

Dissolution of inorganic phosphorous ion from human enamel treated with different concentration of Siwak aqueous extract in comparison with sodium fluoride

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ABSTRACT

Background: The use of Miswak, chewing sticks (*salvadorapersica*) can be traced back to Babylonians some 7000 years ago. It is commonly used throughout the world especially for the purpose of oral hygiene. Muslims are using as the religious view. Current study aimed to test the ability of aqueous siwak extract to increase the resistance of enamel surface against acid dissolution compared to sodium fluoride.

Materials and Method: Twenty maxillary first premolars were treated with the selected solutions included two aqueous siwak extract concentration (5%, 10%) and sodium fluoride (0.05%) as control positive for 2 minutes once daily for 20 days interval, deionized water was used as control negative. The concentration of the dissolved phosphorus ion was measured in etching solution of 2N HCL by flame atomic absorption spectrometer.

Result: 10% aqueous siwak extract test solution was registered the lesser release of phosphorus from etched enamel surface, then 5% aqueous siwak extract followed by sodium fluoride 0.05%. No significant difference between two aqueous siwak extract concentration, while a significant difference was found between mentioned agents and sodium fluoride. Deionized water was recorded a highly significant difference with all test solutions.

Conclusion: Aqueous siwak extract effective in increasing the resistance of enamel surface for acid dissolution and should be used not only for the religious view but also for the benefit of its effects produced.

Key word: Aqueous siwak extract, acid etching, phosphorus dissolution. (J Bagh Coll Dentistry 2013; 25(4):91-94).

INTRODUCTION

Salvadorapersica (S.P.) is a plant that grows in the deserts of the area from west India to Africa. The roots and sticks of S.P. are used widely for cleaning the teeth in these areas. The other names for this plant are Arak tree, Chewing stick, natural toothbrush and Miswak or siwak ⁽¹⁾.

Also Miswak (*Salvadorapersica*) is one of the most commonly used medicinal plants for oral hygiene among global Muslim community ⁽²⁾, and it's widely used as a chewing stick in many countries. The toothbrush tree, *Salvadorapersica*, locally called miswak, is a member of the *Salvadoraceae* family has been used by many Islamic communities as toothbrushes and has been scientifically proven to be very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods ⁽¹⁾. As well as the beneficial effects of miswak in respect of oral hygiene and dental health are partially due to its mechanical action and partially due to pharmacologic action (antibacterial activity). ⁽⁴⁾ A study conducted on

Wood Siwak and reports that it contains natural minerals that can kill and inhibit bacteria growth, erode plaque, prevent cavities and gum care ⁽⁵⁾, this may be attributed to its constituents as calcium, phosphorus, fluoride and other element

may react with the outer enamel surface changing the resistance against caries challenge. At the same time other constituents as trimethylamine may have antibacterial effects changing the microbial composition of the dental plaque, by this Siwak may affect both dental caries and periodontal disease ^(6,7).

Other study revealed that the amount of phosphorus released from *Salvadorapersica* stick soaked in water is 34 µg/ml that represent 26.4% of the total content in the stick, while the amount of calcium release in water is 582 µg/ml which represent 19.6% of the total content in the stick ⁽⁸⁾. For this reason recently these sticks were recommended as an effective tool for oral health by the World Health Organization (WHO). ⁽⁹⁾ So that the purpose of this investigation was to test the effect of aqueous Siwak extract in increasing of the enamel resistance against acid dissolution.

MATERIALS AND METHOD

The sample consisted of 20 teeth which is randomly selected of human maxillary first premolars extracted from (10-13) year old patients for orthodontic purpose. After cleaning of extracted teeth by using conventional hand piece and rubber cup with non-fluoridated pumice and deionized water, then stored in 0.1% thymol solution at 4° C until use, to minimize brittleness of enamel and microbial growth ⁽¹⁰⁾. The preparing of siwak aqueous extract was done by taking 250 gm. of siwak powder and placed in a

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beaker to which de-ionized water was added till reach a volume of one liter, then the beaker was closed tightly and left to boil at 100C for 15 minute, then left to warm . The liquid was then filtered using filter paper (no.1), then this filtered aqueous extract was left to dry at 40C in 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⁽¹¹⁾. 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%) and sodium fluoride (0.05%) which is the approved concentration of daily home- used sodium fluoride⁽¹²⁾.

De-ionized 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 buccal surface of each enamel specimen by applying prepared annular adhesive disc, avoiding microscopic cracks and hypo plastic areas. The rest of specimen was covered with a sticky wax, leaving only the circular enamel window exposed for subsequent etching, then the windows were etched for ten seconds in separated polyethylene tubes, each containing five ml of 2NHCL. The concentration of released phosphorus ion was determined calorimetrically by the Molybdenum – Vanadate method. A ready-made Kit (Bio Maghreb, Tunisia) was supplied. Inorganic phosphorus react with ammonium molybdate in the presence of sulphuric acid to form a phosphomolybdate complex. The color of molybdenum blue was proportional to the phosphorus concentration⁽¹³⁾.

The data was processed with SPSS version (9.0) statistical software. Mean and Standard deviation were calculated. ANOVA (analysis of variance) and LSD (Least significant difference) tests were used to evaluate the significance of difference between different groups. The significance level was accepted at 95% ($P < 0.05$).

RESULTS

The mean values and standard deviation of release phosphorus ion concentration according to different concentration of siwak aqueous extract and sodium fluoride and statistical analysis using ANOVA test were showed in Table(1). The

maximum mean value for dissolved phosphorus ion was recorded for deionized water group followed by sodium fluoride treated group, while the least dissolved phosphorus concentration was registered for siwak aqueous extract (10%) treated group.

The difference in the phosphorus concentration was statistically highly significant among different test groups. The difference between each two groups was illustrated in Table (2) using LSD test. It showed that there was no significant difference between the two siwak aqueous extract concentration (5% and 10%). Concerning Sodium fluoride group (0.05%), a significant difference was recorded with siwak aqueous extract (5%), while non-significant difference with other siwak water extract concentration (10%). On other hand the deionized water immersed group statically showed highly significant difference with all tested solutions.

DISCUSSION

The traditional toothbrush or chewing stick is deeply rooted in Islamic culture. It's apencil-sized sticks of various plants are fashioned from certain plant - parts and are chewed on one end until they become frayed into a brush. So that the brush-end is used to clean the teeth in a manner similar to the use of toothbrush. When used in this manner, they are commonly referred to as chewingsticks or Miswak^(2,14). These sticks have been shown to have a therapeutic effect on the gingiva and surrounding structures in addition to their mechanical effect^(4,15). Many studies were revealed that the aqueous extract of siwak had antimicrobial effect at different concentration^(6,7,16), also *Salvadora persica* miswak contains nearly 1.0µg/g of total fluoride and was found to release significant amounts of calcium and phosphorus into water and these act as anticariogenic agent that may react chemically to the outer surface of enamel^(17,18). In this study aqueous extract of Siwak (*Salvadora Persica*) was used to test its ability in different concentration (5%, 10%) to decrease the loss of phosphorus ions from enamel of teeth which immersed in 2N HCL (to increase enamel hardness) in comparison to sodium fluoride. This fluoridated agent was used as control positive because of their documented re mineralizing potential effect of enamel which increases its resistance to caries⁽¹⁹⁻²¹⁾, while de-ionized water was used as a control negative. Aqueous Siwak extract was selected in this experiment rather than other types of siwak extract (chloroform and ethanol siwak extract) due to the uniform solvent of the used agent. Sticks of siwak were powdered,

and then aqueous extract was prepared following Al-Jeboory technique⁽¹¹⁾. As well as many Iraqi studies used the aqueous extract of siwak due to the easily way for preparing and more effective than other extract types^(7,22). Enamel consists of approximately 97% inorganic minerals. Calcium and phosphorus (as hydroxyapatite) are the main building block of dental hard tissues. Changes in the calcium/phosphate ratio indicate alterations in the inorganic components of Hydroxyapatite^(23,24). In this study the enamel was treated with different percent of aqueous water extract (5% , 10%) and sodium fluoride 0.05% was used due to its stability, well test, not irritant to the gingiva and not cause discoloration of tooth structure⁽²⁵⁾, therefore it was used as control positive in this test. The concentration release of phosphorus ion after etching with acid following treatment of enamel samples with test solutions was higher for deionized water than the selected solutions with highly significant difference. This may be due to incorporation of ions which increase an enamel micro hardness and decrease its porosity against acid demineralization. While the lowest release of phosphorus was found in siwak aqueous extract (10%) followed by (5%) this may related to chemical composition of siwak as it content of anti-cariogenic agents as calcium and phosphorus ions which are the main components of hydroxyapatite crystal, in addition to the large amounts of fluoride present in siwak⁽¹⁷⁾ may be a contributing factor to this effect^(8,26). As these ions incorporated in the outer enamel layer increase the microhardness and may explain why this difference found between the concentration of dissolved phosphorus ion in aqueous siwak extract and de-ionized water. Application of fluoride on the tooth surface directly as a topical agent (sodium fluoride) will react with the hydroxyapatite crystals to form fluoroapatite crystal which in turn makes the enamel surface more resistance to acid dissolution^(22,26), this may explain the lower concentration of phosphorus ion released from teeth treated with sodium fluoride agent than that of de-ionized water, while this release of phosphorus ion was higher than that in aqueous siwak extract (5%,10%) with significant difference, which may be due to the combination of cariostatic ions in siwak⁽¹⁷⁾. The increase in the concentration of aqueous siwak extract result in an increase in these cariostatic ions, which lead to increase in calcium / phosphorus (Ca/P) ratio in the enamel surface thus increase its resistance to acid challenge, in addition to the increase of fluoride concentration in tooth surface made the enamel harder^(24,27), this will explain the

dissolution of phosphorus ions from acid etched teeth immersed with 10% aqueous siwak extract was less than that treated in 5% aqueous extract of siwak with non-significant difference.

Based on results of this study, the conclusion is that the uses of siwak (*salvadorapersica*) in different concentration decrease the dissolution of main ion (phosphorus) from enamel surface when comparing with other test solution, there is no clear idea about the type of reaction took with enamel surface, which at the end lead to decrease the dissolution of phosphorus ion from tooth surface, it may be attributed to a number of chemicals isolated from *salvadorapersica* plants (siwak) like the calcium, phosphorus and fluoride content⁽¹⁷⁾ helps to increase the mineralization of porous enamel surface of the teeth, thus decreasing the dissolution of phosphorus ions from tooth surface. The result of this study showed higher efficacy of 10% miswak extract in reduction the release of phosphorus ions as compared to other test solution. Since miswak is inexpensive, readily available, contains medicinal properties, and is available in most rural areas of the developing countries, so it can be an effective tool in preventing oral diseases.

In this regard, further evaluation studies needs to be carried out on *Salvadorapersica* plant in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

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Table 1: Concentration of phosphorus ion (mean, standard deviation and statistical analysis ANOVA) dissolved in HCL from enamel treated with selected agents

Test Solution	No.	Mean	±SD	F-value	P-value
Aqueous siwak extract 10%	5	0.46	0.026	107.317	0.000**
Aqueous siwak extract 5%	5	0.38	0.047		
Sodium Fluoride	5	0.69	0.068		
De-ionized water	5	2.30	0.381		

** Highly Significant (p<0.01)

Table 2: Least Significant difference (LSD) between each two agents.

Agent 1	Agent 2	Mean Difference	P-value
Aqueous siwak extract 10%	Aqueoussiwak extract 5%	0.80	0.526
	Sodium Fluoride	-0.23	0.081
	Deionized water	-1.84	0.000**
Aqueous siwak extract 5%	Aqueous siwak extract 10%	-0.08	0.526
	Sodium Fluoride	-0.31	0.023*
	Deionized water	-1.92	0.000**
Sodium Fluoride	Aqueous siwak extract 10%	0.23	0.081
	Aqueous siwak extract 5%	0.31	0.023*
	Deionized water	-1.61	0.000**
Deionized water	Aqueous siwak extract 10%	1.84	0.000**
	Aqueous siwak extract 5%	1.92	0.000**
	Sodium Fluoride	1.61	0.000**

* Significant (p<0.05), **Highly Significant (p<0.01)