Efficacy of Miswak toothpaste and mouthwash on cariogenic bacteria

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ABSTRACT

الأهداف: تقييم تأثير منتجات السواك مقارنة بمعجون الأسنان الاعتيادي على البكتريا المسببة للتسوس.

الطريقة: أجريت الدراسة في مدينة زاخو، اقليم كردستان، العراق في الفترة الممتدة من أكتوبر 2013 إلى يناير 2014. أُجريت تجربة منضبطة مغشاة على 40 طالباً ضمن 4 مجموعات وقد طُلب من كل مجموعة استعمال معجون السواك وغسول السواك ومعجون اعتيادي مع الماء أو محلول الملح. أُخذت نماذج لعابية ضمن 3 فترات زمنية مختلفة قبل وبعد الاستعمال وبعد أسبوعين من الاستعمال. تم حساب تأثير كل طريقة على المكورات العقدية الطافرة البكتريا والعصيات اللبنية باستعمال اختبار مخاطر التسوس.

النتائج: أجريت التحليلات الإحصائية باستخدام اختبارات ANOVA باتجاه واحد، ANOVA باتجاه واحد متعدد القياسات، واختبار LSD. كان لغسول السواك تأثير هام في تقليل البكتريا من كلا النوعين مباشرة وبعد أسبوعين من الاستعمال، بينما كان لمعجون السواك تأثير هام في تقليل معدل العصيات اللبنية في كلا الفترتين في حين أظهر نقصاً معنوياً فقط بعد أسبوعين من الاستعمال في معدل المكورات العقدية الطافرة، في حين لم يبد المعجون العادي أي دور ملحوظ في تقليل كلا النوعين في كلا الفترتين بينما كان لاستعمال غسول الملح تأثيراً ملحوظاً على كلا النوعين بعد أسبوعين من الاستعمال.

الخاتمة: كان لمنتجات السواك وخاصة الغسول تأثيرها الفعال في تقليل نمو البكتريا المسببة للتسوس مقارنة مع المعجون العادي.

Objectives: To evaluate the efficacy of *Salvadora persica* (Miswak) products on cariogenic bacteria in comparison with ordinary toothpaste.

Methods: The study was conducted in Zakho city, Kurdistan region, Iraq during the period from October 2013 to January 2014. A randomized controlled clinical trial of 40 students randomly allocated into 4 groups. They were instructed to use Mismark toothpaste, Miswak mouthwash, and ordinary toothpaste with water or with normal saline. Salivary samples were collected at 3-time intervals: before, immediately after use, and after 2 weeks of use. The effect of each method on *Streptococcus mutans* and Lactobacilli was evaluated by using caries risk test.

Results: One-way repeated measure analysis of variance (ANOVA), one-way ANOVA, and least significant difference tests were used. Miswak wash has a significant reduction effect on both bacteria immediately and after 2 weeks of use. Miswak paste has a similar effect on Lactobacilli, while *Streptococcus mutans* showed a significant decrease only after 2 weeks of use. Ordinary paste showed a non significant effect on both bacteria at both time intervals; while the addition of normal saline showed a significant effect on both bacteria only after 2 weeks of use.

Conclusion: Miswak products, especially mouth wash, were more effective in reducing the growth of cariogenic bacteria than ordinary toothpaste.

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Dental caries is a progressive disease of teeth. It is widely distributed globally; as one of the most prevalent chronic oral diseases.[1] The disease is multi factorial in origin that starts with microbiological shifts within the complex biofilm (dental plaque). Oral biofilms are important in the causation of dental caries.[2] The most incriminated caries causing bacteria are *Streptococcus mutans* (*S. mutans*) and Lactobacilli; and their role has been reviewed extensively through many studies.[3,4] It is commonly accepted that *S. mutans* is responsible for the initiation of enamel caries due to its cariogenecity;[4,5] while Lactobacilli are secondary invaders, rather than initiators.[6] The claimed beneficial effects of Miswak in respect to oral hygiene and dental health are due to its mechanical and pharmacological actions.[7] Studies showed that Miswak has antibacterial and anti-inflammatory activities and its use in mouth as rinses or toothpastes has been recommended.[8] Several studies were conducted on measuring its effect on gingival and oral health in general. Despite that, however, few studies have tested such effect on cariogenic bacteria. This is due to the complexity of oral flora and difficulties of measuring such effect. The aim of this study is to examine the effect of Miswak products in comparison with ordinary toothpaste on *S. mutans* and Lactobacilli.

Methods. The study was approved by the Scientific Committee of Duhok college of Medicine and by the Ethical committee of Duhok General Directorate of Health. A search on website was conducted to find prior related works. The study was carried out in Zakho city, Kurdistan region, Iraq. The study period was from the beginning of October 2013 to the end of January 2014. A sample of 40 students (20 males and 20 females) was taken at random from participants in a cross sectional study of 402 secondary school students; who were also selected at random by the same authors for estimation of dental caries index.[9] An inclusion and exclusion criteria were settled for the selection of the 40 students. The inclusion criteria were: age 16-18 years, presence of at least 2 carious teeth, and being with the habit of tooth brushing. The exclusion criteria were: presence of systemic diseases, receiving any kind of antibiotics or mouth washes for the last 2 weeks before initial examination, tobacco smokers, and history of allergy to any component of the products being used. The study was according to the principles of Helsinki declaration. At the start, an oral consent was taken from the selected 40 students who were then allocated by simple random allocation, according to gender, to be in one of the 4 groups. We chose blocks at random to create the allocation sequence. Accordingly, 4 groups of 10 students (5 males and 5 females) were generated as follows: group 1 - Miswak toothpaste group: instructed to use Miswak toothpaste twice daily (morning and before sleeping) for 2 weeks; group 2 - Miswak mouthwash group: instructed to use ordinary toothpaste and to rinse after that with Miswak mouthwash twice daily (morning and before sleeping) for 2 weeks; group 3 - Ordinary toothpaste group: instructed to use ordinary toothpaste twice daily (morning and before sleeping) for 2 weeks; and group 4 - Normal saline group: instructed to use ordinary toothpaste and to rinse after that with normal saline twice daily (morning and before sleeping) for 2 weeks. All randomization and allocations to the groups were carried out by the department statistician. The materials used were: Miswak toothpaste (Siwak.F, toothpaste with Siwak and fluoride, 190 G, Saudia Arabia), Miswak mouthwash (Sarakan antiplaque mouth wash with natural extract of *Salvadora persica*, S. G, R. Lane Health Products Ltd, UK), and ordinary toothpaste (Doctor Toothpaste, 120 G, China). All subjects were supplied with specified tooth brush type (medium size bristle) and taught to use same technique, frequency and duration of brushing or rinsing (3 minutes). All groups were also asked not to rinse with any preventive solution during the study period other than that specified. From each group a pre intervention saliva samples were first collected (2 ml) by stimulation using paraffin chewing wax gum and sterile glass test tube at least 2 hours, or more after the last meal. All participants were asked to use the specified method of intervention then second saliva samples were immediately collected. For the normal saline group the second sample was collected after rinsing with normal saline for 3 minutes. All groups were instructed to keep on using the specified method of cleaning and a third sample was then collected 2 weeks after following the same procedure; also 2 hours, or more after the last meal, but with no rinsing. *Streptococcus mutans* and Lactobacilli counts in saliva were measured by caries risk test (CRT) kit (Ivoclar Vivadent AG, FL-9494 Schaan, Liechtenstein, Europe, Exp. 2014-09). The counts were evaluated according to the kit instructions; where the results expressed quantitatively as colonies per ml. The kit included 2 selective medias incorporated together on each side of the slide: Rogosa agar for Lactobacilli and *S. mutans* agar enriched with sucrose for *S. mutans*. Carbon dioxide tablets were used to increase the CO_2 concentration to favor bacterial growth. Each part of the slide was covered with saliva by pipette and the slide was then inculpated for 2 days at 37°C. Two distinguished morphology were obtained: small black colonies for

S. mutans and white colonies for Lactobacilli. The bacterial growths were also further confirmed by gram stain and biochemical reactions. Growth density of the bacteria was evaluated by the naked eye according to the manufacturer's instructions. Bacterial growth was then scored by comparing with standards expressed in colony forming units (CFU) provided by the manufacturers. For the purpose of this study, a modification of the scores used by Almas and Al-Zeid[10] was adopted. The growth intensities were sub divided into 4 scores for both bacteria as follow: 1= No growth, or very few colonies are detected ≤10^2 CFU; 2= Scanty growth are detected ≤10^4 CFU; 3= Moderate growth are detected ≤10^5 CFU; and 4= Confluent growth are detected >10^5 CFU.

Statistical analyses. Statistical analyses were conducted by using the Statistical Package for Social Sciences version 22 (IBM Corp., Armonk, NY, USA). One way repeated measure analysis of variance (ANOVA) was used for intra group analysis and one way ANOVA was used for inter groups' analyses. Least significant difference (LSD) test was used for pair wise comparisons. The differences were considered significant at $p \leq 0.05$.

Results. **Table 1** shows the mean bacterial scores before and after intervention for the 4 groups. Among Miswak toothpaste users, there was no significant reduction of *S. mutans* immediately post intervention compared with a highly significant decrease at 2 weeks post intervention. Lactobacilli showed highly significant reductions at immediate and 2 weeks post intervention. On the other hand, those who used ordinary toothpaste with Miswak mouthwash had a highly significant decrease in bacterial growth at both immediate and after 2 weeks post intervention for both Lactobacilli and *S. mutans*. **Table 1** also reveals that there was no significant reduction for both bacteria at all times intervals among those using ordinary toothpaste. Finally, normal saline rinse has no significant effect on both bacteria immediately post intervention. However, a significant decrease was detected for both bacteria after 2 weeks of using normal saline rinse with ordinary tooth. **Table 2** illustrates inter-group comparisons for the mean Bacterial scores of Lactobacilli at different time intervals. There was no significant immediate effect between Miswak toothpaste and Miswak mouthwash, while Miswak mouthwash was significantly more effective in reducing Lactobacilli than Miswak toothpaste after 2 weeks. **Table 2** also shows no significant immediate effect if ordinary toothpaste was used with tap water or with normal saline, while after 2 weeks normal saline was more effective in reducing Lactobacilli than tap water used with ordinary toothpaste. Miswak toothpaste was more effective in reducing Lactobacilli immediately and after 2 weeks than ordinary toothpaste. The difference between Miswak toothpaste and ordinary toothpaste used with normal saline wash showed no significant effect on Lactobacilli at all time intervals. The difference between Miswak mouthwash and ordinary toothpaste shows highly significant reduction effect immediately and after 2 weeks. The difference between

Table 1 - Pre and post intervention mean bacterial scores by type and period of intervention among 402 students.

Type of intervention	Pre intervention		Immediate post intervention		2 weeks post intervention	
	Lacto	*S. mutans*	Lacto	*S. mutans*	Lacto	*S. mutans*
Miswak toothpaste	2.8 ± 0.79	2.9 ± 8.8	1.9 ± 0.88	2.4 ± 0.84	2.1 ± 0.88	2.0 ± 0.67
P-value (LSD)			<0.001*	0.174*	0.005†	<0.001†
P-value (ANOVA)‡			(Lacto = 0.001, *Strepto* = 0.003)			
Miswak mouthwash	3.2 ± 0.79	2.9 ± 1.1	1.8 ± 0.92	1.3 ± 0.68	1.3 ± 0.48	1.2 ± 0.42
P-value (LSD)			<0.001*	<0.001*	<0.001†	<0.001†
P-value (ANOVA)‡			(Lacto <0.001, *Strepto* <0.001)			
Ordinary toothpaste	3.4 ± 0.52	3.5 ± 0.53	2.9 ± 0.57	3.3 ± 0.82	3.0 ± 0.82	3.0 ± 0.82
P-value (LSD)			0.067*	0.451*	0.137†	0.070†
P-value (ANOVA)‡			(Lacto = 0.149, *Strepto* = 0.181)			
Normal saline	2.9 ± 1.10	3.2 ± 0.79	2.6 ± 0.84	2.8 ± 1.14	2.0 ± 0.94	2.4 ± 0.97
P-value (LSD)			0.125*	0.217*	<0.001†	0.020†
P-value (ANOVA)‡			(Lacto <0.001, *S. mutans* = 0.061)			

*Immediate post intervention, †2 weeks post intervention, ‡Overall repeated measures one-way analysis of variance (ANOVA), Lacto - Lactobacilli, *Strepto - Streptococcus, S. mutans - Streptococcus mutans,* LSD - least significant difference

Miswak mouthwash and normal saline wash shows no significant effect after 2 weeks, while immediately Miswak mouthwash was significantly more effective. The overall repeated measure one-way ANOVA revealed no significant difference at before, while it was highly significant at both immediate and after 2 weeks intervals. **Table 3** reveals inter-group comparisons for the mean Bacterial scores of *S. mutans* at different time intervals. Miswak mouthwash was significantly more effective in reducing *S. mutans* than Miswak toothpaste immediately and after 2 weeks. The difference between ordinary toothpaste used with tap water, or with normal saline wash showed no significant effect at all times intervals. Miswak toothpaste was more effective than ordinary toothpaste; immediately and after 2 weeks. The difference between Miswak toothpaste and ordinary toothpaste with normal saline wash was not significant at all times intervals, while it was highly significant between Miswak mouthwash and ordinary toothpaste. The difference between Miswak mouthwash and normal saline wash was significant at first, but not after 2 weeks of use. The overall one-way ANOVA

Table 2 - Inter-group comparisons for the mean bacterial scores of Lactobacilli at different time intervals among 402 students.

Study groups	Before intervention		Immediately post intervention		2 weeks post intervention	
	Mean ± SD	*P*-value	Mean ± SD	*P*-value	Mean ± SD	*P*-value
Miswak toothpaste	2.80 ± 0.79	0.905	1.90 ± 0.88	0.778	2.10 ± 0.88	0.031
Miswak mouthwash	3.20 ± 0.79		1.8 ± 0.92		1.30 ± 0.48	
Ordinary toothpaste	3.40 ± 0.52	0.187	2.90 ± 0.57	0.399	3.0 ± 0.82	0.008
Normal saline	2.90 ± 1.10		2.6 ± 0.84		2.0 ± 0.94	
Miswak toothpaste	2.80 ± 0.79	0.115	1.90 ± 0.88	0.007	2.10 ± 0.88	0.016
Ordinary toothpaste	3.40 ± 0.52		2.90 ± 0.57		3.0 ± 0.82	
Miswak toothpaste	2.80 ± 0.79	0.790	1.90 ± 0.88	0.053	2.10 ± 0.88	0.781
Normal saline	2.90 ± 1.10		2.6 ± 0.84		2.0 ± 0.94	
Miswak mouthwash	3.20 ± 0.79	0.595	1.8 ± 0.92	0.003	1.30 ± 0.48	<0.001
Ordinary toothpaste	3.40 ± 0.52		2.90 ± 0.57		3.0 ± 0.82	
Miswak mouthwash	3.20 ± 0.79	0.426	1.8 ± 0.92	0.028	1.30 ± 0.48	0.058
Normal saline	2.90 ± 1.10		2.6 ± 0.84		2.0 ± 0.94	
Overall *p*-value		**0.356**		**0.01**		**<0.001**

P-values using one-way analysis of variance (ANOVA), SD - standard deviation

Table 3 - Inter-group comparisons for the mean bacterial scores of *Streptococcus mutans* at different time intervalsamong 402 students.

Study groups	Pre intervention		Immediate post intervention		2 weeks post intervention	
	Mean ± SD	*P*-value	Mean ± SD	*P*-value	Mean ± SD	*P*-value
Miswak toothpaste	2.90 ± 0.88	1.0	2.40 ± 0.84	0.009	2.0 ± 0.67	0.022
Miswak mouthwash	2.90 ± 1.10		1.30 ± 0.68		1.20 ± 0.42	
Ordinary toothpaste	3.50 ± 0.53	0.422	3.30 ± 0.82	0.219	3.0 ± 0.82	0.080
Normal saline wash	3.20 ± 0.79		2.80 ± 1.14		2.40 ± 0.97	
Miswak toothpaste	2.90 ± 0.88	0.112	2.40 ± 0.84	0.030	2.0 ± 0.67	0.016
Ordinary toothpaste	3.50 ± 0.53		3.30 ± 0.82		3.0 ± 0.82	
Miswak toothpaste	2.90 ± 0.88	0.422	2.40 ± 0.84	0.324	2.0 ± 0.67	0.781
Normal saline	3.20 ± 0.79		2.80 ± 1.14		2.40 ± 0.97	
Miswak mouthwash	2.90 ± 1.10	0.112	1.30 ± 0.68	<0.001	1.20 ± 0.42	<0.001
Ordinary toothpaste	3.50 ± 0.53		3.30 ± 0.82		3.0 ± 0.82	
Miswak mouthwash	2.90 ± 1.10	0.422	1.30 ± 0.68	0.001	1.20 ± 0.42	0.058
Normal saline	3.20 ± 0.79		2.80 ± 1.14		2.40 ± 0.97	
Overall *p*-value		**0.343**		**<0.001**		**<0.001**

P-values using one-way analysis of variance (ANOVA), SD - standard deviation

revealed no significant difference at before while it was highly significant at both immediate and after 2 weeks intervals.

Discussion. Several studies have been conducted on the effect of different Miswak products on oral health. Most of clinical in vivo studies were on gingival health; due to the practicability of measuring gingival indices in a relatively short time intervals.[11,12] Studies of Miswak effect on dental caries and cariogenic bacteria were mainly conducted in vitro.[13,14] This is mainly due to the complexity of oral bacteria and the difficulty of isolating cariogenic bacteria by conventional culture, which is tedious and time-consuming due to the huge diversity of different normal bacterial flora of the mouth.[15] This necessitates the purification of Lactobacilli and *S. mutans* from the anerobic flora, which usually constitute approximately 99% of the total oral flora.[16] The impotance of this study relies on being an in vivo survey conducted on a relatively extended period on cariogenic bacteria using a non conventional technique by using CRT. This method is easy, simple and has been shown to be as accurate and reliable as the conventional culturing. The in vivo antibacterial effect of miswak on cariogenic bacteria has been scarily conducted.[17] A relatively similar study has been carried out in Kingdom of Saudi Arabia among 40 males aged 20-45 years to measure the immediate anti bacterial effect of Miswak products. The study found that Miswak had an immediate antimicrobial effect with *S. mutans*, and to a less extend on lactobacilli. No further follow up was conducted to evaluate the long term effect of Miswak on cariogenic bacteria.[10] This study revealed that the bacterial counts were similar in the 4 randomized groups. This indicates that the randomization was reliable and no baseline variation was present between the groups. The study showed that Miswak mouthwash was more effective in reducing Lactobacilli in comparison with all other methods. A significant difference in reduction was observed between Miswak mouthwash and ordinary toothpaste immediately and after 2 weeks of use. It was also superior to Miswak toothpaste, which was significantly less effective in reducing Lactobacillus after 2 weeks of use. This is in agreement with results reported by other studies.[13,18,19] Miswak mouthwash was also more effective than normal saline used usually with ordinary toothpaste immediately, but the difference did not reach a statistical difference after 2 weeks of use. This might be due to the effect of normal saline in preventing bacterial growth by inhibiting bacterial growth.[20] Miswak mouthwash usually penetrates deeply between teeth with increase in exposed surface area. Also, 2 cleansing methods are used, one for the ordinary toothpaste, which students asked to use and the other for Miswak mouthwash itself. Using 2 methods for cleaning is usually more effective.[21] Miswak toothpaste was significantly more effective in reducing Lactobacill than ordinary toothpaste both immediately and after 2 weeks. This may be due to the strong antibacterial effect of Miswak.[22] This is in agreement with results reported by other studies.[7-10]

The limitation of the study was in its open non-blind nature. This is because participants will eventually have to know the type of paste, or mouthwash used. Normal saline was used initially as an isotonic solution to standardize for the dilution effect of other 3 methods in the first post intervention sample. However, it was not ethical to ask this group to use saline rinse alone with no tooth brushing. No immediate difference was observed when normal saline was used instead of tap water with ordinary toothpaste. While after 2 weeks, a significant reduction was detected; and this might be due to the long effect of salt on bacterial growth.[20] As for *S. mutans* the study showed that Miswak mouthwash was also more effective in reducing it in comparison with all other methods. A significant difference in reduction was observed between Miswak mouthwash and both Miswak toothpaste and ordinary toothpaste immediately and after 2 weeks of use. Miswak mouthwash was also more effective than normal saline used with ordinary toothpaste immediately, but this difference did not reach a statistical significance after 2 weeks of use. This again might be due to the extended effect of normal saline in preventing bacterial growth.[20] Miswak toothpaste was significantly more effective in reducing *S. mutans* than ordinary toothpaste both immediately and after 2 weeks of use. This is also attributed to the antibacterial effect of Miswak.[7,10,22] No significant difference in means *S. mutans* was observed when normal saline was used instead of tap water with ordinary toothpaste immediately and after 2 weeks of use. This indicates that normal saline has been more effective on Lactobaclli than *S. mutans*. The mean bacterial growth for both types of cariogenic bacteria was almost similar, or lower within each group between the 2 times of detection; immediately and after 2 weeks of use. This indicates the powerful antibacterial effect of Miswak as the samples collected after 2 weeks has no dilution effect of water, or saline. Indeed, the bacterial counts after 2 weeks of using Miswak mouthwash was even lower than those detected immediately after use despite the lack of dilution effect. The importance of this study based on being in vivo assessment of Miswak

products on cariogenis bacteria followed up for 2 weeks. Despite that it is highly recommended to extend the period of follow up in future studies to measure the long term effects on Miswak products.

In conclusion, Miswak products were more effective in reducing the growth of cariogenic bacteria than ordinary toothpaste.

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