THE IMMEDIATE TERM EFFECT OF CHEWING COMMERCIALLY AVAILABLE MESWAK *(SALVADORA PERSICA)* ON LEVELS OF CALCIUM, CHLORIDE, PHOSPHATE AND THIOCYANATE IN WHOLE SALIVA

S. Kaur, R. Abdul Jalil, S.L. Akmar. The immediate term effect of chewing commercially available meswak (salvadora persica) *on levels of calcium, chloride, phosphate and thiocyanate in whole saliva. Annal Dent Univ Malaya 2004; 11: 51–59.*

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ABSTRACT

The objective of this study was to determine the effect of chewing commercially available meswak may have on levels of calcium, chloride, phosphate and thiocyanate in stimulated whole saliva. A total of 20 subjects participated in the investigation. They were distributed into two groups. Those in group A (10 individuals) were asked to first chew on a cotton roll (sized #1) followed by the chewing of an equivalent sized 5mm piece of commercially available meswak. Subjects in group B (10 individuals) did the same but, chewed on cotton roll (sized #2) followed by the chewing of an equivalent sized 10mm piece of commercially available meswak. After following a specified chewing protocol, samples of stimulated whole saliva were collected into a graduated tube at the end of every chewing regime.

Calcium, chloride, phosphate and thiocyanate analysis were carried out using colour titration and spectrophotometer. Results from this investigation indicated that commercially available meswak chewing sticks apart from containing high amounts of calcium and chloride may possibly release phosphate and thiocyanate into whole saliva. These findings suggest that the commercially available meswak used as chewing sticks may have the potential of releasing substances into saliva that could influence the state of oral health. Further studies have to be carried out to ascertain the therapeutic benefits of chewing commercially available meswak.

Key words: meswak, calcium, chloride, phosphate, thiocyanate, saliva, oral health.

INTRODUCTION

Saliva plays a major role in the health of the mouth (1,2) and any changes in its amount or quality may alter the oral health status. The ability of saliva to affect demineralization and remineralization is part of an ongoing, homeostatic process. Saliva is generally supersaturated with respect to calcium and phosphate salts as hydroxyapatite, and this inhibits demineralization and promotes remineralization. A high salivary calcium level has been seen to be correlated with the number of intact teeth (3). Recent evidence suggests that high salivary phosphate levels may be associated with lower caries rates (4).

Increasing the rate of flow of saliva increases the degree of saturation with these salts, mainly because an increase in salivary pH enhances remineralization and decrease demineralization of tooth enamel (5). Remineralization is a preventive approach to the treatment of non-cavitated caries lesions. It is now known that carious lesions progress at slower rates than was previously believed; early caries can be arrested, and the affected structure can be remineralized. Active caries lesions can thus revert to "intact" remineralized surfaces. Caries arrest is a well-documented natural repair of the early carious process. When the cariogenic challenge is removed, the natural supersaturated concentration of salivary calcium may contribute to the remineralization process and subsequent maintenance (6,7).

Dairy products such as cheeses and milk have been shown to be anticariogenic in humans. The protective effect was attributed to phosphoprotein casein and calcium phosphate present in dairy products. Recently, *an in-vitro* study reported that casein phosphopeptide-stabilized calcium phosphate solutions remineralized subsurface lesions in human molars. Casein phosphopeptide maintains a high concentration of calcium phosphate on the subsurface lesion, which enhances remineralization (8). Hence, additional remineralization therapies are currently in the product development pipeline.

Chloride concentration usually is linked to sodium concentration except at high flow rates. Its concentration in acinar fluid is similar to interstitial

fluid and plasma, hence damage to acinar cells reduces flow rate but does not affect reabsorption of chloride. This results in inappropriately high concentrations relative to flow rate (3). The major physiological influence on concentration of chloride is the flow rate. Duration of stimulation is important in considering chloride concentrations, which fall over a period of time as bicarbonate concentration rises. A high salivary chloride level was observed in early morning samples, while the lowest level was noted in samples collected in the evening (4). Chloride concentration showed a slightly lower value after a carbohydrate meal (5).

Salivary peroxidase, which is a part of an antibacterial system involves the oxidation of salivary thiocyanate by hydrogen peroxide (generated by oral bacteria) to hypothiocyanite and hypothiocyannous acid. These products affect the bacterial metabolism (especially acid production) involved in glycolysis and sugar transport (6). The salivary peroxidase enzyme involved in this process is synthesized and secreted by the salivary glands. Salivary peroxidase enzyme is an important non-immune defence factor as it is responsible for a significant portion of total peroxidase activity in saliva (7,8). Thiocyanate is concentrated in saliva by the same transport system that secretes iodide. Its concentration falls as flow rates increase, and it also exhibits circadian rhythm (9). Variations due to age has yet to be studied. Its concentration is higher in the saliva of cigarette smokers and is related to diet (10).

The use of chewing sticks to enhance salivary secretion particularly in the management of xerostomia have not been explored. Like chewing gums, chewing sticks too may have a hydrodynamic pumping effect on plaque which might not be expected with other mechanical stimuli. In addition, chewing sticks may have a combined mechanical and chemical stimuli. Products with a hydrodynamic effect might be superior to non-hydrodynamic mechanical and chemical stimuli although no data exist to support this hypothesis.

The objective of this study was to determine the effect of the commercially available meswak chewing stick. The salivary factors investigated were concentrations of calcium, chloride, phosphate and thiocyanate. Flow rate was also included in this study since salivary composition to a large extent is affected by flow rate which also influences the total amount of these salivary components delivered into the oral cavity.

MATERIALS AND METHODS

Subject selection

Twenty subjects (aged 20 – 40 years) participated in the study. Since assessments were made only at the laboratory level, volunteers were accepted regardless of their age, gender, ethnic, oral or medical health status. Before the start of the study all subjects received verbal information about the objective of the study and gave informed consent by filling up the forms provided to them. Participants were instructed not to consume any food and beverages two hours prior to saliva collection.

Procedure

Saliva collection took place between 11.00am to 12.00pm, approximately two hours after the last intake of food or beverages. All subjects first chewed cotton roll at a constant rate of approximately one chew per second changing the chewing sides every 30 seconds for two minutes in order to achieve "maximal" stimulation. This saliva was discarded and not used in the analysis.

Subjects were then divided into two groups. Those in group A (10 subjects) were provided with a new cotton roll (sized #1) and stimulated whole saliva was achieved by cotton roll chewing at a constant rate of about one chew per second and saliva was then transferred into a graduated tube at the end of every 30 seconds. Subjects switched chewing from one side of the mouth to the other every 30 seconds until approximately 4 ml. of stimulated whole saliva was collected. The time taken to collect approximately 4 ml. of saliva was noted for the purpose of calculating the flow rate according to the formula i.e. volume collected / time taken to collect the saliva. The cotton roll was then discarded and subjects were provided with the commercially available meswak (comparable in length to the cotton roll, 5mm) and told to chew using the technique as prescribed earlier until approximately 4ml. of saliva was collected. Once again the time taken to collect the saliva was recorded.

Using the same protocol, subjects in group B (10 subjects) did the same, but using cotton roll sized #2 followed by a sized 10mm commercially available meswak until approximately 4 ml. of saliva was collected.

Procedure of sample analysis

The samples obtained for both groups were then centrifuged at 13,500 rpm and the supernatant stored at 4ºC until analyzed. The components analyzed were calcium, chloride, phosphate and thiocyanate.

For the analysis of calcium, 0.2 ml. of saliva samples were used and analysis was done in duplicate using Calcon as the indicator and titration was carried out with EDTA until the appearance of a blue coloration. The standard used was 10mg. calcium /100 ml.

For the analysis of chloride, 0.2 ml. of saliva samples were used and analysis was done in duplicate using 0.1% diphenyl cabazone as the indicator and titration was carried out with mercuric nitrate until a permanent purple coloration was obtained. Twenty mMols of chloride was used as standard.

For the analysis of phosphate, 0.5 ml. of saliva samples were used and analysis was also done in duplicate comparing the values against a range of standards varying from 0.05mg. – 0.25mg. / ml. of phosphate. After the addition of sodium molybdate and ANSA, the absorbance was read using spectrophotometer at wavelength 680 nm.

For the analysis of thiocyanate, 0.25 ml. of saliva samples were used also in duplicate using the ferric nitrate method (13) ie. 1.25mM stock potassium thiocyanate to give a range of standards containing 0, 0.125, 0.25, 0.375, 0.5 and 0.625mM. The absorbancy was read using spectrophotometer at wavelength 460 nm.

Statistical analysis was carried out using the SPSS (version 9.0) to determine mean values, standard deviations and frequency distributions. Distributions of the original scores were skewed, hence non parametric methods were employed. Analysis of contrast through the use of the Wilcoxan signed rank test was also carried out in order to seek out differences between the means.

RESULTS

In Table 1, using the Wilcoxan signed rank test, the increase in mean concentrations of calcium after chewing with meswak compared to cotton roll in both groups A and B were statistically significant at $p<0.01$. The data was subsequently adjusted to take into account the effect of secretion rates on mean concentrations of calcium. The mean values for calcium were still significantly higher after the chewing of meswak compared to cotton roll in both groups A and B at $p < 0.01$.

In Table 2, using the Wilcoxan signed rank test, the increase in mean concentrations of chloride after chewing with meswak compared to cotton roll in both groups A and B were statistically significant at

Table 1. Mean values, range and standard deviation for calcium in saliva.

Group A, chewing of Cotton roll #1 followed by Meswak 5mm (mg/100ml)

Subject	Cotton roll Mean ±S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	2.018 ± 0.18	10.722 ± 6.49	-2.803	p < 0.01
Range	1.851 – 2.222	5.556 – 27.778		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/100ml)

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group B	2.185 ± 0.37	9.552 ± 5.01	-2.701	p < 0.01
Range	1.481 – 2.592	1.333 – 16.669		

Group A, chewing of Cotton roll #1 followed by Meswak 5mm (mg/100ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	2.104 ± 1.00	10.469 ± 4.29	-2.803	p < 0.01
Range	1.089 – 4.074	4.000 – 18.888		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/100ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group B	1.966 ± 0.52	11.011 ± 5.69	-2.701	p < 0.01
Range	1.333 – 2.955	1.522 – 20.149		

Table 2. Mean values, range and standard deviation for chloride in saliva.

Group A, chewing of Cotton roll #1 followed by Meswak 5mm(mg/ml)

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	5.862 ± 2.03	16.353 ± 6.16	-2.803	$p < 0.01$
Range	3.538 – 10.769	7.385 – 25.692		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/ml)

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group B	5.662 ± 2.54	12.061 ± 3.97	-2.701	$p < 0.01$
Range	0.154 – 9.538	6.615 – 18.462		

Group A, chewing of Cotton roll #1 followed by Meswak 5mm (mg/ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	6.414 ± 4.05	18.562 ± 11.30	-2.803	$p < 0.01$
Range	3.348 – 17.230	5.317 – 37.253		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/100ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group B	5.134 ± 2.49	14.617 ± 6.20	-2.803	$p < 0.01$
Range	0.123 – 8.890	6.615 – 22.708		

$p<0.01$. The data was subsequently adjusted to take into account the effect of secretion rates on mean concentrations of chloride. The mean values for chloride were still significantly higher after the chewing of meswak compared to cotton roll in both groups A and B at $p<0.01$.

In Table 3, no differences were observed with regards to phosphate levels in both groups A and B after chewing with meswak compared to cotton roll. The data was further analyzed using the Wilcoxan signed rank test. The increase in mean phosphate concentration in both groups A and B was not significant at $p > 0.05$. When secretion rate was taken into consideration, both groups A and B showed an increase in mean phosphate concentration after the chewing meswak as compared to cotton roll. However, these differences were only statistically significant at $p < 0.05$ in group B.

In Table 4, the increase in mean thiocyanate concentrations seen after the chewing of meswak compared to cotton roll was apparent in both group A and B. The data was further analyzed using the Wilcoxan signed rank test. The increase in mean thiocyanate concentration was statistically significant at $p<0.05$ in group A only. When secretion rate was taken into consideration, both groups A and B showed an increase in mean thiocyanate concentration after the chewing meswak as compared to cotton roll. However, these differences were statistically significant only in group B at $p<0.05$.

DISCUSSION

The positive role of calcium and phosphate concentrations of dental plaque in the caries process has encouraged investigations of their relationship with environmental sources. Potential sources include saliva, gingival crevicular fluid, diet and tooth

Table 3. Mean values, range and standard deviation for phosphate in saliva.

Group A, chewing of Cotton roll #1 followed by Meswak 5mm (mg/100ml)

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	0.454 ± 0.13	0.430 ± 0.18	-0.561	p > 0.05
Range	0.329 – 0.761	0.221 – 0.856		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/100ml)

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group B	0.374 ± 0.09	0.374 ± 0.09	0.051	p > 0.05
Range	0.282 – 0.566	0.248 – 0.541		

Group A, chewing of Cotton roll #1 followed by Meswak 5mm (mg/100ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	0.473 ± 0.24	0.471 ± 0.25	-0.663	p > 0.05
Range	0.209 – 0.978	0.175 – 0.886		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/100ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group B	0.314 ± 0.13	2.128 ± 0.65	-2.497	p < 0.05
Range	0.184 – 0.606	0.287 – 0.618		

enamel. The precise mechanism by which the levels of calcium and phosphate in saliva may influence plaque concentrations is unclear, although it appears likely that these ions diffuse into plaque from saliva (10,11). However, salivary calcium and phosphate may be precipitated and included in plaque, along with salivary protein during its formation (12). It is also possible that calcium and phosphate concentrations in saliva influence the outward diffusion of these ions from plaque.

Ashley et al. (13), in their study provided some support for the concept that mineral concentrations of saliva influence those in plaque during sugar challenges. During sugar intake, phosphate concentrations in saliva may fall with increases in flow rate, whilst saliva may become desaturated with respect to calcium phosphates due to reductions in pH. Concurrently, mineral concentrations in the fluid phase of plaque may rise and enable calcium and/or phosphate to diffuse out. High concentrations of these constituents in stimulated saliva may reduce outward diffusion, whilst low concentrations may encourage movement of ions out of plaque.

In this study the effect of chewing commercially available meswak on calcium, chloride, phosphate and thiocyanate were investigated. Increase in mean concentrations of calcium were apparent in both groups after the chewing of meswak compared to cotton roll and these differences were statistically significant at $p<0.01$. Gazi et al. (17) studied the immediate term effects of meswak chewing by comparison with saliva samples collected 2 minutes after chewing with pyrogen free rubber before using meswak. As in our study, they reported that meswak produced significant increase in calcium. The increase in mean calcium concentrations in their study is approximately 26 folds. This is much higher than what we found in our study which was 5 folds.

Table 4. Mean values, range and standard deviation for thiocyanate in saliva

Group A, chewing of Cotton roll #1 followed by Meswak 5mm(mg/ml)

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	0.342 ± 0.24	0.473 ± 0.21	-1.988	$p < 0.05$
Range	0.129 – 0.749	0.206 – 0.880		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/ml)

Subject	Meswak Mean ± S.D.	Cotton roll Mean ± S.D.	Z value	Sig. levels
Group B	0.295 ± 0.14	0.331 ± 0.13	-1.938	$p > 0.05$
Range	0.113 – 0.595	0.141 – 0.635		

Group A, chewing of Cotton roll #1 followed by Meswak 5mm (mg/ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group A	0.366 ± 0.32	0.514 ± 0.30	-1.886	$p > 0.05$
Range	0.115 – 1.172	0.165 – 1.056		

Group B, chewing of Cotton roll #2 followed by Meswak 10mm (mg/100ml/min) taking into account the effect of secretion rates

Subject	Cotton roll Mean ± S.D.	Meswak Mean ± S.D.	Z value	Sig. levels
Group B	0.275 ± 0.17	0.384 ± 0.15	-1.989	$p < 0.05$
Range	0.104 – 0.637	0.173 – 0.610		

The mean concentrations of phosphate were higher after the chewing of commercially available meswak compared to cotton roll but the difference was only statistically significant in group B at $p<0.05$. Contrary to our findings, Gazi et al. (17) in their study found no significant changes in phosphate concentrations after the chewing of meswak.

The fact that meswak contains substantial amounts of calcium and possibly phosphates and that these inorganic ions will be released into stimulated whole saliva upon chewing will ensure continuous supply of these two salivary components regardless of the increase in secretion rate. High concentrations of these constituents in stimulated saliva may then reduce the outward diffusion of calcium and phosphates out of plaque and hence limit demineralization particularly in the presence of a cariogenic challenge.

Increase in mean concentrations of chloride were apparent in both groups after the chewing of commercially available meswak compared to cotton roll and these differences were statistically significant at $p<0.01$. Gazi et al. studied the immediate term effects of meswak chewing by comparison with saliva samples collected 2 minutes after chewing with pyrogen free rubber before using meswak. As in our study, they reported that meswak produced significant increase in chloride. The increase in mean chloride concentrations in their study is approximately 6 folds. This is much higher than what we found in our study which was 3 folds.

The role of chloride and thiocyanate concentrations has encouraged investigations of their relationship with environmental sources. In very young subjects, chloride concentrations tend to be high (12). In general, chloride concentration gives diagnostic information relating to the efficiency of ductal transport systems. Variations from normal values are readily detectable in parotid saliva and concentrations in submandibular and whole saliva

are much more predictable. Such measurements however, are of value in linear studies of individuals, when changes over a period of time can be studied eg. in the assessment of recurrent parotitis, in the response to radiation, and following the progress of transplant acceptance or rejection.

Ben – Aryeh et al. (1984) found chloride to decrease with age, this might be due to leakage of immature junctions between cells as possibility of relationship between morphology and permeability of barriers (13). The most regular changes in chloride could arise from rhythms in aldosterone secretion (related to posture and sleep wakefulness) acting directly on salivary gland (14). High chloride content is believed to reduce calculus formation (15) and prevent staining of teeth (16). It assists a - amylase in antibacterial function and lowers pH.

The mean concentrations of thiocyanate were higher after the chewing of fresh meswak compared to cotton roll with statistically significant difference at $p<0.05$. Contrary to our findings, Jalil in 1989 in her study found comparable results in thiocyanate concentrations using paraffin wax.

Thiocyanate concentration in body fluids is related to diet and smoking habits. The salivary glands concentrate thiocyanate from blood through epithelial cells of the striated ducts and secrete thiocyanate at relatively high concentrations[17]. Saliva also contains other peroxidase substrates i.e. iodide but this is not present in high enough concentrations to be of biological significance. Thiocyanate is an essential constituent in the peroxidase-mediated bacteriostatic system in saliva. Salivary lactoperoxidase is synthesized by both parotid and submandibular acinar cells. It utilizes microbially produced hydrogen peroxide to oxidize salivary thiocyanate to hypothiocyanite, which is toxic to bacteria. The hypothiocyanite is the major product and is stable at neutral pH. At low pH, the major product is the neutral molecule hypothiocyanous acid which is in acid base equilibrium with hypothiocyanite (18). The mechanism of action involves oxidation of sulphydryl groups of enzymes involved in glycolysis and sugar transport.

The fact that meswak contains substantial amounts of chloride and thiocyanate and that these inorganic ions will be released into stimulated whole saliva upon chewing will ensure continuous supply of these two salivary components regardless of the increase in secretion rate. In addition, the chewing of meswak as shown in this study has an added benefit as it resulted in an increase in salivary flow rate possibly due to its hydrodynamic pumping effect. Patients should therefore be encouraged to consume foods, which require vigorous chewing. Stimulation of the glands through mastication for example via the use of chewing gums have been shown to be beneficial in terms of promoting clearance of food from the mouth and may help by causing an increase in the unstimulated flow rate on a long term basis. Chewing gum is an extremely effective, rather continuous, sialogogue. Because of the increased risk of dental caries, only the sugarless variety of gum should be used. The possibility of incorporating meswak in chewing gum should be explored since the advantage of chewing with gum is that it is usually kept in the mouth for a longer time than rinses and toothpastes.

The use of cotton roll was preferred over the use of either paraffin wax or corrugated rubber since the physical property of meswak as used in this study (Figure 1) closely resembles the physical property of the cotton roll not only in its size but also in its shape.

Figure 1: Commercially available, mint flavoured meswak used in this study.

Like cotton roll too, meswak loses its cylindrical shape a few minutes after chewing turning into a rather flatten piece of chewing material. The commercially available, mint flavoured meswak used in this study were flown from Doha, QATAR. They were supplied to us in prepacked, precut lengths of approximately 6 inches. For the purpose of this study, each meswak was prepared so as to be of comparable length to the cotton roll. The barks were carefully removed prior to use.

In conclusion, results from this study seemed to suggest that meswak chewing apart from releasing calcium, chloride, phosphate and thiocyanate may contribute to a large extent by flow rate which also influences the total amount of these salivary components delivered into the oral cavity. Because meswak is thought to contain chemical ingredients beneficial to the maintenance of oral health, further studies need to be carried out to ascertain the therapeutic benefits of chewing meswak. Clinical implementations of these chewing sticks should also be tested as it may prove worthwhile to identify active ingredients of meswak for possible future use in chewing gums and mouthwash formulations.

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