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Effect of Siwak Extract on the Microhardness and Microscopic Feature of Initial Caries-Like Lesion of Permanent Teeth, Compared to Fluoridated Agents

Dr. Nibal Mohammed AL-Obaidy M. Sc.*
Dr. Sulafa K. EL – Samarrai M. Sc., Ph. D. **
Dr. Ahlam H. Majeed. M. Sc. ***

Abstract

Siwak is a chewing stick used to clean the teeth and message the gum. Their routine use was shown to be associated with the reduction in severity of dental caries. This study aimed to test the effect of water Siwak extract on the microhardness and microscopic features of artificially initiated carious lesion of the outer enamel surface, in comparison to stannous fluoride and sodium fluoride. Upper first premolars were extracted and subjected to pH cycling procedure. Teeth immerged individually in 20 ml of selected agents for four minutes then rinsed by de ionized water. Agents involved were 5% and 10% water Siwak extract, 8% stannous fluoride and 0.05% sodium fluoride. Samples were subjected to Vickers microhardness test and microscopic examination before and after the pH cycling and following insertion in the agents. Water Siwak extract, stannous fluoride and sodium fluoride were found to increase the microhardness values of de mineralized enamel surfaces, this were statistically highly significant for Siwak extract and stannous fluoride ($P< 0.01$) and significant for sodium fluoride. However none of the mentioned agents was able to increase the micro hardness to approximate the original values of the sound enamel. Under polarized light results revealed that water Siwak extracts and stannous fluoride produce the best remineralization surface zone. Water Siwak extracts is very effective in remineralization of initial carious lesion.

Key words: Siwak, fluoride, microhardness, caries.

Introduction

Siwak or Miswak is a wood stick used for tooth and gum cleaning [1, 2]. Previous studies showed that Siwak may possess a therapeutic beside the mechanical effect, attributed to its constituents [3]. These constituents as calcium, phosphorous, fluoride and other elements may react with the outer enamel surface changing the resistance against caries challenge. At the same time other constituents as tri methylamine may have an antibacterial effects changing the microbial composition of the dental plaque, by this Siwak may affect both dental caries and periodontal disease [4, 5, 6, and 7].

The purpose of the present

* Assistant lecturer, College of Dentistry/ University of Baghdad.
** Professor, College of Dentistry/ University of Baghdad.
*** Professor, College of Dentistry/ University of Baghdad.

experiment was to test the effect of Siwak extracts on the remineralization of caries - like lesion of enamel surface.

Materials and methods

Teeth samples consisted of maxillary first premolars extracted from 11-14- year -old orthodontic patients. Teeth were cleaned and polished using a slowly rotating conventional hand piece and rubber cup with non fluoridated pumice and de-ionized water. Each tooth surface was examined under magnifying lens under good light to check if any crack or defect might be present [8]. An acid resistant nail varnish was used to paint the surfaces of the tooth; leaving a circular window six mm in diameter on the buccal surface. Teeth were adapted in an acrylic model (the size of this model was 30 × 27 mm) using a red wax. The window of each tooth surface was ground and polished using a wet emery paper (grit 400) ten times for each tooth.

Siwak sticks (Salvadora Persica) were left to dry in the incubator at 37^{o}C for 24 hours, then each stick was cut to small pieces. They were ground to powder using commercially food grinder. The Siwak powder was kept in a tightly closed glass container till use. A water extract powder from Siwak was prepared according to Al-Jeboory [(9)].

Teeth samples consisted of 32 maxillary first premolars, two teeth were used for ground section preparation for normal enamel and demineralized enamel, the rest were randomly divided to four study groups and one control group, and each group consisted of five teeth for microhardness test and one tooth for microscopical examination. Then they were subjected to pH cycling procedure [10]. The enamel microhardness was measured initially for normal enamel and after induction of caries lesion by pH cycling procedure; the microhardness measurement was done by Vickers microhardness device in at a load of 500 gm for 30 seconds [(11)].

Teeth were treated by the solutions of the selected agents by emerging each tooth separately in 20 ml of the selected agent solution for four minutes, and then each tooth was rinsed with de-ionized water for two minutes. Teeth were restored in de-ionized water for the next day at temperature of 37^{o}C in the incubator, this procedure was repeated daily for one week, samples were re-examined for the microhardness. Selected agents were 5% and 10% Siwak extracts, 8% stannous fluoride and 0.05% sodium fluoride.

The change in the hardness (D) or the difference between the initial (HVi) and the final (Hvf) micro hardness, was calculated for each specimen and the mean of this difference ($\bar{D}$) was calculated for each of the experimental groups. The ratio of the difference ($\bar{D}$) to the ($\overline{Hvi}$) was also calculated for each experimental group which represents the rate of change in hardness of the specimens [(12)].

Enamel ground sections were made for teeth which were selected for microscopic examination. They were prepared following the procedure described by Guay et al [(13)]. For each enamel sample a longitudinal section with the thickness of 0.5 mm was stacked on the middle of glass slide using Canada balsam. Enamel slabs were examined under polarized light microscope (X25, X63). Microscopic examination involved sound enamel surface, after induction of caries lesion by pH cycling procedure and following treatment with the solutions of the selected agents.

Data processing and analysis were carried out using SPSS program A student's t test was applied to test statistical differences between data at a level of significance of 5%.

Results

The mean values, of the microhardness in Vickers hardness number (VHN) of sound enamel surfaces, after demineralization and following treatment with selected agents and de ionized water are seen in Table (1). For all groups reductions in VHN were notices which were statistically highly significant for all groups (Table 2). Following the insertion in selected agents an elevation in VHN were recorded (Table 1), this were statistically highly significant for all groups except the de ionized water group as it was not significant (Table 2). None of selected agents were able to increase the VHN to approximate the original values of the sound enamel.

Statistical tests were conducted between the mean values of the VHN of the control (treated with de ionized water) and those with selected agents, for all a statistically not significant differences was seen , except for stannous fluoride (8%) difference was highly significant ($t= 2.415$, $df = 8$, $P = 0.042$).

Table 3 represents the changes in the VHN following treatment with agents. Siwak extracts (5%) caused the highest changes, followed by stannous fluoride (8%) while the lowest value was recorded for the de ionized water.

Figure 1 shows the microscopic features of the sound enamel surface appear as a highly mineralized with clear and intact enamel rods. Following the pH cycling procedure a considerable loss of minerals is shown by clear area of de mineralization in outer most layers with a relative irregularity in the enamel rods (Figure 2). A remarkable remineralization of the outer most surface area was seen following the treatment with Siwak extracts and stannous fluoride (Figures 3, and 4). Also samples treated with sodium fluoride showed a mineralization but relatively less than that seen for other agents.

Discussion

In this experiment a water extract of Siwak (Salvadora Persica) was chosen to investigate its ability to re mineralize the initial carious lesion of enamel in comparison to stannous fluoride and sodium fluoride. These fluoridated agents were used as control positive because of their documented re mineralizing potential [14, 15, 16], while de-ionized water was used as a control negative.

The initiation of carious lesion in the present study was conducted in ten days. Under polarized light microscope the ground section of enamel surface indicated a high loss of minerals. The demineralization was found to decrease from the outer to inner layers of enamel. However a clear demarcation between different zones of the carious lesion was not obvious. For natural carious lesion, four zones are present starting from the translucent zone in the inner layer of enamel, dark zone, body of the lesion and intact layer. Increasing the time of pH cycling to weeks or months may allow for more obvious demarcation between zones of carious lesion in similarity to the natural dental caries [17].

Enamel micro hardness was measured for sound enamel, after demineralization and following treatment with the chosen solutions. After treatment of the enamel samples with water Siwak extract (5%, 10%), stannous fluoride 8% and sodium fluoride 0.05%, there was an elevation in the micro hardness values for all agents. This was an indication of incorporation of the ions that decreased porosity and increased the micro hardness of de mineralized enamel; such an observation was not seen for samples treated with de-ionized water. This was also confirmed by microscopic examination.

A microscopic examination showed a remarkable remineralization of the outer enamel surface was shown for teeth samples treated with water Siwak extract and those treated with stannous fluoride, which were nearly comparable to each other. For the teeth treated with sodium fluoride, they also showed a good mineralized enamel surface but somewhat less than samples treated with the other two agents.

Increase in enamel micro hardness after application of sodium fluoride may be related to the reaction of fluoride ion with enamel surface resulted in the formation of fluoride containing compounds mainly calcium fluoride, thus the increase in concentration of fluoride in enamel surface made the tooth surface harder [18]. While in case of stannous fluoride, it is assumed that both fluoride and tin ions reacted with enamel surface leading to the formation of complex compounds as stannous tri-fluorophosphate, tinhydroxy phosphate and calcium trifluorostannate in addition to calcium fluoride [19]. All these may increase mineralization of tooth structure which may explain the increase of micro hardness values of artificially initiated caries. The increase in the micro hardness of demineralized enamel surface was also recorded following application of (5%, 10%) water Siwak extract. It is not well understood what was the type of reaction took place with enamel surface, which finally increase its hardness, it may be attributed to its content of calcium and phosphorous. Both ions are the major components of apatite crystals[18]. 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.

An interesting result recorded in this study was the higher microhardness values for 5% water Siwak extract compared to its 10% concentration. This is difficult to be explained. However Siwak extract may involve elements other than calcium and phosphorous, increase the concentration of extract may increase concentrations of these elements as well. They may substitute calcium ion of hydroxyapatite crystals lead to decrease in the Ca/P molar ratio and forming other crystals that may decrease the microhardness of tooth structure. Siwak extract need to be analyzed chemically to determine the concentrations of different elements that may substitute calcium in the apatite crystals like magnesium and sodium.

One can reveal from all these tests that water Siwak extract 5% is very effective in remineralization of initial carious lesion. These results need to be confirmed by further studies involving *in vivo*, before the recommendation of using Siwak extract in dental practice.

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Table (1): Microhardness (Mean Values and Standard Deviation) of Enamel Surfaces Treated by Different Agents

Siwak Extracts

Variables	Conc.	Mean	S.D.
Normal enamel	5% 10%	325.27 298.10	13.93 55.70
Demineralization	5% 10%	30.35 33.01	7.70 4.76
Remineralization	5% 10%	69.37 62.45	21.87 11.87

Stannous Fluoride

Variables	Mean	S.D.
Normal enamel	299.07	56.71
Demineralization	31.87	5.43
Remineralization	70.73	14.61

Sodium Fluoride

Variables	Mean	S.D.
Normal enamel	265.87	74.54
Demineralization	34.24	7.70
Remineralization	59.43	16.34

De ionized Water

Variables	Mean	S.D.
Normal enamel	284.33	59.00
Demineralization	54.47	3.42
Remineralization	54.55	3.20

Table (2): Student's t–test Between Variables of Micro hardness for Selected Agents

Siwak Extracts

Groups	5% Siwak		10% Siwak	
	t-value	P-value	t-value	P-value
Normal enamel × Demineralization	41.33	0.001 *	10.64	0.001 *
Normal enamel × Remineralization	22.07	0.001 *	9.288	0.001 *
Demineralization× Remineralization	3.76	0.006 *	5.147	0.001 *

Stannous Fluoride

Groups	t-value	P-value
Normal enamel × Demineralization	10.49	0.001 *
Normal enamel ×Remineralization	8.72	0.001 *
Demineralization × Remineralization	5.57	0.001 *

Sodium Fluoride

Groups	t-value	P-value
Normal enamel × Demineralization	6.91	0.001 *
Normal enamel × Remineralization	6.05	0.001 *
Demineralization × Remineralization	3.12	0.014 •

De ionized Water

Groups	t-value	P-value
Normal enamel ×Demineralization	8.70	0.001 *
Normal enamel ×Remineralization	8.70	0.001 *
Demineralization × Remineralization	0.04	0.968 ●

* Highly significant, ● Significant, DF= 8

Table (3): Changes in the Micro hardness after Treatment with the Selected Agents.

Concentration of the selected agents	Change in the micro hardness %
Siwak 5%	128.65
Siwak 10%	89.18
SnF_2 8%	121.90
NaF 0.05%	73.56
De-ionized water	0.16

Figure (1): Normal Sound Enamel Surface.

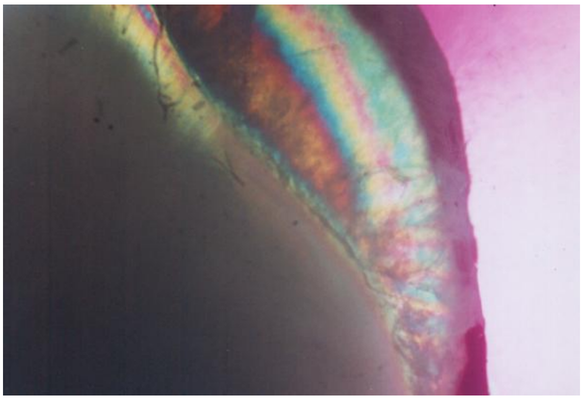

Figure (2): Demineralized Enamel Surface With Analyzer(x25)

Figure (3): Remineralized Enamel Surface after Treatment with Water Siwak Extract

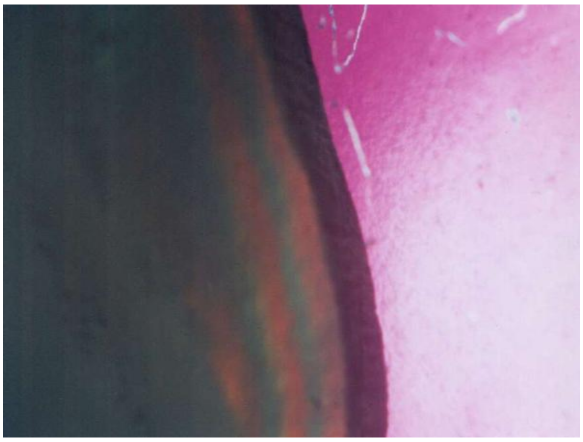

Figure (4) Remineralized Enamel Surface after treatment with SnF