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Effect of Cranberry Extract and Nano-Hydroxy Apatite Powder Mixed with Olive Oil on Microhardness and Micromorphology of White Spot Lesion

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ORIGINAL STUDY

Effect of Cranberry Extract and Nano-hydroxy Apatite Powder Mixed With Olive Oil on Microhardness and Micromorphology of White Spot Lesion

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Abstract

Purpose: This study was performed to examine the effect of cranberry extract and nano-hydroxyapatite (nHA) powder mixed with olive at different times of application on microhardness and micromorphology of white spot lesion. **Patients and methods:** A total of 16 premolars were selected. The samples were sectioned mesiodistaly into two halves to obtain 32 specimens, then divided into two groups according to materials used; A1: Cranberry extract, A2: nHA paste. The two groups were subdivided into two subgroups according to the time of assessment; B1: after 10 min and B2: after 8 days of material application. All the samples were treated by demineralizing solution for 72 h, and then the tested materials were applied. Microhardness test was performed three times for each sample, at baseline, after demineralization, and after treatment for each period (after 10 min and after 8 days). Scanning electron microscope was performed to detect the change of the micromorphology of the specimens. **Results:** Microhardness results revealed that both the two materials show a significant increase in microhardness with no significant difference between them. Scanning electron microscope demonstrated improvement in demineralized enamel surface defects in the two test groups. **Conclusion:** Cranberry extract and nHA powder mixed with olive oil could be considered effective options to re-mineralize the white spot lesion of enamel regardless of the time of application.

Keywords: Cranberry extract, Nano-hydroxy apatite paste, White spot lesion

1. Introduction

Dental caries is the most common dental disease and a significant public health issue [1]. Early detection and treatment are possible, but the condition is irreversible once the incipient lesion turns into cavitation. Because of this, it is crucial to prevent caries from forming in its early stages rather than creating treatment options for its advanced stages [2].

A white patch of demineralized enamel known as a white spot lesion (WSL) can be seen on the surface of the teeth. It is the first stage of a carious lesion, and the activity of bacterial plaque determines its cause. Due to the positioning of orthodontic brackets, which encourages plaque buildup and the development of white spots, this lesion is a frequent adverse reaction for patients wearing fixed orthodontic appliances [3].

Nowadays, remineralization of noncavitated carious lesions is prioritized as a noninvasive treatment option to slow the advancement of the disease and improve the appearance, strength, and functionality of teeth [4].

Herbal extracts rich in Proanthocyanidins (PAs), particularly cranberry extract, are receiving a lot of interest for the prevention of dental caries [5]. PAs, a type of condensed tannins, are the main polyphenols found in cranberry extract which is a phytotherapeutic agent. Fruits and vegetables commonly contain PAs, a naturally occurring plant metabolite. It is said to play a role on the re-mineralization

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process through deposition of minerals over the surface of lesion by forming insoluble complexes [6].

Nano-hydroxyapatite (nHA) was considered promising in tooth re-mineralization due to its resemblance to the bone and mineral composition of teeth, biocompatibility and bioactivity. nHA particles resemble dental apatite in terms of crystal structure and morphology [7]. Due to its tiny size, nHA has a significant potential to penetrate through the enamel rods and has a great ability to repair lesions [8].

Therefore, this in vitro study was conducted to examine the effect of cranberry extract and nHA powder mixed with olive oil after different times of material application on microhardness and micromorphology of white spot lesion.

2. Patients and methods

This research was performed after the approval of research ethical committee of Faculty of Dental Medicine, Al-Azhar University for Girls, in accordance with international guiding principles, Code: REC-OP-23-02.

2.1. Preparation of cranberry extract solution

The maceration procedure was used to extract the alkaloids from the dried cranberry samples. For 48 h at room temperature, 20 gm of dried fruit were macerated with 200 ml of hydroalcoholic solvent, with a solvent ratio of ethanol (70%): water (30%), in a conical flask that was plugged with cotton and kept periodically on a rotating shaker at 190–220 rpm. Whatman Filter Paper was used to filter the macerated liquid. To acquire the crude extracts, the extraction procedures took three days. A vacuum rotary evaporator was used to evaporate and concentrate these extracts under reduced pressure. The residue that was left behind was reddish pink in hue, semi-solid, and water-soluble. At 4 °C, it was kept in a sterile air tight container until use [9].The solution was prepared by measuring 600 mg of cranberry extract powder with sensitive balance. To make a concentration of 0.6% w/v, they were then dissolved in 100 ml of distilled water [6].The primary solution's pH was then measured using a pH metre (AD11), and it was set at pH = 3. The pH was then changed to 7.2 by adding 100 mg of $NaHCO_3$ [10].

2.2. Preparation of nano-hydroxyapatite paste

The nHA powder was obtained from Nano Tech Egypt for Photo-Electronics Company. It was prepared through the wet chemical precipitation technique, in this method of synthesis, 100 ml of 0.3 M H_3PO_4 solution was added to an equal volume of 0.5 M $Ca(OH)_2$ solution at a drip rate of two drops per second, at room temperature. The pH value was kept above 10 by the addition of ammonium hydroxide (NH_4OH) during the precipitation process. The resultant precipitate was left in the mother solution for 5 days and finally was washed with deionized water and dried in an oven at 110 °C for 2 h [11].

The prepared nHA is white in colour, soluble in water and ethanol, has rod-like shape and the size is ranging between 100 ± 30 nm (L), 20 ± 5 nm(D).

To prepare the paste which can be applied on tooth surface, the powder and liquid were measured on a paper pad by using a sensitive balance in a 1 : 1 weight ratio of olive oil to nHA powder [12].

2.3. Teeth selection and preparation

The study involved a total of 16 sound human premolars. From the ages of the patient (18–25) years, all collected teeth were extracted for orthodontic reasons. To rule out fractures, cavities, enamel abnormalities, or other flaws, the chosen teeth were inspected by magnifying lens (Bio Art, Brazil) at 2.5 magnification. In order to get rid of any plaque, blood, or remaining periodontal ligaments, they were scaled by hand scaler [10]. The teeth were prepared by sectioning the crown horizontally to separate the coronal portion from the radicular portion of each tooth under water coolant using low speed double faced diamond disc (Besqualdia −disc NY 11373, USA size 22 mm), mounted in grinding machine (Demco alloy grinder, dental maintenance co., INC..BONSALL, CALIF., U.S.A), after which the crowns were cut in half in a mesiodistal orientation to produce 32 samples [13]. On the buccal and lingual surfaces of the sectioned samples, a window measuring 4 × 4 mm was made in the middle; the remaining surface was then painted with acid-resistant nail polish and left to dry [14]. The samples were mounted with their enamel surface facing upward in a mold filled with self-curing acrylic resin. The samples were taken out of the mold when the acrylic resin had dried.

2.4. Preparation of white spot lesion

All the samples were immersed for 72 h at room temperature in 20 ml of the demineralizing solution. It consisted of 2.2 mM calcium chloride dihydrate, 2.2 mM sodium hydrogen phosphate dihydrate, and 0.05 M lactic acid solution ($2H_2O$, $CaCl_2$ = 2.2 mM;

$2H_2O$, NaH_2PO_4 = 2.2 mM; lactic acid = 0.05 M) at pH 4.5 [12].The solution was exchanged every 24 h to keep the pH constant. All the samples were rinsed with distilled water for 10 min while being stirred after artificial carious lesions were created, and they were then allowed to air dry.

2.5. Remineralization procedure

After demineralization, depending on the re-mineralizing material, the samples were split into two groups of 16 each. Group (A1): cranberry extract solution; the demineralized samples were immersed into 20 ml of cranberry extract solution, group (A2): nHA paste group; the demineralized samples were painted with a paste of nano-hydroxyapatite powder mixed with olive using micro brush. Each group were subdivided into two subgroups (eight each) according to the time of application (B1) for 10 min and (B2) for 8 days. In 8 days subgroups, the solution and the paste were daily exchanged and the process was carried out at room temperature.

2.6. Microhardness assessment

The microhardness measurement of all the samples was performed three times. At base line, following demineralization and after treatment application at the two periods of time (after 10 min and after eight days).The samples' microhardness was measured using the digital display Vickers Micro-hardness tester (Model HVS-50, Laizhou-Huayin Testing Instrument Co., Ltd., China) with a Vickers diamond indenter and a 20× objective lens. The specimen's surfaces were subjected to a 100 g load for 15 s. Each specimen's surface was indented three times, each one evenly spaced around a circle and not more than 0.5 mm apart from one another. Vickers values were transformed into micro-hardness values by measuring the indentations' diagonal length with the built-in scaled microscope.

2.7. Scanning electron microscope (SEM)

Two samples from each group were examined (one from 10 min subgroup and the other from 8 days subgroup). Also one sample of sound enamel and one of demineralized were examined to assess the change in surface morphology after treatment. Images were captured using an ×1000 magnification lens at a distance of 11.8 mm from the sample and an excitation voltage of 20 kV.

2.8. Statistical analysis

All of the information was gathered, collated, and analyzed. In each test, the mean and standard deviation values were computed for each group. Using the Shapiro–Wilk and Kolmogorov–Smirnov tests to determine whether the data were normal, a parametric (normal) distribution of the data was found. To contrast the two groups in unrelated samples, the *t*-test for independent samples was employed. *P* less than or equal to 0.05 was used as the criterion of significance. The statistical analysis was carried out using IBM SPSS Statistics Version 20 for Windows.

3. Results

3.1. Microhardness results

Regarding the effect of the re-mineralizing agents, the comparisons between the microhardness values of each re-mineralizing agents' group within each time of application (10 min and 8 days) are shown in Table 1 and Fig. 1. Results showed that, in the cranberry group, there was a statistically significant difference between baseline, after

Table 1. Descriptive statistics and comparison between microhardness values of each of the two re-mineralizing agents' groups at baseline, after demineralization, and after remineralization within each time of application (10 min and 8 days).

Re-mineralizing agents	Baseline		After demineralization		After remineralization		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Cranberry							
10 min	265.43[a]	7.14	254.78[b]	3.55	256.83[b]	4.94	<0.001*
8 days	264.51[a]	10.06	257.44[b]	2.56	260.30[ab]	5.59	0.047*
Nanohydroxyapatite							
10 min	268.93[a]	4.63	253.89[c]	2.67	257.17[b]	3.72	<0.001*
8 days	264.75[a]	9.79	256.72[b]	3.15	259.54[a]	5.60	0.025*

Means with the same superscript letters in the same row indicate insignificant difference, while means with different superscript letters in the same row indicates significant differences.
*: significant ($P \leq 0.05$).

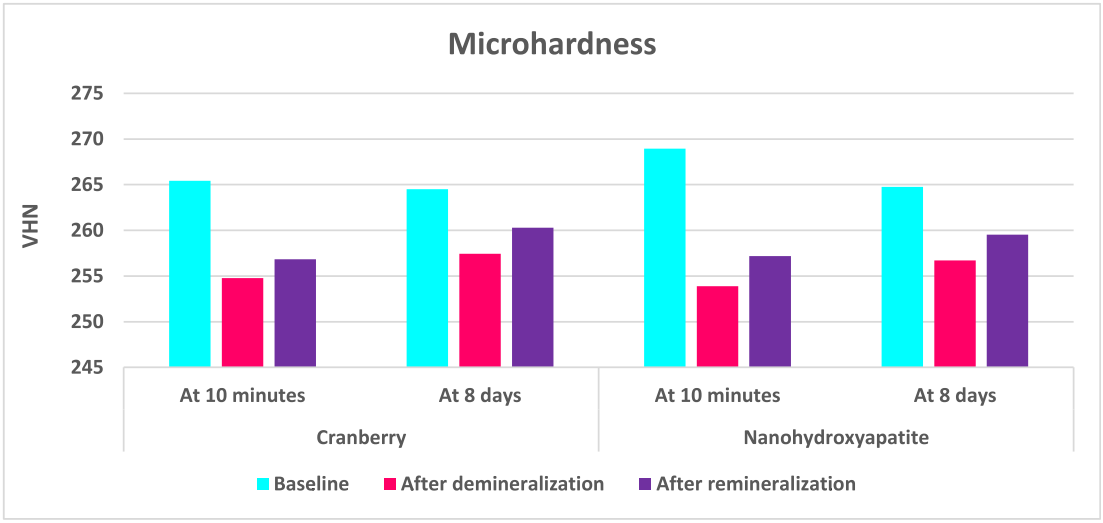

Fig. 1. Bar chart illustrating mean microhardness values at baseline, after demineralization, after remineralization of Cranberry and Nano-hydroxyapatite re-mineralizing agents' groups at 10 min and 8 days times of application.

demineralization and after re-mineralization. Although there were an increase in the microhardness values after re-mineralization comparing with the values after de-mineralization, but it was insignificant. Also, in nHA group, a statistically significant difference was found between baseline, after de-mineralization and after re-mineralization, where ($P < 0.001$) at 10 min and ($P = 0.025$) at 8 days. The highest mean values were found in baseline, while the least mean value was found in after demineralization and there was significant increasing in the microhardness values after re-mineralization comparing with the values after de-mineralization, as the microhardness values of nHA group after demineralization and re-mineralization at 8 days of application were 256.72 ± 3.15 and 259.54 ± 5.60 respectively.

Comparisons between the microhardness values of each re-mineralizing agents group regardless of time of application (overall of 10 min and 8 days) are shown in Table 2 and Fig. 2. In both re-mineralizing agents' groups, cranberry and nHA, A statistically significant difference ($P < 0.001$) existed between the baseline, after demineralization, and after re-mineralization. The considerably least mean value was discovered after demineralization, whereas the significantly greatest mean values were discovered in baseline, followed by after re-mineralization.

Regardless of the time of application, the two tested re-mineralizing agents showed statistically similar re-mineralizing effects, as the microhardness values did not differ statistically significantly between nHA and cranberry, ($P = 0.854$). Cranberry recorded the highest mean value, while nano-hydroxyapatite recorded the lowest mean value, which recorded 258.56 + 5.48 and 258.35 + 4.83 respectively as shown in Table 3 and Fig. 3.

Regarding the effect of the time of application, the comparisons between the microhardness values of the two times of application (10 min and 8 days) within each re-mineralizing agent are shown in Table 4 and Fig. 4. For both cranberry and nHA re-mineralizing agent, no statistically significant difference existed between the application times of 10 min and 8 days ($P = 0.082$ and $P = 0.183$, respectively). The 8-day period had the highest mean value, while the 10 min period had the lowest.

3.2. SEM results

The scanning electron microscope (SEM) pictures are shown in (Fig. 5). SEM of sound enamel showed

Table 2. Descriptive statistics and comparison between microhardness values of each of the two re-mineralizing agents' groups at baseline, after demineralization, and after remineralization regardless of time of application (overall of 10 min and 8 days).

Re-mineralizing agents	Baseline		After demineralization		After remineralization		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Cranberry	264.97[a]	8.58	256.11[c]	3.33	258.56[b]	5.48	<0.001*
Nanohydroxyapatite	266.84[a]	7.82	255.31[c]	3.21	258.35[b]	4.83	<0.001*

Means with different letters in the same row indicates significant difference.
*: significant ($P < 0.05$).

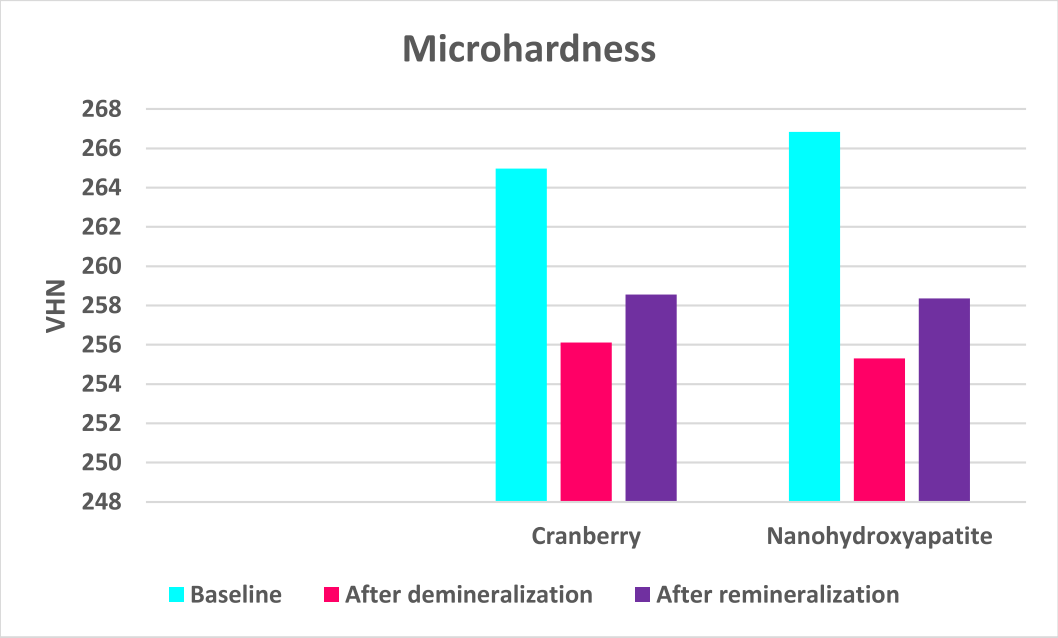

Fig. 2. Bar chart illustrating mean microhardness values at baseline, after demineralization, and after remineralization of Cranberry and Nano-hydroxyapatite re-mineralizing agents' groups regardless of the time of application.

Table 3. Descriptive statistics and comparison between microhardness values of the two re-mineralizing agents after remineralization regardless of time of application (overall of 10 min and 8 days).

Re-mineralizing agents	After remineralization	
	Mean	SD
Cranberry	258.56	5.48
Nanohydroxyapatite	258.35	4.83
P value	0.854 ns	

ns: nonsignificant ($P > 0.05$).

Table 4. Descriptive statistics and comparison between microhardness values after re-mineralization of the two times of application (10 min and 8 days) within each re-mineralizing agent.

Time of application	Cranberry		Nano-hydroxyapatite	
	Mean	SD	Mean	SD
10 min	256.83	4.94	257.17	3.72
8 days	260.30	5.59	259.54	5.60
P value	0.082 ns		0.183 ns	

ns: nonsignificant ($P > 0.05$).

a smooth homogenous surface with some pores which could be easily seen and there were some areas that have scratches; no enamel prisms were observed (Fig. 5a). While demineralized enamel showed loss of typical enamel architecture, as well as micro-porosities and surface irregularities in some areas (Fig. 5b). In the Cranberry extract group after 10 min, there was alteration between pours and

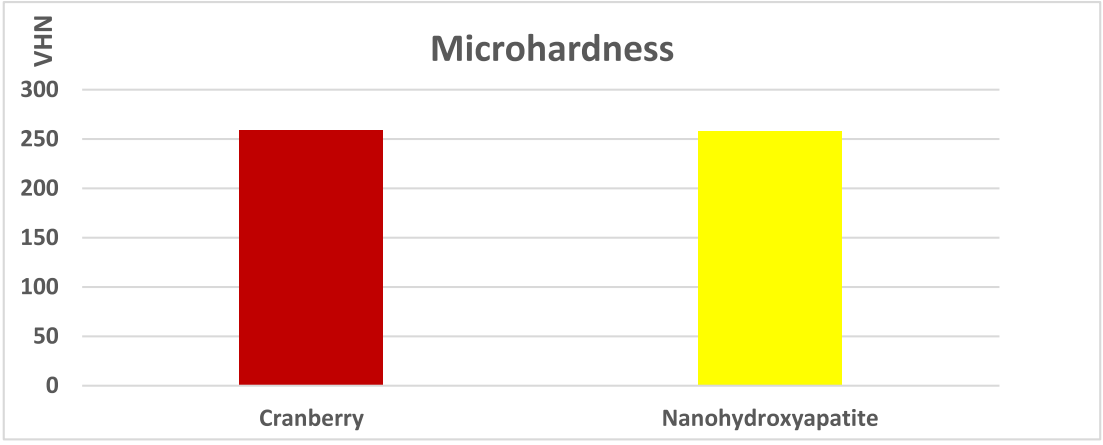

Fig. 3. Bar chart illustrating mean microhardness values, representing effect of re-mineralizing agents regardless of time of application.

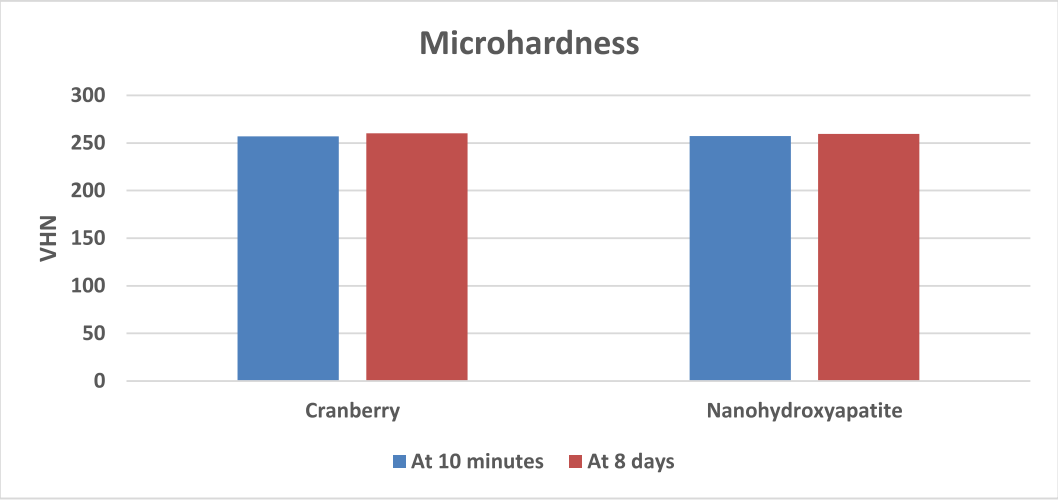

Fig. 4. Bar chart illustrating mean microhardness values, representing the effect of time of application at each re-mineralizing agent.

obliterated rod with the irregular surface (Fig. 5c), while after 8 days there were large areas completely obliterated while the other still porous (Fig. 5d). In nHA group after 10 min, there was a more homogenous surface with obliteration of some rod spaces (Fig. 5e), while after 8 days there was the deposition of minerals on enamel surface with obliteration of rods in some areas (Fig. 5f).

4. Discussion

White spot lesions are likely the early indicators of dental caries disease. These demineralized patches of enamel, which often form as a result of persistent plaque formation, seem opaque due to substantial underlying porosity brought on by demineralization. Demineralization may advance to noncavitated lesions, then cavitated lesions, if the process is not prevented and reversed [15].

The primary technique for avoiding caries and remineralizing early lesions has been fluoride treatment, but caries still develops in high-risk patients regardless of the fluoride amount given [16]. Additionally, dental fluorosis has been criticized as an aesthetic problem and is seen to be an unfavorable side effect of fluoride's preventive routine. Because of this, there is an increasing need to use other safer compounds that have re-mineralizing abilities at least as effective as fluoride [17].

Therefore this study was performed to examine the re-mineralizing effect of cranberry extract and nHA powder mixed with olive oil after different times of application on white spot lesion.

Proanthocyanidin-rich herbal extracts, particularly cranberry and grape seed extracts, are receiving a lot of interest for preventing dental caries. These proanthocyanidin-rich extracts have antibacterial, anti-inflammatory, antioxidant, and anti-adhesion properties. Researchers have demonstrated that PAs, a naturally occurring plant metabolite found in high concentrations in fruits and vegetables provide a wide range of therapeutic values [6].

One of the crystalline calcium phosphate re-mineralizing agents is nano-hydroxyapatite (Nano-HAP). It is among the materials that are highly bioactive and biocompatible. In the crystalline form of hydroxyapatite (HA), it contains calcium (Ca) and nanophosphate [15]. Due to its tiny size nHA has a significant potential to penetrate through enamel rods and has a strong ability to repair lesions [8].

Olive oil is a kind of vegetable oils, it shows a high content of monounsaturated fatty acids up to 85% of its composition, due to its high content in oleic acid which might range between 70 and 85% [18]. It is used in this study instead of water for preparation of nHA paste. Hydroxyapatite powder is soluble in olive oil and the food-grade olive oil is harmless to human health. Olive oil has been used in the production of hydroxyapatite and as an additive agent to hydroxyapatite [12].

In this study microhardness of the samples was assessed three times, at baseline, following demineralization, and following treatment application (after 10 min and after eight days). As substrate-like enamel has a fragile surface that is prone to cracking and a small microstructure, microhardness measurement was used for this study because it is a suitable technique for such materials and is also simple, quick, and nondestructive [19].

Due to its ability to produce highly detailed images of hard objects, the SEM is adapted for the investigation of the composition of tooth enamel [20]. One of the greatest ways to investigate the

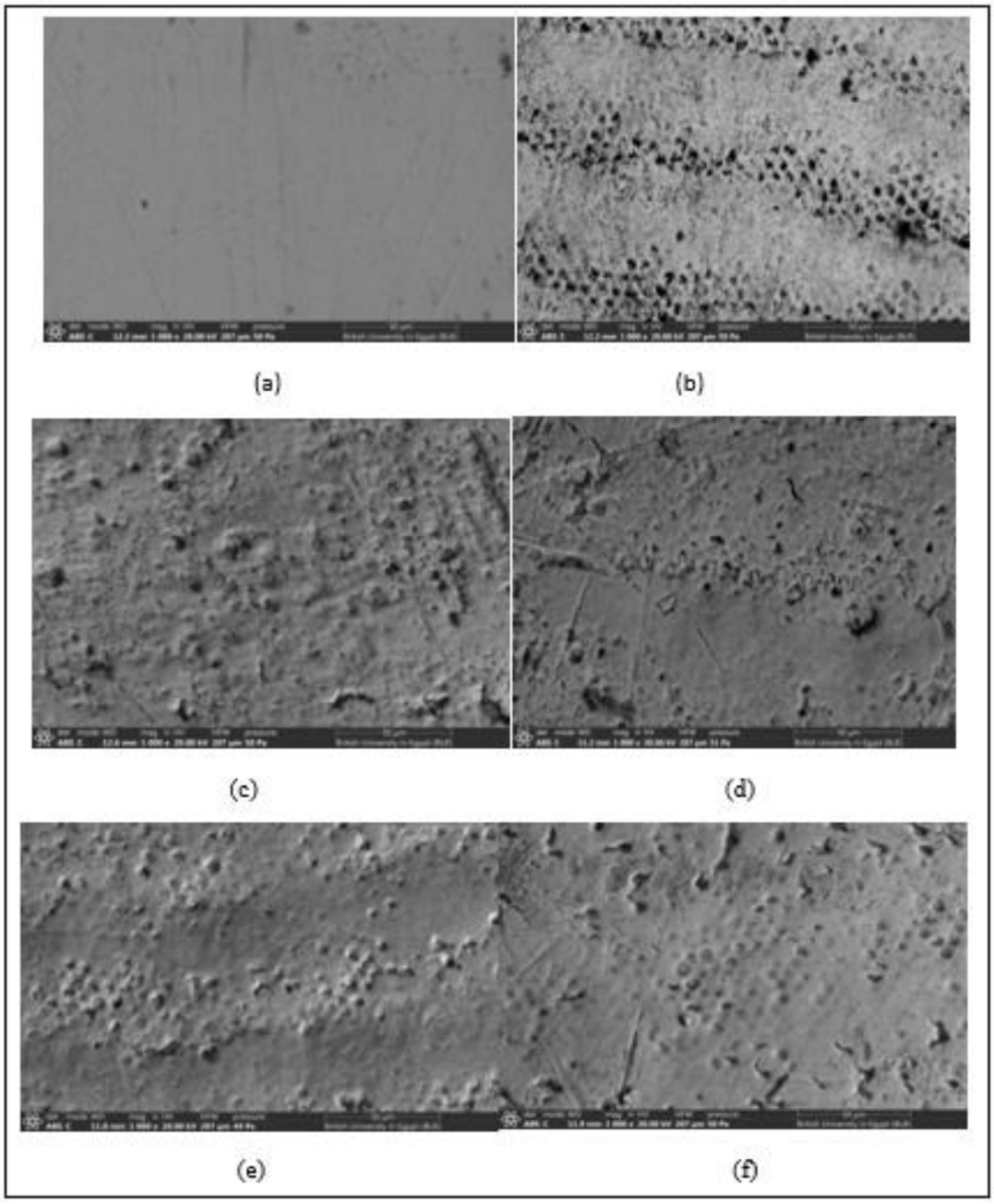

Fig. 5. (a): Sound enamel. (b) Demineralized enamel. (c) Cranberry extract after 10 min. (d) Cranberry extract after 8 days. (e) Nano-hydroxyapatite after 10 min. (f) Nano-hydroxyapatite after 8 days.

enamel surface is by using this technique since it allows you to view structures at extremely high magnification without changing the gross specimen [21].

In this study cranberry extract group exhibited statistically significant increase in the mean value of microhardness. This may be due to the presence of PAs, it is said that PAs affect the re-mineralization process through the superficial deposition of minerals over the lesion by forming insoluble complexes [6].

PAs have phenolic hydroxyl groups that might act as ligands for calcium ions to bind to. The development of calcium–proanthocyanidin complexes may promote mineral deposition and draw in additional calcium phosphate, enhancing re-mineralization and partial recovery of the demineralized lesion [22].

The findings of this study is in agreement with other study which demonstrated the re-mineralization potential of cranberry extract dentifrice on primary teeth and reported that cranberry has a re-mineralizing effect [5]. Also, it is in the same line with another study that demonstrates the role of cranberry extract in preventing enamel erosion and exhibited that cranberry has a role in preventing erosion of enamel [6]. Additionally it is in agreement with another study which evaluated the remineralizing potential of cranberry on demineralized dentin and showed that cranberry has an effect on dentin remineralization [10].

The current results, however, are at odds with another study, which found that dentin microhardness was not significantly increased in demineralized dentin treated with proanthocyanidin. This could be explained by the various procedures and sources of proanthocyanidin. The proanthocyanidin outcomes were highly influenced by their source, the solvent used to extract them, their concentration, and their exposure time [23].

In this study, the microhardness of the enamel surface increased significantly ($P < 0.001$) after treatment with nano-hydroxyapatite paste, demonstrating that after treatment, nHAP causes an even re-mineralization of the enamel by creating a uniform coating apatite on the demineralized surfaces of enamel, which is explained by the hydrophilic and wetting properties of nHAP that enable it to form a thin but securely bounded coating on the tooth surface, leading to higher surface hardness and re-mineralization. Due to the tiny size of the particles that make up nHA, it also functions as a filler and can be used to restore the enamel surface microscopic depressions and holes [24].

Additionally, olive oil has been used in the preparation of nHA paste instead of water. The water provides a suitable environment for bacteria growth and proliferation [12], so the replacement of the water with the oil helps to decrease bacteria accumulation and hence gives a better chance for re-mineralization.

This result coincides with a previous study that assessed how toothpaste containing nano-hydroxyapatite affected white spot lesions in individuals with orthodontics and showed that nano-hydroxyapatite plays a function in remineralization and reducing the severity of the lesion [8]. Also, it is in the same line with the study that evaluated the effect of nHA and olive oil paste on remineralization of early caries lesions and exhibited a positive effect of the paste on early carious lesions [12]. Additionally, it is in agreement with a study that evaluated the effectiveness of remineralizing tri-calcium phosphate, nHA, and resin infiltration system on early caries lesions and determined that the resin infiltration method and the remineralizing agent based on nHA are the most efficient treatments for carious enamel [15].

Results for the time effect showed a considerable rise in microhardness in the two groups after 10 min and after 8 days, and the greatest proportion of microhardness was recorded after 8 days, although this increase was statistically insignificant. This may be due to the ability of PAs to deposit more minerals on the superficial layer of the lesion by the formation of insoluble complexes [25] and the long duration that increases the capacity of nHA to better integrate into the prismatic and interprismatic enamel structure, producing a more homogeneous surface [24].

SEM investigations indicated that the demineralized enamel's surface morphology after being treated with cranberry extract and nHA showed partially obliterated enamel rods and crystals deposition on enamel surface, supporting the microhardness finding.

4.1. Conclusion

Cranberry extract and nHA powder mixed with olive oil are thought to be efficient at re-mineralizing enamel surfaces with white spot lesions regardless of the time of application of each material.

4.2. Recommendations

Additional researches are required to determine the effect of various other natural and synthetic materials on the re-mineralization of white spot lesion; also further *in vivo* researches regarding application of natural re-mineralizing materials are needed.

Ethical statement

This research was performed after the approval of research ethical committee of Faculty of Dental Medicine, Al-Azhar University for Girls, in accordance with international guiding principles, Code: REC-OP-23-02.

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Conflict of interest

There are no conflicts of interest.

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