Evaluation of Antimicrobial Efficacy of Curcumin Analogue-2 on Periodonto-Pathogens

Ramanarayana Boyapati[1], Ravindranath Dhulipalla[2], Chaitanya Adurty[3], Deepa Anumala[4], Tejaswin Polepalle[5], Amar Bhochhibhoya[6]

[1]Professor, Department of Periodontology, Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India.

[2]Professor and Head of the Department, Department of Periodontology, Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India.

[3]Associate Professor, Department of Periodontology, Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India.

[4]Professor, Department of Periodontology, Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India.

[5]Assistant Professor, Department of Periodontology, Faculty of Dentistry, MAHSA University, Jenjarom, Selangor, Malaysia.

[6]Assistant Professor, Department of Prosthodontics, Institute of Medicine, Maharajgunj, Kathmandu, Nepal

ABSTRACT

Introduction: The brilliant yellow phytochemical curcumin, also called diferuloyl methane, is derived from turmeric plant *Curcuma longa* (Zingiberaceae)'s rhizome. Despite decades of research, curcumin's utilization as medicinal agent has been impeded because of limited bioavailability. To overcome the problems, a chemically modified curcumin analogue 2(CA2) has been created. Hence, current research's objective has been to assess antimicrobial efficacy of curcumin analogue 2.

Methods: Antimicrobial efficacy of Curcumin analogue 2 had been evaluated by utilizing MBC (minimal bacterial concentration) as well as MIC(minimal inhibitory concentration) on periodontal pathogenic microorganisms, such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia* as well as *Prevotella intermedia.*

Results: Curcumin analogue-2 has shown both bacteriostatic and bactericidal effects against *A. actinomycetemcomitans* from 6.25 µg/ml concentration and for *P. gingivalis*, CA2 has shown bacteriostatic and bactericidal activity from 12.5 µg /ml and 25µg/ml respectively. Bacteriostatic along with bactericidal effect of CA2 against *T. forsythia* from concentrations of 6.25 and 25µg/ml, as well as from 3.12µg/ml CA2 showed bacteriostatic and bactericidal activity against *P. intermedia*.

Conclusion: The results from the present study indicate Curcumin analogue-2 have shown good antibacterial activity on periodonto-pathogenic microorganisms.

Key words: Antimicrobials, Curcumin analogue-2, Minimum inhibitory concentration, Minimum bactericidal concentration.

INTRODUCTION

Periodontitis is chronic oral condition affecting teeth and its supporting structures. Mechanical and chemical therapy are some of the procedures to decrease the intensity or progression of periodontal disease.[1] Mechanical therapy such as scaling and root planning (SRP) continues to be standard protocol to decrease inflammation in the gingiva and to eliminate local factors such as plaque, some amount of bacteria and stains. Though SRP remains standard procedure, complete elimination of bacteria or biofilm is not possible.[2,3] Therefore,

Conflict of Interest: None

***Corresponding Author**

Dr.Ramanarayana Boyapati, Professor, Department of Periodontology, Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India.
E-mail: dr.ramanarayana@gmail.com

anti-microbial therapy through local drug delivery had been introduced as a supplement to mechanical therapy. These anti-microbial include antibiotics, anti-inflammatory agents and herbal agents.[4] These have enhanced properties of penetration into pocket, less systemic side effects and lower drug resistance. The common agents include tetracycline, metronidazole, minocycline, doxycycline etc.[5]

Currently, use of herbal agents in local drug delivery or as mouthwashes is increasing due to fewer side effects and more benefits. Low cost and easy availability are other advantages. One such herbal agent used as local drug delivery agent, mouth wash etc. is turmeric i.e., *Curcuma longa.*[6]

India remains the global leader in the production, exports, and consumption of Turmeric - "Indian Saffron" (Golden spice)." The active constituent of which was best researched has been Curcumin constituting 0.3-5.4% of raw turmeric. Curcumin (diferuloylmethane) has been a polyphenol compound derived from *Curcuma longa* (turmeric). Other active and strong constituents of Turmeric include Tumerone, Volatile oils, Zingiberone, Atlantone.[7]

Curcumin has been used to treat oral conditions and has an effect of anti-inflammatory, anti-microbial, anti-oxidant, anti-mutagenic and used as soothing agent for pre-cancerous lesions. In chronic periodontitis, it is being used as a sub-gingival irrigant, local drug delivery agent, mouth wash and gum paint.[8] Systemic uses include acting as anti-platelet aggregating agent, it has anti-cancerous properties, in the management of chronic anterior uveitis, has cardiovascular effect and hepato-protective effects.[9]

Curcumin has low bioavailability with low rate of absorption. Only curcumin trace levels are discovered in urine, as well as around 75% of oral consumption is eliminated in the faeces. Within two hours of intravenous treatment, more than half of the curcumin is broken down and released into the bile. Reduced un-metabolized curcumin can be due to extensive intestine and hepatic metabolic biotransformation of conjugation.[10] Curcumin is accessible to tissues and the general blood circulation. Approximately 73% of curcumin metabolites have been eliminated in feces, 11% in urine within 72 hours of intraperitoneal injection. About 85% of the curcumin metabolites were detected in the bile following a 6-hour intravenous infusion.[11]

Therefore, curcumin analogues with high stability and bioavailability have been discovered to act as a therapeutic potential against periodontitis. These have improved zinc binding capacity and can easily replace natural curcumin usage. These chemically modified curcumins or analogues have constant, reliable and definite chemical composition as well as improved pharmacological properties. These also have low toxicity and potential inhibitory activity for matrix metallo proteinases (MMPs) and pro-inflammatory as well as anti-inflammatory cytokines.[12,13]

Curcumin analogue -2 undergoes first-pass metabolism and researchers found out that oral dose of 2000 µg/kg of CA-2 in experimental mouse did not cause any toxicity.[14] The anti-bacterial action and MIC, MBC were determined for curcumin but there are a smaller number of studies or no studies determining the MIC and MBC of curcumin analog-2.[15] MIC and MBC of curcumin analogue can help in implementing these analog in treating periodontal disease in future.

Therefore, research objective has been to determine anti-microbial CA-2 efficacy on Periodonto-pathogens, A. *actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* as well as *P. intermedia*.

METHODS

This was an observational study where we aimed at determining the anti-microbial efficacy of CA-2 on Periodonto-pathogenic bacteria

including, *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* as well as *P. intermedia*. The study was conducted from September 2024 to January 2025, covering the formulation of the Curcumin analogue-2 and its ***in vitro*** evaluation against periodontal pathogens. The research was carried out at the Department of Molecular Biology and Immunology, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum, Karnataka, India. Curcumin analog 2 has been made using methodology reported by Oglah et al. in their published study.[16] MIC as well as MBC assays have been carried out following standard protocols described in previously published literature.[17]

RESULTS

MIC and MBC are definitive concentrations at which a drug inhibits or kills periodontal pathogens. As they represent fixed endpoints, they are not typically subjected to statistical analysis.

This is an observational invitro study where MIC along with MBCs of four microrganisms, *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *P. intermedia* were observed for the herbal synthetic Curcumin analogue-2. This study showed both bacteriostatic and bactericidal effects on micro-organisms.

Table 1: Shows for *A. actinomycetemcomitans*, the bacteria were sensitive at the concentrations 100,50,25,12.5 and 6.25µg/ml. For *P. gingivalis* concentrations where bacteria were sensitive was found to be 100, 50, 25, 12.5µg/ml. Similarly, For *T. forsythia*, it was found to be 100, 50, 25, 12.5 and 6.25 µg/ml. For *P. intermedia*, sensitivity was found to be seen in concentrations100,50,25,12.5,6.25,3.12µg/ml. Therefore, MIC for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *P. intermedia* were 6.25, 12.5, 6.25 and 3.12 µg/ml.

Table 2: Shows MBC of curcumin gel on *A. actinomycetemcomitans*, *P.gingivalis*, *T. forsythia* and *P. intermedia* . For *A. actinomycetemcomitans*, no growth was seen in 100,50,25,12.5,6.25µg/ml concentrations. For *P. gingivalis* and *T. forsythia*, no growth was seen in concentrations of 100, 50, 25µg/ml. For *P. intermedia*, no growth was observed in 100,50,25,12.5,6.25,3.12µg/ml concentrations.

Standard Values used as reference included were *A. actinomycetemcomitans* - Moxifloxacin <0.125µg/ml, *P. gingivalis* - Moxifloxacin <0.125µg/ml, *T. forsythia* - Moxifloxacin <0.125µg/ml, *P. intermedia* - Moxifloxacin <0.125µg/ml.

In brief, Curcumin analogue-2 (CA2) demonstrated both bacteriostatic and bactericidal effects against *A. actinomycetemcomitans* at a concentration of 6.25 µg/ml. Against *P. gingivalis*, CA2 exhibited bacteriostatic activity at 12.5 µg/ml and bactericidal activity at 25 µg/ml. For *T. forsythia*, CA2 showed a bacteriostatic effect at 6.25 µg/ml and a bactericidal effect at 25 µg/ml. Additionally, CA2 exhibited antibacterial activity against *P. intermedia* starting from 3.12 µg/ml.

Table 1: MIC results of Curcumin Analogue-2 on Periodontopathogens

Sl. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	Raw Drug										
01	*Aa*	S	S	S	S	S	R	R	R	R	R
02	*Pg*	S	S	S	S	R	R	R	R	R	R
03	*Tf*	S	S	S	S	S	R	R	R	R	R
04	*Pi*	S	S	S	S	S	S	R	R	R	R

Note: S - Sensitive R - Resistant

Table 2: MBC results of Curcumin Analogue-2 on Periodontopathogens

Sl. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	Raw Drug										
01	*Aa*	NG	NG	NG	NG	NG	13	24	28	46	58
02	*Pg*	NG	NG	NG	42	96	112	126	168	230	254
03	*Tf*	NG	NG	NG	13	28	36	48	82	94	102
04	*Pi*	NG	NG	NG	NG	NG	NG	48	52	69	78

Note: S - Sensitive R - Resistant NG - No Growth

DISCUSSION

This study has observed the efficacy of Curcumin analogue -2 on periodonto-pathogenic bacteria, such as *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *P. intermedia* . Curcumin analog-2 is shown to prevent bacterial strains by disrupting the bacterial membrane. It can show both bacteriostatic and bactericidal activity against periodontopathic bacteria.[18] It can also inhibit the proliferation of red complex bacteria by halting cell division. It is also known to deactivate bacteria by stimulating reactive oxygen species (ROS) and provides an anti-oxidant effect.[19]

According to Socransky et al., there was a strong correlation between red complexes that comprise *T. denticola, P. gingivalis*, as well as *T.* forsythia and clinical indicators of periodontal disease.[20] Therefore, for any agent, to determine its efficacy, MIC and MBC should be measured. This helps in interpreting the agent's minimal value at which the bacteria are destroyed. There are very few studies which have found the MIC and MBC against curcumin and its analogues. Our study has made an attempt to find out curcumin's MIC and MBC analog on periodontopathic bacteria. Results indicated Curcumin analog-2's effect showed strong inhibiting affects against *P. gingivalis*, *A. actinomycetemcomitans*, *P.intermedia* and *T. forsythia's* growth.

In this study, the concentrations of *A. actinomycetemcomitans* where it is sensitive were 100, 50, 25, 12.5 and 6.25 µg/ml. A study found that for *A. actinomycetemcomitans* bacterium, curcumin possessed extensive anti-bacterial activity against varied strains of *P. gingivalis*.

For *P. gingivalis*, the concentrations of curcumin for which the bacteria sensitive was 100, 50, 25, 12.5 µg/ml. This result of our study was similar to study done on rats by Sha and Garib for curcumin. They found out that for clinically isolated *P. gingivalis* curcumin's MBC and MIC were 12 µg/ml each. Additionally, Curcumin suppressed *P. gingivalis* with Type I and Type II FimA's development, with extremely minimal doses. Comparable results had been reported for *P. gingivalis* strains with Types III, IV.[21]

Using gene expression studies, Kumbar and colleagues described how curcumin affected *P. gingivalis* biofilm formation along with virulence factor gene expression. They demonstrated that curcumin's MBC and MIC had been125 as well as 62.5µg/mL, respectively, for clinical strains as well as ATCC of *P. gingivalis*.[17]

Curcumin analog-2 possessed the same action as curcumin during various research. Curcumin prevented bacteria from adhering and forming biofilms in dose-dependent manner.[22] Furthermore, curcumin reduced adhesion expression such as hagA, hagB and fimA and proteinases such as kgp, rgpA, rgpB, which are the primary genes of virulence factors, thereby reducing the virulence of *P. gingivalis.* Curcumin has shown anti-biofilm along with anti-bacterial properties against *P. gingivalis*. Additionally, curcumin's pleiotropic properties

make it a potentially accessible and affordable therapeutic agent for treating periodontal disease.[23]

Chen along with colleagues examined curcumin's anti-inflammatory properties and mode of action in macrophages activated by lipopolysaccharide (LPS) from *P. gingivalis.* They found that when *P. gingivalis* LPS stimulated RAW264.7 cells, curcumin inhibited protein production as well as interleukin -1β as well as tumour necrosis factor TNF-α genes' expression. LPS from P. *gingivalis* induced NF-B-dependent transcription in RAW264.7 cells, that curcumin pre-treatment inhibited.[24]

Very few studies have found MIC and MBC for *T. forsythia* and Pi. Our study has found out that the MIC for *T. forsythia* and Pi are 6.25and 3.12µg/ml respectively whereas MBC was 25 and 3.12µg/ml respectively. This study has found that curcumin analogs are easily available with anti-biofilm activity as well as can inhibit bacteria by modulating the host response. In the in-vivo studies done, so far, it was observed that CA-2 was having more potential than curcumin in TPA (tissue plasminogen activator) inhibition in Induced ear edema and TPA in induced increase of IL-1β.[25] In in- vitro studies, more potent suppression of mouse macrophage proliferation was seen as well as inhibition of TPA-induced Nuclear factor kappa Beta activation and IL-1ß expression, aldose reductase activity, lipid peroxidation and free radical formation was seen.[14]

CONCLUSION

Curcumin has amazing properties of anti-inflammatory, anti-bacterial and antibiotic. Because of curcumin's low bioavailability in the body and rapid modification rate in its chemical structure via keto-enol tautomerism, curcumin has not been actively used in clinical studies. Lack of chemical stability also results in low systemic distribution. Therefore, it has been replaced by synthetic curcumin analogs which have greater advantages of high availability and ease of production.

This study has concluded that curcumin analog-2 has inhibited growth of various periodontopathic biofilm-forming bacteria like *A. actinomycetemcomitans*, *P.gingivalis*, *T. forsythia* and *P.intermedia*. It has also concluded that opting for curcumin analogues can reduce the bio-burden by decreasing the use of natural resources. Curcumin analogs may be a powerful tool for the prevention of periodontal diseases, but more clinical research with larger sample size is needed to prove our studies' positive results.

Ethical clearance/approval:
Ethical approval was not required for this study, as it is an in vitro investigation conducted entirely within a research laboratory setting, with no involvement of human or animal subjects.

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