Original Article

Effects of Three Mastic Gums on the Number of Mutans Streptococci, Lactobacilli and PH of the Saliva

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Abstract

Objective: In the recent years, herbal oral hygiene products have gained increasing attention. The aim of this study was to assess the effects of three types of mastic gums on the level of *Mutans streptococci, Lactobacilli* and pH of the saliva.

Materials and Methods: Forty-two students in the age range of 20-30 years were divided into three parallel groups; each of them separately used pure mastic gum, xylitol mastic gum and probiotic mastic gum for three weeks. Number of microorganisms and pH of the saliva were assessed before and after the intervention. The data were analyzed using Wilcoxon Signed Rank, paired-sample-t, Kruskal-Wallis and Tukey's post-hoc tests and One-way ANOVA.

Results: Level of *Mutans streptococci* showed a significant reduction compared to its baseline value in all three groups (P<0001 for all). Salivary *Lactobacillus* count increased in the groups using pure and xylitol mastic gums but decreased in the group using probiotic type, albeit these changes were only significant in the group using probiotic mastic gum (P<0.001). Use of pure and xylitol mastic gums increased the pH of the saliva but not significantly. In the group using probiotic mastic gum, the pH of the saliva decreased significantly (P=0.029).

Conclusion: Three weeks use of all mastic gums resulted in a significant drop in the number of *Mutans streptococci* in the saliva. However, the drop in the saliva pH due to the use of probiotic mastic gum is not in favor of dental health.

Key words: Mastic gum; Saliva; Mutans streptococci; Lactobacilli

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INTRODUCTION

In the recent years, people are more conscientious of the potential harms and the side effects of conventional chemical drugs, and are encouraged to use natural products. Some herbal products such as Acacia Arabica, Eucalyptus extract and Propolis possess antibacterial agents and other medicinal compounds and thus, may be effective for prevention and treatment of dental caries [1].

Mastic gum is a resinous exudate extracted from the stem and leaves of the Pistacia Lentiscus Linn tree, which possesses many therapeutic properties effective for the treatment of gastrointestinal (GI) diseases such as gastralgia, dyspepsia, and gastritis [2,3,4]. Its antibacterial properties against *Mutans streptococci* (*MS*) have been demonstrated in many researches [5, 6]. Its main antibacterial ingredients include Pinene, Limonene terpinene,

Terpineol, Caryophyllene, Verbenol, Linalool, Camphene and myrcene [2]. Previous studies have reported the positive effects of chewing mastic gum on the decrease of *MS* of the saliva [6] and the increase in the remineralization rate of caries-like lesions [7].

The role of chewing gums containing antiplaque agents such as chlorhexidine, xylitol and sorbitol as an adjunct to daily oral hygiene has been previously examined. The effects of chewing xylitol gums on the increase of the salivary flow and its pH have also been investigated [8] and a reduction in *MS* of the saliva has been reported [9, 10].

Recently, addition of probiotic bacteria to different food products in order to reduce the pathogenic microorganisms in the oral cavity has opened up new horizons in prevention and treatment of dental caries. The most commonly used probiotics include *Lactobacillus* (*LB*) and *Bifidobacterium*, which in fact are part of normal human microflora [11, 12, 13]. *Reuteri lactobacillus* has been used in many studies. *LB* is part of endogenous intestinal flora and is present in all parts of the GI tract including the oral cavity, stomach, small intestine, colon and even stool [14]. Probiotic bacteria are usually added to fermentable milk products such as yoghurt and ice cream and recently to chewing gums. Probiotics generate a biofilm that acts as a protective barrier against pathogenic bacteria [15]. They also secrete different antimicrobial agents such as organic acids, hydrogen peroxide and bacteriocins and are able to modify their surrounding environment through oxidation and balancing the pH [16].

The aim of this study was to assess and compare the effects of pure mastic gum and mastic gums enriched with xylitol and probiotics on the number of *LB* and *MS* and the acidity (pH) of the saliva.

MATERIALS AND METHODS

Forty-two dental students in the age range of 20-30 years willingly participated in this single blind, parallel-design clinical trial. The subjects had no systemic disease, untreated dental caries, gingivitis or periodontitis and had no history of antibiotic therapy or use of products containing xylitol or probiotics in the past four weeks. The study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences and all participants were requested to sign written informed consent before taking part in this study (registry code from IRCT:IRCT201110267910N1).

The mastic gums containing probiotics were prepared in the microbiology laboratory of the Medical faculty of Shahid Beheshti University of Medical Sciences.

In order to do so, Lactobacillus reuteri was obtained in lyophilized form from the Iranian Research Organization for Science and Technology, and was cultivated on MRS agar (Merck, Germany) and finally went through several passages. A modified suspension was then produced using 0.5 McFarland concentration of bacteria; 0.02 ml of this suspension was then injected into pieces of xylitol-containing mastic gums (Van Mastic Gum Industries of Kurdestan, Sanandaj, Iran) using insulin syringes (Sepa, Iran). The mastic gums contained 60% xylitol, maltitol and lactitol as the food source for the microorganisms. Injections were carried out in each newly prepared product at the third day in order to produce fresh probiotic mastic gums for the participants. At 48 hours after the injections, the contents of the probiotic mastic gums were cultivated to ensure the lactobacilli were alive.

Pure mastic gum was used in its natural form with no additives. In order to match the pieces of gum in terms of weight, the manufacturing company (Van Mastic Gum Industries of Kurdestan, Sanandaj, Iran) was ordered to supply pieces of gum equal in weight to be used for all groups of the study.

After the selection of participants, each one was given a toothbrush (Oral B, USA), an Aqua Fresh toothpaste (GlaxoSmithKline, UK), and a fluoride-free dental floss (Orkid, Iran).

In order to match the method of dental care, the participants received instructions on how to brush and floss in the same manner. They were then asked to brush twice a day and floss every night. The participants were asked to avoid using products containing probiotics, xylitol or fluoride during the study. Two weeks later, one hour after brushing with no toothpaste, 0.7 cc of fasting unstimulated saliva of the participants was collected into microtubes that were immediately sent to the microbiology laboratory of the medical faculty of Shahid Beheshti University of Medical Sciences for calculation of the *MS* and *LB* count. *MS* were cultivated on mitis salivarius agar (Merck, Germany) and *LB* were cultivated on MRS agar (Merck, Germany). After colony counting, the rate of growth based on the number of colony forming units (CFU) was determined as follows [17]:

Number of colonies = 0: 0

Number of colonies $\leq 10^3$ CFU: Low

Number of colonies = 10^3-10^4 CFU: Moderate

Number of colonies $\geq 10^5$ CFU: High

After saliva sampling, participants were asked to hold 10% sucrose solution in their mouth for one minute. After five minutes, unstimulated saliva was collected within 10 minutes time and kept at -20° C in order to measure the pH of the collected saliva throughout the study and with one time calibration of the pH-meter (Mettler Toledo, Germany).

Participants were then randomly assigned to three groups in a parallel design (14 each) using stratified permuted block randomization: the group using probiotic mastic gum, the group using xylitol mastic gum and the group using pure mastic gum. The participants used the aforementioned gums for three weeks three times a day. After every meal a piece of mastic gum was chewed for five whole minutes and the participants refrained from brushing for at least one hour after that. After the three weeks, saliva sampling was done again and the collected saliva was sent to the laboratory for microbial cultivation. Then, the participants drank 10% sucrose solution and after five minutes they chewed a piece of the same gum they were using throughout the study for 10 minutes. Then, the saliva samples were collected again and measured for pH [18].

The measured values were compared with the corresponding baseline values and Tukey's post hoc, Kruskal-Wallis, Wilcoxon Signed-Rank, and paired-sample t-test as well as one-way ANOVA were used for data analysis. The level of statistical significance was set at 0.05 ($P<0.05$).

The one-sample Kolmogorov-Smirnov test was used to test the normal distribution of the variables. Because of their non-normal distribution, the Wilcoxon Signed-Rank test was used to make within-group comparisons for the number of *MS* and *LB* colonies in all groups.

Considering the normal distribution of pH, paired-sample t-test was used for within-group comparisons of pH. With respect to the distributions, the Kruskal-Wallis test and the Mann-Whitney test were applied to make inter-group and multiple comparisons, respectively in before and after time points for the number of *MS* and *LB* colonies. ANCOVA was used to adjust the pH differences before the study and compare the mean pH values after the study among groups.

RESULTS

Based on the number of *MS* colonies in the saliva, the percentage of high growth before and after the use of mastic gums was as follows: pure mastic gum group 71.4% and 0, respectively, xylitol mastic gum group 85.7% and 7.1%, respectively and probiotic mastic gum group 64.3% and 0, respectively. The results of Wilcoxon Signed-Rank test indicated that in the three groups the amount of *MS* significantly decreased after using mastic gums ($P<0.001$ for all three).

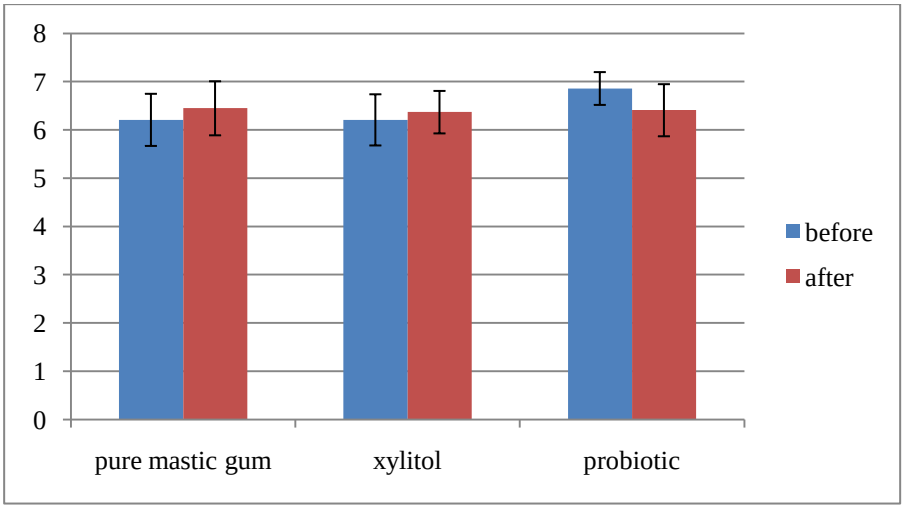

Fig 1. Saliva pH in the three groups

Based on the number of *LB* colonies in the saliva, the percentage of the high growth before and after the use of mastic gums was as follows: pure mastic gum group: 14.3% and 28.6%, respectively, xylitol mastic gum group: 7.1% and 14.3%, respectively and probiotic mastic gum group: 64.3% and 0, respectively.
The results of the paired-sample t-test comparing the amount of *LB* in the saliva showed that in both groups using pure mastic gum and xylitol mastic gum the amount of *LB* increased after chewing the gums but not significantly (P = 0.336 for pure mastic gum and P = 0.512 for xylitol mastic gum). Furthermore, the results of Wilcoxon Signed-Rank test showed that the level of *LB* of the saliva significantly decreased after chewing probiotic mastic gum (P<0.001).
The mean salivary pH before and after chewing the mastic gums was also recorded (Figure 1).
Paired-sample t-test revealed that in both groups using pure mastic gum and xylitol mastic gum no significant difference existed in the pH of saliva before and after chewing the gum (P = 0.286 for pure mastic gum and P = 0.423 for xylitol mastic gum).
The same test showed that the mean pH of the saliva decreased significantly after chewing probiotic mastic gum (P = 0.029).
The Kruskal-Wallis test showed that the amount of *MS* in the saliva before using the mastic gums was not significantly different among the three groups (P = 0.403). Moreover, the amount of *MS* in the three groups was not significantly different after chewing the mastic gums either (P = 0.779).
The Kruskal-Wallis test did not show a significant difference in the amount of *LB* among the three groups before using the mastic gums (P = 0.779) but the results of the test after the use of mastic gums demonstrated a significant difference among the three groups (P = 0.018). Pairwise comparisons of the groups after chewing gums, using Mann-Whitney test indicated that there was no significant difference in the amounts of *LB* in the saliva after chewing pure mastic gum or xylitol mastic gum (P=0.458) but the number of *LB* in the saliva showed a significant difference after chewing probiotic mastic gums compared to both pure mastic gum (P=0.009) and xylitol mastic gum (P=0.043).
One-way ANOVA showed a significant difference in the amount of pH of the saliva among the three groups before chewing mastic gums (P=0.001). Thus, comparison of salivary pH after the use of the three gums and adjusting for pH using ANCOVA indicated statistical differences among the groups (P=0.008).

Multiple comparisons showed that probiotic mastic gums were more effective on the pH of the saliva than xylitol mastic gums (P = 0.018), but the same comparison between probiotic mastic gums and pure mastic gums did not show a significant difference (P = 0.079). Moreover, the same test did not show a significant difference between the effect of xylitol mastic gum and pure mastic gum either (P = 0.849).

DISCUSSION

The growing interest in using natural products and the decreasing tendency towards the use of synthetic materials as well as the increasing resistance of pathogenic microorganisms have made researchers to look for alternative antimicrobial agents from different sources including medicinal plants [19,20]. The superiority of the mastic gum over the use of synthetic materials has been proven in many studies due to the presence of antimicrobial agents in mastic such as α-pinene and its effect on the prevention of dental caries [2]. Replacement therapy with probiotics is also utilized as a means of keeping the number of harmful cariogenic bacteria low. In the recent years, probiotic bacteria have been added to products including hard candies, pastilles, ice creams, sucking pills, dairy products and chewing gums in numerous studies. According to the findings of the current study, after three weeks use of pure mastic gum and xylitol mastic gum the *MS* count reduced significantly (P<0001, P<0.0001). The *LB* count showed an increase but not significantly (P= 0.336 and P= 0. 512), which can be attributed to the equilibrium of the total number of microorganisms due to the reduction of *MS*.

Aksoy et al. [6] demonstrated that in the first 15 minutes of chewing mastic gum, there was a reduction in the *MS* count but not in the amount of *LB*. After 135 minutes, the *MS* and *LB* counts dropped significantly, which is in line with the findings of this study, although Aksoy's study was a short-term, cross sectional study in which mastic gum was used only once for 15 minutes.

Holgerson et al [21], Soldering et al [22] and Rebelles et al [18] also reported reductions in the *MS* count with the use of xylitol gums, which is in line with the findings of the current study. However, none of the mentioned studies investigated the effect of xylitol on the amount of *LB.* Based on the results of our study, the amount of *MS* decreased significantly three weeks after chewing probiotic mastic gums (P<0.001), which is similar to the results of Cildir et al [23], Caglar et al [24] and Nace et al [25] indicating a drop in the amount of *MS* of the saliva after consumption of probiotic products.

According to the results of Montallo et al [26] and Ahola et al [27] the amount of *MS* of the saliva did not show a significant difference after consumption of products containing probiotics.

The difference between the results of these studies can be ascribed to different factors such as the duration of use of probiotics, the age of participants, the type of probiotics and the base product to which probiotics were added. The probiotic agents used were *Bifidobacterium* in Cildir's study, *Lactobacillus rhamnosus* in Ahola and Nace's studies and *Lactobacillus reuteri* in Caglar's study. In the current study, *Lactobacillus reuteri* was used as the probiotic agent, which is proven to be effective on the control of growth of *MS* in various studies [24, 28, 29].

According to the results of this study the number of *LB* decreased significantly after the use of probiotic mastic gums (P<0.001). Montallo et al. [26] reported a significant increase in the number of *LB* after the use of probiotic cheese. Caglar et al. [24] found no significant change in the number of *LB* after the use of probiotic gums. Cildir et al. [23] Investigated the effects of probiotic yoghurts and Caglar et al [30] used probiotic tablets and reported an insignificant decrease in the number of *LB* after the consumption of these probiotic products.

The difference between the results of the current study and the aforementioned studies may be due to the different types of bacteria used or the products to which the bacteria were added.

Furthermore, no other studies have investigated the use of probiotics and mastic gum so far and there is a possibility that the components of mastic itself have influenced the effect of probiotics on *LB* of the saliva but this calls for further research.

Based on the results of our study, pH of the saliva showed an insignificant increase after three weeks of using pure mastic gum and xylitol mastic gum.

Ribelles et al. [18] reported that pH of the saliva increased after a short-term use of xylitol gums. The majority of the studies on the effects of xylitol-containing gums on pH of the saliva have been short term and concerned with the immediate effects after chewing the gum once while the current study measured the pH of saliva after using the gum for three weeks.

According to the findings of the current study, consumption of probiotic mastic gums caused a significant drop in the pH of the saliva (P=0.029). No such research has been conducted on the effects of probiotics on the saliva so far. Considering the type of bacteria used, increasing the acidity of the saliva is intelligible due to the byproducts of the metabolism of *LB.*

All in all, it seems that the effects of pure mastic gum and xylitol mastic gum are similar on the number of *MS, LB* and pH of the saliva. Adding xylitol to mastic gum softens it and improves the taste, which might be appealing to the customers.

Although the reduction in salivary pH in the case of probiotic mastic gum creates some doubts on the beneficial effects of this product for the oral cavity, further research is required to determine the effects of probiotics on other aspects of oral health such as the flow, viscosity and the buffering properties of the saliva.

CONCLUSION

The most important results of this study include:

1) Three weeks use of the three mastic gums resulted in a significant drop in the number of *MS.*

2) During three weeks, the amount of *LB* of the saliva did not increase significantly in the groups using pure mastic gum and xylitol mastic gum but it showed a significant decrease in the group using probiotic mastic gum.

3) After three weeks, the pH of the saliva did not increase significantly in the groups using pure mastic gum and xylitol mastic gum but decreased significantly in the group using probiotic mastic gum.

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