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Comparing Salivary and Dental Plaque Content of Sodium, Calcium and Phosphate after Sucrose Ingestion between *Salvadora persica* Sticks and Tooth Brush Users

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Authors' contributions

This work was carried out in collaboration between all authors. Author WHK designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors YMS and BGG managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Dental caries is a biofilm-dependent oral disorder, resulting in its initiation and development from fermentable dietary carbohydrates as key environmental factors. Sucrose in particular, is considered as the most cariogenic of all carbohydrates; the biofilm forming in its presence is characterized by low concentrations of those critical ions involved in de- and re-mineralization of enamel and dentin, namely calcium, phosphates and fluoride content. Within this context, this present study aimed to compare Sodium, Calcium and Phosphate concentrations in saliva and

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dental plaque after sucrose ingestion among *Salvadorapersica* / *Miswak* chewing sticksusersand tooth brush users. *S*ixty and fifty nine adult males who were using *Salvadorapersica (Miswak* sticks) and tooth brush respectively were recruited in the study, excluding those who had history of smoking tobacco or smokeless tobacco, under certain medications or having chronic or oral diseases as diagnosed by oral examination. Structured face to face standardized questionnaire was performed to assess their dental and nutritional habits. Laboratory investigations were performed for salivary and dental plaque, calcium, phosphate and Na following ingestion of a cooked meal free from sucrose / containing sucrose, or after ingestion of sucrose containing drinks only. Significantly higher levels of Ca, P and Na were found after sucrose ingestion in *Miswak* users compared to brush users, suggesting *Miswak* to be an affordable anti plaque, anti cariogenic measure for providing inorganic minerals needed for re mineralization and combating drop of pH after sucrose ingestion.

Keywords: Salvadorapersica; Siwak/Miswak users; tooth brush; content of Na; Ca and phosphate; dental plaque biofilm; oral homeostasis.

1. INTRODUCTION

Dietary sucrose exposure is thought to lead to deficiency of the calcium binding proteins in the extracellular matrix of the plaque-likebiofilm, hence the low biofilm inorganic concentrations responsible for the cariogenic properties of dietary sucrose [1]. Previous studies had shown that the biofilm formed in the presence of sucrose presents low concentrations of Ca, P, and fluoride, which are critical ions involved in the mineralization of enamel and dentin in the oral environment, as such reduction of ion availability due to sucrose may increase the cariogenic potential of the biofilm [2-7]. A recent study revealed that Miswak users had higher salivary sodium and calcium, higher dental plaque calcium and inorganic phosphate content when compared to brush users, concluding that Miswak use increases dental plaque pH and provides calcium and phosphate at low salivary pH so promoting re-mineralization, and the associated significant positive correlation between plaque sodium and plaque pH in Miswak users and not in brush users again suggests a good role of Miswak, in counteracting the drop of plaque pH [8]). A*Miswak* twig is a tooth cleaning traditional and natural alternative to the modern toothbrush, made from the *Salvadorapersica* tree (known as Arak in Arabia) which is planted in various parts of Africa, Asia and Arabia, and claimed to be an ant-cariogenic agent and as effective as tooth brushing [9-16]. The mechanical effects of its fibers, release of beneficial chemicals, or a combination of both are some of the explanations suggested about its protracted elevating effects on plaque pH following an acidogenic challenge [17]. Similar conclusions have later suggested the possible effectiveness of chewing sticks in promoting oral hygiene and warranting further research [18,19]. This study is a further attempt aiming at comparing Sodium, Calcium and Phosphate content in saliva and dental plaque after 2-4 hours of ingestion of a cooked meal containing sucrose/ no sucrose, or after intake of a sugary meal only, among S.persica sticks/extracts users (Siwak) and tooth brush users, and to propose conclusions as far as oral hygiene is concerned.

2. SUBJECTS, MATERIALS AND METHODS

In this parallel group cross-sectional comparative study, a total of 119 volunteers (59 tooth brush and 60 *Miswask* users) were recruited for the study. Study groups were composed of employees and students from the Faculty of Medicine, Khartoum University, and visitors to faculty of 19 to 65 years. Information about the study, its objectives & invitations to participate were given, a written consent signed, and then, participants were interviewed using a structured questionnaire developed from the WHO manual Oral Health Surveys – Basic Methods, 5th ed 2013 [20] as appropriate, to assess their demographic profiles and oral hygiene habits. Inclusion criteria included regular *Miswak* and tooth brush users, good general health, absence of chronic illnesses such as diabetes and hypertension, no past or present history of smoking or chewing or snuffing or dipping of tobacco between lower gum and cheek or lip (smokeless tobacco or *tomback* as to the Sudanese accent!). Those who had used mouth wash or received antibiotics for at least one month before clinical assessment and also those who have caffeine containing drinks for more than once/day were also excluded from our study. Regular *Miswask* or tooth brush users were defined as individuals who reported using only one of the mentioned oral hygiene methods

at least one time daily for the last year. Individuals were sub grouped into three groups in accordance if whether they had ingested cooked starches only or cooked starches followed with sugary drinks or sugary meal only (e.g. sweets or sweetened juices) 2-4 h before starting the assessment procedures. Supragingival plaque samples were collected from the buccal surfaces of all teeth. Approximately 3 ml of expectorated whole saliva was collected from the selected study subjects. Saliva was usually collected from participants in the afternoons. Volunteers were asked to chew a small piece of paraffin wax for 1 minute as a stimulant after rinsing the mouth with water; collected saliva from each subject was carried in a 15 ml graduated centrifuge tubes, in an ice box to be stored at -20ºC. Immediately after saliva collection, supragingival plaque samples were carefully collected from the selected teeth using sterilized dental curettes, transferred immediately to 1.5 ml Eppendorf tubes filled with 0.5 ml of sterilized (water for injection) and transported in an ice box to be stored at -20ºC. Before electrolyte measurement, the saliva was centrifuged and plaque samples were vortexed and then centrifuged. For calcium and phosphate electrolytes analyzer we used the chemical analyzer full atomization technique of the brand Roche COBAS Integra 400 plus, model 2004. Reading was in mg/dl. For sodium ions electrolyte analyzer we used the selective electrode techniques Roche 9180, values of less than 1.5 mmol/l were not able to read. A diluent was used with a ratio of 0.5 ml (sample):1 ml (diluent) (21462913-2009-01). For pH measurement, Accumet Hand Held pH/MV/ ºC meter (FB68792) and Fisher Brand pH electrode Semi Micro-Glass BNC with 1M Cable (FB68814), were applied.

2.1 Statistical Analysis

Data have been organized and analyzed using the statistical program SPSS (Statistical Packages for Social Sciences). The following tests were used whenever applicable: Test for equality of means; Levene's Test for Equality of Variances; One Way ANOVA for comparing means with categories; Post-Hoc tests (multiple comparisons) with LSD for equal variance; Descriptive statistics: Include cross- tabulations and frequencies; Non parametric tests including independent samples to identify differences between 2 or more (many spaces) groups. Chi-Square test was used to identify difference in a single field, Bivariate and cross correlations between all the parameters were examined, using Two–tailed, Pearson correlation coefficients; Regressions and curve estimations were used to show relationships between different parameters. 0.005 is the proposed P-value significance level.

3. RESULTS

One hundred and nineteen volunteers were investigated for salivary and dental plaque Na, P and Ca after a cooked meal free from sucrose, a cooked meal containing sucrose and a meal of sucrose only. Sixty (50.4%) were *miswak* users for different periods of their life, most were users for more than 10 years while fifty nine volunteers (49.5%) were tooth paste brush users, for nearly the same period of time.

A statistically significant increase in Na plaque was found in *Miswask* users following sucrose containing meal compared to cooked meal without sucrose (P=0.026). Similar significant increase in Na plaque was found in *Miswask* users following sucrose containing meal compared with plaque Na in brush users following sucrose ingestion ($P < 0.0001$). There was a statistically significant rise of Ca and phosphates levels in *Miswak* users' salivary and dental plaque compared to Ca and phosphate dental plaque of brush users' after sucrose ingestion (P=0.037 & $P < 0.0001$respectively) [Table 1].

4. DISCUSSION

In this study authors observed differences on the effects of Miswak users and tooth brush users as far as salivary and plaque electrolyte contents is concerned in relation to sucrose meals. While tooth brush users showing only an increase in salivary calcium, a significant rise was observed in both salivary Ca and plaque Na and calcium, following a sucrose meal among *Miswak* users. This feature was unique for *Miswak*users, since most recent studies have shown a decrease in concentrations of Ca, P and fluoride ions, in the presence of sucrose, so leading to plaque biofilm formation [21-23]. While demineralization is the process of removing minerals ions from the stoichiometric hydroxyapatite crystals of hard tissues (enamel, dentin, cementum, bone), remineralization is a reverse process of restoring these ions. Teeth demineralization results from the acidic effect by two ways: Acid notably content of sugary foods and drinks, and microbial attack from oral bacteria [21-23]. The balance between demineralization and remineralization is

Table 1. Dental plaque electrolytes variations with meal components

Salivary electrolytes	Meal components	*Miswak*			Tooth brush			P-value Brush-*Siwak*
		N	Mean ± SD	p-value	N	Mean±SD	p-value	
Plaque Na	Pro.+lip.+sta.	32	67.06±28.49*	0.026	16	77.69± 3.22	0.054	0.012
	Pro.+lip.+sta.+Sugars.	94	88.11±16.06					
					7	76.711±1.11		
	Sugars		95.50±5.06		9	73.78±7.48		
Salivary Ca	Pro.+lip.+sta.	43	1.67±2.24**	< 0.0001	22	0.59± 0.73	0.054	
	Pro.+lip.+sta.+sug.	10	4.40±1.84*		9	2.11±3.76		< 0.0001
				<0.0001				
	Sugars	6	4.50±2.26		27	1.07± 0.55		
Plaque Ca.	Pro.+lip.+sta.	43	2.51±2.03*		22	1.22±0.55	0.319	Not possible to calculate P-
	Pro+lip+sta.+Sugars	11	4.00±2.24		10	1.00±0.000		value
	Sugars	6	3.50±1.87	.037	8	1.00±.000		because the SD is zero.
Plaque phosphate	Pro.+lip.+sta.	43	2.56±1.33	0.526	22	2.11±1.71	0.680	< 0.0001
	Pro.+lip.+sta.+sug.	11	3.00±0.89		10	1.71±0.48		
					27	2.13±0.99		
	Sugars	5	2.40±0.89					

**pro = Protein *lip = Lipid *sta.= Starch * sug.= Sugar*

kept through calcium, phosphate, and fluoride, and also regulated by salivary pH, which plays an important role in modifying the susceptibility of tooth to caries progression [24]. Calcium releaseprecedes phosphate release from enamel, dentin, and cementum during demineralization, therefore it's suggested that suppression demineralization is more effective by using calcium rather than phosphate [25]. As already discussed, pathophysiology of mineralization and demineralization is a key determinant of dental caries formation, Ca, P, and F been critical ions involved in the mineralization of enamel and dentin in the oral environment, and reduction in their availability increases the cariogenic potential of the biofilm [2-7]. This study has clearly shown this unique feature in *Miswak* users in the provision of Ca, P critical ions for mineralization of enamel and dentin, and with significantly higher values compared to tooth brushes users.

In addition, the study reports another important finding. *Miswak*users reported a significantly higher Na plaque following ingestion of a sucrose meal, compared to other food stuffs, or when compared to brush users. This provides an added merit for the protective role of using *Miswak* sticks in a sugary meal by providing more Na plaque thus counteracting the associated drop in plaque pH with sugary meals and preserving plaque-like biofilm matrix integrity and calcium binding protein, even when challenged by an acid-induced dietary content.

5. CONCLUSION

*Miswak*sticks are suggestedto play a beneficial role in the provision of the critical ions needed for enamel and dentin mineralization thus counteracting the frequent acidic challenge induced by sucrose intake. The findings hence suggest *Miswak* useas promoting factor for plaque biofilm matrix integrity and oral hygiene against the cariogenic effects of sugary meals.

These conclusions invite further research in this issue.

ETHICAL ISSUES

The research protocol has been approved by Research Ethical Committee, Faculty of Dentistry, Khartoum University, dated 11/2/2014, No.: 119. All participating subjects signed a standardized informed consent form as a prerequisite to their participation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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