

**ANTIFUNGAL EFFICACY OF WATER SOLUBLE CHITOSAN BASED DENTURE
CLEANSER – AN INVITRO STUDY**Dr. V. Harshitha M.*¹, Dr. Shruthi Eshwar¹, Dr. Supriya Manvi², Dr. B. K. Srivastava¹ and Dr. Vipin Jain¹¹Department of Public Health Dentistry, K.L.E Society's Institute of Dental Sciences, Bangalore.²Department of Prosthodontics, K.L.E Society's Institute of Dental Sciences, Bangalore.

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ABSTRACT

Introduction: Chitosan (CHS) is a very versatile natural biomaterial that has been explored for a range of bio-dental applications. Many studies have revealed the antimicrobial and anti-fungal property of chitosan but the role of anti-fungal activity of water soluble chitosan was not established. So, the present study aimed at evaluating the antifungal activity of carboxy methyl chitosan and synergistic effect of chitosan and sodium perborate against candida species. **Materials and methods:** An Invitro study was carried out to evaluate the antifungal activity of carboxy methyl chitosan and its synergistic effect with sodium perborate by agar diffusion method. Stock solutions of chitosan and perborate solutions were prepared and different dilutions were loaded into the wells prepared in the Muller Hinton Agar media. The agar plates were then incubated at 37°C for 24 hrs and then examined for zone of inhibition around the wells. **Results:** The agar plates with different dilutions of chitosan, sodium perborate and plain water did not show any zones of inhibition whereas the agar plate with mixture of chitosan and perborate solution showed equal zones of inhibition around the wells irrespective of different dilutions. **Conclusion:** Anti-fungal activity of carboxy methyl chitosan and sodium perborate solution were not found individually whereas the synergistic effect of these two solutions was established.

KEYWORDS: Water soluble chitosan, antifungal, denture cleanser, Invitro study.**INTRODUCTION**

Human body is made up of over 10^{14} cells of which only 10% are mammalian, remaining are micro-organisms which are present on the environmentally exposed sites of the body. Oral cavity is one such site where the micro-organisms like bacteria, archae, fungi, mycoplasmas, protozoa and viral flora lives in harmonious relationship with the host without causing infection.^[1] Candida species which is also a commensal yeast causes candidiasis whenever there is loss of biological equilibrium. 80% of all the candida infections in the oral cavity are attributed to Candida albicans, C. tropicalis and C. glabrata.^[2,3]

Increased life expectancy, increased the population of elderly cohort, resulting in higher prevalence of tooth loss and denture wearing.^[4] The presence of prosthesis in the oral cavity increases the colonization of yeast colonies because of its affinity for acrylic resin resulting in denture associated infections.^[5] Therefore, proper oral and prosthetic hygiene is required to retain the tissue viability and prevent from denture associated infections.^[6] Numerous denture cleansing agents are available for maintaining the hygiene of dentures like enzymes, peroxides, hypochlorites, perborates etc. These

agents are capable of inhibiting candida species activity colonized on the dentures but also have some disadvantages like color change and lowered acrylic resistance.^[7] The cleansing agent selected should have the capacity to dissolve the organic deposits, non-toxic, must not irritate the mucosa, stable for storage, bactericidal, fungicidal and must not harm the denture.^[8] So, the natural biomaterials are preferred over chemically available agents to control oral candidiasis as they possess superior physical, mechanical and biological properties. A few examples of natural biomaterials are collagen, fibrin, natural silk and chitosan.

Chitosan is a natural biomaterial that is purified mainly from chitin. The major source of chitin is from crustacean's (such as crab and shrimp) exoskeleton, insects, fungi and certain plants such as mushrooms.^[9] Chitin on deacetylation produces chitosan which is insoluble in water but soluble only in weak acids such as acetic acid, formic acid, succinic acid, lactic acid and malic acid. So, water soluble chitosan can be prepared by further deacetylation or by sulfation or by carboxy methylation.^[10]

Chitosan is known for its nontoxic, biocompatible and biodegradable properties. Even though the antimicrobial and anti-fungal activity of chitosan is well established,¹¹ the role of water soluble chitosan against *C.albicans* and its synergistic effects with denture cleansing agents has not been explored. This might be because of its insolubility in water, high viscosity, and tendency to coagulate with proteins at high pH.^[11]

So the present *in vitro* study aimed at evaluating the antifungal activity of water soluble chitosan i.e, carboxy methyl chitosan against oral candida species, and the synergistic effect of chitosan with sodium perborate against candida species.

AIM OF THE STUDY

- To evaluate the efficacy of water soluble chitosan against *Candida* species.
- To evaluate the potential synergistic effect of water soluble chitosan and sodium perborate against candida species.

MATERIALS AND METHODS

Antifungal activity of water soluble chitosan (carboxy methyl chitosan) and synergistic effect of chitosan with sodium perborate was evaluated *in vitro* via agar diffusion method. Principle behind the agar diffusion method is that, antimicrobials present in the test material are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Preparation of solutions

- Stock solution of carboxy methyl chitosan was prepared by adding 50mg in 10ml of distilled water and then 75% (750µl stock + 250µl distilled water), 50% (500µl stock + 500µl distilled water), 25% (250µl stock + 750µl distilled water) and 10% (100µl stock + 900µl distilled water) dilutions were prepared.
- Sodium perborate solutions was prepared by adding 50mg to 100ml of distilled water.
- *Candida albicans* strain was obtained from the Department of Microbiology, K.L.E society's Institute of dental Sciences, Bangalore. These isolates were maintained on Sabouraud's dextrose agar SDA media at 4°C.
- Muller Hinton Agar Medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.
- Lawn culture of candida were prepared on the MHA plate. Then MHA plates were taken and 5mm wells

were cut into the agar media. 1st plate was loaded with 60µl of different dilutions (100%, 75%, 50%, 25% and 10%) of chitosan (Fig 1). 2nd plate was loaded with dilutions of chitosan with equal amounts of sodium perborate solution i.e 30µl of each (Fig 2). 3rd plate was loaded with plain sodium perborate solution and plain water (Fig 3). All the plates were loaded with respective solutions using micropipette. The test was done in duplicates and the plates were incubated at 37°C for 24 hours. Zone of inhibition in the agar plates was examined after incubation period.

RESULTS

- The agar plates with chitosan solution dilutions and plate with plain perborate solution and plain water had shown minimum inhibitory zones around the wells which were not significant.
- The agar plate with different dilutions of chitosan solution and sodium perborate solution showed marked zone of inhibition around the wells. But the diameter of zones of inhibition was similar irrespective of different concentrations of chitosan solution used.
- This indicates that there was a synergistic anti-fungal effect of carboxy methyl chitosan solution along with sodium perborate solution, but the carboxy methyl chitosan and sodium perborate solution individually did not show anti-fungal effect.



Fig. 1: Agar plate with different dilutions of carboxy methyl chitosan solution. (100%, 75%, 50%, 25% and 10%).

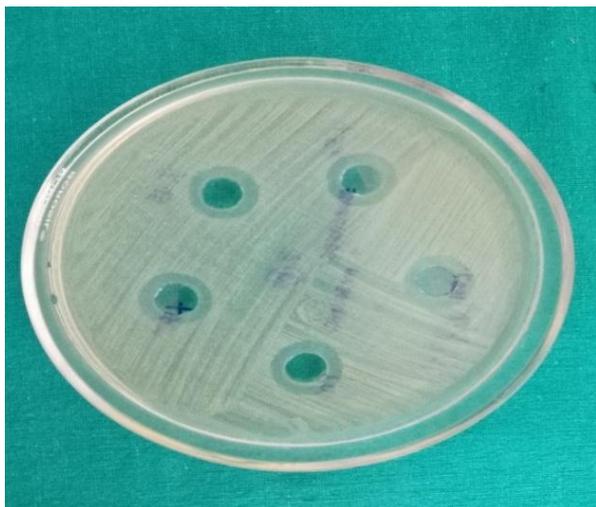


Fig. 2: Agar plate with equal amounts of different dilutions of carboxy methyl chitosan solution (100%, 75%, 50%, 25% and 10%) and sodium perborate solution.



Fig. 3: Agar plate with plain sodium perborate solution and plain water.

DISCUSSION

Oral cavity of healthy individuals with or without teeth may be colonized by yeast and bacteria coexisting in a relationship of commensalism.^[12] Denture wearing and deficient denture hygiene are the predisposing factors for increasing the number of microorganisms in the oral cavity. So, the bacterial colonization increases and becomes more pathogenic, acting as a potential source of infection.^[13] *Candida albicans* adhesion to resin materials is promoted by oral environment temperature and the acquired pellicle formed over dentures.

Ribeiro *et al.* found *Candida* spp. (65.5%) more than *Strep. mutans* and *Staph. aureus* on dentures. Also, Baena-Monroy *et al.* showed the presence of *Candida albicans* on the internal surface of complete dentures. *Candida albicans* is a well-known etiologic agent at denture stomatitis. This inflammatory disorder affects approximately 60% of denture wearers and causes inflammation of the oral mucosa in close contact with the denture.^[14]

The tendency to use natural materials has increased recently. Chitosan is an amino polysaccharide with antifungal effects derived from chitin by alkaline deacetylation. Chitin is the second most abundant polymer in nature after cellulose and has a safe biological profile for patients. It exhibits a therapeutic effect on diabetes along with cholesterol-lowering, wound healing, antitumor, antifungal, and antimicrobial effect. Chitosan is a cationic polysaccharide with a positive charge that can react with the negatively charged cell walls of microorganisms and can damage the targeted cells, causing loss of cell membrane. It prevents the development of fungal diseases by preventing formation and maturation of biofilm and preventing the attachment of *C. albicans* to human mucosal cells.^[15]

So, the present study aimed at evaluating the anti-fungal activity of different dilutions of carboxy methyl chitosan which is a water soluble chitosan, sodium perborate solution and the synergistic effect of chitosan with sodium perborate solution.

The results of the present study indicated that the chitosan along with the sodium perborate solution had shown synergistic effect against *C. albicans* which was not observed when used individually. In contrast to present study, a study done by Albuquerque C *et al.*,^[16] they found that greater antifungal activity of LMWC was observed at pH 4.0 and no evidence of a synergistic effect of the combination of LMWC and fluconazole was found at pH 7.0. Another study done by Costa E *et al.*,^[11] found that Chitosan is capable of inhibiting *C. albicans* (HMW, 1 mg/mL; LMW, 3 mg/mL). Several authors presented various MIC values for the antifungal activity of chitosan upon *C. albicans*. Tayel, Moussa, El-Tras, Knittel, Opwis and Schollmeyer previously reported a MIC of 1.25 mg/mL.^[17] Qin *et al.*^[18] reported an even lower MIC of 0.8 mg/mL, and Şenel *et al.*^[19] reported a MIC of 10 mg/mL.

Considering the present results, where carboxy methyl chitosan did not show any antifungal activity individually at different dilutions, further research is being carried out by us to find the minimum inhibitory concentration of different deacetylation degrees of chitosan against candida species.

CONCLUSION

In conclusion, Carboxy methyl chitosan which is water soluble exhibits antifungal activity when used along with sodium perborate but its role individually against candida species was not established.

REFERENCES

1. Marsh PD, Martin MV, Lewis MA, Williams D. Oral Microbiology. 5th ed., Elsevier health sciences, 2009.
2. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. *J Oral Microbiol*, 2011; 28(3): 1-11.
3. Williams DW, Kuriyama T, Silva S, Malic S, Lewis MA. Candida biofilms and oral candidosis: treatment and prevention. *Periodontol*, 2011; 55(1): 250-65.
4. Gantait S, Bhattacharyya J, Das S, Biswas S, Ghata A, Ghosh S, Goel P. Comparative assessment of the effectiveness of different cleaning methods on the growth of *Candida albicans* over acrylic surface. *Contemp clinical dent*, 2016; 7(3): 336-342.
5. Nikawa H, Nishimura H, Hamada T, Yamashiro H, Samaranyake LP. Effects of modified pellicles on *Candida* biofilm formation on acrylic surfaces. *Mycoses.*, 1999; 42(1-2): 37-40.
6. Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas PG, Dismukes WE, et al. Practice Guidelines for the treatment of candidiasis. *J Infect Dis.*, 2000; 30(4): 662-78.
7. Nalbant AD, Kalkanci A, Filiz B, Kustimur S. Effectiveness of different cleaning agents against the colonization of *Candida* spp and the in vitro detection of the adherence of these yeast cells to denture acrylic surfaces. *Yonsei Med J.*, 2008; 49(4): 647-54.
8. Montagner H, Montagner F, Braun KO, Peres PE, Gomes BP. In vitro antifungal action of different substances over microwaved-cured acrylic resins. *J Appl Oral Sci.*, 2009; 17(5): 432-5.
9. Husain S, Al-Samadani KH, Najeeb S, Zafar MS, Khurshid Z, Zohaib S, Qasim SB. Chitosan biomaterials for current and potential dental applications. *Materials(Basel)*, 2017; 10(6): 602.
10. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules*, 2003; 4(6): 1457-65.
11. Costa E, Silva S, Tavarina F, Pintado M. Antimicrobial and antibiofilm activity of chitosan on the oral pathogen *Candida albicans*. *Pathogens*, 2014; 3(4): 908-19.
12. D. G. Ribeiro, A. C. Pavarina, L. N. Dovigo, A. L. MacHado, E. T. Giampaolo, and C. E. Vergani,. Prevalence of *Candida* spp. associated with bacteria species on complete dentures. *Gerodontology*, 2012; 29(3): 203-8.
13. J. P. Lyon, S. C. da Costa, V. M. G. Totti, M. F. V. Munhoz, and M. A. de Resende. Predisposing conditions for *Candida* spp. carriage in the oral cavity of denture wearers and individuals with natural teeth. *Can J Microbiol*, 2006; 52(5): 462-7
14. Yildirim-Bicer AZ, Peker I, Akca G, Celik I. In vitro antifungal evaluation of seven different disinfectants on acrylic resins. *Bio Med Res Int.*, 2014; 51: 90-98.
15. Atai Z, Atai M, Amini J. In vivo study of antifungal effects of low-molecular-weight chitosan against *Candida albicans*. *J Oral Sci.*, 2017; 59(3): 425-30.
16. Alburquenque C, Bucarey SA, Neira-Carrillo A, Urzúa B, Hermosilla G, Tapia CV. Antifungal activity of low molecular weight chitosan against clinical isolates of *Candida* spp. *Med Mycol*, 2010; 48(8): 1018-23.
17. Tayel, AA, Moussa S, El-Tras WF, Knittel D, Opwis K, Schollmeyer E. Anticandidal action of fungal chitosan against *Candida albicans*. *Int J Biol Macromol*, 2010; 47(4): 454-457.
18. Qin C, Li H, Xiao Q, Liu Y, Zhu J, Du Y. Water-solubility of chitosan and its antimicrobial activity. *Carbohydr Polym*, 2006; 63(3): 367-374.
19. Şenel S, İkinci G, Kaş S, Yousefi-Rad A, Sargon M.F, Hincal AA. Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery. *Int J Pharm*, 2000; 193(2): 197-203.