

## The Effect of Miswak and Toothbrush on Saliva Total Bacterial Count and Cariogenic Bacteria

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**ABSTRACT** Miswak, a chewing stick prepared from the roots, twigs or stems of *Salvadora persica*. The beneficial effects of miswak in respect of oral hygiene and dental health are partly due to its mechanical action and partly due to its pharmacological actions. The aim of this study was to evaluate the effects of miswak sticks, miswak extract and toothbrush/toothpaste as antimicrobial agents on total bacterial count in the saliva, and to study the effect of miswak stick, toothbrush/toothpaste and saline on salivary *Streptococcus mutans* (*S. mutans*) and lactobacilli in vivo. The study clarified that there was a marked reduction in the total bacterial count among all groups. The reduction was 100%, 100%, 97.5% and 67.5% among users of miswak stick, miswak extract, toothbrush/toothpaste and saline (control), respectively. The results of the current study also showed marked reduction of *S. mutans* in 66.7% of the individuals using miswak and among individuals using toothbrush/toothpaste, whereas the reduction was only 29.2% among saline users. The reduction of lactobacilli was 62.5%, 58.3% and 54.2% by using miswak stick, toothbrush/toothpaste and saline, respectively. The difference was not statistically significant. It can be concluded that miswak has an antimicrobial effect comparable to the use of fluorinated toothpaste. It was clear from the current study that the Caries Risk Test (CRT) kit is a rapid and a simple, semi-quantitative method for counting lactobacilli and *S. mutans*, which can be used in dental clinics without the need for trained technicians. It is recommended that further studies can be performed on larger sample size of patients, at high risk of developing dental caries.

### INTRODUCTION

Dental caries and periodontal diseases are the two main afflictions to mankind. Bacterial plaque is solely responsible for the initiation and progression of periodontal diseases. (1,2) Several microorganisms have been characterized as having high cariogenic potential, but two classes of microorganisms, *Streptococcus mutans* (*S.*

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*mutans*) and lactobacilli have been exclusively studied. <sup>(3)</sup>*S. mutans* has been strongly associated with the initiation of caries, while lactobacilli have been mainly connected with the further development of caries lesion. <sup>(4)</sup>

The methods available for maintenance of oral health are mainly mechanical and chemical. Toothbrushes and dentrifices are widely used for cleaning teeth. Miswak , a traditional toothbrush or chewing stick, is deeply rooted in Islamic culture.<sup>(2,5)</sup> It is a chewing stick prepared from the roots, twigs or stems of *Salvadora persica* (*S. persica*) which is an upright evergreen small tree or shrub. The leaves of *S. persica* are small, oval, thick and succulent with a strong smell of cress or mustard. The fresh leaves are eaten as salad and are used in traditional medicine for cough, asthma, scurvy, rheumatism, piles and other diseases. The flowers are small and fragrant and are used as a stimulant and are mildly purgative. <sup>(2)</sup> The beneficial effect

of miswak in respect of oral hygiene and dental health are partly due to its mechanical action and partly due to its pharmacological actions. <sup>(1)</sup>

It has been shown that extracts of miswak posses various biological properties including significant antibacterial and anti-fungal effects. Extracts of *S. persica* and other related plants may be effective against the bacteria that are important for the development of dental plaque.<sup>(6,7)</sup> Therefore, it has been claimed that, miswak sticks may have antiplaque effects and may also affect the pathogenesis of periodontal diseases by reducing the virulence of periodontopathogenic bacteria. <sup>(8)</sup>

The anti-microbial and cleaning effects of miswak have been attributed to various chemicals detectable in its extracts. These effects are believed to be due to its high content of sodium chloride and potassium chloride as well as salvadourea and saponins, tannins, vitamin C, silica, and

resin, in addition to cyanogenic glycoside and benzylisothio-cyanate. (7,9)

It has also been claimed that the vitamin C and sitosterol content of this plant have great roles in strengthening the gum capillaries and preventing gum inflammation. (9,10) Sulfated compounds and isothiocyanate are known to be responsible for antibacterial effects of the plant, while fluoride and calcium salts are quite effective in preventing dental caries. (11,12) Moreover, the silica and calcium salts in the plant act as grinder and detergent.(13) Trimethylamine is known to be effective in reducing surface adhesion and also in decreasing plaque accumulation. Tannins, tannic acid and benzyl isothiocyanate, are reported to have antimicrobial effects and help the healing of gum inflammation. (12,13)

The World Health Organization (WHO) has recommended and encouraged the use of these sticks as an effective tool for oral hygiene, because of tradition, availability and low cost, (1,5) however, its

antibacterial value has not been fully substantiated. Therefore, this study was carried out.

### **AIM OF THE WORK**

The aim of this research is to evaluate the effects of miswak sticks, miswak concentrate , and toothbrush/toothpaste as antimicrobial agents on total bacterial count in the saliva as well as on salivary *S.mutans* and lactobacilli in vivo.

### **MATERIAL AND METHODS**

A randomized controlled trial was carried out in Ajman University for Science and Technology.

#### **MATERIAL:**

- Sealed miswak sticks
- Miswak extract:
  - Preparation: Miswak sticks sealed in plastic bags were purchased from an alternative medicine shop in Sharjah, UAE. Miswak which is sold unsealed with street vendors have been found to be highly contaminated by spore-bearer bacteria which may

exacerbate dental problems, whereas sealed Miswak is completely free of any type of bacteria. Therefore, we used the sealed Miswak in our study.

- The miswak sticks were cut into small pieces and allowed to dry at room temperature for two days. They were then ground to powder by using an Omni mixer ( MFRS and Distrs, Conn. USA). Ten grams of the powdered miswak were transferred to sterile wide-mouthed screw capped bottles of 200 ml value, 100 ml of sterile de-ionized distilled water was added and the miswak powder was allowed to soak for 48 hours at 4 °C. The mixtures were then centrifuged at 2000 rpm for 10 minutes at 4°C. The supernatants were filtered through a 0.45µm membrane (Millipore Corp., Bedford, MA, USA) and the extract was prepared at 50% concentration

in sterile de-ionized distilled water.

The extract was stored at 4°C and used within one week. <sup>(7,9)</sup>

- Toothbrush with toothpaste (Signal-ingredients: water, hydrated silica, sorbitol, Zinc citrate, Sodium Lauryl sulphate, Flavor, Cellulose gum, Sodium fluoride)

#### **SUBJECTS:**

The study subjects were divided into two groups to be studied simultaneously with our aim.

1. **Group A:** One hundred and sixty subjects were chosen randomly from the staff members working at Ajman University, and who accepted to volunteer to participate in this study. The individuals selected were medically healthy with no systemic diseases and did not use any antibiotic or mouth gargle for the past two weeks. They were further subdivided into 4 subgroups of 40 individuals each. Subjects who used more than one

method were excluded.

- a. Miswak stick Group: Forty subjects were asked to use the miswak stick for 6 minutes.
  - b. Miswak extract Group: Forty subjects were asked to rinse their mouth with the miswak extract for 6 minutes.
  - c. Toothbrush with toothpaste Group: Forty subjects were asked to brush their teeth for 6 minutes.
  - d. Saline (control) Group: Forty subjects were asked to rinse their mouth with saline for 6 minutes.
2. **Group B:** Seventy-two healthy individuals (criteria as for Group A) divided into three groups of twenty-four each, were chosen randomly from the staff and the students working at Ajman university. The same procedure was carried on the three groups (the use of miswak stick, toothbrush/toothpaste and saline).

#### **SAMPLES:**

From all studied subjects, a saliva sample

(2ml) was collected in a sterile container before and after the use of the allotted method.

Stimulated saliva was collected from each individual, by giving each a small piece of paraffin wax (1cm long) and was asked to chew for a period of 30 seconds, then, to swallow any saliva but not the paraffin wax. Thereafter, the patients continued to chew the paraffin wax and saliva was collected at 2 minutes intervals for a period of six minutes.

#### **METHODS**

1. **Group A:** 10 $\mu$ l of saliva of each sample was evenly distributed on Brain heart infusion agar with 5% sheep blood. The plates were incubated at 37°C for 24 hours. Following incubation, the number of the isolated colonies present was determined and multiplied by a factor that converts the volume of saliva to one ml. The final calculation was then equal to the number of organisms per ml of sample. <sup>(10)</sup>

2. **Group B:** The levels of *S. mutans* and lactobacilli in salivary samples were measured using commercial Caries Risk Test (CRT -Vivacare line CRT two bacteria in 1 kit, Vivadent, Liechlenstein/Europe).
- The kit comprised a slide attached to the cover of the vial.
  - The commercial product had one side of the slide coated with a solid selective culture medium (mitis salivarius agar enriched with sucrose) for the cultivation of *S. mutans*, while the medium on the other side of the slide (Rogosa agar) was for the cultivation of lactobacilli.
  - The salivary samples were used as per instructions of the manufacturer. The samples were incubated at 37°C for 48 hours.
  - Growth density of the bacteria was evaluated under good lighting condition by naked eyes and as per manufacturer's instructions. (Figure 1)

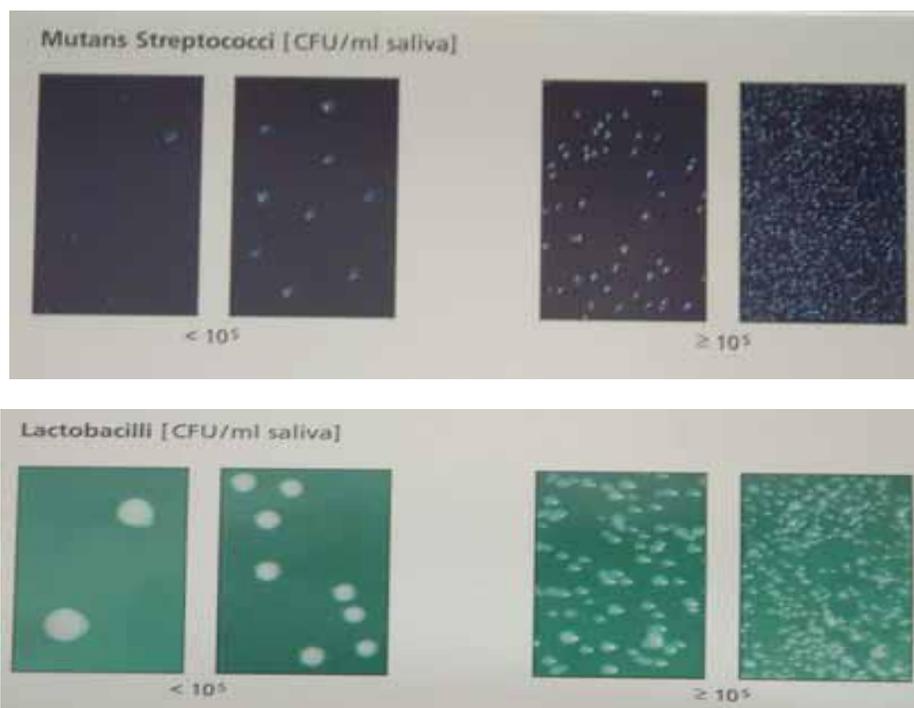


Figure (1)

### Statistical analysis

The data were entered and analyzed using computer program SPSS version "13". The following was performed: <sup>(14)</sup>

- Descriptive statistics as mean and standard deviation for the age was carried out.
- Kolmogorov-Smirnov test was used to test the distribution of the data.

The data were not normally distributed so the nonparametric statistics was used.

- Wilcoxon signed rank test was used to test the difference in bacterial counts before and after use of various agents( toothbrush, miswak , miswak concentrate and saline)
- Kurskal Wallis-H test with post hoc Turkey test was used for comparison of

bacterial counts after use of various agents.

- Chi-square test was used for categorical data.
- Z test was used for comparison of two proportions.
- $P < 0.05$  was set as the cut of level of significance

## RESULTS

### ***Total bacterial counts among users of various agents***

The present study comprised four groups of 40 participants each according to the use of toothbrush, miswak, miswak concentrate and saline. Among the miswak and toothbrush users, females outnumbered males (80%), the mean age was  $29.78 \pm 6.60$  years,. Regarding the miswak concentrate or saline users, 72.5 % were females, mean age was  $30.07 \pm 3.20$  years and  $30.5 \pm 4.82$  years, respectively.

Table (1) shows the total bacterial counts before and after use of various

agents. The table clarified that there was a marked significant reduction in the total bacterial counts among all groups. Kurskal Wallis test ( H test) and post hoc turkey test yielded significant difference in the bacterial counts after use of miswak stick, miswak concentrate and the toothbrush with toothpaste compared to the saline (  $H = 45.92$  ,  $p < 0.05$ ).

Table (2) represents the frequency of distribution of bacterial reduction among participants after use of various agents. The table portrayed that 82.5% ,70 % , 52.5% and 32.5 % of miswak stick , toothbrush, miswak concentrate, and saline users, respectively showed two log reduction in the bacterial counts . Four logs reduction was only noticed among miswak concentrate users (10%). All participants whom used miswak or miswak concentrate (100.0%) and 97.5 % of toothbrush users experienced reduction in the bacterial counts, compared to the saline users (67.5 %). This was

statistically significant ( $Z = 3.64$  and  $3.24$ , respectively,  $P < 0.05$ ).

**Table (1): Bacterial count among participants before and after use of various agents**

Group	Bacterial counts		Wilcoxon signed rank test
	Before	After	
	median	median	
Toothbrush	$10^5$	$10^3$	5.52*
Miswak stick	$10^5$	$10^3$	5.61*
Miswak stick concentrate	$10^5$	$10^3$	5.56*
Saline	$10^5$	$10^4$	4.58*
H test	3.45	54.92*	

**Table (2): Frequency distribution of total bacterial count reduction among participants after use of various agents**

Bacterial counts reduction		used Agents			
		Toothbrush (n=40)	Miswak stick (n = 40)	Miswak stick concentrate (n = 40)	Saline (n = 40)
<b>No reduction</b>	<b>No %</b>	<b>1 2.5</b>	<b>0 .00</b>	<b>0 .00</b>	<b>13 32.5</b>
One log reduction	No %	4 10.0	6 15.0	2 5.0	14 35.0
Two log reduction	No %	28 70.0	33 82.5	21 52.5	13 32.5
Three log reduction	No %	7 17.5	1 2.5	13 32.5	0 .00
Four log reduction	No %	0 .00	0 .00	4 10.0	0 .00
<b>Number with reduced bacterial count</b>	<b>No %</b>	<b>39 97.5</b>	<b>40 100.0</b>	<b>40 100.0</b>	<b>27 67.5</b>
<b>Test of significance</b>		<b>Z = 3.24*•</b>		<b>Z = 3.64*#</b>	

\* $P < 0.05$

• Significant difference between toothbrush and saline

# Significant difference between miswak , miswak concentrate and saline

***Streptococcal and lactobacilli counts among users of various agents***

Three groups of 24 subjects each were the sample used to test the effect of using toothbrush, miswak and saline on *Streptococci mutans* and lactobacilli counts. Among the toothbrush users, the mean age was  $27.5 \pm 8.10$  years and 66.7 % were females, For the miswak group, there was 75 % females, mean age was  $29.17 \pm 6.51$  years. Regarding the saline users group, mean age was  $31.17 \pm 6.45$  years and more than 90 % were females (91.7 %).

Table (3) revealed that there was one log reduction of *mutans streptococci* (median 90 % of change) after using

different agents as toothbrush, miswak stick and saline. It was also clear that among the toothbrush or miswak users, 66.7 % showed reduction in *Streptococcus mutans* counts while 33.3 % did not show any change. Among the saline users the reduction was only noticed among 29.2 %. This difference was statistically significant ( $X^2_2 = 9.06, P < 0.05$ )

Reduction of lactobacilli is presented in table (4). The one log reduction was also 90 % of change). It was noticed that, 62.5 % of miswak users showed change in antibacterial activity (reduction) compared to toothbrush or saline (58.3% and 54.2%, respectively), but the difference was not statistically significant,  $P > 0.05$ .

**Table (3) : Frequency distribution of reduction in mutans streptococcal counts among users of various agents**

Mutans streptococci counts		Agents used		
		Toothbrush	Saline	Miswak stick
One log reduction	No	16	7	16
	%	66.7	29.2	66.7
No reduction	No	8	17	8
	%	33.3	70.8	33.3
Total	No	24	24	24
	%	100.0	100.0	100.0

$X^2_2 = 9.06, P < 0.05$

**Table (4): Frequency distribution of reduction in lactobacilli counts among users of various agents**

Lactobacilli counts		Agents used		
		Toothbrush	Saline	Miswak stick
One log reduction	No	14	13	15
	%	58.3	54.2	62.5
No reduction	No	10	11	9
	%	41.7	45.8	37.5
Total	No	24	24	24
	%	100.0	100.0	100.0

$X^2_2 = 0.34, p > 0.05$

## DISCUSSION

A number of factors are necessary for caries to occur. These factors include the interaction of the susceptible host, oral flora, and the substrate which is primarily sucrose which should be present for a sufficient length of time. Removal of dental Plaque is effective in treating gingivitis, preventing periodontal disease and dental caries. Plaque covering early caries lesions usually contains high levels of *S. mutans* and lactobacilli.<sup>(15)</sup>

Tooth brushing can reduce the likelihood of caries by reducing number of cariogenic organisms and removing the substrate. Tooth brushing is the most common method used to remove plaque. However, 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 strongly needed.<sup>(16)</sup>

The present study revealed a one log reduction in the total bacterial counts

among all groups and as clear from table 2, there was significant total bacterial count reduction 82.5%, 70%, 52.5% and 32.5% by the use of miswak stick, toothbrush/toothpaste, miswak concentrate, and saline, respectively. Batwa et al have demonstrated, in vitro, that the aqueous extracts of miswak have growth inhibitory effects on several oral microorganisms.<sup>(15)</sup> Darout and Skaug also reported that the periodontal status of miswak users was better than that of toothbrush users.<sup>(7)</sup> Also, our results are strongly supported by that reported by Socransky et al that lower caries is experienced among miswak users.<sup>(17)</sup> In addition, this is also in agreement with previous epidemiological studies.<sup>(18, 19, 20)</sup> On the other hand, some studies found that there were more plaque formation and gingival bleeding among individuals who used chewing sticks in comparison with toothbrush users. The difference between one study from another may be attributed to the different groups of

patients or subjects involved in each study, method of miswak used and the source of miswak. In the present study, miswak which is found with street vendors and sealed Miswak were tested for the presence of bacteria.

The present study results (table 3) revealed that 66.7 % of the samples showed reduction in *mutans streptococci* counts by the use of miswak and toothbrush/toothpaste. Among the saline users the reduction was only noticed among 29.2 %. This difference was statistically significant. Whereas, reduction of lactobacilli as presented in table (4) was 62.5 % among miswak users compared to toothbrush or saline (58.3% and 54.2% , respectively). But the difference was not statistically significant. The difference in the reduction between *mutans streptococci* and lactobacilli may be attributed to that lactobacilli are not as susceptible to the miswak ,and toothbrush as much as the *Streptococcus mutans*. Epstein et al.,

reported in their study that a trend toward reduction of *S. mutans* counts was seen in those patients who used the fluoride gel regularly. On the other hand, in the same study high lactobacilli counts were found in the patients with high and low compliance with fluoride gel use, which partly explains our findings that lactobacilli was not reduced by the use of toothbrush/toothpaste as much as by the use of miswak. <sup>(21)</sup> The present study results are in agreement with Darout and Skaug results regarding lactobacilli, that Miswak users had lower results of cariogenic bacteria than toothbrush users.<sup>(7)</sup> Almas and Al Zeid reported similar findings to our results, in which the results showed a marked reduction of *S.mutans* among all groups, but there was no significant difference for lactobacilli. They explained that this may be due to the amount of lactobacilli present in saliva at the time of collection. <sup>(9)</sup>

Although saliva is thought to possess no

distinctive microbiota of its own, it harbors as much as  $10^8$  or  $10^8$  bacteria/ml. These high numbers reflect micro-organisms derived from oral biofilms adhering to teeth and oral mucosal surfaces, gingival crevices, and periodontal pockets <sup>(22)</sup>. In addition to the previous reason, saliva was used for the bacterial count instead of plaque samples because saliva represents the oral load of the microorganism as well as their average colonization in the dentition. In addition, plaque samples represent only a single site, and there is a wide variation in bacterial numbers among different tooth surfaces. Moreover, saliva samples are less time consuming and easier to collect outside dental clinics. <sup>(9)</sup> It was also reported by Darout et al, that *S. mutans* and lactobacilli occurred significantly more frequently in saliva than in subgingival plaque. <sup>(22)</sup>

### CONCLUSION & RECOMMENDATIONS

It can be concluded from the present study that Miswak has an antimicrobial and

prophylactic effect comparable to the use of toothbrushing with fluorinated toothpaste. Therefore, miswak can be a good alternative to the toothbrush since it is inexpensive, and readily available. Miswak contains many medicinal properties, and is available in most rural areas of the poor countries. In addition, miswak does not need expertise or expensive resources to manufacture it. Thus it is recommended as an important and effective tool for oral hygiene.

It was also clear from the current study that the CRT kit is a rapid and a simple, semi-quantitative method for counting lactobacilli and *S. mutans*, which can be used in dental clinics without the need for trained technicians.

It is recommended that further studies can be performed by the use of serial dilution of the saliva samples to get accurate counts of cariogenic bacteria. More studies can also be performed on larger samples of patients that are

diagnosed to be a high risk group of developing dental caries.

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