Antimicrobial efficacy of curcumin on periodontopathic microorganisms: An In-vitro study

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Abstract

Objectives: The present study was designed to analyse the effect of curcumin on certain periodontopathic pathogens in vitro.

Material and Methods: 25% Curcumin was employed to analyze its Minimal Inhibitory Concentration (MIC) potential against the selected micro-organisms.Gram negative oral bacterial strains which included primary colonizers namely, *Aggregatebacter actinomycetemcomitans, Streptococcus mitis, Streptococcus sanguis* and secondary colonizers which are *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum* and *Prevotellainter media* were cultured using thioglycollate growth medium under anaerobic conditions. Broth microdilution susceptibility test method was employed in order to determine the antimicrobial effectiveness of bacterial responses at growing dilutions of curcumin. After incubation in tubes, the antibacterial activity of curcumin was detected by the lack of turbidity, which indicated the inhibition of bacterial growth.

Results: The curcumin MIC values were 12.6 µg/mL, 25 µg/mL, 50 µg/mL, 50 µg/mL, 0.4 µg/mL, 25 µg/mL and 50 µg/mL against *P. gingivalis, P. intermedia, T. forsythia, F. nucleatum, A. actinomycetemcomitans, S. sanguis* and *S. mitis* respectively.

Conclusions: Curcumin inhibited the growth of all the selected bacterial strains at growing dilutions and is therefore a potent agent against periodontal diseases. There is a need for further in vivo studies to assess the experimental observations on antibacterial effects of curcumin.

Keywords: Curcumin, Mean Inhibitory Concentration, Periodontitis, Anti inflammatory, Optical Density.

Introduction

Periodontal disease is a chronic inflammatory disease of the supporting tissues of the teeth, triggered by the host's immune response to microbial plaque. Gingivitis and periodontitis, which are the two most common forms of periodontal diseases, are caused by the bacteria present in the plaque biofilm. This biofilm community is initially formed through bacterial interactions with the tooth and then through physical and physiologic interactions among different species within the microbial mass.[1]

Various pathognomic microorganisms are known to be associated with periodontitis.[2] Scaling, root planning (SRP) and meticulous oral hygiene techniques aim at reducing the pathogenic bacteria levels to proportions manageable by the host innate immune system.[3,4] SRP sometimes fails to eliminate the bacteria in inaccessible areas, leading to recurrence of disease.

Hence, the use of antimicrobial agents, both systemic and topical, has eventually increased because of the realization that periodontal disease is not only an overgrowth of bacteria, but also a shift in bacterial species.[5]

Systemic administration of antimicrobial drugs has been useful in treating periodontal diseases. However, it involves consumption of a relatively high dose with repeated intake over a prolonged period of thus increasing the possibility of development of resistance, alteration of commensal flora and increased potential for adverse effects.[6] Local administration of these antimicrobial drug provides answers to problems of systemic administration with no systemic side-effects. At the same time, this approach again relies on the ability to control and prolong the release rate of the therapeutic agent from the device.

In recent years, the use of natural plant products has attracted increased attention among clinicians in the treatment of various chronic diseases. In this context, Curcuma longa, a medicinal plant that botanically is related to Zingiberaceae family,[7] was the need of the hour. Curcuma longa, commonly known as 'turmeric', is widely used as a spice and coloring agent, and is well known for its medicinal properties[8] Components of turmeric are called curcuminoids, which mainly include curcumin (diferuloylmethane), demethoxycurcumin and bisdemethoxycurcumin.[9] Among the three components, curcumin is the most important fraction which is responsible for the biological activities of turmeric.[10] Curcumin action in suppressing the activity of Toll like receptors (TLRs) has initiated great interest in identifying and expanding its therapeutic potential in limiting or halting the destruction in periodontitis. It has been shown that curcumin has a wide spectrum of biological actions, such as antibacterial, anti-inflammatory, antioxidant, antidiabetic, anti cancer.

Hence, the current study was undertaken to check the efficacy of Curcumin against primary and secondary colonizers in plaque.

Material and Methods

The present study was conducted at Department of Microbiology and Immunology, NGH Institute of Dental Sciences and Research Centre, Karnataka. The study was approved by the ethics board of Krishna Institute of

Medical Sciences, Maharashtra, India. [KIMSDU/IEC/04/2016].

In the current study, anti-microbial effectiveness was assessed in terms of MIC and OD assays using curcumin against periodontopathic microrganisms. The study focused on the effectiveness of curcumin on primary and secondary colonizers which included *Aggregatebacter actinomycetemcomitans (Aa), Streptococcus mitis (Sm), Streptococcus sanguis (Ss), Porphyromonas gingivalis (Pg), Tannerella forsythia* (*Tf*), *Prevotella intermedia (Pi)* and *Fusobacterium nucleatum* (*Fn).*

Bacterial strains and culture conditions: Seven representative strains of gram negative periodontopathic bacteria comprising of primary colonizers which included, *Aggregatebacter actinomycetemcomitans* (ATCC® 43718™), *Streptococcus mitis* (ATCC® 49456™), *Streptococcus sanguis* (ATCC® 10556™) and secondary colonizers which are *Porphyromonas gingivalis* (ATCC® 33277™), *Tannerella forsythia* (ATCC® 43037™), *Fusobacterium nucleatum* (ATCC® 25586™) and *Prevotella intermedia* (ATCC® 25611™) were procured from NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka. Periodontopathic bacteria were grown in thioglycollate medium containing 15g/L tryptose, 10 g/L yeast extract, 0.5 g/L Sodium thioglycollate, 2.5 g/L sodium chloride, 0.5 g/L L-cystine HCl, 0.4 g/L Sodium bicarbonate, 0.001 g/L Resazurine, 0.005 g/L hemin, 0.0005 g/L Vitamin K and 0.75 g/L agar.

MIC assays (Broth dilution method)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration which results in reduction of inoculums' viability**.**

Antimicrobial agent: The test agent used in the study is 25% curcumin (Melzer Chemicals Private Limited Pune). The rhizomes of *Curcumin longa* were extracted using acetone as a solvent and the extract was then treated with isopropyl alcohol.

Preparation of antimicrobial stock solution and serial dilution: A master test tube was prepared by combining 380 µL of Brain Heart Infusion Broth (BHI) and 20 µL of antimicrobial agent. 9 MIC dilution blanks of 200 µL, BHI (HIMEDIA M210-500G) with a final pH of 7.2-7.6 at 25°C were prepared. Two fold serial dilution of each antimicrobial agent was achieved at different concentrations of 0.2, 0.4, 0.8, 1.6, 3.2, 6.25, 12.5, 25, 50, 100 µg/mL. Three different sets of 9 tubes were used per dilution set for different strains of micro organisms. Nutrient broth without curcumin in turbidity measurement method was used as a negative control.[11]

Preparation of standard bacterial suspension: From the maintained stock cultures of each organism, 5 µL of each was taken and added into 2ml of BHI broth (with dextrose 2g/L, supplemented with protease peptone). The inoculums size was adjusted to match 0.5 McFarland turbidity standards. The final inoculums concentration of the periodontopathic bacteria was 3 x 10^5 CFU/mL. Each serial dilution was then challenged with small inoculums (200µL) of an overnight sterile diluted broth culture of the test organisms. The last tube or the last dilution of mouthwash at which turbidity was not observed after 24h incubation, was considered as the MIC.[12]

Statistical analysis

The present study is just simple and straightforward. The aim (null hypothesis) is to test curcumin against standard strains of periopathogens; hence, no statistical intervention is needed to draw any sensible conclusion.

Results

Antibacterial Activity of Curcumin against Periodontopathic Bacteria

Curcumin significantly inhibited the growth of the tested periodontopathic bacteria. All the organisms, primary and secondary colonizers are sensitive at minimum concentration of 50µg/mL. The MIC of curcumin against tested periodontopathic bacteria.

This inhibition resulted in an outgrowth of S. mitis of OD up to 1.4112 (± 0.4265) $\log_{10}$ CFU/ml and T. forsythia,S. sanguis and *P. gingivalis* respectively of up to 1.3791 (±0.5827), 1.352 (±0.4183) and 1.3287 (±0.66299) $\log_{10}$ CFU/ml.

At 25 µg/mL, *P. gingivalis*, P. intermedia, *A. actinomycetemcomitans and S. sanguis were sensitive while* T. forsythia, S. mitis and F. nucleatum are resistant.

S. *sanguis* comprised a larger proportion than *P. gingivalis.* However, both the bacterial species showed an increased susceptibility with decrease in concentration of the drug curcumin.

Among the periodontopathic bacteria, *A. actinomycetemcomitans* is considered to be the most influential microorganism. Their growth was inhibited by curcumin at all dilutions except at 0.2 µg/mL, there was a significant inhibition of the bacterial growth. These results indicate that curcumin exerted strong antibacterial activity against *A. actinomycetemcomitans* even at low concentrations.

The data obtained for P. intermedia strain after 48 h of incubation showed that for all tested dilutions from 12.6 µg/mL up to 0.2 µg/mL, only a non-significant reduction of bacteria growth was observed, indicating a relative resistance of P. intermedia strain to Curcumin below 12.6 µg/mL dilution. In addition, a graphical comparison between P. intermedia and A. *actinomycetemcomitans* depicted that he drug curcumin was more effective against A. *actinomycetemcomitans.*

Table 1: MICs and Optical density of Curcumin

Curcumin	100µg/ml	50	25	12.6	6.25	3.12	1.6	0.8	0.4	0.2
Pg	S	S	S	S	R	R	R	R	R	R
	2.623	1.899	1.742	1.436	1.374	1.279	0.933	0.900	0.880	0.221
Pi	S	S	S	R	R	R	R	R	R	R
	2.478	1.460	1.375	1.012	0.860	0.704	0.688	0.586	0.421	0.210
Tf	S	S	R	R	R	R	R	R	R	R
	2.554	2.041	1.727	1.556	1.179	1.043	1.143	0.995	0.812	0.741
Fn	S	S	R	R	R	R	R	R	R	R
	3.346	2.170	1.436	0.979	0.817	0.757	0.715	0.688	0.610	0.586
Aa	S	S	S	S	S	S	S	S	S	R
	2.236	1.656	1.522	1.319	1.289	1.189	0.990	0.861	0.714	0.621
Ss	S	S	S	R	R	R	R	R	R	R
	2.009	1.691	1.591	1.587	1.468	1.260	1.190	0.961	0.871	0.712
Sm	S	S	R	R	R	R	R	R	R	R
	2.180	1.983	1.721	1.353	1.345	1.33	1.203	1.175	0.981	0.841
S, Sensitive; R, Resistant										

Table 2: MICs and Mean and Standard Deviation of curcumin against test organisms

Curcumin	MIC (µg/mL)	Mean ± SD
Pg	12.6	1.3287±0.66299
Pi	25	0.9794±0.6555
Tf	50	1.3791±0.5827
Fn	50	1.2104±0.8953
Aa	0.4	1.2352±0.4896
Ss	25	1.352±0.4183
Sm	50	1.4112±0.4265

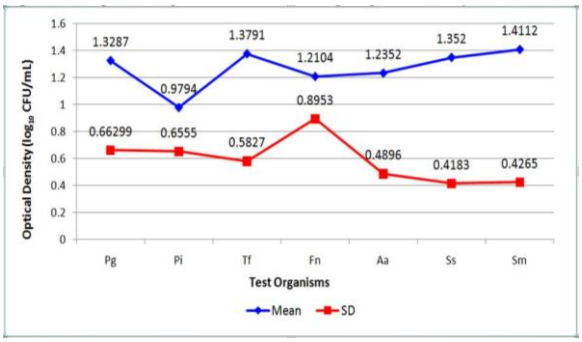

Fig. 1: Graphical representation of average optical density

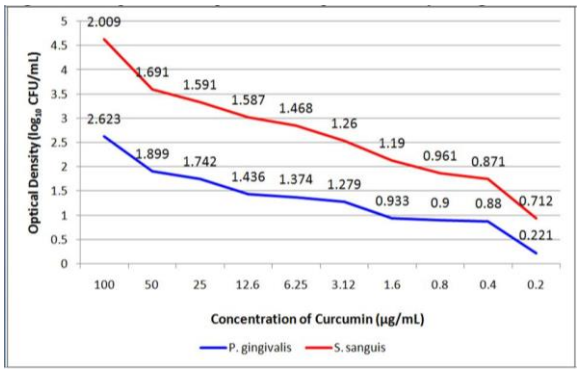

Fig. 2: Graphical comparison of optical density in S. *sanguis* and *P. gingivalis*

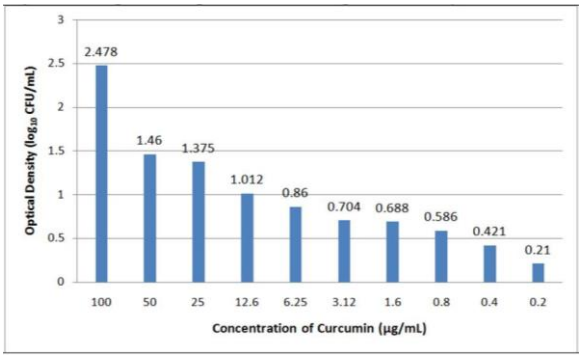

Fig. 3: Graphical representation of optical density in P. intermedia

Discussion

The present analysis is focused on the study inhibitory potentials of curcumin at different concentrations against certain microbial isolates like *Aggregatebacter actinomycetemcomitans, Streptococcus mitis, Streptococcus sanguis, Porphyromonas gingivalis, Tannerella forsythia*, *Fusobacterium nucleatum* and *Prevotella intermedia*. The results of the present study showed that curcumin extract was able to show antimicrobial activity against the selected oral pathogenic bacteria *in vitro*. On comparing the different concentrations of curcumin it was possible to produce a significant 6 log reduction of *bacterial* counts (significantly higher percentages of bacterial viability) at higher dilutions for all bacterial samples and decreased on lower dilutions. The previous literature reviews use of Curcumin in dental practice as an adjunct to mechanical periodontal therapy (SRP), Sub gingival irrigant, pit and fissure sealant, dental- plaque detection system, local drug delivery system, obturating material for primary teeth and as a treatment modality in various oral infections and periodontal diseases.[13] It has been known to possess antioxidant, anti-tumor, anti-inflammatory, antibacterial, antifungal properties, analgesic, anti-allergic properties.

The properties of Curcumin are widely documented by many in vitro studies assessing primarily its anti-inflammatory properties including inflammatory bone resorption.[14] Research has revealed curcumin to be a highly pleiotropic molecule capable of numerous molecular actions including the regulation of numerous transcription factors, cytokines, protein kinases, adhesion molecules, redox status and enzymes that has been linked to inflammation.[15] The primary mechanism of action of Curcumin is NF-κB modulation following Toll like receptor (TLR4) inactivation by lipopolysaccharide (LPS) in affecting periodontal disease. Guimaraes MR *et al*, (2011) reported that systemic administration of curcumin in lipid vehicle inhibited activation of NF-kB infammatory bone resorption in LPS-induced experimental model of periodontal disease. [16] Similar results were observed in ligature induced periodontal disease after administering curcumin.[17,18] The advent of chemically modified curcumin (CMC) has demonstrated better inhibition of MMPs and proinflammatory cytokines including MMP9, 13, TNFα, IL1β, MCP1, IL6, PGE2 compared to free curcumin.[19] Although previous studies have investigated the effects of curcumin on microorganisms, available knowledge on the effects of curcumin on periodontopathic bacteria is still limited. According to current study curcumin exhibited a broad inhibition spectrum toward all selected gram negative bacterial strains based on serial dilution method. The extract curcumin showed an effective antimicrobial activity on the oral flora with a progressive recovery in bacterial viability at higher dilutions. Studies have also demonstrated an equivalent antibacterial effectiveness of 1% curcumin when compared to 0.2 % chlorhexidine containing mouthwashes.[20- 22]

Various studies have reported MIC values of curcumin but most of these studies are in vivo studies. The current study demonstrated that Curcumin has the ability to exert antibacterial activity aganst one of the most tissue invasive periodontal pathogen – A. actinomycetemcomitans at a minimum inhibitory concentration of 0.4 µg/mL. However, reports by Abdulpur MS et al (2015) suggested the ability of curcumin to achieve MIC even at a dilution of 0.2 µg/mL.[23] In addition, previous studies have also reported a MIC of 12.5 µg/mL against Aa.[24] Curcumin was also found to be more effective against A. actinomycetemcomitans as compared to P. *intermedia.* A study by Rai et al suggested that curcumin strongly inhibited the formation of cytokine Z-ring, which would prove lethal to bacteria, thus, accounting for curcumin's antibacterial activity.[25] Another reason which might be suggested is the property of polyphenolic compound of curcumin which aids in possessing antibiofilm activity.[26]

In addition, the results of the present study demonstrated an MIC of 0.4 µg/mL of curcumin against Aa and 25 µg/mL against Ss. In contrast, Savita AM et al (2015) reported that curcumin showed an inhibitory potential against Aactinomycetemcomitans at a dilution of 100 µg/mL.[27] Also, Vieira et al (2018) observed that curcumin displayed a moderate antibacterial activity against Ss (MIC= 100 µg/mL) which is in contrast with the present observation. However, the results are in agreement for the organism Sm in the current study (MIC= 50 µg/mL).[28] Further observations revealed that at a dilution of 25 µg/mL, Pi, Pg, Aa and Ss were susceptible whereas Tf, Fn and Sm were resistant. This observation puts light on its application in a dose dependent manner. Meanwhile, both the primary and secondary colonizers were found to be sensitive to curcumin at a minimum dilution of 50 µg/mL. Lipopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria, including *P.intermedia*. LPS have the potential to induce host cells to release prionflammatory cytokines, including tumor necrosis factor alpha (TNF-á), IL-1â, IL- 6, and IL-8. According to Sung-Jo Kim et al (2011) curcumin significantly suppressed the activation of IL-6 production induced by LPS from P. intermedia in Macrophages which in turn could be the explanation for the reduced levels P. intermedia in the present study. [29] Hence, the present study demonstrated the microbial efficacy of curcumin on the periodontopathic microorganisms; however one must consider the limitations of the experimental approach.

Conclusion

In conclusion, this proof of principle study demonstrates that local application of curcumin in cases of experimental periodontal disease inhibits bacterial growth, which is associated with destructive changes. The clinical application of curcumin is based on its non-invasive, topical application to the diseased areas of gingival sulcus as an adjunct of mechanical periodontal treatment. This perspective of clinical application requires further research and development regarding the local absorption of the curcumin that may improve its efficacy to enhance its anti-microbial properties, such as photo activation.

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