Original Article

Comparison of Changes in Salivary pH after Chewing Guava Leaves (*Psidium Guajava*) and Xylitol Gum: A Randomized, Single-Blind, Crossover Study

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Abstract

Background: The medicinal benefits of guava have been discussed in many ethnopharmacological studies. Extract from the guava leaves is known for its spasmolytic, antioxidant, antimicrobial, anti-inflammatory, and antibacterial properties. Its effects on saliva have been least explored. The study aimed at evaluating the effect of chewing guava leaves and xylitol gum on salivary pH. **Materials and Methods:** The study design was a randomized, single-blind, crossover type. Forty-five volunteers were chosen based on selection criteria for the study. The participants were asked to chew guava leaf and sugar free xylitol chewing gum for about 90 s and the salivary pH was assessed. **Results:** There was no statistically significant difference in pH on comparing the two groups. pH comparisons between different time intervals showed significant differences in both groups (P = 0.001). *Post hoc* comparisons of pH after chewing guava leaves showed significant differences between different time intervals (P = 0.001) except between 30 and 60 min (P = 0.377). *Post hoc* comparisons in the xylitol group showed significant differences between different time intervals (P = 0.001) except between baseline and 30 min (P = 0.071) and between 30 and 60 min (P = 0.83). **Conclusion:** Chewing guava leaves showed a similar effect and had a better short-term effect when compared to xylitol chewing gum at different time intervals.

Keywords: Chewing gum, pH, Psidium, saliva, xylitol

Introduction

Saliva plays an important role in maintenance of good oral health. It prevents bacterial invasion, growth, and metabolism through various mechanisms. The constant salivary flow is one such mechanism that can efficiently dilute and eliminate the products of bacterial metabolism within the oral cavity. Saliva also has buffering capacity; the pH of saliva ranging between 6.2 and 7.6, which neutralizes acids in the mouth.[1] Ingestion of carbohydrate-rich foods such as breads, pastas, animal proteins, candies, and sodas enhances bacterial glycolysis, thereby inducing demineralization of tooth enamel. This demineralization phase is followed by a period of alkalinization that restores the integrity of enamel by remineralization.[2] Restoration of neutral pH occurs 30 min after food consumption.[3] Hence, prolonged maintenance of low pH values due to frequent consumption of carbohydrate-rich foods is believed to cause caries.

A number of methods have been studied to neutralize the pH immediately after food consumption. Chewing gums have been known to act as gustatory and mechanical stimuli increasing salivary flow and also elevating salivary pH, thereby reducing the risk for dental caries. Distinctively, the salivary pH remains elevated for 15–20 min.[4]

The medicinal benefits of guava have been discussed in many ethnopharmacological studies.[5] Extract from the guava leaves is known for its spasmolytic, antioxidant, antimicrobial,

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anti-inflammatory, and antibacterial properties.[6,7] Paste of guava leaves has been used in the past for maintenance of oral hygiene, to treat bleeding gums and bad breath.[8] Recent *in vitro* studies have showed antibacterial activity of guava leaves against *Streptococcus mutans*.[8-12]

The aim of the present study was to assess and compare the salivary pH changes after chewing guava leaves and xylitol chewing gum.

Materials and Methods

The research was conducted in full accordance with the World Medical Association Declaration of Helsinki. The study protocol was approved by the institution's ethics committee. The study was conducted during the months of November and December 2018.

Study participants

Participants were chosen from the institution (ages between 19 and 24) by a convenience sampling method. Only those who voluntarily agreed to participate after having understood the purpose of the study were included. It was also made clear to the participants that it was acceptable if they decided to withdraw at any time during the process. Sample size was based on estimates from previous literature. Among 80 volunteers, forty-five were chosen based on selection criteria. Inclusion criteria: Participants free from dental caries decayed, missing, and filled teeth score = 0; free from gingival and periodontal diseases (clinically examined); provided written consent to participate. Exclusion criteria: Under orthodontic treatment; suffering from any systemic illnesses. Participants were standardized for age, dietary habits, and oral hygiene practices.

Collection of salivary samples and pH determination

Saliva collection was done between 9 a.m. and 12 p.m. Procedure for collecting saliva was as suggested by Navazesh *et al.*[13] Participants were asked to avoid rinsing the mouth and food intake 1 h before commencement of the study. The subjects were asked to relax and sit comfortably in a dental chair. Saliva samples were collected in sterile test tubes. One milliliter of saliva was collected at baseline and during each follow-up. Salivary pH was estimated within 5 min of collecting samples using litmus test strips (Bexco pH test indicator). pH estimation was done by a single examiner blinded from the intervention being used by the participant. The test strips were dipped in the saliva collected for about 2–5 s. pH estimates were determined by comparing the color change of litmus strips over a gradient scale.

Intervention

Healthy guava leaves (without signs of necrosis and color changes) and a commercially available xylitol chewing gum (Happydent White Xylit chewing gum manufactured by Perfetti Van Melle, Italy) were used. Intervention (Group 1: Guava leaves or Group 2: Xylitol chewing gum) to be used during the first phase was chosen by lottery method. Xylitol chewing gum (1 gum weighing about 1.1 g) or guava leaves (weighed to about 1.1 g) were chewed for about 90 s. Stimulated saliva samples were collected (a) immediately after chewing, (b) after 15 min of chewing, (c) after 30 min of chewing, and (d) after 60 min of chewing and pH was estimated. The study was a single-blinded crossover trial. 28 participants were allocated to Group 1 (guava leaves) during the first phase of pH assessment and then allocated to Group 2 (xylitol gum) after a washout period of 1 week. Likewise, 17 participants were initially allocated to Group 2 (xylitol gum) and then to Group 1 (guava leaves) after a period of 1 week. This ensured blinding of the researcher involved in pH estimation [Figure 1].

Data analysis

The data were analyzed using SPSS (Version 22.0; SPSS Inc., Chicago, IL, USA). The data showed near-normal distribution and therefore parametric tests were used. Comparisons at different time intervals were done using repeated measures ANOVA and *post hoc* tests. Comparisons between the different interventions were done using independent samples *t*-test. Statistical significance was fixed at $P \leq 0.05$.

Results

A total of 45 volunteers participated in the study. All 45 participants were followed up till the end of the study and hence there were no dropouts. All participants showed almost similar pH at baseline (mean = 6.89; standard deviation [SD] = 0.57). Salivary pH immediately after chewing guava leaves was 8.36 (SD = 0.68), after 30 min was 7.20 (SD = 0.69), and after 60 min was 7.11 (SD = 0.61). Salivary pH immediately after chewing xylitol gum was 8.24 (SD = 0.48), after 30 min was 7.09 (SD = 0.67), and after 60 min was 7.11 (SD = 0.44). There was no statistically significant difference in pH between the two groups at different time intervals [Table 1 and Figure 2].

In our study, pH comparisons between different time intervals showed significant differences in both groups (after chewing guava leaves and xylitol gum) [Table 2]. *Post hoc* comparisons of pH after chewing guava leaves showed significant differences between different time intervals except between 30 and 60 min. *Post hoc* comparisons in the xylitol group showed significant differences in all except 2 time intervals (between baseline and 30 min; between 30 and 60 min) [Table 3]. The results indicate that chewing guava leaves had better short-term effect (baseline to 30 min) than that of xylitol chewing gum.

Discussion

Salivary pH reductions are observed immediately after consuming foods. It reduces rapidly in approximately 5–10 min followed by a gradual recovery taking over 30–60 min. Acidic pH is of major concern to oral health owing to demineralization of tooth structure, thereby leading to dental caries. The pH is maintained by two mechanisms namely through salivary flow and buffering activity.[14] Chewing gum stimulates salivary flow enhancing protection by neutralizing pH. This

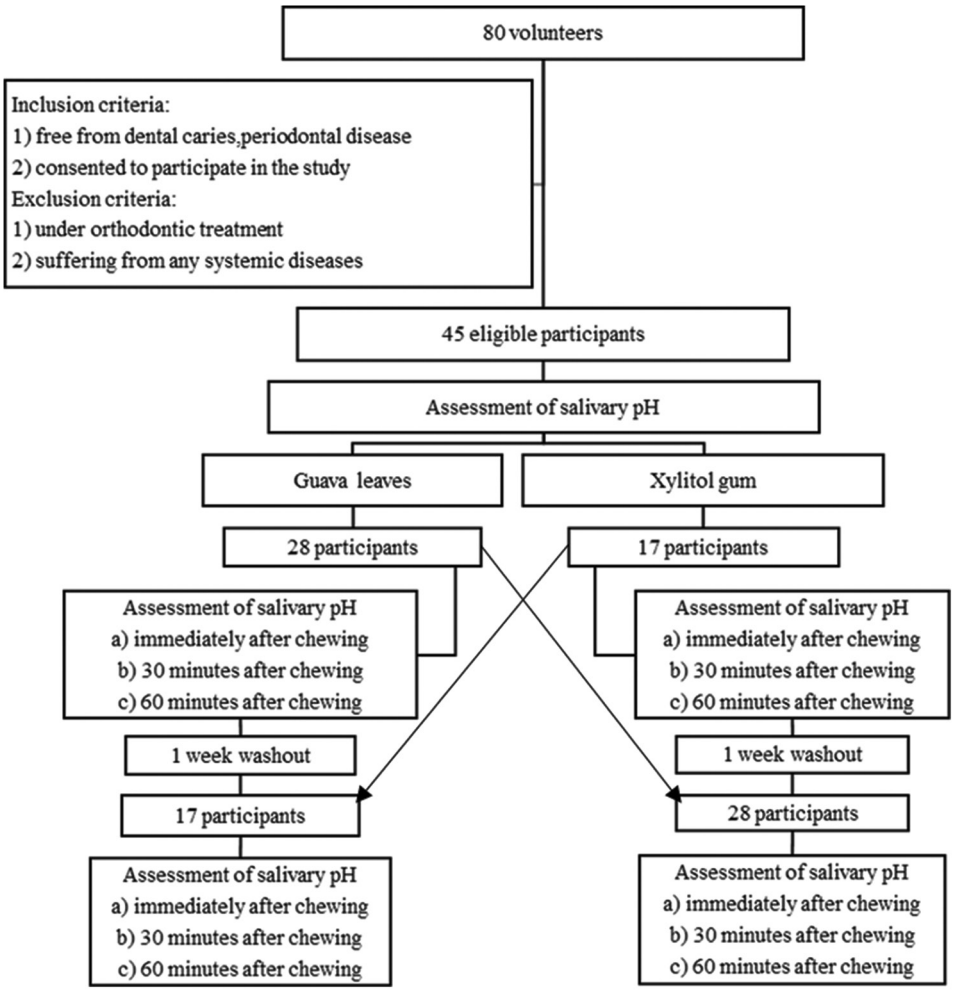

Figure 1: Algorithm of study design

Table 1: Comparison of salivary pH change after chewing guava leaf and xylitol chewing gum at different time intervals

Time intervals	Group	*n*	Mean	SD	*t*	*P*
Immediately after chewing	Guava leaf	45	8.36	0.68	1.40	0.17
	Xylitol chewing gum	45	8.24	0.48		
30 min after chewing	Guava leaf	45	7.20	0.69	0.96	0.34
	Xylitol chewing gum	45	7.09	0.67		
60 min after chewing	Guava leaf	45	7.11	0.61	0	1
	Xylitol chewing gum	45	7.11	0.44		

SD – Standard deviation

Table 2: Comparison of salivary pH change within groups at different time intervals

Groups	Time intervals	Mean	SD	*F*	*P*
Guava leaf	At baseline	6.89	0.57	100.25	0.001*
	Immediately after chewing	8.36	0.68		
	30 min after chewing	7.20	0.69		
	60 min after chewing	7.11	0.61		
Xylitol chewing gum	At baseline	6.89	0.57	76.70	0.001*
	Immediately after chewing	8.24	0.48		
	30 min after chewing	7.09	0.67		
	60 min after chewing	7.11	0.44		

*Statistically significant. SD – Standard deviation

Table 3: *Post hoc* comparison within groups using Bonferroni correction

Groups	Pairwise comparison	Mean difference	95% CI (lower bound-upper bound)	*P*
Guavaleaf	Baseline versus immediate	−1.47	−1.64 - −1.29	0.001*
	Baseline versus 30 min	−0.31	−0.45 - −0.17	0.001*
	Baseline versus 60 min	−0.22	−0.40 - −0.04	0.017*
	Immediate versus 30 min	1.16	0.94 - 1.37	0.001*
	Immediate versus 60 min	1.24	1.04 - 1.45	0.001*
	30 min versus 60 min	0.09	−0.11 - 0.29	0.377
Xylitol chewing gum	Baseline versus immediate	−1.36	−1.51 - −1.20	0.001*
	Baseline versus 30 min	−0.20	−0.42 - 0.02	0.071
	Baseline versus 60 min	−0.22	−0.41 - −0.03	0.024*
	Immediate versus 30 min	1.16	0.93 - 1.39	0.001*
	Immediate versus 60 min	1.13	0.95 - 1.32	0.001*
	30 min versus 60 min	−0.02	−0.23 - 0.19	0.83

*Statistically significant. CI – Confidence interval

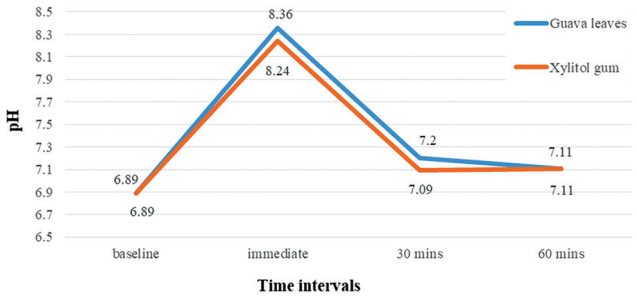

Figure 2: Change in salivary pH at different time intervals after chewing guava leaves and xylitol chewing gum pH

suggests beneficial effects of chewing on oral health. Certain ingredients added to the gums provide additional benefits besides increasing salivary flow. This has constituted to in-depth research on a number of compounds such as fluorides, carbamides, bicarbonates, and polyols, that when incorporated in chewing gums could prevent tooth demineralization by neutralizing pH.[15] Our study attempted to compare such changes in salivary pH after chewing xylitol gum and guava leaves.

Salivary pH increased markedly after chewing guava leaves and xylitol chewing gum. Various studies have been conducted earlier on oral effects of xylitol. It is known to have anticariogenic properties. It acts on *S. mutans* in the oral cavity and gets metabolized to xylitol 5-phosphate which cannot be further utilized by bacteria. The levels of *S. mutans* on tooth surfaces are lowered during long term habitual consumption of xylitol.[16,17] Salivary pH after chewing xylitol chewing gum shows alkalinity immediately and also for 30 min and it begins to drop after that and become neutral after 60 min. Similar changes in pH was observed among children in a study conducted by Kumar *et al.* (2013) and Hegde *et al.* (2017).[18,19]

Guava is well known for its medicinal properties. The guava leaves contains active ingredients such as flavonoids, guavavolic acid, guavanoic acid, guajadial, guaijaveri, and other active components that have significant antibacterial, antioxidant, anti-inflammatory, antiplaque, and anticarcinogenic properties.[5] A flavonoid guaijaverin obtained from guava leaves is found to have potential effect against *S. mutans*.[20-22] pH changes after using guava extracts have not been assessed till date. In our study, the salivary pH after chewing guava leaves showed significant rise that is maintained for about 30 min and then dropped after 60 min.

Comparison of salivary pH changes after chewing xylitol gum and guava leaves showed no significant differences at different time intervals. Chewing guava leaves showed slightly more alkalinity immediately and after 30 min as compared to that of xylitol.

Strengths and limitations

Although sufficient literature is available on the various medicinal properties of guava leaves, salivary pH changes have not been assessed till date. Furthermore, comparisons with xylitol gums have not been made. The study had a crossover pattern and hence the influence of confounding covariates is reduced. The participants were also standardized for dietary habits and oral hygiene practices before commencement of the study. The study did not use a negative control, considering the fact that salivary pH changes by mere stimulation, which may be considered a limitation. Although method of estimation of pH was subjective, a single-blinded examiner conducted the procedure. The study did not assess other physiochemical properties of saliva such as flow rate and buffering capacity. Furthermore, the mechanism of action of individual constituents of guava leaves in bringing about such changes was not studied, which provides scope for further research.

Conclusion

Chewing guava leaves showed a similar effect when compared to xylitol chewing gum at different time intervals. Chewing guava leaves also showed a better short-term effect at baseline and 30 min than xylitol.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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