Original paper

Comparative evaluation of the remineralizing potential of *Salvadora persica* and probiotic yogurt on incipient enamel lesions: An ex-vivo study

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Abstract

Background. *Salvadora persica* (miswak) is known to exert antibacterial, antifungal, antioxidant, and anticariogenic effects by elevating the pH of plaque after the consumption of sucrose.

Objectives. The study aimed to compare the effectiveness of *S. persica* and probiotic yogurt in the remineralization of tooth enamel on artificially produced enamel lesions.

Material and methods. A total of 40 intact human premolars were collected and each tooth was sectioned longitudinally into 2 identical halves in a buccolingual direction. The buccal halves were selected for inclusion in this study, and standardized windows (5 mm × 3 mm) were isolated on the buccal surface of the enamel. The samples were incubated in a demineralizing solution at 37°C for 96 h. Subsequently, they were randomly selected for treatment with one of the experimental remineralizing solutions (*S. persica* or probiotic yogurt). After treatment, the samples were examined using scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and polarized light microscopy at baseline, after demineralization and after remineralization.

Results. The remineralizing effect of *S. persica* was found to be greater than that of probiotic yogurt. With regard to mineral content, *S. persica* exhibited the highest calcium and phosphorus levels among all groups. No significant differences were observed between the samples treated with *S. persica* and normal enamel.

Conclusions. *Salvadora persica* extract has been demonstrated to effectively reduce the demineralization of enamel in experimental conditions. Furthermore, it has the potential to restore the mineral content to its original level.

Keywords: scanning electron microscopy, probiotic yogurt, *Salvadora persica*, energy dispersive X-ray, polarized light microscope

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Introduction

Dental caries is a dynamic process initiated by the demineralization of enamel, followed by the involvement of the deeper layers and resulting in cavitation.[1] White spot lesions, also known as surface-softened defects or incipient lesions, represent the initial stage of carious lesions. The lesions clinically manifest as opaque white areas when air-dried. Histologically, they exhibit an area of demineralization with an intact surface layer.[2] The presence of adequate amounts of calcium, phosphate and fluoride ions in saliva promotes the formation of fluorapatite, which enhances the process of remineralization. Unfortunately, in patients at high risk, the calcium and phosphate levels in saliva may not be sufficient for complete remineralization. Consequently, the supplementation of calcium and phosphate ions is essential for an effective remineralization process.[3]

Numerous non-invasive remineralization strategies were used in the clinical management of caries.[1] In recent years, several remineralizing agents have been developed and investigated. Milk and milk products play a unique role in remineralization due to their protective effects against the development of dental caries.[4] Additionally, the use of probiotics against bacteria-mediated diseases has recently been highlighted. The term "probiotic" was first introduced by Ferdinand Vergin in 1954 and originates from a Greek word meaning "for life".[5] According to the Food and Agriculture Organization/World Health Organization (FAO/WHO) (2001),[6] probiotics are defined as live bacteria that confer a benefit to the host when consumed in an appropriate amount. The harmless bacteria can compete with pathogens that threaten the host.[7] Most probiotics belonging to the *Lactobacillus* or *Bifidobacterium* genera have been shown to have a significant effect on the prevention of dental caries by inhibiting the growth properties of *Streptococcus mutans* bacteria.[8–10] Moreover, the concentration of calcium in the saliva is used as an indicator to estimate the balance between the demineralization and remineralization stages of enamel.[11] Many authors have asserted that the presence of a high concentration of calcium in yogurt can stabilize the oral biofilm and reduce the probability of dental caries formation.[7,9,12]

Miswak (*Salvadora persica*) is a natural product that has been widely used in many Muslim countries for the maintenance of oral health since ancient times. The product is also known by several other names, including the Arak tree, the mustard tree and the natural toothbrush tree. The miswak is a wooden stick that is used to clean the teeth and gums. It has been demonstrated to exert antibacterial, antifungal, antioxidant, and anticariogenic effects by elevating the pH of the plaque after the consumption of sucrose.[13]

The therapeutic and mechanical effects of miswak may be attributed to its constituents, such as calcium, phosphorus and fluoride, which can harden the outer surface of enamel and make it more resistant to caries. Similarly, trimethylamine, another constituent of miswak, can alter the bacterial contents of dental plaque due to its antibacterial effects.[14]

This study aimed to compare the remineralizing effect of *S. persica* and probiotic yogurt on artificially produced enamel lesions in order to identify the most effective natural product that can inhibit demineralization and promote the remineralization process in a carious lesion.

Material and methods

Sample size calculation

A sample size of 20 was calculated for each group, with a confidence interval (*CI*) of 95%, power of 80% and a significance level of 0.001.

Miswak extract

The roots of fresh miswak were chopped into tiny pieces and dried for 3 days at room temperature. Then, the pieces were ground using an electric grinder (Brightsail Industries Group Co., Ltd., Jiangyin, China). Subsequently, 10 g of the powder was added to 100 mL of ethanol in a sterile bottle and allowed to soak at 4°C for 48 h. Thereafter, centrifugation (Thermo IEC CL40R; Thermo Fisher Scientific, Waltham, USA) of all samples was conducted at 2,000 rpm for 15 min. The liquid content was then evaporated using a rotary evaporator (R-1010; Keda Machinery and Instrument Equipment Co., Ltd., Zhengzhou, China). The supernatant extract was collected and stored in the refrigerator in order to be used within 1 week.[15]

Preparation of casein phosphopeptide additives

A probiotic yogurt (including Bifidobacterium lactis BB-12 and *Lactobacillus acidophilus*) was used due to its high concentration of casein phosphopeptides (CPP). The yogurt (4,000 g) was centrifuged (Thermo IEC CL40R; Thermo Fisher Scientific) at 25°C for 10 min, resulting in the formation of 2 distinct portions: an insoluble portion precipitated at the base of the test tube; and a soluble portion (the supernatant containing CPP). The suspension was collected after 3 rounds of centrifugation and stored for further use.[16]

Tooth preparation

A total of 40 maxillary premolar teeth, extracted for orthodontic purposes, were collected from the outpatient dental clinic at the Faculty of Oral and Dental Medicine, Nahda University in Beni Suef, Egypt. The teeth were cleaned to remove debris and blood immediately after

extraction and sterilized using an autoclave (40 min at 120°C and 776 mmHg). Teeth exhibiting obvious signs of caries, restorations or cracks were excluded from the study. The selected teeth were soaked in 1 mL of sodium hydroxide (NaOH) for 48 h to remove any residual pellicle and kept in 0.1% thymol solution until further use. Each tooth was sectioned longitudinally into 2 identical halves in a buccolingual direction using a microtome (Leica SP 1600; Leica Microsystems, Wetzlar, Germany). Only the buccal halves were included in this study. Standardized windows (5 mm × 3 mm) were isolated on the buccal surface of enamel in each tooth using nail polish.

Grouping of teeth

The samples (N = 40) were divided into 4 groups, as follows: a negative control group (–ve CG) (n = 40), which was comprised of normal teeth before demineralization; a positive control group (+ve CG) (n = 40), which consisted of demineralized teeth without treatment; an experimental group I (EGI) (n = 20), which consisted of teeth remineralized with *S. persica*; and an experimental group II (EGII) (n = 20), which was comprised of teeth remineralized with probiotic yogurt.

Before demineralization, the samples were examined using scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and polarized light microscopy. White spot lesions were induced by soaking the samples in a demineralizing solution (2.2 mM of $CaCl_2 \cdot 2H_2O$, 2.2 mM of KH_2PO_2 and 45 mM of acetate; pH = 4.6) and incubating them at 37°C for 96 h.[17] The demineralized samples were randomly divided into 2 experimental groups, according to the type of remineralizing solution used (*S. persica* extract or probiotic yogurt). The samples were soaked in the remineralizing solutions at 37°C for 8 days, and the solution was changed every 2 days. The treated samples were then immersed in distilled water until testing.

SEM

The buccal segments were anchored on stubs without coating and examined using SEM (JSM-IT200 InTouchScope™; JEOL USA, Inc., Peabody, USA) to evaluate the morphological changes on the tooth surface. The analysis was performed using the following parameters: an acceleration voltage of 25 kV, a working distance of 15 mm and pressure in the pulp chamber of 5–10 Pa.

Polarized light microscopic assessment

Longitudinal sections (200-µm thickness) of the samples were obtained using a microtome (Leica SP 1600; Leica Microsystems) and observed under a polarized light microscope (PriorLux POL™; PRIOR scientific, Cambridge, UK) using Canada balsam as the imbibition medium. Photomicrographs were taken at ×100 magnification and analyzed using the Image J software version v. 1.48q (https://imagej.net/ij/index.html).

EDX analysis

The quantitative mineral content of the surfaces was calculated using the EDX analysis. The amount of calcium and phosphorus (wt%) was calculated for all groups at baseline, after demineralization and after remineralization.

Statistical analysis

The IBM SPSS Statistics for Windows software v. 18 (SPSS Inc., Chicago, USA) was used to analyze the results. Data is presented as mean and standard deviation. The normality of the data was examined using the Kolmogorov–Smirnov and Shapiro–Wilk tests. One-way analysis of variance (ANOVA) was used to compare the tested elements in different groups, and pairwise differences were detected by Tukey's post hoc test. Two-sided p-values ≤0.005 were considered statistically significant.

Results

SEM observations in different groups

–ve CG

Scanning electron microscopy examination of the –ve CG samples revealed normal enamel surface morphology, enamel rod ends in some areas with horizontal lines at equal intervals (perikymata grooves), and ridges. Additionally, multiple fine scratches and scattered pores were observed on the enamel surface (Fig. 1A,B).

+ve CG

In the +ve CG, SEM examination after demineralization revealed a porous enamel surface (Fig. 1C). The prism cores exhibited erosion, with the prism peripheries remaining intact. Additionally, relatively narrow and shallow cracks were observed. Some areas demonstrated type I etching patterns with erosion of the rod cores and preservation of the prism peripheries. Areas of type II etching patterns were observed, with predominantly eroded interrod regions, leaving behind raised bumps representing the enamel rods (Fig. 1D).

EGI

An apparent reduction in the porosity of the enamel surface with a decrease in the number of type I etching patterns was observed in the EGI (Fig. 1E). Some rod holes were completely obliterated with globular precipitates, while others were empty (Fig. 1E,F).

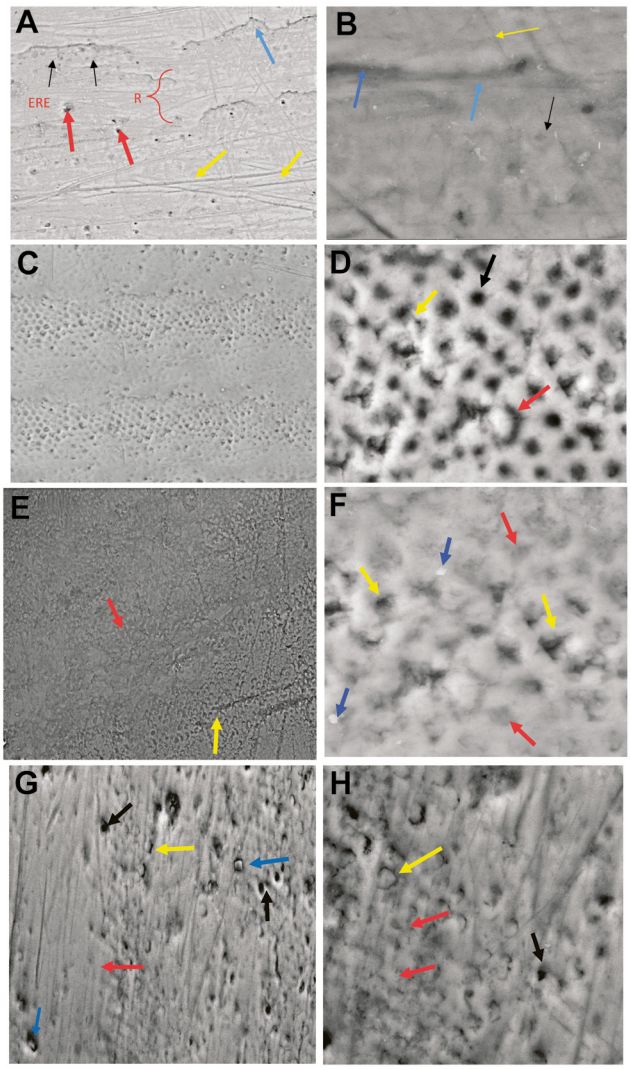

Fig. 1. Scanning electron micrographs of the enamel surfaces of specimens treated with miswak

A. Negative control group (–ve CG) sample showing enamel rod ends (ERE) in some areas (black arrows), perikymata grooves (blue arrow) and ridges (R). Multiple scratches (yellow arrows) and scattered pores (red arrows) were observed on the surface (×1000 magnification); B. Higher magnification of the previous image shows ERE (black arrow) and prikymata grooves (blue arrows). The surface contains a few scratches (yellow arrow) (×5000 magnification); C. Positive control group (+ve CG) sample showing a porous enamel surface (×1000 magnification); D. Higher magnification of the previous image shows the erosion of the prism cores with preserved prism peripheries (type I; black arrow), type II etching pattern with preferential loss of interrod enamel (red arrow), and narrow and shallow cracks (yellow arrow) (×5000 magnification); E. Experimental group I (EGI) sample showing a reduction in surface porosity, with completely obliterated prism cores (red arrow) and empty prism cores (yellow arrow) (×1000 magnification); F. Higher magnification of the previous image shows completely obliterated prism cores (red arrows), globular precipitation within the prism core (blue arrows) and empty prism cores (yellow arrows) (×5000 magnification); G. Experimental group II (EGII) sample showing completely obliterated prism cores (red arrow) and incompletely obliterated prism cores (yellow arrow). Globular precipitates were observed inside the prism cores (blue arrows), and pores were present in the samples (black arrows) (×1000 magnification); H. Higher magnification of the previous image shows completely obliterated prism cores (red arrows), a reduction in areas with type II etching pattern (yellow arrow), and enamel rod erosion as a result of demineralization (black arrow) (×5000 magnification).

EGII

In the EGII, prism cores completely obliterated with globular precipitates were observed on the enamel surfaces. Additionally, some incompletely obliterated prism cores were detected. The surface area of the type II etching pattern was reduced due to the thickening of the interrod enamel (Fig. 1G,H).

Polarized light microscopy observations in different groups

–ve CG

The samples in the –ve CG had a sound enamel surface and no signs of demineralization. An area of translucency was observed on the surface (Fig. 2A).

+ve CG

The samples in the +ve CG exhibited a relatively high degree of positive birefringence with the loss of the typical enamel structure within the lesion. This revealed a dark brown stain associated with the demineralization effect caused by the acid on the outer enamel surface (Fig. 2B).

EGI

The EGI presented with a birefringent zone, known as the remineralizing zone (RZ), on the surface of the treated

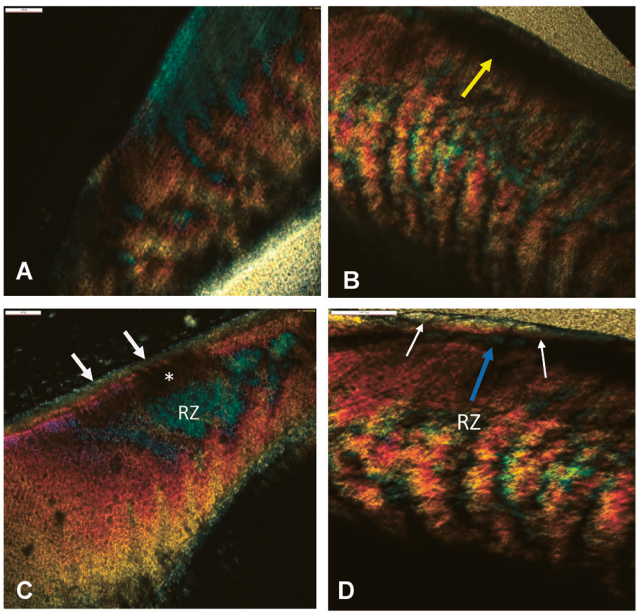

Fig. 2. Polarized light microscopy images of the samples (×40 magnification)

A. –ve CG sample showing an area of translucency with no signs of demineralization on the enamel surface; B. +ve CG sample showing a positive birefringence lesion area (yellow arrow); C. Remineralizing zone (RZ), an isolated area of demineralization (*) and a mineral precipitation band (white arrows) in the EGI sample; D. EGII sample showing the RZ (blue arrow) and a mineral precipitation band (white arrows).

enamel lesion. The zone was thick and more prominent, indicating a decrease in the body of the lesion. Additionally, mineral precipitation bands and isolated areas of demineralization were observed on the enamel surface (Fig. 2C).

EGII

In the EGII, a thin and less prominent negative birefringent zone was observed on the treated enamel surface, accompanied by the presence of a mineral precipitation band (Fig. 2D).

EDX calcium analysis

The –ve CG recorded the highest mean calcium value (49.1 ±1.85), followed by the EGI (48.1 ±1.28) and EGII (46.12 ±1.36), with the lowest value recorded in the +ve CG (42.82 ±1.84). The results of the ANOVA revealed a statistically significant difference between the groups (p = 0.000). The mean value in the EGI was significantly different from those in the +ve CG and EGII, but no significant difference was observed between the –ve CG and EGI, as determined by Tukey's test (Table 1) (Fig. 3).

EDX phosphorus analysis

The –ve CG and EGI recorded the highest mean phosphorus values (20.26 ±0.45), followed by the EGII (20.00 ±0.74), with the lowest value recorded in the +ve CG (18.65 ±0.43). A statistically significant difference between the groups was observed using ANOVA (p = 0.000). The mean value in the +ve CG was found to be significantly different from those observed in the other groups. However, no significant differences were identified between the –ve CG, EGI and EGII (Table 1) (Fig. 3,4).

Table 1. Descriptive statistics and significant differences in calcium (Ca) and phosphorus (P) levels between the groups (analysis of variance (ANOVA) test)

Group		*M*	*SD*	*SE*	95% *CI* for *M*		Min	Max	*F*	*p*-value
					lower bound	upper bound				
Ca	–ve CG	49.10[a]	1.85	0.58	47.78	50.42	46.59	50.98	29.86	0.000*
	+ve CG	42.82[c]	1.84	0.58	41.5	44.13	40.36	44.78		
	EGI	48.10[a]	1.28	0.40	47.18	49.01	46.59	49.72		
	EGII	46.12[b]	1.36	0.43	45.15	47.09	44.78	47.98		
P	–ve CG	20.26[a]	0.45	0.14	19.94	20.59	19.88	20.9	20.94	0.000*
	+ve CG	18.65[b]	0.43	0.14	18.34	18.96	18.07	19.09		
	EGI	20.26[a]	0.45	0.14	19.94	20.59	19.88	20.9		
	EGII	20.00[a]	0.74	0.23	19.47	20.53	19.09	20.9		

Data presented as wt%. *M* – mean; *SD* – standard deviation; *SE* – standard error; *CI* – confidence interval; –ve CG – negative control group; +ve CG – positive control group; EGI – experimental group I; EGII – experimental group II; * statistically significant ($p \leq 0.005$, Tukey's post hoc test). Different superscript letters indicate statistically significant differences between the groups.

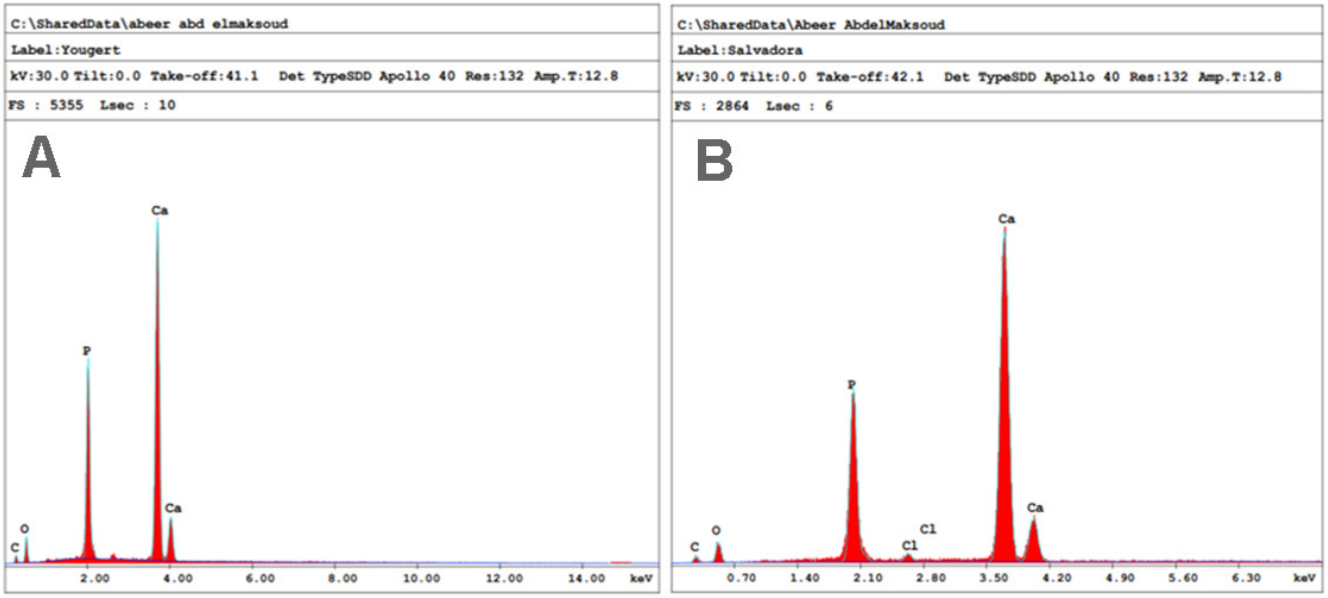

Fig. 3. Energy dispersive X-ray (EDX) element analysis in different groups

A. Probiotic yogurt; B. *Salvadora persica*.

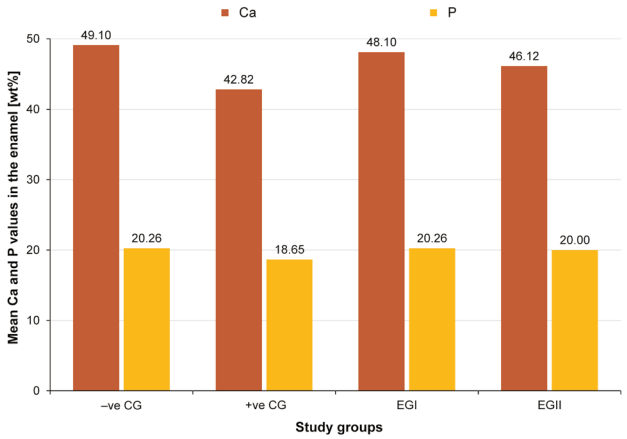

Fig. 4. Mean calcium (Ca) and phosphorus (P) values in the enamel in different groups

Discussion

Initial caries pass through different stages, starting from changes in the molecular structure of the tooth's hydroxyapatite crystal and ending with the formation of white spot lesions. The progression of these stages is enhanced by a continuous imbalance between protective and pathological variables, which may result in the dissolution of the hydroxyapatite crystals and the initiation of the demineralization process.[18]

Modern dentistry has focused on the treatment of initial caries (non-cavitated or white spot lesions) and the replenishment of minerals lost during remineralization. This process helps to precipitate minerals on the demineralized enamel surface, thereby reducing the progression of the disease and enhancing the aesthetics.[19]

This study aimed to evaluate the effectiveness of miswak in remineralizing initial carious lesions in enamel compared to that of probiotics. The SEM and polarized light microscopic results indicate a significant increase in the level of remineralization in all the tested samples treated with miswak. This finding is consistent with another study, which reported an increase in enamel hardness after brushing with miswak.[20] Fluoride has a high affinity for binding with OH ions in hydroxyapatite, forming fluorapatite, which replaces the demineralized hydroxyapatite.[21] In another study, different concentrations (5% and 10%) of miswak were applied to demineralized enamel surfaces for 7 days, and a remineralizing effect was observed, attributed to the presence of fluoride in the extract.[22]

Another potential explanation for the defensive effects of miswak versus citric acid is the immediate formation of a polymer on the enamel surface. This polymer is reported to have a protective effect against further demineralization.[15] One study evaluated the effect of fluoridated miswak sticks on demineralized lesions that appeared after removing an orthodontic appliance and reported enhanced remineralizing effects on the demineralized areas.[23]

The antibacterial effect of miswak is attributed to the presence of many active compounds such as trimethylamine, fluoride, vitamin C, salvadorine chlorides, silica, sulfur, and small amounts of saponins, flavonoids, sterols, and tannins. Studies have demonstrated that *S. persica* inhibits the growth and colonization of *S. mutans*.[13,24] Al-Sohaibani and Murugan showed that the antibacterial effect of *S. persica*, which is extracted from the bark, pulp, or both, may be due to the presence of trimethylamine, sodium chloride, potassium chloride, and salvadourea.[24] Darout et al. correlated the antimicrobial effect of miswak with the presence of anionic components, including thiocyanate.[25] The leaching of thiocyanate from *S. persica* has been demonstrated to elevate salivary thiocyanate levels and improve the effectiveness of the hydrogen peroxide–peroxidase–thiocyanate system in the saliva.[25,26] Another study has shown that the aqueous extraction of miswak released more ionic concentrations of calcium, phosphorus and fluoride than the alcoholic extract, which may account for its superior remineralization effect.[27] To the best of our knowledge, there is no evidence that miswak has an allergenic effect. However, 1 case report published in 2017 reported the occurrence of allergic contact stomatitis after the use of *S. persica* toothpaste. The author concluded that it is a very rare condition.[28]

The probiotic yogurt used in this study was free from any artificial sugars, in order to avoid their cariogenic effects. Even though yogurt contains the intrinsic lactose sugar (cariostatic), it was proven to be the least cariogenic sugar of all mono- and disaccharides. Milk yogurts may have a protective effect on the teeth, but only when no sugars are added to them.[29] However, the results of the current study indicated that the probiotic yogurt extract may also have a protective effect against caries. This finding is in accordance with those of previous studies, which correlated a decrease in dental caries with daily consumption of dairy products.[16,30–32] Further analysis of the various constituents of dairy products revealed that each constituent plays a unique role in enhancing the remineralization effect. The protein and fat content in milk may inhibit the demineralization effect of acidogenic bacteria and promote the formation of a protective barrier against further mineral loss.[16] On the other hand, the enzymes present in milk may play a key role in diminishing the growth of cariogenic bacteria.[30–32]

The protective action of yogurt against dental caries may be attributed to the similarity between the components in yogurt and milk. Furthermore, yogurt has additional properties that enhance its remineralization effect. The high protein concentration in yogurt results in the incorporation of dry milk with low or no fat during processing. Moreover, the concentrations of calcium and phosphorus in yogurt are the same as those in milk. However, calcium remains in its ionic form due to the characteristic low pH of yogurt. Furthermore, yogurt contains a higher concentration of CPP than milk. These differences enhance the remineralization effects of yogurt compared to dairy milk.[33]

The EDX analysis revealed that the mineral content of the 2 remineralizing solutions differed significantly. In particular, the concentrations of calcium and phosphorus in *S. persica* were found to be significantly higher than those in yogurt. Furthermore, the application of *S. persica* on demineralized surfaces can elevate calcium and phosphorus levels to their original values. These findings indicate that miswak is a suitable remineralizing agent for the treatment of white spot lesions. Further studies are necessary to assess the remineralization effects of various casein derivatives and natural products on the enamel surface. Similar experiments should be conducted in vivo (on animals) to evaluate the action of these elements on surrounding vital structures. Further studies are required on both experimental groups (*S. persica* and yogurt) using different methodologies.

Conclusions

In the present study, *S. persica* effectively restored the minerals that had been lost during the demineralization of enamel. The effects of *S. persica* were superior to those of probiotic yogurt.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Research Ethics Committee of the Faculty of Oral and Dental Medicine at Nahda University in Beni Suef, Egypt (approval No. 010722). Written informed consent was obtained from the study participants prior to their inclusion in the study.

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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