ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antimicrobial Activity of Water Soluble Chitosan Lactate and Carboxymethyl Chitosan on *Aggregatibacter Actinomycetemcomitans* and *Porphyromonas Gingivalis*.

Paranjyothi Magadi Visveswaraiah*, and Deepak Prasad.

*****Research Scholar, Faculty of Dentistry, Pacific University, Udaipur, Rajasthan India.
HOD, Department Of Periodontics. Farooqia Dental College , Mysore , Karnataka, India.

ABSTRACT

A strong relationship exists between the indigenous microbiota of the human body and their influence on systemic disease. *Porphyromonas gingivalis (Pg)* and *Aggregatibacter actinomycetemcomitans (Aa)* are the main etiologic agents of periodontal diseases. Periodontal therapy aims to maintain oral hygiene by the elimination of pathogenic microorganisms. This is achieved mechanically by tooth brushing, oral prophylaxis and by using antimicrobial agents such as chlorhexidine. The adverse effects of chitosan necessitates the search for a superior alternative. Chitosan, a deacetylated polymer of chitin which forms the structural components in the exoskeleton of arthropods or in the cell walls of certain fungi, has wide-ranging antimicrobial activity against Gram positive and Gram negative bacteria, as well as fungi. The insolublility of chitosan in water and organic solvents limits its utilization which can be overcome by chemical modification of the structure of chitosan. Antimicrobial activity of water soluble chitosan lactate and carboxymethyl chitosan was evaluated against *Aa* and *Pg* by agar well diffusion method and minimum inhibitory concentration (MIC) was done for the same. Chitosan lactate and carboxymethyl chitosan exhibited significant antimicrobial activity against *Aa* and *Pg* and could be used as a potential antimicrobial in periodontal therapy.
Keywords*:* chitosan, periodontitis, antimicrobial.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Oral health is a window to one's overall health. Diseases in the mouth can affect the rest of the body and many systemic diseases produce changes within the oral environment. The oral cavity is a host to a number of microorganisms — most of them commensals. Good oral hygiene habits and the body's immune system maintain the oral environment in a healthy state. However, without proper oral hygiene, an increase in pathogenic microorganisms leads to oral infections, such as dental caries and periodontitis.

Periodontitis is a chronic immunoinflammatory disease of the periodontium, leading to progressive loss of gingival tissue, periodontal ligament and adjacent supporting alveolar bone leading to loss of teeth.[1] It is caused by the complex action of periodontal pathogens harboured in dental plaque like *Porphyromonasgingivalis*, *Prevotellaintermedia*, *Prevotellabuccae*, *Tanerellaforsythensis*and *Aggregatibacteractinomycetemcomitans*among which Aa and Pg play an important role.[2]

The constant presence of microorganisms, chronic inflammation and inflammatory mediators associated with periodontitis are associated with increased risk for systemic diseases such as cardiovascular disease, diabetes mellitus, arthritis, preterm low birth weight babies and adverse pregnancy outcomes such as chorioamnionitis, neonatal infection and premature delivery.[1-7]

The periodontium is maintained in a healthy state mechanically by tooth brushing and by chemical adjuncts in the form of antimicrobial mouth washes to improve the outcome of mechanical oral hygiene procedures. [8]

The current gold standard for an oral antimicrobial, chlorhexidine, has an appreciable antimicrobial effect, but its use is limited due to its various adverse effects such as staining of teeth, restorations and prosthesis, taste alterations, formation of calculus and on rare occasions, reversible swelling in the lips or parotid glands, peeling of the oral mucosa, hives, dyspnea and anaphylactic shock. [9]

Hence, a safe antimicrobial substance which overcomes the deleterious effect of chlorhexidine is the need of the hour. Chitosan is a natural co-polymer which possesses antimicrobial activity against a wide variety of gram negative and gram positive microorganisms and fungi. This polysaccharide is largely distributed in nature, being the main component of the exoskeleton of crustaceans and insects, also occurs, in nematodes and in the cell wall of yeast and fungi mainly in the order Mucorales.[10]

Chitosan is insoluble in water, in alkaline medium and even in organic solvents. The poor solubility in water and organic solvents limit its utilization which can be overcome by its chemical modification [11] and hence water soluble chitosan lactate and carboxymethyl chitosan are used in this study.

The aim of our study is to evaluate and compare the antimicrobial activity of water soluble Chitosan lactate and carboxymethyl chitosan on Aa and Pg by agar well Diffusion Method and determination of Minimum Inhibitory Concentration (MIC) by broth dilution method.

MATERIAL AND METHODS

Chitosan lactate and Carboxy methyl Chitosan was procured from Everest labs, Bangalore. Strains of *Aa(ATCC43718)* and *Pg (*ATCC-3327) were obtained from Skanda lab, Bangalore and the cultures and strains were maintained in nutrient agar slants in refrigerator at 4 °c.

Agar well diffusion method

Bacterial inoculum were prepared to match 0.5 McFarland standard. 5 mm diameter wells was punched into the freshly prepared brain heart infusion agar for Aa and blood agar for *Pg* respectively. All plates were inoculated by streaking the organisms on the plates with the test bacterium. Different concentrations of extracts from 5µg/ µl, 10 µg / µl, 25 µg / µl, 50 µg / µl and 75 µg /µl was dispensed into each well after the inoculation of the plates with bacteria. 150 µg / µl chlorhexidine was used as control. Inoculated plates were sealed, labeled and incubated at 37°C for 24 hr. After incubation the plates were examined for the inhibition zones and the experiments were carried out in triplicates.

ISSN: 0975-8585

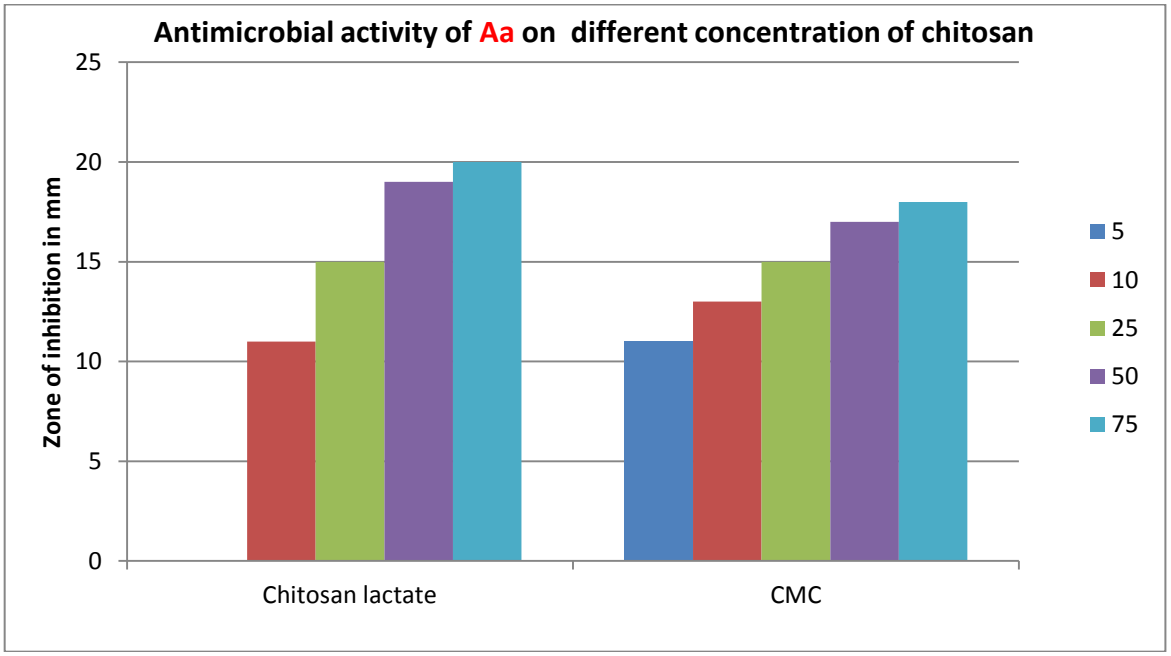
MIC

Minimum inhibitory concentrations (MIC) refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. Serial dilution method was done where in 9 dilutions of each drug was prepared with BHI. To determine the MIC of the extracts the starting concentration was 100 µg/ml. Serial dilutions were made with sterile saline to further achieve the following concentrations of 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, 0.2 µg/ml. The tubes were incubated for 24 hours and observed for turbidity.

RESULTS

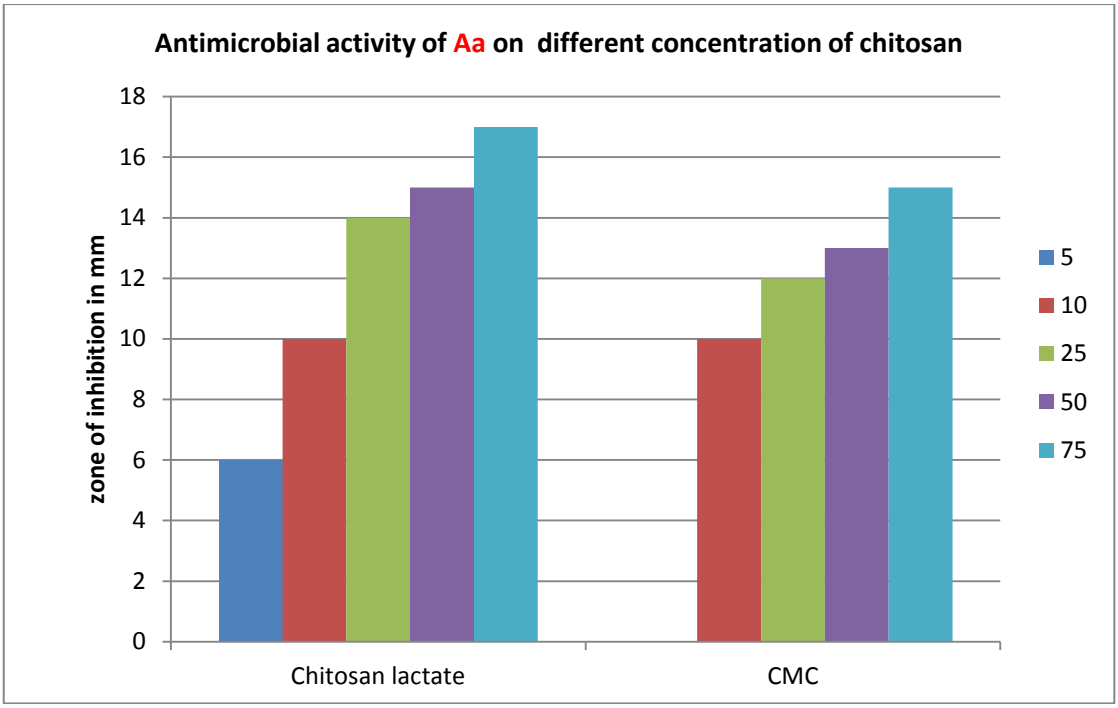
The zones of inhibition observed against test extracts are summarized in Table 1 , Graph 1,Graph 2. Both chitosan lactate and carboxymethyl chitosan exhibited antimicrobial activity against both organisms, Pg and Aa. There was no statistically significant difference in the effect of the different concentrations of chitosan against the microorganisms. The zones of inhibition obtained by both variants of chitosan was statistically significant ($p<0.01$) against the microorganisms, hence proving its antimicrobial capabilities.

	Test Compound	Concentration µg/ml	Zone of Inhibition (in mm) Aa	Zone of Inhibition (in mm) P g
	Chitosan lactate	5	-	6
		10	11	10
		25	15	14
		50	19	15
		75	**20**	**17**
	Carboxy methyl Chitosan	5	11	-
		10	13	10
		25	15	12
		50	17	13
		75	**18**	**15**



Graph 1: zone of inhibition of *Chitosan lactate and carboxy methyl chitosan*on *Aa on and Pg*

ISSN: 0975-8585



Graph 2: zone of inhibition of chitosan lactate and carboxy methyl chitosan on Pg and Aa

MIC for chitosan lactate was recorded at 3.12g/mL for AA and 25µg/mL for Pg and Carboxymethylchitosan showed MIC at 1.6µg/mL for AA and 6.25µg/mL for Pg. Thus carboxymethylchitosan inhibited Aa and Pg at lower concentration as compared to chitosan lactate.

DISCUSSION

Periodontal disease is associated with oral anaerobic species such as black pigmented Porphyromonasgingivalis (Pg) a Gram negative rod and Aggregatibacteractimomycetemcomitans (Aa) a facultative Gram negative rod in subgingival environment. Tissue destruction seen in periodontal disease is caused by the harmful effects of host immune response to the action of the sepathogenic bacteria.[8]

Periodontal pathogens are translocated and released from the sulcus into the bloodstream, after preventive and therapeutic dental procedures, and sometimes while tooth brushing and chewing, with frequencies ranging from 17% – 100% in infected individuals.[2]

Periodontal therapy aims to eliminate periodontal pathogens through subgingival debridement, but few microorganisms are inaccessible to mechanical periodontal therapy. Hence antimicrobial agents act as adjuncts which results in qualitative as well as quantitative changes in microflora.

The microbial organization in biofilms increases the resistance to almost all type of antimicrobial agents and hence the minimum inhibitory concentrations to be considered are below the ideal one for clinical practice. This factor promotes natural products as antimicrobial agents due to their biological activity with lower risk of deleterious effect.[12]

Hence the search for a safe antimicrobial substance which overcomes the deleterious effect of chlorhexidine is required. Chitosan is a polysaccharide composed of units of 2-amino-2-desoxi-D-glycopyranose interconnected by glycosidic bonds β-1, 4 in variable proportions. Chitosan is insoluble in water, in alkaline medium and even in organic solvents. The poor solubility in water or in organic solvents, limit its utilization which can be overcome by the chemical modification of the chain, conserving its original properties. [11,13]

Hence chitosan lactate and carboymethyl chitosan which are water soluble are used in this study. The exact mechanism of the antimicrobial activity of chitosan is poorly defined . Proposed actions include the action of chitosan on the cell wall of the microorganism by modification of the electric potential of the cell membrane.

The amino groups of the chitosan when in contact with physiological fluids are protonated and bind to anionic groups of the microorganisms, causing agglutination of the microbial cells and inhibition of growth. Yadav, Bhise (2004) report that when interacting with the bacterial cell, chitosan, promotes displacement of Ca++ of the anionic sites of the membrane resulting in cell damages. Chitosan also potentiates the action of other inhibition drugs, such as chlorhexidine gel.[14,15]

According to Kong et al, chitosan has demonstrated low toxicity and development of resistance has not occurred′ Chitosan exhibits anti-inflammatory activity by modulating PGE_2 levels through the JNK pathway, which may be useful in the prevention or treatment of periodontal inflammation. [16]′

CONCLUSION

There is an intimate connection between oral health and overall health. Chronic inflammation in the oral cavity can exacerbate few systemic diseases and hence, though the diseases of the oral cavity are perceived as non-life-threatening, one can argue that we are being short sighted. Foreseeing the adverse effects of periodontal diseases on oral as well as general health, preventive and curative measures are recommended. Chitosan is a natural antimicrobial which combines several favorable biological characteristics, including biodegradability, biocompatibility and non-toxicity; properties which renders it superior over present-day chlorhexidine. Thus water soluble chitosan promises to be a potent antimicrobial agent in preventing and combating periodontal disease.
AA

REFERENCES

[1] Oz HeliehS , Puleo D A . J Biomed Biotechnol 2011; 2011 :754857.
[2] Wada K, Kamisaki Y . J PhamacolSci 2010: 113 :115-119.
[3] Cugini C, CerajVK, Rackaityte E, Riggs JE, . DaveyME . J Oral Microbiol 2013; 5:(19804):1-10
[4] Agarwal V, Khatri M, Singh G, Gupta G, Marya CM, Kumar V. J Oral Health Comm Dent 2010 ; 4: 7-16 .
[5] Salah R, Dar- Odeh N, AbuHammad O ,Shehabi AA. BMC Oral Healt 2008;1:(8):17
[6] American Society for Microbiology. (2006, May 29)" ScienceDailyretrived Aug 26 ,2015 from www.science daily .com/releases /2006/05/060529082223.htm
[7] Lopez E. Raymond J, Patkai J, El Ayoubi m, Schmitz T, Moriette G, .Jarreau P. ClinMicrobiolInfect 2010;16 (10): 1539–1543
[8] León R, Silva N, Ovalle A, Chaparro A, Ahumada A, Gajardo M, Martinez M, Gamonal J. J Periodontol 2007; 78(7):1249-55
[9] Kshitish D, Laxman VK. Indian J.Dent Res. 2010;21(3):341-348
[10] McCoy LC, Wehler CJ, Rich SE, Garcia RI, Miller DR, Jones JA,M .J Am Dent Assoc. 2008 ;139(2):178-83.
[11] Rinaudo M. 2006. Prog.Polym. Sci., 31: 603 632
[12] Riva R, Ragelle H, Rieux A, Duhem N, Jerome C, Preat V. Adv Polym Sci 2011; 244: 19–44
[13] .deCarvalho MMSG, Stamford TCM , dos Santos EP , Tenório P, Sampaio F .In : Science against microbial pathogens; communicating current research and technological advances. Méndez-Vilas A(Ed.) 2011 FORMATEX 542-50
[14] Kerch G. Mar. Drugs 2015; 13:(4) 2158-2182
[15] Rabeae E I, Badawy ME, Stevens CV, SmaggheG, Steurbaut W .Journal: Biomacromolecules 2003; 4 (6): 1457-1465,
[16] IkincI G, Senel S, A kaincibay H, KasS,Ercis S, Wilson CG, Himcala AA . Int J PharmInternational Journal of Pharmaceutics 2002; 20 235(1-2): 121-7
[17] Arancibia R , Maturana C, Silva D, Tobar N , Tapia C, Salazar JC. Martínez J. Smith PC. J Dent Res, Effects of Chitosan Particles in Periodontal Pathogens and Gingival Fibroblasts 2013 92(8) 740-5.