

Comparative oral health status of an adult Sudanese population using miswak or toothbrush regularly

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الدراسة شملت مجموعتين من السودانيين إحداهما تمثل مستخدمي المسواك بشكل منتظم بينما تمثل الأخرى مستخدمي الفرشاة بشكل منتظم. خضع أفراد المجموعتين لفحص لنوي إكلينيكي وجمع عينات اللعاب واللويحات الجرثومية السنوية للتحليل الجرثومي. كذلك تم فحص مادة المسواك الحامئة للبحث عن بعض العناصر المضادة للجراثيم. النتائج المستخلصة من هذا البحث والمشورة في هذا المقال تبين أن معدل حدوث التسوس بين مستخدمي المسواك أقل مقارنة بمستخدمي الفرشاة. لم يلاحظ أي فرق بين المجموعتين فيما يتعلق بمؤشرات الصحة اللثوية عدا أن الترسبات الجيرية ظهرت بشكل أقل في الأسنان الخلفية لمستخدمي المسواك. أظهرت النتائج أيضاً أن استخدام المسواك بشكل منتظم له دور فعال في تقليص نسبة الجراثيم في اللعاب واللويحات الجرثومية السنوية تحت اللثوية. مادة المسواك الحام تحتوي على مجموعات مضادة للبكتيريا تشمل Cl^- , SO_4^{2-} , SCN^- , NO_3^- . هذه النتائج تدعم جزئياً الفرضية ١ - بأن السودانيين الذين يستخدمون المسواك بشكل منتظم يتمتعون بصحة فموية وبمستويات بكتيرية فموية أفضل من اللذين يستخدمون الفرشاة بشكل منتظم. ٢ - وأن هذه الآثار المقيدة قد تكون نتيجة لشرب - من المشطال.

The objective of the present study was to conduct a systematic evaluation of miswak as an alternative tool to the modern toothbrush in preventing oral diseases. This involved clinical, microbial and chemical assessment using modern scientific methods. An adult Sudanese population using miswak or a modern toothbrush regularly was examined using clinical and microbial parameters. Freeze-dried extract of miswak was analyzed for antimicrobial components. The results showed a lower caries experience in the miswak users than in the subjects who used a modern toothbrush. There were no significant differences between the two groups in the periodontal variables examined except for less calculus in the posterior sextants of miswak users. The results also indicated that regular use of miswak had a significant inhibitory effect on the levels of some salivary and subgingival plaque bacteria. The chemical analysis of miswak extracts showed that miswak contained a number of antimicrobial components including Cl^- , SO_4^{2-} , SCN^- , NO_3^- . These findings partially support the hypothesis that (1) adult Sudanese regular miswak users have better oral health and lower levels of oral pathogens than have adult Sudanese who use a modern toothbrush regularly and (2) these beneficial effects may be due to leachable SCN^- in miswak.

Introduction

The use of miswak could be traced back at least to pre-Islamic times.¹ Currently, many of the world populations in India, Pakistan, several African countries, the Arab countries and most of the Muslim world still use miswak.² In geographical regions in which the Arak (Araak) shrub or tree (botanical name *Salvadora persica* L) grows, miswak is interpreted as tooth sticks prepared from this plant. Where *S. persica* is not growing, miswak is prepared from other suitable plants. Miswak is a pencil-sized stick 15-20 cm long and with diameter 1-1.5 cm that is prepared from the root, stem, twigs or bark. The stick is chewed or tapered at one end until it becomes frayed into a brush.

Cleansing Efficacy of Miswak

Various explanations³ for the cleansing efficacy of miswak have been offered including (i) the mechanical effects of its fibers, (ii) its release of beneficial chemicals and (iii) a combination of (i) and (ii).³ Also, when the mouth cleaning

procedure that includes brushing of teeth, gums and tongue is completed, miswak is removed from or may be left in the mouth for some additional time. Left in the mouth, it will stimulate salivation and thus, there may be a better cleansing effect.

Mechanical Effect of Miswak

Miswak is generally used for a longer period of time than is a modern toothbrush and the cleaning is usually implemented for 5 to 10 min each time.⁴ The plant fibers remove plaque and simultaneously massage the gum. Unlike a modern toothbrush, the bristles of miswak are situated along the long axis of its handle. Consequently, the facial surfaces of the teeth can be reached more easily than the lingual surfaces or the interdental spaces. Eid *et al.*⁵ reported that the majority of miswak users applied miswak to both aspects of their teeth and no significant differences in facial plaque scores were noted between the miswak and toothbrush users. Additional studies suggested that miswak efficacy was comparable to that of the conventional

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toothbrush⁶ or demonstrated plaque scores to be significantly lower following the use of miswak when compared with the conventional toothbrush used without toothpaste.⁷

Beneficial Substances in *S. Persica* Extracts

A variety of chemical components have been identified in *S. persica* extracts. Some of these have been suggested to contribute to the cleansing efficacy of miswak. Miswak extracts have also been shown to inhibit the growth of different microorganisms. In addition, a decoction of *S. persica* has been used for the treatment of many diseases. These topics have been extensively reviewed by others.⁸ It remains, however, to be shown that beneficial substances are leaching out in saliva while miswak is kept in the mouth in amounts that will benefit oral health.

Epidemiological Studies

Periodontal Disease

Low periodontal treatment needs have been reported among Saudi adults who use miswak.⁹ Furthermore, Gazi *et al.*⁷ compared the periodontal status of habitual users of miswak or toothbrush and showed that the former had lower gingival bleeding and interproximal bone height than the toothbrush users. The same authors also indicated that there were no significant differences in plaque scores and pocket depths between the two groups. Carl and Zambon¹⁰ suggested that in northern Kenya advanced periodontal disease was very rare among persons over the age of 50 years who used miswak for teeth brushing. Eid *et al.*¹¹ reported that there were no significant differences in gingival or bleeding indices between miswak and modern toothbrush users.

Caries

In a dental health survey in Sudan, Emslie¹² reported for the first time less caries in people using chewing sticks than in those using a modern toothbrush. In a controlled clinical study, Baghdady and Ghose¹³ compared the caries prevalence between Iraqi and Sudanese schoolchildren using the WHO DMFT (diseased, missing, filled teeth) index.¹⁴ They reported that Sudanese schoolchildren showed lower caries prevalence due to the use of miswak and their

diet. Similar results were noted in Saudi children aged 13 to 15 years when compared with children in western countries.¹⁵ Again, the main preventive factor reported was miswak use by these children. Carl and Zambon¹⁰ reported that dental caries was relatively rare among Kenyan primary school children who were using only miswak as an oral hygiene tool. The authors concluded that caries in adults was mostly present in older persons and usually involved the maxillary and mandibular second and first molars, which are difficult to reach for cleaning with miswak. It has also been demonstrated that users of chewing sticks not prepared from *S. persica*, had low caries prevalence compared to modern toothbrush users.¹⁶

Rationales of our Study

Surprisingly, despite the widespread use of miswak since ancient times, relatively little scientific attention has been paid to its oral health beneficial effects. In 1987, the WHO¹⁷ encouraged developing nations to use miswak for oral hygiene because of tradition, availability and low cost. Recently, it was concluded that chewing sticks may have a role to play in the promotion of oral hygiene and that evaluation of miswak effectiveness warranted further research.¹⁸

Study Hypothesis

Based on our experience from the Sudan and available information in the literature, we formulated a working hypothesis that miswak users have a better oral health and lower levels of oral pathogens than modern toothbrush users and that thiocyanate contributes to the oral health promoting effect of miswak.

Objective and Aims of the Study

The objective was to conduct a systematic evaluation of miswak as an alternative to the modern toothbrush in preventing oral diseases by using modern scientific methods of clinical, microbial and chemical assessments. Specific aims were:

1. To assess and compare in a population of adult Sudanese habitual miswak users and toothbrush users: (a) their periodontal status using prevalence of gingival bleeding, dental calculus, probing pocket depth (PPD) and clinical attachment level (CAL) as clinical

parameters; (b) salivary bacterial levels and their relationships with the periodontal status and experience of caries, respectively, of the test subjects; (c) levels and associations of subgingival plaque bacteria with respect to oral hygiene and periodontal status at the sampled sites.

2. To identify and quantify some potentially antimicrobial anionic components of *S. persica* root and stem aqueous extracts.

Materials and Methods

Study Group

The participants were employees and students at the Medical Sciences Campus, University of Khartoum, Khartoum, Sudan. A total of 213 individuals volunteered to participate in the study. Their age ranged between 19 and 65 years (mean 36.6 ± 8.7 years), and they included 201 males (mean age 36.6 years) and 12 females (mean age 35.6 years).

Inclusion Criteria

The selection criteria for inclusion in the study were dentate subjects ≥ 18 years of age who had 18 or more teeth present, had good general health, and used either miswak or toothbrush regularly as their main oral hygiene tool. In addition, a subject must not have used antibiotics during the preceding 3 months and had no present or past history of smoking cigarettes or using other tobacco products. Regular users of miswak or toothbrush were defined as individuals who reported using miswak or toothbrush, respectively, as their main oral hygiene method at least once a day for the past year. Consenting subjects were then subjected to a structured interview to assess their demographic profile and oral hygiene habits. In all, 109 subjects (age range 20-61 years, mean 36.1 years), 11 females and 98 males were regular miswak users, and 104 subjects (age range 20-65 years, mean 35.7 years), 1 female and 103 males, used toothbrush regularly. The periodontal status of the study group has been published elsewhere.¹⁹

Clinical Examinations

The periodontal status of the study subjects was assessed using the Community Periodontal Index (CPI).²⁰ Each sextant was given the highest score of examined teeth. CAL was then assessed

and was defined as the distance from the cement-enamel junction (CEJ) to the bottom of a pocket/sulcus. Caries experience was recorded as present or absent according to the WHO caries criteria.²⁰

Selection of Subjects for Biological Sampling

The inclusion criteria of this study group required at least one maxillary and one mandibular tooth with PPD ≥ 4 mm that showed gingival bleeding on probing. Using the measurements of PPD, one maxillary and one mandibular posterior tooth exhibiting 4-5 mm or ≥ 6 mm PPD and bleeding on probing were selected for bacterial sampling. If posterior teeth were missing, anterior teeth were used instead. Seventy-four subjects were sampled. These included 38 miswak users (27 males and 11 females) and 36 toothbrush users (35 males and 1 female). Fifty-six of the subjects also donated saliva samples; 30 were miswak users (19 males and 11 females) and 26 toothbrush users (25 males and 1 female).

Collection of Biological Samples

Collection of saliva from the study subjects has been previously reported.²¹ Immediately after saliva collection, the teeth of each individual selected for subgingival plaque sampling were isolated with cotton rolls, carefully scaled supragingivally with sterile Gracey curettes, cleaned with sterile cotton pellets and dried with air. A sterile curette was then inserted into the pocket and subgingival plaque was collected by multiple scaling strokes of the 6 probing sites per selected tooth. The plaque collected from one maxillary and one mandibular tooth (totally 12 sites) of each subject was pooled and immediately transferred into one Eppendorf tube containing 150 μ l of sterile TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 7.6) and the plaque samples were then suspended into the buffer by vigorous shaking.

Identification and Quantification of Bacteria

Handling of the biological samples and identification and quantification of bacteria using whole genomic DNA probes from 28 bacteria (plaque samples) or 25 bacteria (saliva samples) and checkerboard DNA-DNA hybridization²² have been previously published.²¹

Chemical Analysis of *S. Persica* Freeze-Dried Extract

Aliquots of the powdered extracts were reconstituted in sterile distilled water, filtered through a 0.2 mm membrane (Millipore Corp.) and, used for identification and quantification of potentially antimicrobial anionic components by using capillary electrophoresis.²³

Data Analysis

The percentage of subjects and the mean number of sextants per subject with gingival bleeding, calculus, PPD 4-5 mm and PPD ≥ 6 mm, and CAL as well as the number and percentage of subjects with sound teeth or having teeth with caries experience were calculated according to Darout *et al.*^{19,21} The mean values of pocket depths 4-5 mm and ≥ 6 mm was computed for each tooth and the teeth values were averaged for each subject. The analysis of variance for unbalanced data was used to compare the study subjects, and the model was adjusted for age groups (20-39 years and 40-65 years) as described by Darout *et al.*¹⁹. The percentages of subjects with various bacterial levels grouped by age, gender, periodontal status, and oral hygiene were compared by the Wilcoxon Signed Rank test. The *t* test was used to compare the percentages of subjects by caries status, as well as, the concentrations of different anionic components in miswak extracts. The Spearman correlation coefficient was utilized to assess the strength of correlation between cariogenic species ($\geq 10^5$ cells) and the caries scores and between bacterial levels. *P* value < 0.05 were considered statistically significant. A α value of 0.01 was used when multiple comparisons were made, and this adjustment was in accordance with the method of Hochberg.²⁴ The statistical analysis was performed by the SAS statistical package (SAS version 6.12, SAS Institute Inc., Cary, NC, USA).

Results

Periodontal Status

Fifty-four percent of the total study groups had one or more sextants with gingival bleeding and 31.9% dental calculus in one or more sextants. Approximately, 10% of the subjects had one or more sextants with PPD 4-5 mm and about 2% had sextants with PPD ≥ 6 mm. Fifty-one percent of the subjects had CAL ≥ 4 mm in one or more of the sextants. Subjects in the age group 40-65 years had significantly ($P=0.004$) higher numbers of sextants

with gingival bleeding and PPD 4-5 mm ($P=0.03$) and CAL ≥ 4 mm ($P=0.02$) than had the 30-39 year group. The overall effect of the two oral hygiene methods showed no marked differences as assessed by the periodontal variables (i.e. PPD and CAL) used in this population. However, when the data were reanalyzed in order to test the effect of tooth type, the results demonstrated that miswak users had significantly ($P=0.002$) lower numbers of sextants with dental calculus in the posterior sextants than had toothbrush users (Table 1).

Table 1. The mean number of sextants per subject with gingival bleeding, supragingival calculus, probing depth, and clinical attachment loss adjusted for age, by oral hygiene group and tooth type.

Variables	Oral hygiene group	Mean	S.E.	<i>P</i> -value
Anterior teeth				
Gingival bleeding	miswak	0.30	0.05	0.5
	toothbrush	0.34	0.05	
Dental calculus	miswak	0.22	0.04	0.4
	toothbrush	0.17	0.04	
Probing depth ≥ 4 mm	miswak	0.04	0.01	0.05*
	toothbrush	0.009	0.01	
Attachment loss ≥ 4 mm	miswak	0.53	0.06	0.2
	toothbrush	0.42	0.07	
Posterior teeth				
Gingival bleeding	miswak	0.94	0.12	0.09
	toothbrush	1.22	0.12	
Dental calculus	miswak	0.11	0.05	0.002*
	toothbrush	0.35	0.06	
Probing depth ≥ 4 mm	miswak	0.10	0.05	0.057
	toothbrush	0.23	0.05	
Attachment loss ≥ 4 mm	miswak	0.64	0.04	0.20
	toothbrush	0.55	0.04	

* Statistically significant

Salivary Bacteria

Subjects with one or more sextants with PPD 4-5 mm or two or more sextants with CAL ≥ 4 mm had similar levels of most salivary bacteria compared to subjects without attachment loss or deep ≥ 6 mm PPD. Presence of $\geq 10^5$ *Lactobacillus acidophilus* bacterial cells in saliva was significantly correlated with the subject's caries score ($P=0.02$). The percentages of subjects with detectable levels of *Actinobacillus actinomycetemcomitans*, *Prevotella melaninogenica*, *Campylobacter rectus*, *Peptostreptococcus micros*, *Veillonella parvula*, *Streptococcus mutans*, *Streptococcus anginosus*, *Actinomyces israelii*, *Capnocytophaga sputigena*, and *Capnocytophaga gingivalis* were significantly higher in the miswak group whereas for *Prevotella intermedia*, *Fusobacterium*

nucleatum, *Selenomonas sputigena*, *Eikenella corrodens*, *Lactobacillus acidophilus*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus oralis*, and *Streptococcus mitis* the percentages of subjects were significantly higher in the toothbrush group (Table 2). In the miswak group, 16 (53.3%) subjects had one or more teeth with primary or recurrent caries score as compared

Table 2. Percentage of subjects showing detectable levels of bacteria in saliva, by type of oral hygiene habit

Bacterial species*	Miwak (n=30) No. of bacteria		Toothbrush (n=26) No. of bacteria		P
	10 ⁵	≥10 ⁶	10 ⁵	≥10 ⁶	
<i>P. gingivalis</i>	56.7	0	50.0	7.7	0.7
<i>A. actinomycetemcomitans</i>	33.3	30.0	11.5	0	0.0001
<i>P. intermedia</i>	56.7	3.3	50.0	26.9	0.03
<i>P. melaninogenica</i>	40.0	30.0	0.0	0	0.0001
<i>F. nucleatum</i>	20.0	0	42.3	15.4	0.002
<i>T. denticola</i>	20.0	0	8.0	4.0	0.5
<i>C. rectus</i>	73.3	13.3	23.1	11.5	0.001
<i>P. micros</i>	53.3	0	0.0	0	0.0001
<i>S. sputigena</i>	60.0	10.0	34.6	57.7	0.0002
<i>S. intermedius</i>	26.7	0	34.6	0	0.5
<i>E. corrodens</i>	40.0	0	34.6	30.8	0.01
<i>V. parvula</i>	60.0	13.3	11.5	3.9	0.0001
<i>S. mutans</i>	26.7	0	3.9	0	0.03
<i>S. sobrinus</i>	3.3	0	0.0	0	0.4
<i>L. acidophilus</i>	13.3	0	46.2	0	0.01
<i>S. anginosus</i>	50.0	46.7	19.2	19.2	0.0001
<i>S. sanguis</i>	53.3	3.3	30.8	57.7	0.0001
<i>S. salivarius</i>	70.0	3.3	57.7	34.6	0.002
<i>S. oralis</i>	20.0	0	57.7	7.7	0.0005
<i>S. mitis</i>	0	0	19.2	0	0.02
<i>A. israelii</i>	50.0	0	7.7	0	0.001
<i>C. sputigena</i>	73.3	3.3	11.5	0	0.0001
<i>C. gingivalis</i>	66.7	0	7.7	0	0.0001
<i>C. gracilis</i>	46.7	0	26.9	7.7	0.6
<i>L. buccalis</i>	30.0	0	19.2	0	0.4

*Subdivided into 3 categories of bacteria: periodontitis-associated ($n=12$), cariogenic species ($n=3$), and species associated with dental health ($n=10$).

$P < 0.05$ = statistically significant.

to 36 (76.9%) subjects among the toothbrush users ($P=0.03$).

Subgingival Bacteria

The detection frequencies of the 28 investigated species at $<10^5$, 10^5 and $\geq 10^6$ bacterial cells varied between 33.8% and 100%, 1.4% and 37.8%, and 1.4 and 41.9%, respectively. Small percentages of subjects had 10^5 bacterial cells of *S. mutans* (1.4%) and *S. sobrinus* (5.4%). At 10^5 bacterial cells threshold, the detection

frequencies of the investigated species varied between 2.6% and 47.4% in the miswak group and between 2.8% and 36.1% in the toothbrush group. Similarly, the prevalences of periodontopathic species including *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythensis* (*Bacteriodes forsythus*), *P. intermedia*, *A. actinomycetemcomitans*, and *V. parvula* were between 10.5% and 36.8%, and between 2.8% and 19.4% in the two groups. There were significantly ($P=0.05$) more miswak users than toothbrush users harboring *Streptococcus intermedius*, *A. actinomycetemcomitans*, *V. parvula*, *A. israelii* and *C. gingivalis*, and significantly fewer of the former group harboring *S. sputigena*, *S. salivarius*, *Actinomyces naeslundii* and *S. oralis*. Significantly more subjects with PPD ≥ 6 mm harbored *P. gingivalis*, *T. denticola*, *T. forsythensis*, *F. nucleatum* and *V. parvula* than did the subjects with PPD 4-5 mm.

Correlations Between Salivary and Subgingival Bacteria in Autologous Samples

There were significantly higher percentages of subjects with $\geq 10^5$ bacterial cells of *P. intermedia*, *C. rectus*, *V. parvula*, *S. mutans*, *L. acidophilus*, *S. anginosus*, *S. salivarius*, and *L. buccalis*, and significantly lower percentages of subjects with *T. denticola* in saliva than in subgingival plaque ($P \leq 0.01$). Significantly higher percentages of subjects demonstrated $\geq 10^6$ bacterial cells of *S. sputigena*, *S. anginosus*, *S. sanguis* and *S. salivarius* while significantly lower percentages of subjects showed *P. gingivalis* in saliva than in subgingival plaque ($P \leq 0.01$). Significant correlations between the levels of salivary and subgingival plaque bacteria were exhibited between *P. gingivalis*, *F. nucleatum*, *S. sputigena*, *S. sanguis*, and *Streptococcus mitis* ($P \leq 0.05$) as seen in Table 3. When such correlations were made separately for miswak users and toothbrush users, the former group demonstrated significant correlation between the levels of *F. nucleatum* and *S. oralis* ($P \leq 0.01$). No significant correlations were shown between these bacterial levels in the toothbrush users.

Identification and Quantification of Some Potentially Antimicrobial Anionic Components of Miswak Extracts

The results showed that *S. persica* root and stem deionized distilled water extracts contained

Cl⁻, SO₄²⁻, SCN⁻, and NO₃⁻. However, the concentrations of these four anionic components differed considerably; stem extract contained more chloride (6.84%), sulphate (20.1%), and thiocyanate (0.38%) than did the root extracts (chloride 4.64%, sulphate 19.85%, thiocyanate

Table 3. The correlations between the levels of autologous bacteria in intra-subject subgingival plaque and saliva samples.

Bacterial species	r-values	P-values
Opportunistic bacteria		
<i>P. gingivalis</i>	0.27	0.04*
A	0.16	0.22
<i>actinomycetemcomitans</i>	0.25	0.06
<i>P. intermedia</i>	0.23	0.08
<i>P. melaninogenica</i>	0.29	0.03*
<i>F. nucleatum</i>	0.10	0.46
<i>T. denticola</i>	0.05	0.71
<i>C. rectus</i>	-0.04	0.78
<i>P. micros</i>	0.31	0.02*
<i>S. sputigena</i>	0.26	0.06
<i>S. intermedius</i>	-0.12	0.38
<i>E. corrodens</i>	-0.12	0.39
<i>V. parvula</i>	-0.14	0.29
<i>C. sputigena</i>	-0.10	0.45
<i>C. gingivalis</i>	N.c.	N.c.
<i>S. mutans</i>	N.c.	N.c.
<i>S. sobrinus</i>	N.c.	N.c.
<i>L. acidophilus</i>	-0.02	0.89
Commensal bacteria		
<i>S. anginosus</i>		
<i>S. sanguis</i>	0.23	0.09
<i>S. salivarius</i>	0.30	0.02*
<i>S. oralis</i>	N.c.	N.c.
<i>S. mitis</i>	0.25	0.07
<i>A. israelii</i>	0.26	0.05*
<i>L. buccalis</i>	-0.02	0.89
	-0.03	0.81

N.c.= no correlation

*Statistically significant

0.28%). Nitrate concentration was 0.05% in both extracts. The % values express w/w % of reconstituted freeze-dried extract.

Discussion

Resources for oral health care are limited in many developing countries and the need to explore and test easily available and inexpensive traditional preventive measures is recognized and supported by the WHO.¹⁷ This is also in line with a recent consensus¹⁸ stating that "chewing sticks may have a role to play in the promotion of oral hygiene" and that "evaluation of their effectiveness warrants further research". Our hypothesis that habitual miswak users have better oral health and lower levels of oral pathogens than individuals who use modern toothbrush regularly was not fully supported by the results of this study. The overall effect of the two oral hygiene methods

showed no significant differences regarding the periodontal variables assessed in this Sudanese population. However, in order to see if tooth type may have influenced the results, the data were re-analyzed using a bi-variate table, which is somewhat similar to the analysis of co-variance. The results demonstrated that miswak users had significantly lower numbers of sextants with dental calculus in the posterior sextants than the modern toothbrush users had. This is in line with Almas and Al-Lafi.²⁵ Miswak extracts contains high amounts of chloride²⁵ and substantial amounts of silica.²⁶ Recently, it has been shown that the commercially available dentifrice (Whitening Toothpaste, Colgate) which contains 10% silica is efficacious for control of supragingival calculus formation.²⁷ Furthermore, miswak is generally used for a longer period of time than is the modern toothbrush, the cleaning is usually done for 5 to 10 min each time,⁴ and the plant fibers remove plaque and simultaneously massage the gum. Thus, our finding that miswak users had lower numbers of posterior sextants with dental calculus than toothbrush users had may be attributed to miswak's chemical components and/or the differences in frequency and duration of brushing between the two methods.

It has been suggested that the level of supragingival calculus is a fairly good measure of the oral hygiene level and the frequency of professional dental care in a population.²⁸ Our observation that there were no significant differences in the pocket depths between habitual miswak and toothbrush users is consistent with previous reports. Gazi *et al.*⁷ had demonstrated that there were no significant differences in plaque scores and PPD measurements between habitual miswak and toothbrush users. Eid *et al.*¹¹ also indicated that there were no significant differences in plaque scores and attachment loss between habitual miswak and toothbrush users.

The lower caries experience in miswak users²¹ partially supports our working hypothesis of better oral health in the miswak group. This is also in agreement with previous epidemiological studies.^{10,13,15,16} The lower caries experience in the miswak users may possibly be explained by the results of a previous study,²³ which demonstrated that miswak extract contained thiocyanate (SCN⁻). Tenovuo *et al.*²⁹ showed *in vitro* that acid production by *S. mutans* in human dental plaque was almost totally inhibited when supplementing saliva with SCN⁻ and hydrogen peroxide. The finding of a lower caries experience of miswak

users may also be referred to the cleansing effect of miswak. For example, when the mouth cleaning procedure is completed, miswak is often left in the mouth for some additional time. Left in the mouth, it will stimulate salivation and thus promoting a better cleansing and anti-cariogenic effect.

Darout *et al.*³⁶ is the first reported applying the checkerboard DNA-DNA hybridization method²² to assess bacterial levels in saliva, in which they showed that several bacterial species including periodontitis-associated ones were detectable. This was in agreement with previous reports which used different methods to detect various bacterial species including periodontitis-associated species in saliva.^{30,31} Our results also showed that the method of oral hygiene had a significant effect on the salivary levels of 19 out of the 25 bacterial species investigated. Ten of these species were present in significantly higher numbers, and 9 were found in significantly lower numbers in the saliva of miswak users than of toothbrush users. These microbial differences may be due to release of antimicrobial substances of miswak or other factors.

Our finding that four out of the six *Streptococcus* spp. examined were detectable in significantly lower levels in the miswak group can be explained by the results reported by Darout *et al.*²³ Several of the anionic components detected in miswak extract are known to have antimicrobial effects. The higher levels of some periodontal pathogens in the saliva of miswak users²¹ may be due to a microbial shift from more streptococci to more periodontitis-associated species. If so, this would be in line with the ecological plaque hypothesis.³² This may explain the weak effect of miswak use on some oral anaerobic (*P. gingivalis*, *E. corrodens*, *S. sputigena*, *P. micros*, *T. denticola*, *C. rectus*) and facultative (*C. sputigena*, *C. gingivalis*, *A. actinomycetemcomitans*) species.

This is the first report on detection frequencies and levels of subgingival bacteria and their associations in adult Sudanese habitual miswak users and toothbrush users.³³ The results showed that the type of oral hygiene practice had a significant effect on the subgingival plaque levels of 11 out of the 28 bacterial species investigated. Using 10⁵ bacterial cells threshold, *S. intermedius*, *A. actinomycetemcomitans*, *V. parvula*, *A. israelii* and *C. gingivalis* were present in significantly higher numbers in subgingival plaque of the miswak than of the toothbrush group. *S. sputigena*, *S. salivarius*,

A. naeslundii and *S. oralis* were found in significantly lower numbers in the miswak group. When we used 10⁶ bacterial cells threshold, *P. intermedia* and *S. mitis* were significantly higher in the toothbrush group than in the miswak group. The results comply with the levels of 25 of these bacterial species in salivary samples from the same individuals.

Darout *et al.*^{23,36} showed that species including *P. gingivalis*, *T. denticola*, *C. rectus*, *F. nucleatum* and *L. buccalis* did not seem to be influenced by the type of oral hygiene used. This may suggest that the two oral hygiene methods had similar effects on the levels of these species. This is consistent with the findings of studies which showed that supragingival plaque control had little or no effect on the levels of subgingival species, at least in sites with deeper probing depths.^{34,35}

Darout *et al.*³⁶ was the first to report the use of the checkerboard DNA-DNA hybridization method to assess bacterial levels and their correlations in autologous saliva and subgingival plaque samples. Previously, Kononen *et al.*³⁷ correlated gram-negative anaerobes recovered by culture in saliva and subgingival samples of a group of young women. A relatively high percentage of the study group had detectable levels of several of the examined opportunistic and commensal bacteria in their paired saliva and subgingival plaque samples. The results of Darout *et al.*³⁶ showed that species including *P. intermedia*, *T. denticola* and *P. gingivalis* were more frequently detected in saliva than in subgingival plaque. This was consistent with a study that used PCR to assess the frequencies of six oral bacteria in paired samples of unstimulated saliva and subgingival plaque of adult subjects in the USA.³⁰ The latter study showed that *P. gingivalis*, *P. intermedia*, *P. nigrescens*, and *T. denticola* were detected more frequently in saliva than in periodontal pockets. The cariogenic bacteria *S. mutans* and *L. acidophilus* were demonstrated at detectable (10⁵ bacterial cells) levels but not at high (10⁶ bacterial cells) levels.²¹ Usually, high *S. mutans* and *L. acidophilus* counts indicate active caries or a high caries risk. This may not always be valid for Sudanese subjects who have been shown to have high counts of mutans streptococci even in populations with extremely low prevalence of dental caries.³⁸

The results of Darout *et al.*³⁶ showed that *S. mutans* and *L. acidophilus* occurred significantly more frequently in saliva than in

subgingival plaque. This was in agreement with the previous studies.^{39,40} The lower detection frequencies of mutans streptococci reported in a study³⁶ may be attributed to the difference in the threshold levels of detection frequencies used in the two studies as well as to ethnic differences.

Darout *et al.*³⁶ showed significant positive correlations between levels of *P. gingivalis*, *F. nucleatum*, *S. sputigena*, *S. sanguis* and *S. mitis*. Umeda *et al.*³⁰ used kappa statistics to estimate the agreement between bacterial levels in paired samples of saliva and pooled subgingival plaque. They found a fair agreement between saliva and plaque samples for *P. gingivalis*, *P. intermedia* and *T. denticola*, and a poor agreement for *A. actinomycetemcomitans*, *B. forsythus* and *P. nigrescens*. Except for *P. gingivalis*, our data did not support their findings.

Miswak aqueous extracts contain potentially antimicrobial anionic components including Cl^- , SO_4^{2-} , NO_3^- and SCN^- . The antibacterial and weak anti-inflammatory effects of *S. persica* root and twig extracts have been attributed to their content of beta-sitosterol, SO_4^{2-} compounds and Cl^- .⁴³ In addition, Cl^- , I^- and SCN^- (pseudohalides) are substrates for salivary peroxidase and/or the myeloperoxidase hydrogen peroxide antimicrobial system. The lower levels of *P. intermedia* and *F. nucleatum* in the miswak users²⁴ may be attributed to its Cl^- and SCN^- content.

NO_3^- in *S. persica* root and stem water extracts may be released from the residual nitrate ions taken up by the *S. persica* plant or from the oxidation of ammonia and other nitrogen compounds. Recently, Allaker *et al.*⁴⁴ reported that acidified nitrite exhibited growth-inhibitory effect on *F. nucleatum*, *E. corrodens* and *P. gingivalis*. Furthermore, SCN^- leaching out from miswak while in the oral cavity may lead to an elevated level of salivary SCN^- . This in turn may enhance the efficacy of the salivary peroxidase-thiocyanate and hydrogen peroxide system, a known innate antimicrobial component of human saliva. There are data showing that the most susceptible bacteria to this antimicrobial system are the oral streptococci, whereas other anaerobic oral bacteria (*C. sputigena*, *C. gingivalis*, *P. gingivalis*, *E. corrodens*, *S. sputigena*, *P. micros*, *T. denticola*, and *C. rectus*) were less affected.⁴⁵

Our study showed for the first time that miswak aqueous extracts contain potential anionic components including SCN^- in both free and

bound forms and for which we postulate their possible mode of action against oral bacteria. If our working hypothesis on the contribution of endogenous SCN^- to the antimicrobial effect of miswak were correct, this implies that miswak, when in the mouth, may represent an external source of leachable SCN^- . However, it remains to be shown *in vivo* that saliva extracts SCN^- from miswak in adequate amounts and that such additional SCN^- really results in a more efficient peroxidase-thiocyanate and hydrogen peroxide antimicrobial system.

Conclusions

1. The periodontal status of miswak users in this Sudanese population was similar to that of the toothbrush users, suggesting that the efficacy of miswak use for oral hygiene was comparable to that of the modern toothbrush.
2. The miswak users had significantly less calculus in their posterial sextants than had the toothbrush users which may be due to anti-calculus effect of miswak.
3. The findings suggested that miswak may also have a selective inhibitory effect on the level of certain bacteria in saliva, particularly several oral Streptococcus species.
4. The results indicated that the type of oral hygiene method used had a significant effect on the levels of 11 out of the 28 species investigated and that the effect was also dependent on type of bacteria and probing pocket depth.
5. This study indicated that the levels of *P. gingivalis*, *F. nucleatum*, *S. sputigena*, *S. sanguis* and *S. mitis* are correlated significantly in autologous saliva and subgingival plaque.
6. Miswak users showed lower caries experience and lower levels of some oral pathogens (*P. intermedia*, *F. nucleatum*, *S. sputigena*, *E. corrodens*, *L. acidophilus*, *S. sanguis*, *S. salivarius*, *S. oralis*, and *S. mitis*). This is in support of our hypothesis of better oral health and lower levels of oral pathogens in miswak users than modern toothbrush users.
7. Demonstration of high levels of thiocyanate in aqueous miswak extracts complies with our hypothesis that antimicrobial effect of miswak may be due to its thiocyanate content.

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References

1. Ring M. Dentistry. An illustrated history. New York: Abrams, 1985.
2. Corbet EF, Zee KY, Lo EC. Periodontal diseases in Asia and Oceania. *Periodontol* 2000;29:122-152.
3. Hardie J, Ahmed K. The miswak as an aid in oral hygiene. *FDI World* 1995;4:5-8 & 10.
4. Akhtar MS, Ajmal M. Significance of chewing-sticks (miswaks) in oral hygiene from a pharmacological viewpoint. *J Pak Med Assoc* 1981;31:89-95.
5. Eid MA, Selim HA, Al-Shammery AR. Relationship between chewing sticks (Miswak) and periodontal health. Part 1. Review of the literature and profile of the subjects. *Quintessence Int* 1990;21:913-917.
6. Sote EO. The relative effectiveness of chewing sticks and toothbrush on plaque removal. *Afr Dent J* 1987;1:48-53.
7. Gazi M, Saini T, Ashri N, Lambourne A. Miswak chewing stick versus conventional toothbrush as an oral hygiene aid. *Clin Prev Dent* 1990;12:19-23.
8. Wu CD, Darout IA, Skaug N. Chewing sticks: Timeless natural toothbrushes for oral cleansing. *J Periodontal Res* 2001;36:275-284.
9. Al-Khateeb TL, O'Mullane DM, Whelton H, Sulaiman MI. Periodontal treatment needs among Saudi Arabian adults and their relationship to the use of the Miswak. *Community Dent Health* 1991;8:323-328.
10. Carl W, Zambon JJ. Dental health of the Rendille and Samburu of the northern frontier district of Kenya. *N Y State Dent J* 1993;59:35-39.
11. Eid MA, Al-Shammery AR, Selim HA. The relationship between chewing sticks (Miswak) and periodontal health. 2. Relationship to plaque, gingivitis, pocket depth, and attachment loss. *Quintessence Int* 1990;21:1019-1022.
12. Emslie RD. A dental health survey in the Republic of the Sudan. *Br Dent J* 1966;120:167-178.
13. Baghdady VS, Ghose LJ. Comparison of the severity of caries attack in permanent first molars in Iraqi and Sudanese schoolchildren. *Community Dent Oral Epidemiol* 1979;7:346-348.
14. World Health Organization. Oral health surveys. Basic methods. Geneva: WHO, 1979.
15. Younes SA, El-Angbawi MF. Dental caries prevalence in intermediate Saudi schoolchildren in Riyadh. *Community Dent Oral Epidemiol* 1982;10:74-76.
16. Sathananthan K, Vos T, Bango G. Dental caries, fluoride levels and oral hygiene practices of school children in Matebeleland South, Zimbabwe. *Community Dent Oral Epidemiol* 1996;24:21-24.
17. World Health Organization. Prevention of diseases. Geneva: WHO, 1987.
18. Loe H. Oral hygiene in the prevention of carier and periodontal disease. *Int Dent J* 2000; 50:139
19. Darout IA, Albandar JM, Skaug N. Periodontal status of adult Sudanese habitual users of miswak chewing sticks or toothbrushes. *Acta Odontol Scand* 2000;58:25-30.
20. World Health Organization. Oral health surveys. Basic methods. Geneva: WHO, 1997.
21. Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE. "Checkerboard" DNA-DNA hybridization. *Biotechniques* 1994;17:788-92.
22. Darout IA, Albandar JM, Skaug N, Ali RW. Salivary microbiota levels in relation to periodontal status, experience of caries and miswak use in Sudanese adults. *J Clin Periodontol* 2002;29:411-420.
23. Darout IA, Christy AA, Skaug N, Egeberg PK. Identification and quantification of some potentially antimicrobial anionic components in miswak extract. *Ind J Pharmacol* 2000;32:11-14.
24. Hochberg, Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 1988;75:800-802.
25. Almas K, Al-Lafi T. The natural toothbrush. *World Health Forum*. 1995;16:206-210.
26. Ezmirly ST, Cheng JC, Wilson SR. Saudi Arabian medicinal plants *Salvadora persica*. *Planta Medica* 1979;35:191-192.
27. Sowinski JA, Battista GW, Petrone DM, Petrone ME, DeVizio W, *et al*. Clinical study to assess the anticalculus efficacy of a new dentifrice containing a special grade of silica (Colgate Total Plus Whitening Toothpaste): A clinical trial on adults. *J Clin Dent* 2002;13:65-68.
28. Mandel ID, Gaffar A. Calculus revisited: A review. *J Clin Periodontol* 1986;13:249-257.
29. Tenovuo J, Mansson-Raheamtulla B, Pruitt KM, Arnold R. Inhibition of dental plaque acid production by the salivary lactoperoxidase antimicrobial system. *Infect Immun* 1981;34:208-212.
30. Umeda M, Contreras A, Chen C, Bakker I, Slots J. The utility of whole saliva to detect the oral presence of periodontopathic bacteria. *J Periodontol* 1998;69:828-833.
31. Sakamoto M, Umeda M, Ishikawa I, Benno Y. Comparison of the oral bacterial flora in saliva from a healthy subject and two periodontitis patients by sequence analysis of 16S rDNA libraries. *Microbiol Immunol* 2000;44:643-652.
32. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. *Adv Dent Res* 1994; 8:263-271.
33. Darout IA, Skaug N, Albandar JM. Subgingival microbiota levels and their associations with periodontal status at the sampled sites in an adult Sudanese population using miswak or toothbrush regularly. *Acta Odontol Scand* 2003;61:115-122.
34. Heijl L, Dahlen G, Sundin Y, Wenander A, Goodson JM. A 4-quadrant comparative study of periodontal treatment using tetracycline-containing drug

- delivery fibers and scaling. *J Clin Periodontol* 1991;18:111-116.
35. Edwardsson S, Bing M, Axtelius B, Lindberg B, Soderfeldt B, Attstrom R. The microbiota of periodontal pockets with different depths in therapy-resistant periodontitis. *J Clin Periodontol* 1999;26:143-152.
 36. Darout IA, Albandar JM, Skaug N. Correlations between bacterial levels in autologous subgingival plaque and saliva of adult Sudanses. *Clin Oral Investig* 2002;6:210-216.
 37. Kononen E, Jousimies-Somer H, Asikainen S. The most frequently isolated gram-negative anaerobes in saliva and subgingival samples taken from young women. *Oral Microbiol Immunol* 1994;9:126-128.
 38. Carlsson P, Gandour IA, Olsson B, Rickardsson B, Abbas K. High prevalence of mutans streptococci in a population with extremely low prevalence of dental caries. *Oral Microbiol Immunol* 1987;2:121-124.
 39. Beighton D. The value of salivary bacterial counts in the prediction of caries activity: In: Risk markers for oral diseases. Johnson NW ed. Cambridge: Press Syndicate of the University of Cambridge; 1991, p. 313-326.
 40. Van der Reijden WA, DelleMijn-Kippuw N, Stijne-van Nes AM, de Soet JJ, van Winkelhoff AJ. Mutans streptococci in subgingival plaque of treated and untreated patients with periodontitis. *J Clin Periodontol* 2001;28:686-691.
 41. Al Lafi T, Ababneh H. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. *Int Dent J* 1995;45:218-222.
 42. Allaker RP, Silva Mendez LS, Hardie JM, Benjamin N. Antimicrobial effect of acidified nitrite on periodontal bacteria. *Oral Microbiol Immunol* 2001;16:253-256.
 43. Courtois P, Majerus P, Labbe M, Vanden Abbeele A, Yourassowsky E, Pourtois M. Susceptibility of anaerobic microorganisms to hypothiocyanite produced by lactoperoxidase. *Acta Stomatol Belg*