Original Article
Journal of Korean Society of Dental Hygiene 2016; 16(3): 463-9
http://dx.doi.org/10.13065/jksdh.2016.16.03.463
ISSN 2287-1705(Print)
ISSN 2288-2294(Online)
JKSDH 한국치위생학회

Effect of *Prunus mume* extract(PME)-containing chewing gum mastication on the change of saliva ingredients

Jong-Hwa Jang · Young-Soo Lee[1]

Department of Dental Hygien Science, Hanseo University · [1]Department of Dental Hygien Science, Sunmoon University

***Corresponding Author: Jong-Hwa Jang,** Dept. of Dental Hygiene Science, Hanseo University, 46 Hanseo 1-ro, Haemi-myun, Seosan, Chungcheongnam-do, 356-706, Korea, Tel: +82-41-660-1574, Fax: +82-41-660-1579, E-mail: jhjang@hanseo.ac.kr
Received: 16 May 2016; Revised: 14 June 2016; Accepted: 15 June 2016

ABSTRACT

Objectives: The purpose of the study is to investigate the effect of chewing gum containing *Prunus mume* extract(PME) on the change of saliva ingredients. On the basis of the biological background of molecules and diagnostic indices in the use of saliva, the mastication effect of chewing gum containing PME was demonstrated in terms of secretory IgA concentration and total protein concentration in stimulated saliva.

Methods: This study is an experimental research on the use of a research design before and after applying a randomized control group. Participants were distributed randomly to the experimental group and the control group, respectively. The experiment group was instructed to masticate the chewing gum containing PME for 10 minutes for one month after each meal within 30 minutes. Salivary secretion was collected by the participants between 8 and 10 a.m in the morning in the research office. For the measurement of secretory IgA and total protein concentrations in the saliva, indirect enzyme-linked immunosorbent assay(ELISA) was used.

Results: The salivation stimulation rate was significantly increased after four weeks of masticating chewing gum containing PME after each meal($p<0.001$). Mastication of chewing gum containing PME for four weeks decreased the concentration of secretory IgA much more significantly than that after mastication for one week($p=0.003$). The concentration of total protein in the saliva was decreased after four weeks in the experimental and control groups.

Conclusions: Mastication of chewing gum containing PME stimulated salivary secretion and led to oral disease prevention in patients with xerostomia. Furthermore, it seems to be urgent to seek measures that can be utilized in intervention for patients with xerostomia.

Key Words: saliva, *Prunus mume extract*, chewing gum, total protein, Secretory IgA(SlgA)

Introduction

Saliva plays an important role in maintaining a constant oral cavity milieu[1]. Saliva performs biological functions including lubrication, cleaning process, protection of the mucous membrane of mouth, anti-bacterial function, re-calcification of teeth, and digestive process. Saliva also protects the oral cavity by means of secretion of protein and immunoglobulin, pH maintenance, and acid-base balance in body fluids[1,2].

Salivary secretion consists of water and electrolytes including sodium, potassium, calcium, chloride, and phosphate. The electrolytes play an important role in oral health and immune reaction against bacterial metabolites from the food[3,4]. Body fluids is a general term for all fluids flowing within the human body and it represents the plasma fraction of blood. Body fluids mainly consist of water and show osmotic pressure depending on adjusting the ion and electrolyte concentration, and contribute to transport of oxygen, nutrition and bodily waste, and to an even distribution of body heat[5]. Most of the disease burdenprimarily arises

from respiratory infectious diseases[5]. Therefore, as attention to prevention and treatment of various oral diseases for promoting and maintaining oral health has increased, attention to naturally occurring materials that have an excellent effect of anti-bacterial function with less side effects is also increasing[6].

Prunus mume is a fruit tree that belongs to cherry genus of the rose family and its origin is known to be in the Tstuan Province and mountainous region of the Hubei Province of China, being significantly distributed in Southeastern areas like Korea, China and Japan, and especially, a report suggested that *Prunus mume* cultivated in Korea has an excellent antioxidative activity[7-9] and anti-bacterial properties[10,11]. Organic acid is the main ingredient in Japanese apricot and has strong antibacterial activity that leads to sterilization, detoxification, deduction, purification of the intestine, liver function activation, and prevention of acidification. Organic acid facilitates gastric juice and saliva secretion[12]. As mentioned above, Japanese apricot is known to have various types of effects within the human body, but a study on the mechanism that can reveal the anti-bacterial process and immunity reaction within the mouth is needed.

The purpose of the study is to investigate the amount of salivary secretion by measuring secretory IgA and total protein amount using chewing gum containing PME. Through this study, we will suggest the use of Japanese apricot extracts as a preventive treatment tool for maintaining dental hygiene.

Materials and Methods

1. Study design

This study is an analogue experiment before and after application of a random comparison group. The design of the study is as follows<Table 1>.

2. Subjects

The subjects comprised adults in the age range of 50 to 60 years who were included in the study on the first-come, first-served basis through an advertisement on the website of H University from October 11, 2015 through October 19, 2015. The informed consent document was approved by the Institutional Review Board (IRB) of K University after providing an explanation for the study. Twenty- eight persons who agreed to participate in the experiment after re-explanation regarding the purpose of the study was provided to the recruited applicants were included in the study, and classification was performed into the experimental group and the comparison group by using a random method. Assessment of the sample size revealed that a minimum of 5 persons were needed in each group based on the calculation by G*Power program for determining the sample size.

Criteria for selecting the subjects of this study were healthy ordinary adults who understood the purpose of the study and voluntarily agreed to participate in the study, who did not have any general diseases like diabetes, thyroid disease, adrenal function disorder, liver ailment, kidney ailment, oral ailment, and who had not taken any drugs for a long time that can affect xerostomia and can chew and react when the secretion rate of saliva was measured.

3. Saliva sample collection

Saliva was collected from 8 to 10 a.m in the morning to prevent the error caused by midday changes, and it was stored in a container for exactly 5 minutes for each person at 1 hour after of chewing gum. Education was given regarding restriction of oral activity like consuming food, smoking, and tooth brushing 1 hour before the visit on the

Table 1. Study design

Group	Baseline	Treatment	10 days	20 days	30 days
Experimental	- saliva flow rate - perceived xerostomia - total protein - SIgA	masticating chewing gum mastication containing PME (masticating for 5 min 30 min after meal 3 times a day) 2.60 gm/1 time, 7.80 gm/1 day	- saliva flow rate - perceived xerostomia - total protein - SIgA		
Control	- saliva flow rate - perceived xerostomia - total protein - SIgA	no treatment	- saliva flow rate - perceived xerostomia - total protein - SIgA		

day of the experiment. The practice of mastication of chewing gum was recorded in a diary. The experimental group was instructed to masticate 1 tablet of chewing gum containing Japanese apricot extracts(PME) for 10 minutes after a meal within 30 minutes, three times a day. The experimental procedure included measurement of the flow rate of saliva and total volume before treatment. Total volume of entire saliva at 10 days, 20 days, and 30 days after each treatment was compared, The experimental procedure for the comparison group included measurement of the flow rate of saliva, total volume before treatment in the same way as in the experimental group by a researcher, and measurement of the flow rate and total volume of entire saliva at 10 days, 20 days, and 30 days each after treatment without conducting any treatment.

4. Saliva sample processing and analysis

Measurement of the concentration in irritating saliva after masticating chewing gum was performed using salivary secretory IgA indirect enzyme immunoassay kit(Salimetrics, USA). Optical density was measured at 450nm after stopping the reaction with stop solution by making each sample react with phased application of tetramethylbenzidine(TMB) after individuation of antibodies with added samples of saliva prepared in 96-well high affinity binding plate coated with highly purified human SIgA. Measurement of the concentration of total protein within saliva was performed using total protein ELISA kit(Antibody-Online, USA). Saliva samples was assessed by centrifuging for 15 minutes at 3,000 rpm taking the fluid after removing the impurities, and salivary total protein concentration was measured using a control in the kit.

5. Statistical analysis

Collected data were analyzed with Chi-square test and Fisher's exact test in order to assess homogeneity between groups by using IBM(SPSS 22.0 for Windows, SPSS Inc, Chicago, USA). Comparison of differences in stimulated saliva flow rate and total volume between the experimental group and the comparison group of patients who chewed gums containing apricot extract before treatment was performed with Mann-Whitney test, and changes occurring with passage of time after treatment were analyzed with repeated measures ANOVA. Differences in the content of irritating saliva and concentration of SIgA were analyzed with Wilcoxon signed rank test at 4 weeks after treatment.

Results

1. Verification of homogeneity of subjects

Gender and subjective awareness of oral health and smoking and alcohol drinking were analyzed in order to verify homogeneity between the group masticating chewing gum containing apricot extract and the comparison group, targeting adults in the age range of 50's-60's <Table 2>.

Differences were not observed between gender distribution and subjective awareness of oral health, and homogeneity was secured as there was no significant difference in characteristics between groups with 10 drinkers(62.5%) and 3 smokers(50.0%)(P>0.05).

As shown in <Table 3>, as a result of measuring the secretion rate of irritating saliva and xerostomia in the experimental group and comparison group for 5 minutes before treatment by masticating chewing gum, homogeneity was verified as there was no significant difference between the groups(P>0.05).

2. Change in the stimulated salivary volume flow rate after treatment by masticating chewing gum

As a result of verification by repeated measures ANOVA shown in <Table 4> by measuring irritating saliva for 5 minutes in the group masticating chewing gum containing apricot extract and the comparison group after treatment by masticating chewing gum, both between groups(P=0.002) and within groups(P<0.001) showed a significant difference.

3. Concentration of SIgA within saliva after masticating chewing gum

As a result of measuring the concentration of SIgA in irritating saliva at 30 days after treatment by masticating chewing gum <Table 5>, the concentration of SIgA in the experimental group was significantly reduced compared to that at 10 days post treatment(P=0.003).

4. Concentration of total protein after masticating chewing gum

<Table 6> shows the result of comparison and analysis of containment of total protein with comparison within saliva 30 days after treatment by masticating chewing gum. Concentration of total protein in saliva was significantly reduced at 30 days after masticating chewing gum compared to that before the experiment(P=0.006).

Table 2. General characteristics of the subjects Unit: N(%)

Variables	Experimental group	Control group	p-value*
Gender			
Male	4(50.0)	6(60.0)	0.695
Female	10(55.6)	8(44.4)	
Perceived oral health			
Good	3(25.0)	9(75.0)	0.144
Moderate	8(66.7)	4(33.3)	
Poor	3(75.0)	1(25.0)	
Alcohol drinking			
Yes	10(62.5)	6(37.5)	0.252
No	4(33.3)	8(37.5)	
Smoking experience			
Ex-smoker	3(50.0)	3(50.0)	0.891
Present smoker	1(33.3)	2(66.7)	
Non-smoker	6(42.9)	8(57.1)	

*by the Chi-square test and Fisher's exact test at α=0.05

Table 3. Stimulated salivary volume flow rate and perceived xerostomia in subjects before treatment Unit: Mean±SD

Variable	Experimental group(n=14)	Control group (n=14)	p-value**
SSFR*	10.73±3.39	9.50±2.19	0.283
Perceived xerostomia	4.62±1.12	5.00±1.88	0.529

*Stimulated salivary flow rate, **by Mann-Whitney test

Table 4. Variation of the stimulated salivary flow rate after treatment Unit: Mean±SD

Group	N	Baseline	10 days	20 days	30 days	p-value*
Experimental	14	10.73±3.39	15.19±4.533	18.23±5.68	15.69±5.31	<0.001
Control	14	9.50±2.19	11.50±2.20	11.50±3.44	9.63±2.51	

*by the repeated measures ANOVA at α=0.05

Table 5. Concentration of SIgA at 30 days after treatment by masticating chewing gum Unit: Mean±SD, μg/ml

Group	N	10 days post treatment	30 days post treatment	p-value*
Experimental	14	53.67 ± 0.13	13.45 ± 0.19	0.003
Control	14	48.27 ± 0.09	50.97 ± 0.18	0.182

*by the Wilcoxon Signed rank test at α=0.05

Table 6. Containment of total protein at 30 days after masticating chewing gum Unit: Mean±SD, μg/ml

Group	N	10 days post treatment	30 days post treatment	p-value*
Experimental	14	860.42 ± 419.87	404.13 ± 248.52	0.006
Control	14	493.50 ± 417.29	458.00 ± 248.68	0.875

*by the Wilcoxon Signed rank test at α=0.05

Discussion

Oral health, an essential element of health, has been emphasized for maintaining general health. Recently, the importance of preventive treatment rather than direct treatment of disease using restorative treatment has been emphasized; hence, attention has increased towards probiotics using lactobacillus in addition to chemical drugs that have been used so far[13].

Reduction in the intake of sucrose for prevention of oral disease is important, but it is realistically difficult to practice this in life as modern people prefer a snack between meals

mixed with sugar[14]. With respect to sugar alternatives, attention has been paid to xylitol and sorbitol, which are the ingredients in chewing gum, known to be effective in reducing the numerical value of S. mutans through blocking and preventing formation of biofilms from sugar as well as mechanical cleaning by facilitating secretion of saliva interaction with protein of saliva prevention of dental caries by buffering acidity(pH) of orak biofilm mainly used as ingredient for chewing gum. This also has a relation with the ingredient in chewing gum as well as taking the physical cleaning activity of chewing gum itself[14]. Many diseases that cause health problems are infectious, which first arise through the respiratory tract, and oral substances that contain antibacterial materials for restricting bacterial multiplication within the mouth are used for preventing and treating oral diseases. In addition to this, attention is increasing towards naturally occurring materials that can be safely used as replacement for existing chemical synthetic antibacterial materials that have many side effects within the mouth[6,16,17].

Plant extract, specific protein and enzymes, isozyme, bacteriocin, organic acids, fatty acids are developed as natural antibacterials. But, natural materials contain various ingredients that have not yet been confirmed or not yet known, and they have not been industrialized and commercialized properly as their effectiveness has not been evaluated[10].

Prunus mume used in this study has been known to show effectiveness with the human body, but the study is still lacks information on the effect for prevention and treatment of oral diseases according to the antibacterial process and immunity reaction mechanism. Generally, *Prunus mume* is reported to have an excellent effect of quenching thirst, facilitating saliva secretion, and antibacterial activity and antioxidative activity in various Chinese medical literatures[18-20], and it is regarded to be a useful ingredient in chewing gum. In other words, when a basic study is conducted on the antibacterial effect of *Prunus mume* within the mouth for development of oral substances, it can have various utilities as a natural antibacterial agent and will enhance the value as a cash crop for local apricot farmers.

As a result of this study, the secretion rate of irritating saliva in the group masticating chewing gum containing apricot extract was gradually increased($p<0.001$). Mastication of apricot extract-containing chewing gum was found to be helpful in maintaining oral health with a significant reduction in the concentration of SIgA in irritating saliva at 30 days after treatment by masticating chewing gum compared to the first week. In addition to this, containment of total protein within saliva was significantly reduced at 30 days after masticating chewing gum, which is assumed to have an association with the immune system within the mouth.

Mouth is an organ with a concentration of various microorganisms within the human body, which cause dental caries and periodontal diseases and become a direct and indirect cause of general physical infection, leading to various diseases in the respiratory and digestive organs[4]. Oral microorganisms essentially attach themselves to the surface of the mouth in general, and these adhered microorganisms at an early stage of dental plaque formation form a dental plaque while producing a microcell colony by bacterial organisms like polysaccharide, saliva protein and glucoprotein[21]. The number of germs existing in the dental plaque changes with time, and as the dental plaque matures, gram-negative anaerobic germs will increase, and early existing types include *Streptococcus oralis, S. mitis* and *S. sanguinis, and S. mutans* and *Porphyromonas gingivalis* mainly cause dental caries and periodontal disease as they there are germs that cause diseases within the mouth[22]. Jang et al.[23] identified the antibacterial effect of apricot extract within the mouth, and considering the increase in secreting materials in irritating saliva and concentration of SIgA and change in the content of total protein in this study, masticating chewing gum containing apricot extract is suggested to contribute to prevention of oral diseases and a strategy for xerostomia.

Conclusions

This study is an analogous experimental study that uses an experiment design before and after application of a random comparison group in order to analyze the change of content like the secretion rate of irritating saliva after masticating chewing gum containing *Prunus mume* extract that has widely been used as medication and health food for a long time, concentration of SIgA and the content of total protein, and the following results were obtained:

1. A significant difference was observed between the group masticating chewing gum containing *Prunus mume* extract with passage of time after treatment by masticating chewing gum and comparison group as a result of measuring irritating saliva for 5 minutes.

2. As a result of comparing and analyzing the content of total protein within saliva at 30 days after treatment by masticating chewing gum, the concentration of total protein within saliva was significantly reduced compared to that before experiment(p=0.006).
3. As a result of measuring the concentration of SIgA, targeting the experimental group masticating chewing gum containing *Prunus mume* extract and the comparison group, a significant difference was observed between the groups at 30 days after masticating chewing gum.

Based on above results, masticating chewing gum containing *Prunus mume* extract is useful for solving oral problems in patients suffering from xerostomia, and furthermore, the antibacterial activity of saliva and immune reaction that were observed, are suggested to contribute to the development of a diagnostic indicator for oral health.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning(2014R1A1A3051084) and was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Small and Medium Business Administration in 2013(C0029783).

References

1. Mandel ID. The functions of saliva. J Den Res 1987; 66(Spec Iss): 623-7.
2. Mandel ID. The role of saliva in maintaining oral homeostasis. J Am Dent Assoc 1987; 119(2): 298-304.
3. Lamkin MS, Oppenheim FG. Structural features of salivary function. Crit Rev Oral Biol Med 1993; 4: 251-9.
4. Scannapieco FA. Saliva-bacterium interactions in oral microbial ecology. Crit Rev Oral Biol Med 1994; 5: 203-48.
5. Sato EF, Choudhury T, Nishikawa T, Inoue M. Dynamic aspect of reactive oxygen and nitric oxide in oral cavity. J Clin Biochem Nutr 2008; 42: 8-13.
6. Wong RWK, Samaranayake HL, Yuen MKZ, Seneviratne CJ, Kao R. Antimicrobial activity of Chinese medicine herbs against common bacteria in oral biofilm. A pilot study. Int J Oral Maxillofac Surg 2010; 39: 599-605.
7. Han JT, Lee SY, Kim KN, Baek NI. Rutin, antioxidant compound isolated from the fruit of *Prunus mume*. J Korean Soc Agri Chem Biotechnol 2001; 44: 35-7.
8. Shim JH, Park MW, Kim MR, Lim KT, Park ST. Screening of antioxidants in mume(*Prunus mume* Seib. et. Zucc) extract. J Korean Soc Agri Chem Biotechnol 2002; 45: 119-23.
9. Seo KS, Huh CK, Kim YD. Comparison of antimicrobial and antioxidant activities of *Prunus mume* fruit in different cultivars. Korean J Food Preserv 2008; 15: 288-92.
10. Ha MH, Park WP, Lee SC, Choi SG, Cho SH. Antimicrobial characteristic of *Prunus mume* extract. J Food Preserv 2006; 13(2): 198-203.
11. Choi MY, Won HR, Park HJ. Antimicrobial activities of Maesil(*Prunus mume*) extracts. Korean J Community Living Sci 2004; 15: 61-6.
12. Kim GS. Studies on the antimicrobial activities and substances of *Prunus mume*[Doctoral dissertation]. Seoul: Univ. of Ewha Womans, 1985.
13. Lee SJ, Lee SH, Choi BK, Kim JW, Kim YJ, Kim CC. The influence of Lactobacillus rhamnosus GG on the binding ability of Streptococcus mutans. J Korean Acad Pediatr Dent 2011; 38(2): 155-60.
14. Baik SH. The effects of xylitol and sorbitol on oral health and xerostomia in Korean elderly[Doctoral dissertation]. Seoul: Univ. of Ewha Womans, 2004.
15. Lee HS, Lee KH. The influence of gum chewing on the caries, periodontium, saliva, plaque and oral hygiene. J Korean Acad Dent Health 1990; 14(2): 185-92.
16. Jang JH, You SY, Oh TJ. Antimicrobial activity of jaborandi extract and sorbitol to oral microbes. J Korean Soc Dent Hyg 2013; 13(3): 517-23. http://dx.doi.org/10.13065/jksdh.2013.13.3.517.
17. Oh HN, Hong SJ, Lee HJ, Choi CH. The effects of bamboo salt solution on remineralization of artifical caries-like lesions. J Korean Soc Dent Hyg 2012; 12(2): 335-43.
18. Han JT, Lee SY, Kim KN, Baek NI. Rutin, antioxidant compound isolated from the fruit of *Prunus mume*. J Korean Soc Agri Chem Biotechnol 2001; 44: 35-7.
19. Shim JH, Park MW, Kim MR, Lim KT, Park ST. Screening of antioxidants in mume(*Prunus mume* Seib. et. Zucc) extract. J Korean Soc Agri Chem Biotechnol 2002; 45: 119-23.
20. Seo KS, Huh CK, Kim YD. Comparison of antimicrobial and antioxidant activities of *Prunus mume* fruit in different cultivars. Korean J Food Preserv 2008; 15: 288-92.
21. Yang KH, Han SJ. The effect of xylitol and carbohydrates

on the replication of *Streptococcus oralis* and *Streptococcus salivarius*. J Korean Acad Pediatr Dent 2003; 30(4): 722-7.
22. Samaranyake LP. Essential Microbiology for Dentistry. 3rd ed. Edinburgh: Churchill Livingstone/Elsevier; 2006: 34.
23. Jang JH, Kim YI, Lee H. Antimicrobial activity of *Prunus mume* extract to oral microbes. J Korean Soc Dent Hyg 2014; 14(2): 109-15. http://dx.doi.org/10.13065/jksdh.2014.14.01.109.