA-DNA hybridization. *Int Endod J*, 2011;44: 225-35.

Rai D, Singh JK, Roy N, Panda D. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. *Biochem J*, 2008;410:147-55.

Holland GR, Davis SB. 2008. Pulpal Pathosis. In:Ingle JJ, Bakland LK, Baumgartner. JC, eds. *Endodontics*. Hamilton Ontario:BC Decker Inc. pp. 468.

Aggarwal BB, Harikumar KB. Potential Therapeutic Effects of Curcumin, the Anti-inflammatory Agent, Against Neurodegenerative, Cardiovascular, Pulmonary, Metabolic, Autoimmune and Neoplastic Diseases. *Int J Biochem Cell Biol*, 2009;41:40-59.

Yeon KY, Kim SA, Kim YH, et al. Curcumin produces an anti-hyperalgesic effect via antagonism of TRPV1. *J Dent Res* 2010;89:170-74.

Portenier I, Waltimo TMT, Haapasalo M. Enterococcus faecalis – the root canal survivor and ‘star’ in post-treatment disease. *Endodontic Topics*, 2003;6:135-59.

Moghaddam KM, Iranshahi M, Yazdi MC, Shahverdi AR. The combination effect of curcumin with different antibiotics against staphylococcus aureus. *International Journal of Green Pharmacy*, 2009; 3:141-43.

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