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The effect of Mastic Chewing Gums on Salivary Streptococcus Mutans Count

Abstract

Introduction: *Streptococcus Mutans* is one of the most common cariogenic microorganisms. Use of natural anti-cariogenic agents, such as Xylitol has been well established in literature. On the other hand, there is a scarcity of studies that have reported the antimicrobial potential of Mastic Gum as an anti-cariogenic chewing agent; hence the present study was designed. To evaluate and compare the anti-cariogenic action of two commercial chewing gums Mastic and Xylitol on the salivary *Streptococcus Mutans* count in a group of children from Residental School in Bangalore City. ***Study Design:*** Clinical Setting and Experimental Design. ***Methods and Material:*** Sixty healthy children aged 5-8 yrs with dmft/DMFT index score ≥ 3 were included in the study. Before the test, unstimulated saliva was collected. Children divided in Group I and II were given Mastic and Xylitol chewing gums respectively; to chew for fifteen minutes. Saliva samples were then collected at fifteen mins (just after spitting) 45 mins, 5th day and 60th day. The amount of *Streptococcus Mutans* in saliva was evaluated using a selective media (MSAB). Statistical analysis use; Mann-Whitney test. ***Results:*** The total number of bacterial colonies was significantly reduced when compared to baseline in both the groups. Mastic Chewing gum showed statistically significant reduction in the number of colonies as compared to Xylitol. ***Conclusions:*** Mastic chewing gum can be used as an anti-cariogenic agent in children.

Key Words

Mastic chewing gum; xylitol chewing gum; anti-cariogenic agent; *Streptococcus Mutans*

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INTRODUCTION

Dental caries continues to be an important public health problem and the most prevalent disease affecting the human oral cavity.[1] The incidence is particularly high during childhood.[2,3] The tooth enamel and dentin are demineralized by acids, such as lactic acid, which are produced as a by-product of carbohydrate metabolism by cariogenic bacteria in dental plaque.[4] *Streptococcus mutans* is the leading cause of dental caries worldwide, and is considered to be the most cariogenic amongst the oral streptococci.[5] Numerous antimicrobials and antibiotics including chlorhexidine, spiramycin and vancomycin have been used for against *Streptococcus mutans* to reduce plaque mediated diseases including dental caries.[6] However, antibotics have several adverse effects such as vomiting, diarrhoea and teeth staining. In addition, the development of antimicrobial resistant strains is a growing cause of concern. These drawbacks justify further research and development of natural antimicrobial agents targeting specific oral pathogens while being safe for the host.[7,8] Natural products have recently been investigated more thoroughly as promising agents to prevent oral diseases, especially plaque-related diseases such as dental caries.[9-11] Recent studies have demonstrated antimicrobial activity of natural products against selected oral pathogens one such natural product is Mastic gum. It is a natural resin derived from the

Comparison of Streptococcus mutans between Group A and Group B at different time intervals

Time Interval	Group	Mean	Std Dev	SE of Mean	Mean Difference	Z	P-Value
Baseline	Group A	6.97	1.38	0.25	-0.133	-0.792	0.428
	Group B	7.10	1.21	0.22			
15 Mins	Group A	5.23	2.16	0.39	-0.500	-2.743	0.006*
	Group B	5.73	0.69	0.13			
45 Mins	Group A	4.93	1.17	0.21	0.233	-1.136	0.256
	Group B	4.70	1.37	0.25			
5 Days	Group A	4.10	0.92	0.17	0.767	-2.600	0.009*
	Group B	3.33	1.06	0.19			
60 Days	Group A	1.83	0.95	0.17	-1.233	-4.900	<0.001*
	Group B	3.07	0.83	0.15			

**denotes significant difference*

Comparison of Streptococcus mutans at different time intervals within Group A: (Wilcoxon Signed Ranks Test)

Time Interval	Mean	Std Dev	SE of Mean	Mean Difference	Z	P-Value
Baseline	6.97	1.38	0.25	1.733	-3.747	<0.001*
15 Mins	5.23	2.16	0.39			
Baseline	6.97	1.38	0.25	2.033	-3.978	<0.001*
45 Mins	4.93	1.17	0.21			
Baseline	6.97	1.38	0.25	2.867	-4.646	<0.001*
5 Days	4.10	0.92	0.17			
Baseline	6.97	1.38	0.25	5.133	-4.810	<0.001*
60 Days	1.83	0.95	0.17			
15 Mins	5.23	2.16	0.39	0.300	-0.602	0.547
45 Mins	4.93	1.17	0.21			
15 Mins	5.23	2.16	0.39	1.133	-2.838	0.005*
5 Days	4.10	0.92	0.17			
15 Mins	5.23	2.16	0.39	3.400	-4.808	<0.001*
60 Days	1.83	0.95	0.17			
45 Mins	4.93	1.17	0.21	0.833	-2.939	0.003*
5 Days	4.10	0.92	0.17			
45 Mins	4.93	1.17	0.21	3.100	-4.773	<0.001*
60 Days	1.83	0.95	0.17			
5 Days	4.10	0.92	0.17	2.267	-4.586	<0.001*
60 Days	1.83	0.95	0.17			

**denotes significant difference*

stem and the leaves of the mastic tree, Pistacia lentiscus Linn, native to Mediterranean areas.[12,13] It has been used by traditional healers for the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer.[14 15] It has also been shown in numerous studies to have impressive antibacterial and antimicrobial properties.[16 17] In vitro Studies have also demonstrated that a short treatment time is required for the bacteriostatic effect of mastic gum against *S. mutans*. Although the antibacterial activity of mastic chewing gum has already been demonstrated, very few studies have been conducted on bacteria of clinical relevance in dentistry especially in children. Xylitol a naturally occuring sugar substitute is a promising anti-caries agent in children. Maternal xylitol-gum use during early infancy has been well documented to successfully reduce *S. mutans* (MS) colonization followed by caries prevention in their children.[18-21] Recent studies also showed that use of xylitol in high-caries-risk infants successfully reduced early childhood caries (ECC).[22,23] Hence, in this study, we aimed to evaluate the antibacterial activity of mastic chewing gum and Xylitol chewing gum against *Streptococcus Mutans* in vivo conditions.

MATERIALS AND METHODS

Hundred and fifty children, aged 5-8 years, from a Residental school in South Bangalore city were screened as a part of routine dental examination and Sixty children with an age range of 5-8 (mean age

Comparison of Streptococcus mutans at different time intervals within Group B: (Wilcoxon Signed Ranks Test)

Time Interval	Mean	Std Dev	SE of Mean	Mean Difference	Z	P-Value
Baseline	7.10	1.21	0.22	1.367	-3.708	<0.001*
15 Mins	5.73	0.69	0.13			
Baseline	7.10	1.21	0.22	2.400	-4.125	<0.001*
45 Mins	4.70	1.37	0.25			
Baseline	7.10	1.21	0.22	3.767	-4.794	<0.001*
5 Days	3.33	1.06	0.19			
Baseline	7.10	1.21	0.22	4.033	-4.738	<0.001*
60 Days	3.07	0.83	0.15			
15 Mins	5.73	0.69	0.13	1.033	-3.344	0.001*
45 Mins	4.70	1.37	0.25			
15 Mins	5.73	0.69	0.13	2.400	-4.752	<0.001*
5 Days	3.33	1.06	0.19			
15 Mins	5.73	0.69	0.13	2.667	-4.774	<0.001*
60 Days	3.07	0.83	0.15			
45 Mins	4.70	1.37	0.25	1.367	-3.673	<0.001*
5 Days	3.33	1.06	0.19			
45 Mins	4.70	1.37	0.25	1.633	-4.184	<0.001*
60 Days	3.07	0.83	0.15			
5 Days	3.33	1.06	0.19	0.267	-1.197	0.231
60 Days	3.07	0.83	0.15			

**denotes significant difference*

The inclusion criteria comprised of children recorded with a dental caries score (dmft/DMFT) equal to or greater than 3, those who were willing to comply with the procedures and children with written consent signed from parents. The exclusion criteria included medically compromised children, children with any systemic diseases or allergies, those with a history of taking antibiotics three months prior to and during the study period, children undergoing orthodontic treatment or with an intraoral prosthesis and those with presence of any intraoral soft tissue pathology. The investigation that was carried out over duration of eighty days had a randomized double-blind prospective design that was approved by the Ethical committee of the institution. The study and intervention involved were explained in detail to the school authorities, children and the parents of the included children in the study. No modifications were made in the diet or oral hygiene measures of the children. The school authorities were however advised not to provide any food two hours prior to the procedure. Prior to the commencement of the chewing regime, at 11.30 a.m. (which was more than two hours after the morning breakfast); about 2ml of unstimulated saliva samples were collected from all the children in sterile falcon tubes and were transferred immediately to the laboratory in an temeperature controlled cold chain cartoon boxes with the help of critical logistics (Sequel Logistics ^{R}). The saliva samples were vortexed and serial dilutions were prepared, 100 µl was inoculated on selective media for *Streptococcus Mutans,* (Mitis Salivarius Bacitracin Agar, HiMedia Laboratories, Pvt. Ltd, Mumbai, India) and incubated at 37°C for 48 hours. Colony forming units were counted and recorded. The children were then assigned with numbers from one to sixty. They were randomly allocated into two groups and the confounding factors were taken into consideration. The total study population consisted of forty males and twenty females. Both the participants and the examiner were blinded. The chewing gums were wrapped in green and yellow packages, which were prepared by an assistant. The male subjects given Forty packages (20 green and 20 yellow); and the female subjects was given ten packages (10 green and 10 yellow). Children were asked to pick the colour they wanted and their number and the colour were recorded. Thus two groups, each of 30 children (20 males and 10 females) were formed. The green group was assigned as Group I and the yellow group as Group II. Group I was given a commercially available Mastic chewing gum (ELma Mastic-Greece) and Group II was given a commercially available Xylitol chewing gum.

Children were asked to chew the gums for fifteen minutes under supervision of the examiner. Saliva samples were collected at fifteen minutes (immediately after spitting the gum) 45min, 5th day and 60th day. In between the time intervals, the children were not allowed to eat or drink anything on Day 1. The saliva samples were transferred to the laboratory and processed. *Streptococcus Mutans* count was evaluated and compared with the baseline values. The colour codes of the gums were revealed after the results had been fed into SPSS V16 software package.

RESULTS

Samples were collected from all the 60 children included in the study. The overall mean dmft/DMFT score was found to be 4±1.14. A significant positive correlation was found between the dmft/DMFT score and the baseline *Streptococcus Mutans* count Mann-Whitney test showed no statistical difference in the baseline *Streptococcus Mutans* count between Group A and Group B. However, in both the groups there was a significant reduction in mean *Streptococcus Mutans* count at different time intervals (15 minutes, 5 days, 60th day) when compared with the baseline values. No significant difference is observed between Group A and Group B at baseline ($P>0.05$) and 45 minutes ($P>0.05$). Statistically significant difference was observed between Group A and Group B at 15 minutes ($P<0.01$), at 5 days ($P<0.01$) and at 60 days ($P<0.001$) (Table 1). The reduction in mean *Streptococcus mutans* was found to be significant from baseline to 15 mins ($P<0.001$), baseline to 45 mins ($P<0.001$), baseline to 5 days ($P<0.001$), baseline to 60 days ($P<0.001$), 15 mins to 5 days ($P<0.01$), 15 mins to 60 days ($P<0.001$), 45 mins to 5 days ($P<0.01$), 45 mins to 60 days ($P<0.001$) and from 5 days to 60 days ($P<0.001$). The reduction was not significant from 15 mins to 45 mins ($P>0.05$) (Table 2). The reduction in mean *Streptococcus mutans* was found to be significant from baseline to 15 mins ($P<0.001$), baseline to 45 mins ($P<0.001$), baseline to 5 days ($P<0.001$), baseline to 60 days ($P<0.001$), 15 mins to 45 mins ($P<0.01$), 15 mins to 5 days ($P<0.001$), 15 mins to 60 days ($P<0.001$), 45 mins to 5 days ($P<0.001$) and from 45 mins to 60 days ($P<0.001$). The reduction was not significant from 5 days to 60 days ($P>0.05$) (Table 3).

DISCUSSION

Prevention of dental caries in children is one of the hallmarks of contemporary pediatric dental practice. The concept of dentistry has recently changed from conventional dental treatment to prevention of dental caries to a large extent with the use of various novel techniques apart from the traditional ones. It is an established fact that *Streptococcus Mutans* plays a primary role in causing dental caries, specifically targeted antimicrobial agents have been researched extensively so as to achieve effective dental caries prevention. With the increased penchant for natural products, researchers are currently interested in the promising perspectives that these natural substances have to offer as alternatives for the control of dental caries in terms of antimicrobial response and lower associated risks. The results of our study have clearly shown the antibacterial effect of mastic gum on total bacteria, which is in compliance with similar study by Alev Aksoy 2007,[24] mastic gum inhibited de novo plaque accumulation compared with a placebo gum, indicating that mastic itself has an anti-plaque-formation activity. The present study's results is also parallel to the study of Takashashi *et al*.[13] In agreement with the studies of Magiatis *et al*., and Takahashi *et al*., it is suggest that regular use of mastic gum may be useful in controlling dental caries because of its antibacterial effect and anti-plaque-formation activity. Routine use of mastic gum may also include situations where mechanical hygiene measures or mouthwashes would be impractical but where a chewable antiplaque agent would be desirable. *Mutans streptococci* are considered to be predominant species isolated from human saliva and dental plaque, and have been identified as the major aetiological agent for caries. Individuals heavily colonised by *Mutans streptococci* were thought to be at high risk for caries. Hence, eradication of these cariogenic bacteria is off importance for the treatment of human dental caries. Several antiseptic agents including chlorhexidine, cetylpyridinium chloride, fluorides and phenol derivatives have been used widely in dentistry to inhibit bacterial growth. Nevertheless, dental scientists have still been searching for new applications of therapeutic drugs to prevent or treat dental plaque- related diseases.[18-25] Lately, mastic gum has attracted much attention as a natural useful substance in folk medicine to treat a variety of ailments for its antibacterial, anti-inflammatory and antiulcer activities.[29-32] Because of this broad spectrum of biological activities, mastic gum has attracted much attention as a natural useful substance in medicine, health food, and

cosmetic industries. These results support the possibility that mastic gum also has potential antibacterial activity against oral bacteria. Although mastic gum appears promising as a potential antibacterial agent against oral bacteria, it has not been explored widely in dentistry. In this study, the mastic gum showed significantly reduced bacterial growth in saliva compared with Xylitol chewing gum. In routine clinical works, saliva samples are often preferred owing to easier handling.[34] Therefore, we investigated the effect of mastic gum on the salivary bacterial numbers in vivo. In the present study, the saliva samples were collected in Baseline, 15 min, 45 min after chewing gum on Day 1 and 5 th Day and 60 th day after regular chewing of one gum per day there had been significant reduction in the total number of *Mutans streptococci* compared with substantial samples taken after Xylitol chewing ($p < 0.001$). Generally gum chewing has a mechanical effect washing out the bacteria by increasing the salivary flow rate. The Xylitol gum was used in the present study as a comparative chewing gum against mastic. Previous studies have shown that chewing gums containing xylitoland sorbitol had antibacterial effects.[34,35] In our study, mastic gum was more effective reducing the number of *Mutans streptococci* in saliva compared to Xylitol chewing gum. In this study, our results indicate that mastic gum had significant antibacterial activity and support the possibility that mastic may be a useful ingredient to aid oral health. When the mastic gum analysed is seen to have the main constituents of leaves of mastic tree (*P. lentiscus*) which are terpinen-4-ol and a-terpineol. These constituents are believed to be active compounds of many essential oils, and particularly teatree oil.[30] In the study by Magiatis *et al*.,[36] the in vitro antimicrobial activity of the three essential oils of *P. lentiscus* and of the resin (total, acid and neutral fraction) against six bacteria and three fungi was reported, our study parallel to the study of Magiatis *et al*., it was found that mastic gum obtained from *P. lentiscus* had serious antibacterial effect on *S. mutans* in vitro conditions. The number of in vivo studies on this subject is limited in dentistry. In the study of Takahashi *et al*.,[13] which was published on 2003, mastic gum was found to inhibit the plaque accumulation compared with placebo gum, indicating that mastic itself has antidental plaque formation activity which is an important factor for total *Streptococccci* counts in the oral environment. In agreement with the results of the studies of Takahashi *et al*.,[13] our results suggest that regular use of mastic gum may be useful to control dental caries via its antibacterial effect and antiplaque formation activity. The idea of adding additional mechanical hygiene measures to patients' oral hygiene methods has significant appeal from a convenience and compliance perspective. Routine usage of mastic gum may also be useful where mechanical hygiene measures or mouthrinses would be impractical but where a chewable anti-plaque agent would be desirable. Considering its antibacterial effect; our results suggest that mastic gum has an antibacterial activity against one of the most important cariogenic oral bacteria (*S. mutans*), which yields to decalcification of enamel and surface caries, and it may be useful for maintaining oral hygiene by reducing the bacterial growth (*Mutans streptococci*) in saliva.

CONCLUSION

Current study results were significant with respect to mastic chewing gum. Nevertheless further studies are needed to identify and purify its active ingredients for future use in trials using toothpastes and mouth rinse formulas. In addition longer term studies will be required to evaluate the usefulness of this material more exactly, and its bacteriostatic mechanism and specificity against this cariogenic bacterium need to be further researched.

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