



The effect of chitosan and fluoride varnish on *Streptococcus mutans* count in saliva

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General Note



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ABSTRACT

Background and Objective: Dental caries is considered as the major oral health problem caused by oral bacteria. The aim of the present study was to compare the effect of chitosan and fluoride varnishes on *Streptococcus mutans* count in saliva. **Materials and**

methods: A pre-structured questionnaire was prepared to collect data. A total of 90 children without caries or primary caries within the age group of 4-6 years were selected for the study. The subjects were divided in three groups of 30 (n=30) based on the three studied varnishes. Following eating and brushing (1hr), the samples of unstimulated saliva were collected. Group I was treated with 5% chitosan varnish and group II received 3% chitosan varnish and group III was treated with sodium fluoride varnish. Assessment of *Streptococcus mutans* in saliva was carried out at the baseline and 24h after varnish application. Data were analyzed using t-test, one-way ANOVA, Tukey test, and SPSS ver.15. **Results:** According the obtained results the number of *Streptococcus mutans* in saliva was significantly reduced in all three groups ($p < 0.05$). The highest *Streptococcus mutans* reduction was found in 5% chitosan varnish, 3% chitosan varnish, and sodium fluoride varnish, respectively. The difference between 5% chitosan varnish and sodium fluoride varnish was statistically significant ($P < 0.05$), but no statistically significant difference was found between 3% chitosan varnish and the two other varnish groups ($P > 0.05$). **Conclusion:** All three varnish groups, 5% chitosan varnish, 3% chitosan varnish, and sodium fluoride varnish can significantly decrease *S. mutans* count in saliva. 5% chitosan varnish has the satisfactory antimicrobial activity compared the two other varnishes.

Keywords: *Streptococcus mutans*, Fluoride varnish, Chitosan varnish

1. INTRODUCTION

Dental caries is the most global and frequent chronic infectious oral disease that affects people of all ages specially children (Mohsin et al., 2015). Dental caries is considered as a bacterial infection which causes demineralization and obliteration of the hard tissues (Kumar et al., 2015). Microorganisms reside in the oral cavity are equipped with receptors that allow it to adhere to the surface of the tooth and contribute to tooth decay. Fastidious organisms such as *Streptococcus mutans* (*S. mutans*), *Lactobacillus* and some species of *Actinomyces* colonize and cause the formation of dental plaque. In the initial phase of biofilm formation, *S. mutans* plays a major role in dental caries. A positive correlation exists between salivary levels of *S. mutans* and dental caries. The level of *S. mutans* in saliva can be used as risk indicators for dental caries. So, regular removal of biofilm and reducing the number of oral bacteria are the most common methods for caries management and inhibiting *S. mutans* proliferation (de Freitas-Fernandes et al., 2015). Although mechanical plaque control is the most dependable oral hygiene methods, chemical plaque control is also essential for controlling plaque formation and preventing dental caries (Dean & Hughes, 2010).

Fluoride is the most commonly used chemical materials for prevention of dental caries. Fluoride due to its effect on the calcified tissues of teeth and ability to diminish specific types of acid-producing bacteria, including *streptococcus mutans* plays an important role in dental caries prevention (Erdem et al., 2012). Fluoride at low concentration has bacteriostatic property and its bactericidal property is found at high concentration. Fluoride at high concentration inhibits oral bacterial activity and reduces the amount of specific types of acid-producing bacteria (Deepti et al., 2008). However, there is little evidence that fluoride and its relative concentrations cause dramatic changes in the oral bacterial activity (Anderson et al., 2016). In recent years, much attention has been paid to new dental plaque control materials, including chitosan. Chitosan is a natural polysaccharide originated from complete or partial deacetylation of chitin which is widely present in crab and shrimp shells (Kim & Shin, 2013). This non-toxic material due to its polycationic nature inherent antibacterial activity, as well as it is known to have biocompatible and biodegradable properties (Ikinci et al., 2002). Chitosan with low molecular weight have the ability to adhere to the dental analogue and prevent adhesion of *S. mutans* to saliva-coated hydroxyapatite (Helander et al., 2001). Chitosan interferes with the demineralization process of the tooth enamel and inhibits the mineral loss (Franca et al., 2014). Since chitosan is soluble only in acid conditions, the application of chitosan in toothpastes and mouth washes is limited due to its insolubility in water (Chen & Chung, 2012).

In dental products, preventive caries supplements are usually found in the form of toothpaste, gel or mouthwash, but these supplements can provide short term activity. Recent studies have found that varnishes have long lasting efficacy to prevent caries, i.e. all varnishes comprise film formers, which impart the desired measure of solidity and persistence on the surface (Franca et al., 2014; Chen & Chung, 2012). Varnish systems exhibit a wide range of applications and allow for the gentle and effective care of both natural teeth and high-quality restorations (Chen & Chung, 2012; Matthijs & Adriaens, 2002). The plaque mutans *streptococci* can be suppressed efficiently by topical applications of antibacterial varnishes (Twetman et al., 1995). Therefore, varnishes should be considered as an option for preventing caries (Bratthall et al., 1995; Chandak et al., 2016). The aim of the present study was to compare the effect of chitosan and fluoride varnish on *Streptococcus mutans* count in saliva.

2. MATERIALS AND METHODS

The present study was a clinical trial study. A total of 90 children within the age group of 4-6 years were selected for the study. A pre-structured questionnaire was prepared to collect data. All the participants were in the primary and mixed dentition stage. The inclusion criteria included: children without caries or primary caries, no history of antibiotic use in the last month, and no history of fluoride therapy in the last six months. As well as, the children did not have any medical contraindication for receiving varnishes. A written informed consent was obtained from the parents of all participants. The study was approved by research ethics committee, Ahvaz Jundishapur University of Medical Sciences. The subjects were divided in three groups of 30 (n=30): Group I: Subjects who received 5% chitosan varnish, Group II: Subjects who received 3% chitosan varnish, Group III: Subjects who received fluoride varnish. 3% and 5% chitosan varnish with pH ranging from 6.0 to 6.5 were prepared using a mixture of 3g and 5g low-molecule-weight chitosan powder (sigma – Aldrich, America) in 100cc acetic acid 1% for 24h at room temperature. At the baseline of the study children were told to brush and skip eating and drinking 1hr before beginning the study. Initial samples of non-stimulated saliva were collected one hour after eating and brushing in sterile tubes (walhin YB, 1991) (Figure 1). Following saliva collection, the teeth were cleaned using cotton swab. Varnishes were applied quadrant wise consecutively beginning from the lower arches and then continued to the upper arch using small disposable soft brush. Varnish application was performed as follow: Group I was treated with 5% chitosan varnish and group II received 3% chitosan varnish and group III was treated with fluoride varnish (Pascal, America). For more uniformly successful results the subjects were asked to avoid eating sticky and hard foods for 24h and also avoid brushing for 10hr. After 24h the samples of saliva were collected, accordingly. In order to blinding and avoiding chaos in counting samples a code was specified to each plastic vial of saliva. The collected samples of saliva were sent on the same day for *S. mutans* analysis to the microbiology laboratory.



Figure 1 collecting non-stimulated saliva one hour after eating and brushing

Microbiological analysis

All the collected salivary samples were transferred immediately on the same day to microbiological laboratory in Ahvaz Jundishapur University of Medical Sciences in sterile containers within one hour for *S. mutans* analysis. Salivary Samples were vortexed during 1 min and diluted 1:10, 1:100 and 1:1000 with sterile phosphate buffer saline. One loop (1/1000th ml of sample) was inoculated on the Modified Mitis Salivarius agar "Mitis-Salivarius bacitracin" (MSB) agar. This medium was prepared by solving Mitis Salivarius agar (HIMEDIA, India) according to the manufacturer's recommendation with 20% sucrose. After autoclaving for 20 min at 121°C and cooling to approximately 50°C, Bacitracin and potassium tellurite were added to a final concentration of 0.2 U per ml agar and %1 respectively for detection *S. mutans*. The inoculated plates were incubated for 48hrs at 37°C in microaerophilic condition using the candle jar with 5% CO₂. The colonies of the developed Bacteria on Modified MSB agar were isolated and further identification of *mutans streptococci* carried out by using diagnostic tests including Gram staining, catalase test, ability to ferment mannitol, sorbitol, Inulin, raffinose, sucrose, arginine dihydrolase and hydrolysis bileesculine test. Finally characteristics of colonies and biochemical tests were studied and saliva's number of colony forming units of *S. mutans* (CFU/ml) was determined by using a colony counter. To

determine the number of colony-forming unit (CFU) the colony number was multiplied by the dilution factor. Data were analyzed using *t*-test, one-way ANOVA, Tukey test, and SPSS ver.15. Significance level was set at $\alpha = 0.05$.

3. RESULTS

The present study compared the effect of chitosan (3%, 5%) and fluoride varnishes on *Streptococcus mutans* count in saliva. The obtained data were statistically analyzed. The mean and the standard deviation for *Streptococcus mutans* count in saliva before and after three varnishes application is reported in Table 1 and Fig. 2.

Table 1 Mean \pm SD of *Streptococcus mutans* count before and after use of three types of varnishes

Group	Number	Time Interval	Mean (CFU/ml)	SD	Median Difference	SD	P-value
5% chitosan varnish	30	Before	17266.66	14236.75	9666.66	10752.17	0.00
		After	7600.00	4796.55			
3% chitosan varnish	30	Before	16033.33	12271.31	8000.00	8300.39	0.00
		After	8033.33	6239.38			
Sodium fluoride varnish	30	Before	15166.66	10415.78	3533.33	4216.00	0.00
		After	11633.33	8235.68			

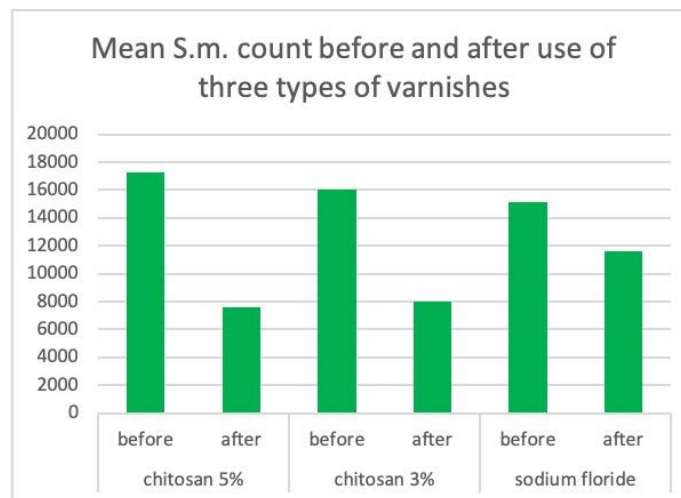


Figure 2 Mean *streptococcus mutans* before and after use of three types of varnishes

According the obtained results (Table 1 and Fig 2), the number of *Streptococcus mutans* in saliva was significantly reduced in all three groups ($p < 0.05$). The highest *Streptococcus mutans* reduction was found in 5% chitosan varnish, 3% chitosan varnish, and sodium fluoride varnish, respectively. Table 2 shows the difference among three varnishes using one-way ANOVA and Tukey test.

Table 2 Comparison of the 3% chitosan varnish, 5% chitosan varnish, and sodium fluoride varnish

Group	Standardized mean difference	P-value
5% chitosan varnish 3% chitosan varnish	1666.66	0.713
5% chitosan varnish Sodium fluoride varnish	6133.33	0.013
3% chitosan varnish Sodium fluoride varnish	4466.66	0.094

Referring to the results of Table 2, The difference between 5% chitosan varnish and sodium fluoride varnish was statistically significant ($P < 0.05$), but no statistically significant difference was found between 3% chitosan varnish and the two other varnish groups ($P > 0.05$).

4. DISCUSSION

Dental caries is one of the most common infectious and bacterial diseases. Dental caries is multifactorial and contagious disease and bacterial plaque is considered as one of the leading factors of dental decay (Mohsin et al., 2015). Dental caries can be prevented by inhibiting risk factors, i.e. removing bacteria involved in the dental caries process (Nowak & Mabry, 2013). Various products have been marketed for the control of dental decay includes sodium fluoride. Fluoride enhances remineralization and at high concentration has been proven to be an effective antibacterial agent (Erdem et al., 2012). Although topical fluoride has been successfully used for prevention of dental caries, high-level exposure to fluoride can result in skeletal fluorosis (Patel et al., 2017). Antibacterial therapy is another accepted technique for caries prevention. Chlorhexidine is the most important and multipurpose antimicrobial agent inhibits oral bacteria and commonly is used in periodontal diseases, however side effects such as tooth/tongue staining, dry mouth, and decreased taste sensation limit the long term use of this product and are not recommended for regular caries preventive program (Franca et al., 2014). Due to the side effects of chemical drugs on the one hand, safety, availability and lower prices of natural products on the other hand, new material such as chitosan is introduced as cost-effective product in dentistry (Wassel & Khattab, 2017).

The present study evaluated the effect of three varnishes, chitosan (3%, 5%) and fluoride, on *Streptococcus mutans* count in saliva. According the results of present study all three varnishes reduced the number of *Streptococcus mutans* in saliva. Various studies examined the antibacterial activity of chitosan, but most of them applied it as mouth wash rather than varnish. As well as, several studies suggested that chitosan could be efficacious in reducing plaque and salivary levels of *Streptococcus mutans* (Erdem et al., 2012; Deepti et al., 2008; Mortazavi et al., 2007). Kim et al.'s study recommended chitosan composite resin as a feasible antibacterial restorative due to its antibacterial nature and mechanical properties (Kim & Shin, 2013). Franca et al., 2014 in a study examined the effect of 5%, 10%, 15% sustained-release propolis-based chitosan varnish (PCV) on dental cariogenic biofilm prevention. The results of the study showed that propolis-chitosan varnish at all concentrations inhibit the growth of all microorganisms tested. However, no significant difference was observed among the 5%, 10% and 15% PVC varnishes. Franca et al. concluded that PCV 5%, PCV 10%, and PCV 15%, into products suitable for clinical examination on dental caries prevention field (Franca et al., 2014). The results of present study was consistent with Franca et al.'s study regarding the efficacy of chitosan varnish, however the present study suggested that 5% chitosan varnish have the highest efficacy on salivary levels of *Streptococcus mutans* compared to fluoride varnish.

Wassel et al. (2017) in an in vitro study evaluated *S. mutans* susceptibility to natural formulated dental varnishes comprising propolis, miswak, and chitosan nanoparticles (CS-NPs) with or without sodium fluoride (NaF). Medium molecular weight chitosan was converted to nanoparticles via the ionotropic gelation process. The results of the study showed that the tested natural products without sodium fluoride can effectively prevent dental caries compared to sodium fluoride. The result of the study was in agreement with the result of the present study. In addition, the highest antimicrobial activity was observed in the chitosan nanoparticles varnish; however, it didn't significantly differ than other varnishes. Although in the Wassel et al.'s study varnishes containing natural products combined with sodium fluoride had higher antibacterial influence; they didn't considerably differ in antibacterial activity compared to varnishes containing just natural products (Wassel et al., 2017).

According the results of the present study the difference between sodium fluoride and 5% chitosan varnish was statistically significant, but no significant difference was found between 3% chitosan varnish and those two other varnishes. Therefore, high concentrations of chitosan display noticeable antibacterial activity.

5. CONCLUSION

Chitosan varnish and sodium fluoride varnish both have antimicrobial activity against salivary *Streptococcus mutans*. Chitosan varnish at high concentration has potent antibacterial effect on *S. mutans*, and recommended for caries prevention.

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Conflict of interest

The authors declare that there is no conflict regarding the publication of this manuscript.

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Ethical Code

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REFERENCE

- Anderson M, Grindefjord M, Dahllöf G, Dahlén G, Twetman S. Oral microflora in preschool children attending a fluoride varnish program: a cross-sectional study. *BMC Oral Health*. 2016;16:130.
- Bratthall D, Serinirach R, Rapisuwon S, Kuratana M, Luangjarmekorn V, Luksila K. A study into the prevention of fissure caries using an antimicrobial varnish. *Int Dent J*. 1995;45:245-54.
- Chandak S, Bhondey A, Bhardwaj A, Pimpale J, Chandwani M. Comparative evaluation of the efficacy of fluoride varnish and casein phosphopeptide - Amorphous calcium phosphate in reducing *Streptococcus mutans* counts in dental plaque of children: An in vivo study. *J Int Soc Prev Community Dent*. 2016 ;6:423-429.
- Chen CY, Chung YC. Antibacterial effect of water-soluble chitosan on representative dental pathogens streptococcus mutans and lactobacilli brevis. *J Appl Oral Sci*. 2012;20:620-7.
- de Freitas-Fernandes FS, da Silva RA, Fernandes JD, Gabardo MC, Souza SD, Jansen MV. Effect of Chlorhexidine Gel in Mentally Handicapped Children highly Infected with *Streptococcus Mutans*. *Braz Res Pediatr dent Integ Clin*. 2015;15:49-55.
- Dean JA, Hughes CV. Mechanical and chemotherapeutic home oral hygiene. McDonald and Avery Dentistry for the Child and Adolescent-E-Book. 2010:205.
- Deepti A, Jeevarathan J, Muthu M, Prabhu VR. Effect of Fluoride Varnish on *Streptococcus mutans* Count in Saliva of Caries Free Children Using Dentocult SM Strip Mutans Test: A Randomized Controlled Triple Blind Study. *Int J Clin Pediatr Dent*. 2008 ;1:1-9.
- Erdem A, Sepet E, Kulekci G, Trosola S, Guven Y. Effects of two fluoride varnishes and one fluoride/chlorhexidine varnish on *Streptococcus mutans* and *Streptococcus sobrinus* biofilm formation in vitro. *Int J Med Sci*. 2012;9:129-136.
- Franca JR, De Luca MP, Ribeiro TG, Castilho RO, Moreira AN, Santos VR. Propolis-based chitosan varnish: drug delivery, controlled release and antimicrobial activity against oral pathogen bacteria. *BMC Complement Altern Med*. 2014; 14: 478.
- Helander IM, Nurmiaho-Lassila EL, Ahvenainen R, Rhoades J, Roller S. Chitosan disrupts the barrier properties of the outer membranes of gram –negative bacteria. *Int J Food Microbiol*. 2001 ;71:235-44
- Ikinci G, Senel S, Akincibay H, Kaş S, Erciş S, Wilson CG. Effect of chitosan on a periodontal pathogen *Porphyromonas gingivalis*. *Int J Pharm*. 2002 ;235:121-7.
- Kim JS, Shin DH. Inhibitory effect on streptococcus mutans and mechanical properties of the chitosan containing composite resin. *Restor Dent Endod*. 2013 ;38:36-42 .
- Kumar KS, Asokan S, John JB, Priya PRG. Antimicrobial efficacy of two commercially available toothpastes on salivary *Streptococcus mutans* , *Lactobacillus* , and *Candida*: A randomized controlled trial. *J Indian Acad Dent Res*. 2015;2:13-15.
- Matthijs S, Adriaens PA. chlorhexidine varnishes: a review. *J Clin Periodontol*. 2002 ;29:1-8.
- Mohsin S, Manohar B, Rajesh S, Asif Y. The effects of a dentifrice containing propolis on Mutans streptococci: A clinico-microbiological study. *Ethiop J Health Sci*. 2015;25:9-16.
- Mortazavi M, Kohanteb J, Jahanimoghaddam F. Inhibitory of NaF_varnish and APF_Gel on cariogenic Bacteria: an invitro study. *Shiraz univ Dent J*. 2007;15:64-73.
- Nowak AJ, Mabry TR. 19 Prevention of Dental Disease. *Pediatric Dentistry-E-Book: Infancy through Adolescence*. 2013:279.
- Patel PM, Hugar SM, Halikerimath S, Badakar CM, Gokhale NS. Comparison of the Effect of Fluoride Varnish, Chlorhexidine Varnish and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) Varnish on Salivary *Streptococcus mutans* Level: A Six Month Clinical Study. *J Clin Diagn Res*. 2017;11:ZC53-ZC59.
- Twetman S, Hallgren A, Petersson LG. Effect of an antibacterial varnish on mutans streptococci in plaque from enamel adjacent to orthodontic appliances. *Caries Res*. 1995;29:188-91.
- Wassel MO, Khat tab MA. Antibacterial activity against *Streptococcus mutans* and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes. *J Adv Res*. 2017;8:387-392.