Original Article

Persica Chewing Gum Effects on Saliva Fluoride Concentration and Flow Rate: A Triple-Blind Randomized Clinical Trial

Abstract

Context: Dental caries is a prevalent disease worldwide. *Salvadora persica* or Miswak could be a source of fluoride and has caries preventive effects. **Aims:** The aim of this study was to compare saliva fluoride concentration and flow rate after using Persica and sodium fluoride chewing gums. **Settings and Design:** In a triple-blind crossover randomized trial, 44 healthy volunteers (21–25 year old) were recruited according to the inclusion criteria and were randomly allocated into two groups (22 each). **Participants and Methods:** This study was performed within two sets of trial with a 10-day washout period. Participants (subjects) were followed a running period and matched for trial confounders. Saliva samples were collected under controlled conditions at similar time (11 am) within baseline, 5, 10, 20, and 45 min intervals preceded by 5-min chewing of Persica or sodium fluoride chewing gum. Saliva samples were analyzed for fluoride ion using hexamethyldisiloxane diffusion method. **Statistical Analysis Used:** Saliva flow rate (ml/min) and mean fluoride concentration (ppm) during different time periods in two types of gum were compared using ANOVA-repeated measures ($P < 0.05$). **Results:** The difference in total means of stimulated saliva flow rates between two Persica and sodium fluoride gum intervention groups was statistically significant ($P = 0.048$); however, difference of fluoride concentrations was not statistically significant ($P = 0.244$). **Conclusions:** Chewing Persica containing gum released fluoride ions in saliva and increased saliva flow rate comparable with sodium fluoride chewing gum use as a gold standard.

Keywords: *Chewing gum, dental caries, fluorides, oral health, saliva, Salvadora persica L.*

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Introduction

Dental caries is still one of the main global public health issues. Different fluoride compounds and products have been extensively used as an effective factor in dental caries control and prevention.[1-3] Strong evidence have proven the efficacy of frequent use of home care fluoride agents in dental caries control.[4] Continuous presence of low fluoride ion concentrations in saliva with other salivary favorable qualitative and quantitative factors increases the chance of enamel remineralization and reduces the risk of demineralization.[5,6]

Using chewing gum is an accepted and pleasurable habit among all age groups. Saliva flow rate will increase with the use of chewing gum. Teeth surface access to sufficient saliva which has been disregarded during brushing has a crucial role in oral health stability. Hence, using chewing gums could be considered as a supplementary measure to toothbrushing action. Chewing gums have been increasingly accepted as an oral care product.[7]

Synergic effects on dental and periodontal health can be achieved by incorporating some minerals and/or organic agents to chewing gums. Fluoride-containing chewing gums increase saliva secretion while speed up dental plaque pH elevation.[8] Using this type of chewing gums results in higher saliva calcium and phosphate concentrations and reinforces enamel remineralization. *Salvadora persica* is a type shrub that from the old days because of the fiber texture of its roots and stems has been used as a tool for cleaning teeth. In different Middle Eastern languages, the local name (Miswak) of this plant is being used to call contemporary toothbrushes.[9] In addition to the physical qualities, *S. persica* has peculiar chemical characteristics. These characteristics make this plant able to inhibit periodontal pathogenic and cariogenic bacterial growth and acid production.[10] *S. persica* has also antifungal effects[11,12] and has been used

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widely as an established periodontal therapeutic agent mainly in the form of mouth rinse.[13]

Silica is one of the chemical ingredients of *S. persica*.[14] There are some reports about Persica extracts fluoride concentrations.[14-16] However, similar to other organic materials, fluoride is in the compound forms and in the oral environment may not be in the form of bioavailable ions. Therefore, theoretically, if *S. persica* is added to chewing gum, both the released fluoride from Persica and the mechanical effects of chewing gum can enhance the remineralization process and clean the tooth surfaces. This hypothesis has been assessed in an *in vitro* study by Aslani *et al*. The organoleptic, physicochemical, and mechanical characteristics of the *S. persica* chewing gum were evaluated. They concluded that *S. persica* can be formulated in the form of medicinal gum to deliver fluoride to the mouth.[17] Here, any clinical trial quantifying the fluoride release and its durability of fluoride in saliva derived from Persica could be helpful. The aim of this study was to measure saliva fluoride concentration (primary outcome) and flow rate (secondary outcome) at consecutive periods of time, following chewing gums containing Persica extract or sodium fluoride in adults.

Participants and Methods

Ethics

The present clinical trial was registered at IRCT (IRCT2012091810872n1) and was approved by the Ethic Committee code 391307.

Participants

During October 2012, all students who had attended pediatric dentistry clinic were invited to participate in this triple-blind randomized clinical, crossover study. An invitation letter provided to the students with their daily handout files. At the beginning, 84 students volunteered to participate in the study. Among them, finally, 44 students (15 men, 29 women) were eligible or consent to participate [Figure 1]. Exclusion criteria were the presence of any systemic or oral disease during the study phases, using medications/Persica mouthwash, recent systemic/topical fluoride (except for toothpaste), excessive tea drinking, and smoking habit.

After providing a verbal explanation about the trial, the volunteers were requested to sign informed written consent.

Participants were instructed to refrain from drinking tea (as a possible source of fluoride) and using oral care products containing fluoride. To maintain their oral hygiene habits during all the steps of the study, all the participants were provided by a toothpaste containing 1450 ppm fluoride (Crest, Procter and Gamble, UK).

At the morning of the intervention, participants refrained from using toothpaste and eating or drinking (except for water) during the last 3 h before the trial.

Participants were randomly assigned into two intervention groups using random numbers by a practitioner with no clinical and laboratorial interest. Participants, data analyst, and laboratory technician were blinded during all the steps about the type of chewing gum used.

Clinical procedures

This study was designed with a running period followed by two crossover intervention phases, while participants had a 10-day washout between the two trials. Saliva sample collection and participant's (subject's) instructions and other clinical affairs were carried out by one practitioner who had no interference in other parts of this study. Saliva sampling was performed for all participants (subjects) at 11 am in preweighed, precoded plastic tubes. Saliva samples were collected at baseline (before chewing the gums) and 5, 10, 20, 4, and 45 miuntes after 5 minutes of chewing the gums. During the saliva sample collection, participants remained seated in a calm and comfortable position and were not allowed to eat food or drink. Then, saliva samples were immediately sent to the fluoride laboratory. Tubes were reweighed; aliquots were taken, coded, and stored at −18°C until fluoride analysis. Each saliva sample had labels with participants' code, sample collection date, and time of collection.

Salivary flow rate calculation

$$\text{Flow rate}\left(\text{ml}/\text{min}\right) = \frac{\text{Volume of stimulated saliva*}}{\text{Time interval of saliva collection}}$$

* Final weight − initial weight

Then, the total means of salivary flow rate calculated for each participant and group.

Chewing gum characteristics

Both *S. persica* and sodium fluoride chewing gums produced at pharmaceutics' laboratory. All gum sticks had the same color, shape, size, and taste and both contained the same concentration of fluoride per weight of gum.

Each chewing gum contained 0.01 ppm fluoride/stick (diffusion method fluoride analyses).[17]

Fluoride analysis in saliva samples

After defrosting and homogenizing, 1 ml of saliva sample was mixed with 2 ml of $DDiH_2O$ in a plastic petri dish with a hole in the lid. About 50 µl of 0.05 N, NaOH was used as F^- trap in 3–5 drops inside the surface of the lid. About 1 ml of 3N hexamethyldisiloxane saturated with H_2SO_4 pipetted through the hole. Petri dish lid was sealed using one layer Parafilm. After 16–24 h diffusion in the room temperature, 25 µl acetic acid 0.1 N was added to buffer the solution. Fluoride concentration of drops was measured using a combination fluoride ion-selective electrode while directly placed on the collected drops and buffer solution. Orion #96-909-00 and an Orion 720A+ advanced ISE/pH/mv/ORP

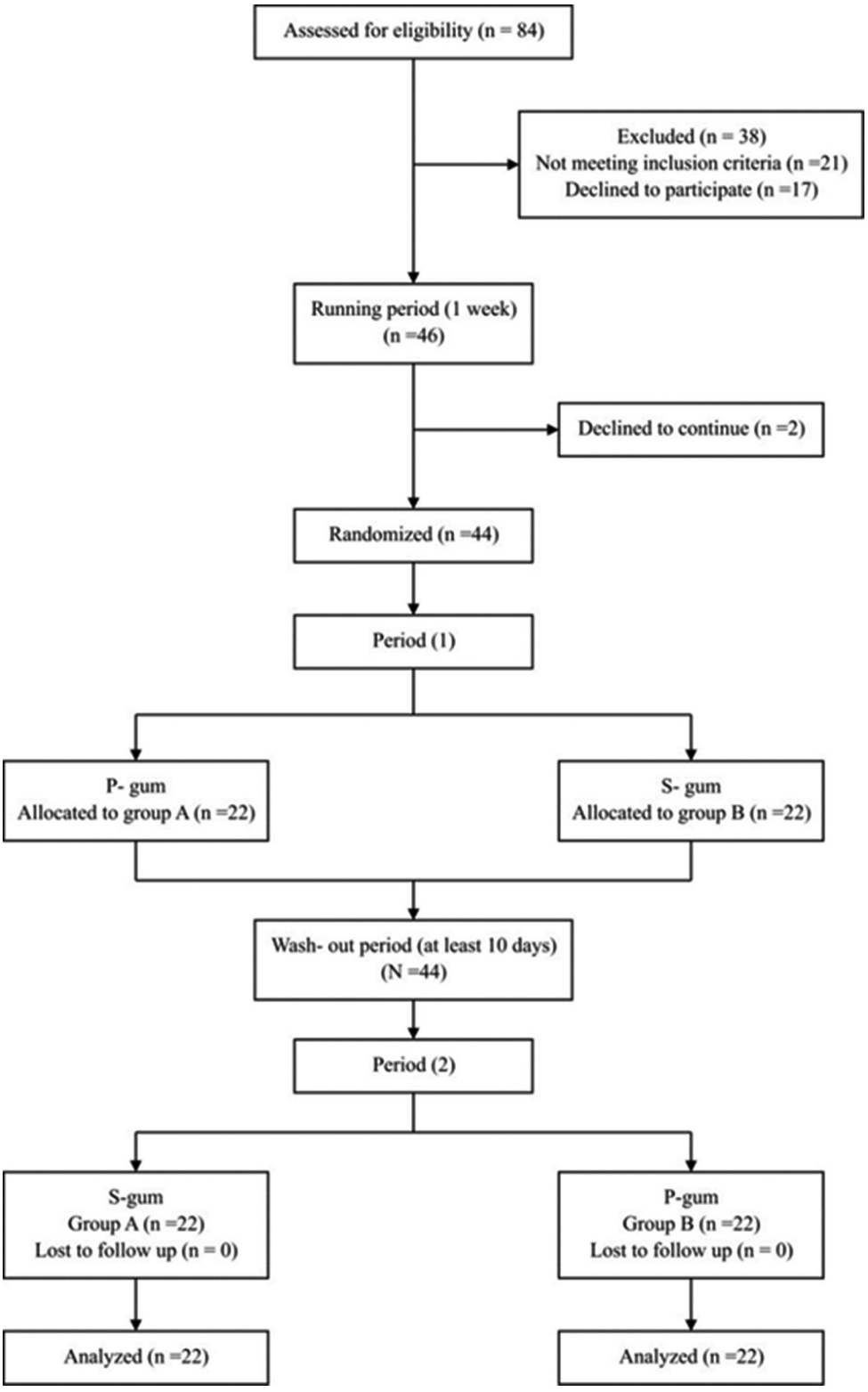

Figure 1: Diagram of the study design

(Thermo electron corporation, USA)[18] 0.01, 0.02, 0.04, and 0.1 ppm fluoride standards were used to plot the calibration curve. Each saliva sample was analyzed for fluoride in three times. The average of measured fluoride concentrations (with <30% variation) was considered as the final fluoride concentration. The trueness of fluoride analyses was estimated by percentages of recovered fluoride standards, using similar analyzing method for saliva samples. The recovery test was performed for 10% of samples with 100 ± 2% recovery for trueness.[19]

Statistical analysis

ANOVA repeated measures were used to assess the validity of crossover study design by comparing saliva flow rate means and fluoride concentration after the 10-day washout period (carryover effect). ANOVA test was used to compare mean saliva flow rate and mean fluoride concentration in different time intervals of both intragroup and intergroup in Persica and sodium fluoride chewing gums. A one-side statistical significance level of 5% and a power of 80% were selected. Statistical analysis was performed using the Statistical Package for the Social Sciences version 18.0 (SPSS Inc., Chicago IL, USA). $P < 0.05$ was considered as a significant difference between the groups.

Results

All the 44 participants who were randomly allocated to two groups completed all the study phases [Figure 1].

Carryover effect analyses showed that there was no significant difference between crossover groups ($P > 0.05$) [Tables 1 and 2]. Hence, participants after 10 days were matched for second Persica or sodium fluoride intervention.

The repeated measures showed that the difference in total means of stimulated salivary flow rates at different time intervals was significant between groups ($P = 0.048$) [Table 1].

Salivary fluoride concentration

There was no significant difference between saliva fluoride concentrations in two groups of Persica and sodium fluoride chewing gums ($P = 0.244$).

Salivary fluoride concentration peaked during first 5 min after chewing both types of gums and decreased rapidly afterward but was still higher than base levels after 45 min [Table 2].

Saliva fluoride concentration after different intervals of using two types of gum followed a similar pattern.

Discussion

Surprisingly, the amount of fluoride released in saliva after chewing Persica-containing gum was statistically comparable with fluoride release by sodium fluoride chewing gum as a gold standard. We found that fluoride present in Persica extract could be ionized in the mouth environment in comparable amounts with sodium fluoride. Under favorable conditions, sodium fluoride as a soluble fluoride compound could completely be hydrolyzed into fluoride ion.

Bruun *et al*.[20] showed that chewing only 0.5 mg fluoride in one stick of sodium fluoride can keep high levels of fluoride in the mouth for at least 60 min. In our study, amounts of fluoride in saliva were still slightly higher at 45 min than base in both types of gum. Based on Sjögren *et al*. study,[21] the highest amounts of fluoride in saliva were between 5 and 10 min following using fluoridated chewing gum and then decreased with time in the subsequent periods. Our observation supports the latter study results. In the *in vitro* study of the synthetic Persica chewing gum (with the similar Persica gum and under simulated mouth conditions), fluoride ion release in artificial saliva increased sharply in first 5 min and a steady increase was observed for 50 min of the study.[17] Different patterns of saliva fluoride concentrations were observed between *in vivo* and *in vitro* studies. It seems that dynamic saliva secretion and circulation in the mouth could be the cause of this difference.

Among the literature, we did not find any research about fluoride release from Persica as an ingredient of chewing gum. Chemical caries preventive effects of Persica mostly were attributed to antibacterial effects of *S. persica*. The organic nature of *S. persica* with complex compounds needs more precise laboratory pretreatment and fluoride analysis methods. While saliva innately has several compounds that interfere with the ionization of fluoride, we used the recommended fluoride analysis method for organic base complex samples. We followed the fluoride analysis trueness steps, including fluoride recovery tests (for 10% of samples). Other trial cofounders were eliminated by the trial design including the washout period and the running period for all the participants before the trial. However, more *in vivo* fluoride-releasing studies are needed to justify Persica as a constant source of fluoride in the mouth. These

Table 1: Crossover effect of estimated salivary flow rate means between two groups

Cross-over	Period (1) (mean±SD)					Wash out period	Period (2) (mean±SD)				
	Base	T5’	T10’	T20’	T45’		Base	T5’	T10’	T20’	T45’
P-gum	0.58±0.32	0.73±0.39	0.80±0.41	0.81±0.46	0.90±0.41	10 days	0.91±0.44	0.83±0.38	0.89±0.40	0.90±0.35	0.89±0.33
S-gum	0.88±0.68	0.90±0.38	0.85±0.31	0.95±0.42	0.91±0.44		0.98±0.52	0.88±0.31	0.88±0.31	0.87±0.44	0.94±0.38
Period effect‡ (*P*)						0.119					
Carry-over effect‡ (*P*)						0.41					
Main effect* (*P*)						0.048					

‡ANOVA repeated measure test was used to check period and carryover effects, *Statistical difference within the two P-gum and S-gum intervention groups using repeated measure test. SD: Standard deviation; P-gum: Persica chewing gums; S-gum: Sodium fluoride chewing gums

Table 2: Crossover effect of estimated fluoride means between two groups

Cross-over	Period (1) (mean±SD)					Wash out period	Period (2) (mean±SD)				
	Base	T5'	T10'	T20'	T45'		Base	T5'	T10'	T20'	T45'
P-gum	0.041±0.013	0.103±0.142	0.056±0.026	0.05±0.017	0.049±0.014	10 days	0.036±0.014	0.06±0.023	0.055±0.015	0.043±0.021	0.035±0.017
S-gum	0.038±0.011	0.067±0.028	0.052±0.015	0.047±0.014	0.049±0.024		0.04±0.014	0.059±0.021	0.072±0.087	0.050±0.016	0.043±0.012
Period effect‡ (*P*)						0.082					
Carryover effect‡ (*P*)						0.703					
Main effect* (*P*)						0.244					

‡ANOVA-repeated measure test was used to check period and carryover effect, *Statistical difference within two P-gum and S-gum intervention groups using repeated measure test. SD: Standard deviation; P-gum: Persica chewing gums; S-gum: Sodium fluoride chewing gums

studies are especially necessary for children and with other Persica products.

Chewing sugarless gums result in increasing salivary flow rates significantly at short term and its long-term effects show caries preventive impacts.[22] In our study, sodium fluoride gum comparing to Persica chewing gum had better effect on stimulated salivary flow rate. The effect of chewing both types of gums on saliva flow rate was dropped to the baseline levels after 45 min. Flavored and sweetened gums can modify saliva discharge rate and stimulate saliva secretion sufficiently from the salivary glands.[23,24] Studies have shown that Persica extract has a considerable effect on parotid salivary flow rate. This effect has been attributed to its strong taste.[25] In our study, sodium fluoride had more influence on the stimulation of saliva comparing to Persica gum. This observation might be due to similar taste and consistency of sodium fluoride and Persica gums and individual differences between saliva collection steps among the participants.

Suyama *et al*.[26] concluded that 50 μg fluoride-containing chewing gum resulted in higher level of remineralization and subsequent more acid-resistant enamel and recommended consistent use of fluoride chewing gum to prevent dental caries. One of the side effects of fluoride products is dental fluorosis. Hattab *et al*.[27] showed slight increase in fluoride plasma levels following using a fluoride chewing gum and considered the gum as a safe vehicle for fluoride with the least adverse effects. For ethical purposes, this study was performed in adults since using fluoride-containing products during the active eruption periods in children need special care.

The participants (subjects) selection with regard to inclusion criteria, especially drinking tea (frequent tea drinking habit is very common in the studied community), participants cooperation to comply with investigator's request until the end of the study procedures, and lack of any nonfluoridated toothpaste in the market (we gave the participants subjects the same toothpaste with predetermined fluoride concentration), were our study limitations. Releasing fluoride ion from saliva and Persica compounds needed meticulous methods and precision.

Conclusions

Chewing Persica-containing gum released fluoride ions in saliva and increased saliva flow rate comparable with sodium fluoride chewing gum use as a gold standard.

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Conflicts of interest

There are no conflicts of interest.

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