## Dissolution of calcium ion from teeth treated with different concentrations of siwak water extract in comparison with sodium fluoride

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### ABSTRACT

**Background:** The miswak is a teeth cleaning twig made from a twig of the Salvadora persica tree (known as arak in Arabic). A traditional alternative to the modern toothbrush, it has a long, well-documented history and is reputed for its medicinal benefits. It also features prominently in Islamic hygienical jurisprudence.

**Materials and methods:** Twenty maxillary first premolars were treated with the selected solutions which included siwak water extract (5%, 10%) and sodium fluoride 0.05% for 2minutes once daily for 20 days interval, deionized water was used as control negative. Then the concentration of the dissolved calcium ion in the etching solution of 2N HCL was measured.

**Results:** The least amount of the dissolved calcium ion was registered for water siwak extract 10%, then sodium fluoride0.05% followed by water siwak extract5%. A significant difference was found between the two concentrations of siwak water extract, while no significant difference was recorded between the mentioned agents and sodium fluoride. There was highly significant difference between deionized water group and other groups except between water and siwak water extract5% the difference was significant.

**Conclusions:** Siwak water extract is successful in improving tooth resistance against caries challenge as it mineralize and harden enamel surface.

Keywords: Siwak water extract, calcium dissolution, acid etching. (J Bagh Coll Dentistry 2014; 26(1):166-170).

#### **INTRODUCTION**

The use of plants as medicines is an ancient and reliable practice <sup>(1)</sup>.Tooth brush tree, Salvadora persica locally called siwak is a member of Salvadoraceae family has been used by many Islamic communities as tooth brush and has been scientifically proven to be very useful in the prevention of tooth decay ,even when used without any other tooth cleaning method <sup>(2)</sup>. The wide spread use of siwak among Muslims was attributed to the prophet Mohammed (Peace and Blessing of Allah be upon Him) who recommended its use for tooth cleaning before each of the five daily prayer <sup>(3,4).</sup>

Chemical analysis of Salvadora persica has demonstrated the presence of sulfur that has a bactericidal effect <sup>(5)</sup> and vitamin C was found to help in tissue healing and repair <sup>(6)</sup>. Silica acts as an abrasive and was found to help in removing stains from tooth surfaces <sup>(3,7)</sup>. The astringent effect of tannins may help to reduce clinically detectable gingivitis. Tannins were found to inhibit the action of glucosyltransferase, thereby reducing plaque and gingivitis <sup>(8)</sup>. Resins may form a layer on enamel that protects against dental caries. Salvadorine, an alkaloid present in S. persica miswak, may exert a bactericidal effect and stimulate the gingiva <sup>(6,7)</sup>. The mildly bitter taste of the essential oils in S. persica miswak stimulates the flow of saliva, which acts as a buffering agent. High concentrations of chloride inhibit the formation of calculus (9) and aid in removing stains from tooth surfaces <sup>(6)</sup>. It contains nearly 1.0µglg of total fluoride and found to release significant amount of calcium and phosphorous into water <sup>(10)</sup>. Saturation of calcium in saliva due to the use of chewing sticks was found to inhibit demineralization and promote enamel remineralization (8,11). In 2006, Al-Obaidy measured the concentration of calcium and phosphorous ions in the crude water Siwak extract and found that the concentration of calcium was higher as compared to phosphorous, an interesting result in Al-Obaidy study was that the water siwak extract at a concentration (5%) was very effective in remineralization of initial carious lesion even better than stannous fluoride <sup>(12)</sup>.

Studies found that salvadora persica extract is somewhat comparable to other oral disinfectants and antiplaque agents such as triclosan and chlorhexidine gluconate and the use of such extract in tooth paste will protect teeth and gums <sup>(13-15)</sup>.Aqueous extract of siwak could be used to reduce the growth of candida albicans <sup>(16)</sup>. The values of these sticks are due to their components and cleaning mechanism. Recently

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these sticks were recommended as an effective tool for oral health by World Health Organization (WHO) <sup>(17).</sup>

Because siwak contain minerals like calcium that may react with outer enamel surface improving its resistance against acid dissolution therefore, this study was designed to test the ability of water siwak extract to increase the enamel resistance against caries challenge.

#### **MATERIALS AND METHODS**

Teeth sample consisted of twenty randomly selected human maxillary first premolars extracted from (10-13) year old patients for orthodontic purpose. The extracted teeth were cleaned using conventional hand piece and rubber cup with non-fluoridated pumice and deionized water and stored in 0.1% thymol solution at 4°C until use, to minimize brittleness of enamel and microbial growth <sup>(18)</sup>.

The siwak water extract was prepared by taking a 250 gm of Siwak powder and placed in a beaker to which de-ionized water was added till reach a volume of one liter. The beaker was closed tightly and left to boil at 100°C for 15 minutes, then left to warm, the liquid was then filtered using filter paper (no.1). The filtered water extract was left to dry at 40°C in the incubator for 24 hours, to allow the evaporation of water and to obtain a powder of Siwak extract. The powder was collected and kept in tightly closed glass container and kept in refrigerator until use (19). Teeth were divided randomly into four equal groups, each group consisted of five teeth and then the teeth were immersed individually for two minutes once daily over twenty days in thirty ml of their assigned test solution which included, Siwak water extract (5%, 10%)  $^{(12)}$  and sodium fluoride (0.05%) which is the approved concentration of a daily home-used sodium fluoride (20). Deionized water group was used as a control negative. After each immersion, the specimens were water washed in deionized water for 5 minutes, then stored in humid condition of deionized water to which 0.1% thymol was added until the next immersion. After the twenty day treatment period, a circular area, 3 mm in diameter were selected on each enamel specimen by applying prepared annular adhesive discs, avoiding macroscopic cracks and hypoplastic areas .The rest of the specimen was covered with a sticky wax, leaving only the circular enamel window exposed for subsequent etching. The windows were etched for ten seconds in separate polyethylene tubes; each containing five ml of 2NHCl .The concentration of dissolved calcium ion was determined by flame atomic

absorption spectrophotometer <sup>(18)</sup>. Statistical parameters mean and standard deviation were calculated. Analysis of variance (ANOVA) and least significant difference (LSD) tests were used to evaluate the significance of difference between different variables. The confidence limit was accepted at 95%.

#### RESULTS

After acid etching the mean values of the released calcium concentration were illustrated in Table(1). The maximum amounts was recorded for deionized water group followed by siwak water extract5% immersed group ,then sodium fluoride treated teeth group, while the least amount of the dissolved calcium was registered for siwak water extract 10% treated group. Table (2) showed by ANOVA test the difference in the calcium concentration was statistically highly significant among different groups. In Table (3) LSD test was presented to evaluate the difference between each two groups. It showed that there was significant difference between the two concentrations of siwak water extract where as the difference was statistically not significant between the mentioned solutions and sodium fluoride. The difference between water immersed group and the tested solutions was statistically highly significant except between deionized water and siwak water extract solution 5% the difference was significant.

#### DISCUSSION

Siwak has been used by many Islamic communities as tooth brush and has been scientifically proven to be very useful in the prevention of tooth decay. The mechanical action of the stick fibers was proved to be effective by many studies <sup>(21,22)</sup>. It was documented that the extracts of these sticks had a drastic antimicrobial effect <sup>(7,14,23)</sup>. Many investigators found that siwak contains efficient chemical substances as alkaloids, fluoride, calcium, phosphorous, etc, these agents may have antibacterial action in addition it may chemically react with the outer enamel surface <sup>(24,25)</sup>.

In this experiment a water extract of Siwak (Salvadora Persica) was chosen to investigate its ability to decrease the loss of calcium ions from enamel of teeth which immersed in 2N HCl (increase enamel hardness) in comparison to sodium fluoride and deionized water. Sodium fluoride was used as a control positive because of its well documented ability to increase the enamel resistance to acid dissolution that protect tooth against dental caries <sup>(26,27)</sup>, while de-ionized water was used as a control negative. Siwak sticks were

powdered and water extract was prepared according to Al-Jeboory technique (19). Many types of Siwak extract are present beside water Siwak extract, as ethanol and chloroform and ethanol Siwak extracts, but the aqueous one was selected due to the uniform solvent of the used agents. It was shown by previous Iraqi study that water Siwak extract can be easily prepared and effective even more than other types of extracts <sup>(14)</sup>. Calcium is one of the enamel major elements that comprise about 33.6%-39.4% of hydroxyapatite crystal (28,29), therefore it was chosen to test its dissolution upon exposure to acid attack.

After treatment of the enamel samples with water Siwak extract (5%, 10%) and sodium fluoride 0.05%, an acid etching revealed that the released calcium ion concentration was higher for deionized water than the selected solutions and the difference was statistically highly significant except between water and siwak water extract 5% the difference was statistically significant .This may be an indication of incorporation of ions that decrease porosity, and increase enamel microhardness against demineralizing action of acid. When sodium fluoride solution is applied on the tooth surface as topical agent, it reacts with the enamel to form calcium fluoride or fluoridated hydroxyapatite crystal and these will increase the concentration of fluoride on enamel surface which in turn makes the tooth surface more resistance to acid attack (29).

Concerning Siwak water extract, its mineralizing ability may be related to its content of calcium and phosphorus ions which are the major components of hydroxyapatite crystal, in addition to other anticariogenic ions like fluoride <sup>(30)</sup>. It was proposed that these ions incorporated in the outer enamel surface harden it and explain this difference in the concentration of dissolved calcium between siwak water extract and deionized water. This combination of cariostatic ions in siwak could also explain the higher amount of released calcium from teeth treated with sodium fluoride compared with those treated with siwak water extract 10% and the difference was not significant.

The concentration of the dissolved calcium ions from the acid-etched teeth immersed with 10% siwak water extract was less compared with that after treatment in 5% of the extract and the difference was significant. An increase in the concentration of siwak water extract result in an increase in the concentrations of calcium, phosphorus and fluoride, the increase in calcium and phosphorus in the extract led to an increase in Ca/P ratio thus increase the resistance of enamel to acid dissolution and increase in concentration of fluoride in enamel surface made the tooth surface harder  $^{(12,31)}$ .

It is not well understood what was the type of reaction took place with enamel surface, which finally decrease the concentration of the dissolved calcium ion, it may be attributed to its content of calcium and phosphorous. Both ions are the major components of apatite crystals. Other studies reported a presence of a small amount of fluoride. Presence of calcium, phosphorous and fluoride ions in Siwak extract make the assumption of production of calcium fluoride, in addition to fluoroapatite crystals and fluorohydroxyapatite crystals, all these may increase the mineralization of porous enamel, thus decreasing calcium release from enamel surface.

An interesting result recorded in this study was the lowest concentration of calcium ions released from enamel surface was for 10% water siwak extract compared to its 5% concentration.

As the concentration of calcium ions from enamel surface was measured, water siwak extract at 10% was found to produce minimum demineralization as the lowest calcium concentration was recorded for it. Sodium fluoride came the next then 5% water Siwak extract and finally de-ionized water.

One can reveal from above results that water siwak extract at a concentration 10% was very effective in increasing the resistance of enamel to demineralization even better than sodium fluoride. These results need to be confirmed by further studies involving in vivo, before the recommendations of using water siwak extract in dental practice as an active way for prevention of dental caries.

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# Table 1: Concentration of calcium ion (mean and standard deviation) dissolved in 2 N HCl from enamel treated with selected agents

Agent	No.	Mean (mmol/ L)	±SD
Siwak5%	5	2.92	1.182
Siwak10%	5	1.80	0.132
NaF0.05%	5	2.05	0.732
<b>Deionized</b> Water	5	4.15	0.99

#### Table 2: ANOVA test among different solutions

	Sum of Squares	df	Mean Square	F-value	P-value.		
<b>Between Groups</b>	16.88	3	5.62				
Within Groups	11.77	16	.73	7.64	.002**		
Total	28.65	19					
** 11::							

**\*\*** Highly significant

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Agent(1)	Agent (2)	Mean Difference	Sig.
	Siwak10%	1.11	.056*
Siwak 5%	NaF0.05%	0.81	N.S.
	<b>Deionized Water</b>	$1.2^{3}$	.038*
Siwak10%	Siwak5%	1.11	.056*
	NaF0.05%	0.24	N.S.
	<b>Deionized Water</b>	2.3 4	.001**
	Siwak5%	0.87	N.S.
Siwak 5% Siwak10% NaF 0.05% Deionized Water	Siwak10%	0.24	N.S.
	<b>Deionized Water</b>	2.1 0	.001**
Deionized Water	Siwak5%	1.23	.038*
	Siwak10%	2.34	.001**
	NaF0.05%	2.1 0	.001**

#### Table 3: Least significant difference (LSD) between each two agents

\*. significant \*\* highly significant