REMINERALIZATION EFFECT OF FLUORIDATED MISWAK VARNISH ON INITIAL CARIES-LIKE LESIONS IN PERMANENT TEETH (IN-VITRO STUDY)

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ABSTRACT

INTRODUCTION: New remineralization therapies aim to improve the existing fluoride treatments rather than replacing them. Fluoridated miswak varnish was proven to have remarkable antimicrobial and remineralizing potentials on primary teeth in experimental studies.

OBJECTIVES: To evaluate the remineralizing effect of fluoridated miswak varnish on initial caries-like lesions in permanent teeth and compare its remineralizing effect to fluoride varnish (DURAPHAT).

MATERIALS AND METHODS: Forty four premolars were selected, prepared and sectioned into two halves buccolingually,giving mesial and distal halves. One half received the treatment varnish and the other half served as its negative control. Teeth were randomly assigned into two groups; group I (fluoridated miswak varnish), group II (Duraphat varnish). Specimens were evaluated both quantitatively and qualitatively. From each group, half of the specimens and their corresponding controls were examined with Vickers Microhardness Device. While the remaining specimens were prepared and evaluated using Polarized Light Microscopy. Data were analyzed using Paired t test, independent t test and repeated Measures ANOVA followed by post hoc test with Bonferroni correction for microhardness test results. Wilcoxon Sign Rank test and Mann Whitney U test were used for polarized light quantitative results analysis. Significance level was set at p value of 0.05.

RESULTS: Both groups demonstrated a statistically significant increase in enamel surface microhardness compared to their controls ($P<0.0001$). As for polarized light microscopy, both groups; fluoridated miswak and Duraphat, showed a statistically significant increase in the negative birefringence ($P=0.047$), ($p=0.003$) respectively. By comparing both test groups, there was no statistically significant difference in mean percent recovery of microhardness and mean lesion depth percent reduction ($P=0.088$), ($P=0.949$) respectively.

CONCLUSION: Both fluoridated miswak and Duraphat varnishes had comparable remineralizing potential on initial caries-like lesions in permanent teeth. However, Duraphat varnish was more effective in remineralizing the lesion body.

KEYWORDS: Miswak varnish, Fluoride, Duraphat, Initial caries lesions, Remineralization.

RUNNING TITLE: Remineralization effect of fluoridated miswak varnish.

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INTRODUCTION

Natural products are presenting a comeback all over the world, as they symbolize safety compared to the synthetic chemical products which have hazardous effects (1). From a primitive toothbrush to a contemporary aid for enhancing oral hygiene, miswak has proven its efficacy (2). Miswak has been reported to contain diverse inorganic and organic components within its extract. Moreover, many scientific researches verified that miswak has antiseptic, antimicrobial, antiplaque, anticariogenic, antifungal and analgesic effects (2). Miswak sticks discharge notable amounts of calcium and phosphorus when immersed into water.

It has low fluoride content, it contains nearly 1.0 µg/g of total fluoride (3). However, another study by Hattab (4) reported that an insignificant amount (0.07 µg/ml) of fluoride was discharged from water soaked miswak. Gazi et al. (5) investigated miswak effects on the mixed saliva composition, it yielded significant increase in calcium (22-fold), which resulted in salivary saturation that suppressed demineralization

and promoted minerals gain of tooth enamel. Miswak also increased chloride concentration (6-folds), which inhibited calculus formation. Calcium and chloride hamper the attachment of the bacteria on the tooth enamel therefore offering a protective medium.

Manufacturing dental varnishes that incorporate miswak with fluoride, can be a pragmatic preventive measure against caries disease, particularly in communities with deficient financial resources (6).

Wassel and Sherief (6) studied the remineralizing effect and ions releasing of many natural-based dental varnishes, including miswak, propolis and chitosan nano-particles with and without 5% sodium fluoride and the study was conducted on primary teeth enamel. Miswak-based dental varnishes showed considerable ions release and promising results at rehardening enamel surfaces after pH cycles. The miswak fluoride combination showed promising results other than miswak or fluoride varnish alone.

This in- vitro study aimed to investigate the remineralizing effect of fluoridated miswak varnish on initial caries- like lesions in permanent teeth and compare its remineralizing potential with the fluoride varnish (DURAPHAT). The null hypothesis is that there is no difference in the remineralizing effect between fluoridated miswak varnish and sodium fluoride varnish (Duraphat) on initial caries- like enamel lesions in permanent teeth.

MATERIALS AND METHODS

This study was an experimental in- vitro study, approved by the Scientific Research Ethical Committee (0166-10/2020). The minimal sample size was calculated based on the mean difference in percentage of surface microhardness recovery (%SMHR) between group I (fluoridated miswak varnish), obtained from a pilot study that has been conducted for 10 days, and group II (Duraphat varnish), obtained from a previous study by Kooshki et al (7). The percentage of SMH recovery (%SMHR) was calculated as follows: %SMHR= 100 * [(SMHremin - SMHdemin)/ (SMHbaseline - SMHdemin)] (8). The %SMHR for the fluoridated miswak varnish and Duraphat varnish were 76.43±47.26% and 27.58±14.96% respectively. The minimum sample size was 10 halves per subgroup. This was increased to 11 specimens to make up for processing errors. The total sample size = number of groups × number per group= 8×11= 88 halves (44 teeth), based on assuming 95% confidence level and 80% study power.

Forty four caries free human premolars, extracted for orthodontic reasons, were collected from public hospitals, Department of Orthodontics, Faculty of Dentistry, Alexandria University and private dental clinics. Selected teeth were examined using magnifying lens for any previous restorations, enamel cracks or developmental defects.

Premolars were cleaned using fluoride free pumice then washed with distilled water and air dried. The study sample was randomly allocated using a computer-generated list of random numbers to one of the two arms; group I (fluoridated miswak varnish) and group II (Duraphat varnish). Allocation was performed by a trial independent individual and allocation ratio was intended to be equal. Allocation was in blocks of 4 to insure that all study groups have equal number of teeth.

Materials

- **Fluoridated miswak varnish (6)** was prepared according to Wassel and Sherief. It contained 50% miswak ethanolic extract (7.5 ml), distilled deionized water (2.5 ml), colophony resin (2.0 gm) and NaF powder (0.5 gm).
- **Duraphat varnish (9):** 1mL suspension contains 50mg sodium fluoride (5%w/v), in an alcoholic solution (95% ethanol) of natural resins (colophony resin). Other ingredients include: white beeswax, shellac, mastic, sodium saccharin and flavour.
- **Demineralizing solution (10):** It was prepared by mixing 2.2 mM Calcium Chloride ($CaCl_2$), 2.2 mM Potassium dihydrogen Phosphate (KH_2PO_4), 0.05 M acetic acid (CH_3COOH) and 1 M Potassium hydroxide KOH to adjust the pH to 4.4.
- **Remineralizing solution (11):** It was prepared by mixing 1.5 mM Calcium Chloride ($CaCl_2$), 0.15 M Potassium Chloride (KCl) and 0.9 mM Sodium dihydrogen Phosphate (NaH_2PO_4), at pH 7.0.
- **Artificial Saliva (11):** It was prepared by mixing 200 g Methyl-p-hydroxy methyl cellulose, 10.00 g Sodium carboxy methyl cellulose, 0.625 g Potassium Chloride (KCL), 0.059 g Magnesium Chloride hydrated ($MgCL_2$-6H), 0.166 g Calcium Chloride hydrated ($CaCL_2$ & H_2O), 0.804 g Dipotassium Hydrogen Phosphate (K_2HPO_4), 0.326 g KH_2PO, one liter of distilled water and Sodium Hydroxide (0.05 M) was added to the mixture to have a pH 7.0.

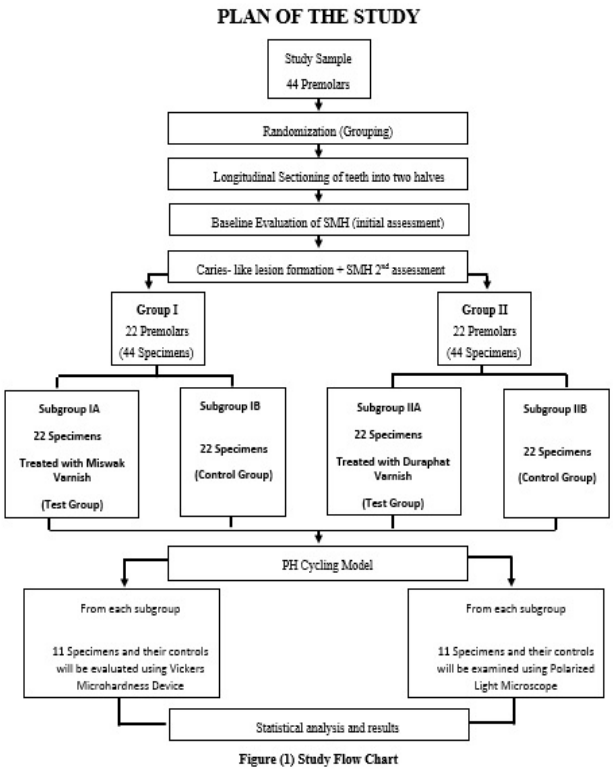

Figure (1) Study Flow Chart

Methods

Teeth preparation

Squares of 4X4 self-adhesive stickers were centrally positioned on the buccal surface of each tooth. Acid-resistant nail varnish was used to paint all teeth surfaces. Then, the stickers were removed exposing only standardized enamel windows. The crown of each tooth was longitudinally sectioned through the center of the enamel window in a buccolingual direction into two halves; a mesial half and a distal one. Each half was regarded as a specimen (88 specimens). One half was treated with the varnish; subgroup IA (22 specimens) to receive fluoridated miswak varnish and subgroup IIA (22 specimens) to receive Duraphat varnish. While the other half was left untreated and considered as its negative control; subgroup IB (22 specimens) and subgroup IIB (22 specimens).

All specimens were re-painted with the nail varnish to coat any surface that has been uncovered after teeth sectioning (if required). Specimens that were evaluated by Vickers Microhardness tester were mounted in a self-cure acrylic resin inside cylindrical plastic mold with their buccal surfaces were mounted perpendicular to the long axis of the block. Specimens that were evaluated by Polarized Light Microscope were also re-painted to cover the cut surface too.

Enamel lesions were created by soaking each specimen in10 mL of demineralizing solution for 96 hours without stirring (11). Then, teeth were washed with deionized water for 30 seconds and kept in artificial saliva till being evaluated.

Miswak Ethanolic Extract Preparation (12)

Fresh cut miswak sticks were obtained from the roots of Arak (Salvadora persica) trees from a commercial market in Saudi Arabia. They were examined and identified by a professor at the Botany department, Faculty of Science, Alexandria University. The miswak extract was prepared at the Pharmacognosy Department, Faculty of Pharmacy, Alexandria University.

1 kilogram of miswak chewing sticks were dried and cut into small pieces with a sharp knife. The resulting pieces were ground to a fine powder using a high speed grinder. For each 40 gram of the powder 120 ml of 95% ethanol was added in a sterile well capped flask, macerated for 3 days at room temperature and then filtered using number 1 whatman filter paper. The solvent was then evaporated in a rotary evaporator at 40^{o} C until complete ethanol evaporation. The crude extract was stored at 4^{o} C refrigerator until further use.

Inductively coupled plasma optical emission spectrometry analysis

Inductively coupled plasma optical emission spectrometry (ICP-OES) is a pioneering technology that is used for elemental analysis of biological samples. It detects and quantifies the present elements using the emission spectrum of the excited atoms (13).

Three samples of the crude extract, one gram each, were analyzed using the Inductively Coupled Plasma Emission Spectrometry (ICP-OES 5100 VDV by Agilent, Australia) at the Institute of Graduate Studies and Research, Alexandria University. Analysis was done on the ethanolic extract rather than the dry plant samples, as it represents the raw material of our varnish.

- Samples preparation for ICP-OES analysis included three main steps: digestion, evaporation and extraction (13).
- Nitric acid/ hydrogen peroxide (HNO_3/H_2O_2) Wet Digestion method (14) was recommended to breakdown the extract organic substances.
- Nitric acid (HNO3), 9 ml and hydrogen peroxide (H_2O_2), 1 ml were added to each sample separately on a hot plate till complete digestion and evaporation.
- Deionized water was added to the dry material, extract was filtered with Whatman number 42-filter paper. The resulting solutions were diluted to 50 ml with distilled water.
- Quantitative determination of the Calcium and Phosphorus elements was done by the ICP-OES device and the mean values of the three samples were recorded.

Treatment phase

For Subgroups IA: Each specimen received a thin uniform coating of fluoridated miswak varnish on the exposed enamel window.

For subgroup IIA: Each specimen received a thin uniform coating of Duraphat varnish on the exposed window of the buccal surface according to the manufacturer's instruction.

The varnishes were left in contact with the teeth for 4 minutes then all specimens were washed with deionized water. All specimens were kept in artificial saliva for 24 hours. Afterward, the varnish was removed from the specimens' surfaces with a scalpel.

For Subgroups IB and IIB: Each specimen was left untreated and stored in artificial saliva till being evaluated.

PH Cycling

pH cycling model was performed over a 10 days period according to ten Cate and Duijsters (10). To imitate caries process, all the specimens were individually soaked in a demineralizing solution for 6 hours, washed with distilled water for 10 seconds. After which, specimens were individually soaked in a remineralizing solution for 18 hours. Along the pH cycling, specimens were stored separately in falcon tubes inside an incubator at 37º C. Demineralizing and remineralizing solutions were daily changed.

Surface microhardness evaluation (Quantitative evaluation)

From each test subgroup (IA and IIA) and their corresponding controls (IB and IIB), half of the specimens were evaluated using Vickers Microhardness tester (Wolpert Wilson Instruments, 402MVD, ITW Test & Measurement (Shanghai) CO., Ltd). Quantitative evaluation of surface microhardness was done before any cariogenic challenge (initial baseline assessment), after demineralization (second assessment) and after PH cycling (final assessment). A load of 50 g was applied to the surface of each specimen for 5 seconds (15). Three indentations at a distance of 100 µm were made at the center of the each enamel specimen, the average was calculated and recorded as Vickers microhardness number (VHN) for each specimen (15).

Polarized Light Microscopy

The remaining specimens from each subgroup were evaluated by Polarized Light Microscope qualitatively and quantitatively. Specimens were prepared by grinding them to thin longitudinal ground sections of about 15 µm thickness, first on a rotary lathe and then by manual grinding on wet glass plate with different granulations of aluminum oxide (AL_2O_3) powder. Each ground section was washed under running water, passed in ascending grades of alcohol (50, 70, 90 and 100%) for dehydration, then cleared using Xylol. Ground sections were mounted using Canada balsam. A longitudinal ground section of sound enamel was prepared as a reference section to be compared histologically with the experimental sections of the study (16).

A-Lesion depth evaluation (Quantitative evaluation) (17)

Using software (image J.46), lesion depth of each ground section was analyzed. The mean depth of the enamel lesion of each specimen was measured by averaging of three lines: one at each side and one at the center of the lesion within the subsurface of the lesion body, perpendicular to the outer layer of the enamel surface and extending to the translucent band.

B-Histological evaluation (Qualitative evaluation) (18)

The test and control ground sections were examined under the polarized microscope to interpret the changes in enamel rods and enamel birefringence as a result of demineralization and remineralization. Photomicrographs were taken at a magnification of X40 to compare the histological features in both test and control specimens. Ground sections were also compared with the reference section.

Statistical analysis

Differences between test and control in each varnish regarding microhardness values were assessed using Paired t test while comparisons between the two types of varnishes were done using independent t test. Within-subgroup differences were compared using Repeated Measures ANOVA followed by post hoc test. Percentage of surface microhardness recovery was calculated according to the following formula: $\%SMHR = 100 \times [(SMH_{Treatment} - SMH_{lesion}) / (SMH_{sound} - SMH_{lesion})]$

Differences between test and control in each varnish regarding lesion depth were assessed using Non-parametric Wilcoxon Sign Rank Test while comparisons between the two types of varnishes were done using Mann Whitney U test. Percent reduction in lesion depth values was calculated according to the following formula: % Reduction= 100 x [(Lesion depth $_{test}$ – Lesion depth $_{control}$) / Lesion depth $_{control}$). Significance level was set at p value of 0.05. Data were analyzed using SPSS version 25.

RESULTS

Results of ICP-OES analysis

Mean (%) concentrations of Ca and P were found to be 16.964 ± 0.84 (%) and 1.091 ± 0.15 (%) respectively.

Results of microhardness (displayed in Table 1)

Table 1: Comparison of microhardness values at different phases between test and control in Miswak and Duraphat varnish

	Miswak Varnish			Duraphat varnish		
	Test (n=11)	Control (n=11)	P value	Test (n=11)	Control (n=11)	P value
	Mean (SD)			Mean (SD)		

Baseline	300.95 (17.67)^{a,A}	291.40 (36.31)a	0.245	267.28 (29.43)a,B	264.43 (39.04)^{a}	0.694
Demineralization	158.15 (34.77)b,A	159.36 (25.14)b	0.891	130.55 (33.88)b,A	135.51 (30.60)b	0.707
Remineralization	250.13 (17.70)c,A	184.34 (24.93)c	<0.0001*	230.68 (27.05)c,A	179.00 (27.92)^{c}	**<0.0001**
P value	<0.0001*	0.0001*		<0.0001*	**<0.0001***	
% Recovery	63.72 (14.73)	18.41 (7.33)	<0.0001*	74.83 (14.33)	35.07 12.43)	**<0.0001**

*Statistically significant at p value≤0.05
Lowercase different letters denote statistically significant difference between phases within each subgroup
Uppercase different letters denote statistically significant difference between Miswak and Duraphat (Test groups) at each phase.

Following demineralization, the microhardness values of both groups decreased significantly as compared to their baseline values (P<0.0001). After treatment, using either fluoridated miswak or Duraphat varnish, the microhardness values of the two groups increased significantly as compared to the demineralization phase (P<0.0001). Nevertheless, it was significantly lower as compared to baseline values (P<0.0001). By comparing the percentage of surface microhardness recovery (%SMHR) of both test subgroups relative to their controls, fluoridated miswak varnish (%SMHR) was 63.72 % while (%SMHR) of its control was 18.41 %. Meanwhile, Duraphat varnish (%SMHR) was 74.83 % compared to 35.07 % for its control. By comparing miswak varnish with Duraphat, there was a statistically significant difference in the microhardness values at baseline while there was no statistically significant difference after demineralization and after treatment. Although percentage of surface microhardness recovery (%SMHR) showed no statistically significant difference between the two groups (P=0.088), miswak varnish achieved a higher surface microhardness recovery relative to its control.

Results of lesion depth evaluation (Quantitative evaluation) (displayed in Table 2)

Table 2: Comparison of lesion depth values at different phases between test and control in Miswak and Duraphat varnish

	Miswak Varnish			**Duraphat varnish**		
	Test (n=11)	**Control (n=11)**	**P value**	**Test (n=11)**	**Control (n=11)**	**P value**
	Mean (SD)			Mean (SD)		
Lesion depth	142.27 (110.9)^{A}	234.18 (144.11)	**0.047***	166.09 (102.10)^{A}	247.82 (115.29)	**0.003***
% Recovery	31.90 (36.49)^{A}			35.23 (16.95)^{A}		

*Statistically significant at p value≤0.05
Uppercase different letters denote statistically significant difference between Miswak and Duraphat (Test groups) at each phase.

After treatment, using either fluoridated miswak or Duraphat varnish, the lesion depth of the two groups decreased significantly as compared to the control ground sections P=0.047 , P=0.003 respectively. By comparing the percent reduction of lesion depth in both groups there was no statistically significant difference (p=0.003), with 31.90 % of lesion depth reduction for fluoridated miswak varnish and 35.23 % for Duraphat varnish. However, Duraphat varnish achieved a greater percent reduction in lesion depth.

Histological evaluation (Qualitative evaluation)
Subgroup IA (Fluoridated miswak varnish)
Examination of specimens treated with fluoridated miswak varnish (subgroup IA) revealed improvement of the caries-like lesions in enamel specimens. Evaluated specimens showed marked reduction in lesion depth. Most of the specimens showed remineralization of the enamel surface layer noted by negative birefringence, however the subsurface layer of enamel was still showing positive birefringence denoting demineralization in most of the specimens (Figure 2).

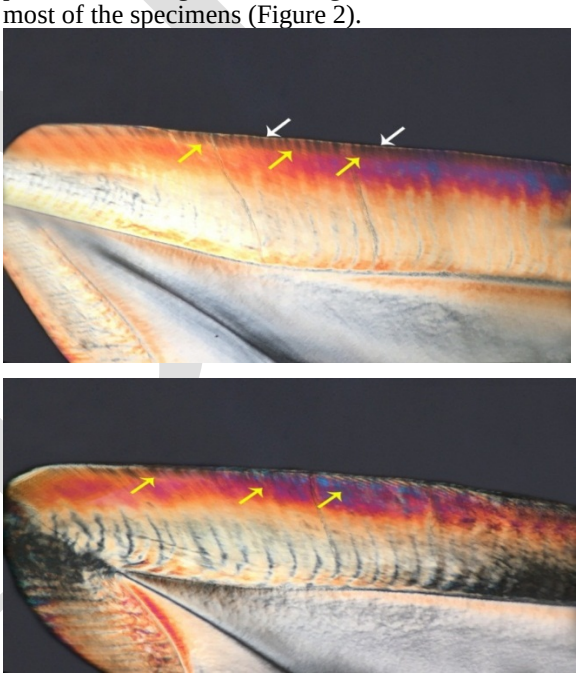

Figure 2A: Polarized light photomicrograph of a longitudinal ground section of the control specimen (subgroup IB) showing obvious surface and subsurface demineralization affecting most of the surface and subsurface of the specimen (yellow arrows) – Mag. X40

Figure 2B: Polarized light photomicrograph of a longitudinal ground section of the test specimen (subgroup IA) (miswak group) showing slight remineralization of the surface layer of enamel noted by negative birefringence (white arrows). However, persistence of the subsurface dark areas of demineralization can evidently by detected (yellow arrows) – Mag. X40

Subgroup IIA (Duraphat varnish)
In this subgroup the evaluated specimens showed an outstanding increase in the degree of remineralization. Some specimens illustrated

almost complete remineralization of the surface layer of enamel denoted by clear negative birefringence especially at the surface layer. However, few specimens presented a narrow zone of demineralization in the subsurface layer (Figure 3). Meanwhile most of specimens revealed marked disappearance of the dark zones of demineralization in the surface and subsurface enamel layers that were seen prior to remineralization.

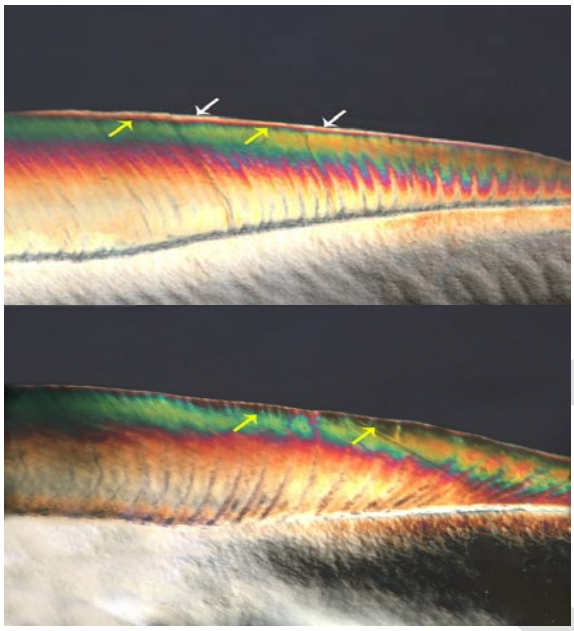

Figure 3A: Polarized light photomicrograph of a longitudinal ground section of the control specimen (subgroup IIB) showing extensive surface and subsurface demineralization increasing in cervical direction (yellow arrows) marked by strong positive birefringence – Mag. X40

Figure 3B: Polarized light photomicrograph of a longitudinal ground section of the test specimen (subgroup IIA) (Duraphat group) showing almost complete remineralization of the surface layer of enamel denoted by clear negative birefringence (white arrows). However, a narrow zone of demineralization can still be seen in the subsurface layer (yellow arrows) – Mag. X40

DISCUSSION

ICP-OES technique has been frequently used by biological research laboratories because of its high precision, synchronous multi-elements scanning and low cost (13). To the best of the authors' knowledge, this was the first study to use ICP-OES method to detect and quantify calcium and phosphorus elements in miswak. ICP-OES analysis allows quantification of minerals content in medicinal plants and their extracts (13) but because of their high organic content which can block metals analysis, Nitric acid (HNO_3) is used for acid digestion to breakdown the organic compounds (14). Accordingly, these results do not reflect the freely bioavailable Ca and P ions but rather give an estimate of the total minerals content.

In the present study, quantitative evaluation of enamel surface was carried out by measuring the surface microhardness. Though the study sample was randomly allocated to either groups by a trial independent individual, there was a statistically significant difference at baseline (SMH) between specimens of both groups. This could be explained because of the selection criteria of the study sample, as the premolars extracted for orthodontic reasons could have considerable variations in the developmental stage and accordingly a mutable level of enamel maturation and surface porosity, which was evident in baseline (SMH). This was overcome as each test specimen was compared to its other half which served as a negative control, in addition to calculating the percentage recovery of surface microhardness (SMHR %), which included the baseline of each specimen separately.

Within groups comparison results revealed that there was a statistically significant difference in the mean enamel surface microhardness between subgroup IA (treated with fluoridated miswak varnish) and subgroup IB (control group). This finding is in agreement with Wassel and Sherief (6) who concluded that 10% miswak fluoride varnish has the potential to favor remineralization and increase SMHR% due to its high F^-, Ca^{++} and PO_4^{---}release.

Similarly, there was a significant difference in the mean enamel surface microhardness between subgroup IIA (treated with Duraphat varnish) and subgroup IIB (control group). This result is in agreement with Mohd Said (19) who concluded that Duraphat varnish, which contains 5% sodium fluoride alone, could achieve remarkable remineralization of carious non-cavitated WSLs.

By comparing both study groups, a slight increase in enamel surface microhardness in group I (miswak group) than in group II (Duraphat group) was noted, but was insignificant. This finding is in agreement with Wassel and Sherief (6) who concluded that 10% miswak fluoride varnish had a higher SMHR% compared to experimental 5% sodium fluoride varnish with no significant difference.

Since a higher miswak extract concentration (50%) was used in the study varnish, a superior remineralizing effect was anticipated. This postulation did not hold true. However, there are many possible explanations for the results. Ekambaram et al. (20) argued that the remineralizing potential of different calcium and phosphate based remineralizing systems is drastically affected by the nature of the incorporated calcium and phosphate ions and their stabilization method.

This is in agreement with Al Dehailan et al. (21) who observed that different varnish compositions may interfere with enamel lesions remineralization,

fluoride release and deposition on tooth surface. Furthermore, Godoi et al. (22) clarified that the hydrophobic nature of colophony resin can affect the varnish solubility.

Al Dehailan et al. (23) agreed that adding calcium and phosphate ingredients to the varnish formulation had a superior effect on enamel lesions rehardening, as long as it will not hinder the fluoride ions bioavailability. Another possible explanation is that Duraphat varnish, unlike our varnish, contained other stiffening agents, aside from colophony resin, like shellac and beeswax (9) which extended the contact time with enamel surface.

In addition to the microhardness analysis, polarized light microscopy (PLM) was used for qualitative and quantitative lesion change evaluation. Each specimen was compared with its other half which served as a control. The advantage of this design is that the depth of the artificial enamel lesion can be determined for any tooth before lesion treatment and then compared with lesion post treatment.

In the present study, qualitative data obtained from PLM showed that most of the representative lesions, treated by either fluoridated miswak varnish or Duraphat varnish, exhibited a shift from positive to negative birefringence with a marked reduction in the depth of the lesion that is present in the control specimens. However, Duraphat varnish was more effective in remineralizing the lesion body.

This was proven quantitatively by the significant reduction in the mean lesion depth of subgroups IA (fluoridated miswak) and IIA (Duraphat) in comparison with their corresponding controls. However, subgroup IIA (Duraphat) showed greater percent reduction in the mean lesion depth compared to subgroup IA (fluoridated miswak) though the difference was not significant.

Although there was no significant difference in the remineralizing potential of both varnishes, fluoridated miswak varnish superseded the Duraphat varnish with respect to the mean percent recovery of enamel microhardness while the later had greater reduction in the mean percent values of the lesion depth.

One of the possible explanation of these results was proposed by ten Cate and Arends (24) who confirmed that enamel surface remineralization hinders the subsurface lesion remineralization. Remineralization of the body lesion takes more time than surface remineralization (25).

Balakrishnan et al. (26) supported the same explanation that minerals restoration occurs to a great extent at the surface level than the underlying body lesion. Another explanation was proposed by Ekambaram et al. (20) who claimed that low calcium and phosphate solubility retards subsurface remineralization.

Based on the overall quantitative and qualitative results, it could be concluded that both fluoridated miswak and Duraphat varnishes had comparable remineralizing potentials. Accordingly, the null hypothesis of the present study was accepted.

In vitro models are frequently used in cariology research as their controlled scientific settings increase sensitivity and limit variability compared to clinical models (23). Yet they have their own shortcomings as they lack the full simulation of the complex oral environment, specifically the dental caries contributing circumstances (27).

Within the limitations of the current study, it was noticed that both fluoridated miswak and Duraphat varnishes can be used as remineralizing agents. It is worth noting that clinically, the antibacterial and remineralizing potentials of this experimental varnish, when combined together, may yield different outcomes.

CONCLUSION

Based on the results of the present study and its limitations, it could be concluded that:

1. Fluoridated miswak varnish can be used as a remineralizing agent.
2. Both fluoridated miswak and Duraphat varnishes had comparable surface remineralizing potentials
3. Duraphat varnish was more effective in remineralizing the lesion body.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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