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Effect of Different Natural Herbal Products on Microhardness of Eroded Enamel Surface: An in Vitro Study

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Abstract

Aims: The study aimed to evaluate and compare the effects of the herbal medicaments (Neem, Ginger, Green tea, Clove oil, and peppermint oil) and traditional fluoride gel on the surface microhardness of the eroded enamel samples of permanent teeth in vitro study. **Materials and methods:** A total of (80) sound maxillary first premolars were used in the study. Enamel blocks were prepared and divided into eight groups: Negative control group (C-ve) (n=10) not exposed to Pepsi drink. The remaining samples exposed to Pepsi drink then subdivided into (7) subgroups: Positive control group (C+ve) (Pepsi group), the remaining groups representing different experimental remineralizing agents used as follows: Group 3 (NaF gel), Group 4 (Neem), Group 5 (Ginger+Honey), Group 6 (Green tea), group 7 (Clove oil) and group 8 (Peppermint oil). The Microhardness of enamel blocks was measured before and after the PH cycle by using a Vickers microhardness measurement machine. **Results:** The microhardness of enamel surface in all study groups was decreased after eroding Pepsi drink due to the demineralization, then increased after treatment with remineralizing agents but the highest increase of the surface microhardness measurements belonged to the Ginger+Manuka group followed by Neem and NaF group, while the control negative group of deionized water which not exposed to Pepsi drink had the minimum decrease in the surface microhardness measurements. **Conclusions:** Ginger+Manuka honey was significantly better than other groups against demineralization and preserving enamel microhardness.

الخلاصة

الاهداف: ان الهدف من الدراسة هو لتقييم ومقارنة تاثير المواد العشبية (النيم، الزنجبيل، الشاي الاخضر، زيت النعناع، زيت القرنفل) و الفلورايد جيل على صلادة سطح مينا الاسنان الدائمية مختبريا. **المواد و طرائق العمل:** تم الاختبار على (80) عينة من الاسنان الضواحك العلوية الدائمية السليمة والتي قلعت لاسباب التقويم , تم تحضير عينات اسطح المينا وتقسيمها عشوائيا الى 8 مجاميع: مجموعة السيطرة السالبة متمثلة بالماء منزوع الايونات (10 عينات) , مجموعة السيطرة الموجبة متمثلة بالعينات المعرضة للببسي فقط (10عينات),مجموعة صوديوم فلورايد جيل (10عينات) ,مجموعة النيم (10عينات), مجموعة الزنجبيل و عسل المانوكا (10عينات) مجموعة زيت القرنفل (10عينات) مجموعة زيت النعناع (10عينات) ، تم ادخال العينات الى دورة ازالة واعادة المعادن الى السن وقياس صلادة سطح المينا قبل الدورة و بعد ازالة المعادن و بعد اعادة المعادن عن طريق جهاز قياس الصلادة. **النتائج:** كان هناك اختلافات ذات دلالة احصائية عالية في مجموعات الدراسة بعد دورة ازالة و اعادة المعادن الى السن ، وكان هناك نقصان في صلادة الاسطح في جميع المجموعات بعد تعرضها لمشروب الببسي بسبب فقدان العناصر المعدنية و بعد تعرض العينات لمواد اعادة المعادن للسن فان الصلادة زادت ولكن بنسب مختلفة فكانت الزيادة العظمى في مجموعة الزنجبيل وعسل المانوكا ثم صوديوم فلورايد جيل ثم النيم ، ، الجاي الاخضر ، زيت القرنفل واخيرا زيت النعناع. **الاستنتاجات:** من خلال الدراسة تبين ان مجموعة الزنجبيل وعسل المانوكا هي افضل مجموعة في زيادة صلادة سطح المينا للعينات المعرضة لازالة المعادن منها بواسطة مشروب الببسي.

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INTRODUCTION

Dental enamel erosion is a real dental problem associated with the increase in the consumption of soft drinks, fruit juices, and sports drinks in many countries [1]. Carbonated drinks especially cola are associated with erosion and most likely due to their low pH [2].

Dental erosive wear is the chemical dissolution of the dental hard tissues by acids without the involvement of bacteria. Hydrogen ions from acidic solutions can replace the calcium ions of the enamel, consequently breaking the crystal structure of the enamel and initiating dental erosion and this problem led to severe impairment of esthetics along with loss of hardness and function of teeth [1]

Many preventive measures have been suggested for the control of dental enamel erosion and the use of fluoride is one of them [2]. Fluoride increases the hardness of the tooth surface and decreases the depth of dental erosive lesions [3].

A topical fluorides system can be used to prevent the progression of dental erosion [4]. The fluoride application remains the best method for remineralizing the early enamel demineralization and it has been well documented. Unfortunately, fluoride could not guide the formation of mineral crystals and failed to form oriented and ordered mineral crystals on the enamel surface [5]. Dental fluorosis and skeletal fluorosis in severe cases result from chronic consumption of a high dose of fluoride [6].

In recent years, attention has been focused on the use of natural products (herbal) as they have both advantages of minimal side effects and being sugar and/alcohol-free, which are the two most common ingredients found in over-the-counter products [5,7].

The purpose of the current study was to evaluate and compare the effects of the herbal medicaments (Neem, Ginger, Green tea, Clove oil, and Peppermint oil) and traditional fluoride gel (NaF) on the surface microhardness of the eroded enamel samples of permanent teeth in vitro study.

MATERIALS AND METHODS

The study was approved by the Research Ethics Committee board (University of Mosul, College of Dentistry, REC reference No. UoM.Dent/H.L.31/21.

Martials

The tested materials used in this study are listed in table (1) and other materials used in the study show in table (2).

Table (1): Tested material used in study.

Materials	Ingredients
Ginger	Rhizomes are carbohydrates (50–70%), lipids (3–8%), phenolic compounds and terpenes. Terpene components of ginger include β-bisabolene, α-farnesene, zingiberene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, shogaol and paradols (Prasad and Tyagi, 2015).[10]
Manuka Honey	Caffeic acid, Phenyllactic acid, Isoferulic acid, 4Methoxyphenolactic acid, Kojic acid, Gallic acid p-Coumaric acid, , 5-Hydroxymethylfurfural, 4-Hydrobenzoic acid, 2-Methoxybenzoic acid, Quercetin, Syringin acid, Phenylacetic acid, Methyl syringate, Luteolin, Dehydrovomifoliol, 8-Methoxykaempferol, Leptosin, Pinocembrin, Glyoxal, Isorhamnetin, Methylglyoxal (MGO), Chrysin, Kaempferol, 3Deoxyglucosulose, Galangin, Pinobanksin (José *et al.*, 2014).[11]
Neem oil	Azadirachtin (azadirachtin A-G and azadirachtin E), limonoids, volatile oils, meliantriol, nimbin, nimbidin, nimbinin, gedunin, nimbolides and mahmoodin, fatty acids (stearic, oleic and palmitic) and salannin (Campos *et al.*, 2016).[12]
Clove oil	Eugenol, β-caryophyllene α-humulene, eugenol acetate and Caryophylene oxide (Sohilait *et al.*, 2018). [13]
Peppermint oil	Menthol and menthone together with limonene several other minor constituents as menthofuran, menthyl acetate, 1,8-cineole, neoisomenthol, viridiflorol, germacrene-D and β-caryophyllene (Beigi *etal.*, 2018).[14]
Green tea	Protein, Amino acids, Fiber, carbohydrates, Lipids, Pigments, Minerals, Phenolic compounds, Oxidized phenolic compounds and flavonoids (catechins) (Chacko *et al*., 2010).[15]
Fluoride gel	Water, O- phosphoric acid (<3%), flavors and fragrances, additives, sodium fluoride, Free from aspartame, gluten, saccharine, Xylitol.

Table (2): Other Materials used in the Study.

Materials	Composition
Artificial Saliva	NaCl 0.4 mg/L, CaCl2.2H2O 0.79mg/L, KCl 0.4 mg/L, Na2S9H2O 0.005 mg /L, CH4N2O 1.0 g, distilled water 1 L. [16].
Pepsi (Erbil)	Carbonated water, sugar, caramel color, phosphoric acid, natural flavors, caffeine.

Teeth Samples Collection:

Eighty (80) sound maxillary first premolars were collected from patients aged between (12-18) years extracted for orthodontic treatment from Mosul city.

A tooth with specific criteria: Intact upper first premolars are collected, being free of caries, having no fillings, no developmental anomalies, no enamel hypoplasia, no cracks, wears, or fractures. Also, the enamel surface should be unaffected by a chemical agent as a bleaching agent or acid etching.

Teeth Samples Preparation:

Before using the teeth, they were cleaned with non-fluoridated pumice and

white rubber prophylactic cup using a low-speed handpiece. Then by using a diamond disc bur in the high-speed handpiece the crowns separated from the roots and cooled with water to prevent damaging of enamel and the crown of the teeth collected, figure (1).

Figure (1): **Sound intact teeth sample after separation of roots.**

All crowns were thoroughly washed with deionized water and kept in a 0.1 % thymol solution in the refrigerator at 4 ^{o}C to maintain the structural integrity of enamel samples until being mounted in a chemical cured resin in plastic rings [8]. Then the crowns were mounted in cylindrical plastic tubes (16 mm diameter ×14 mm depth) with cold cure acrylic resin with the outer buccal enamel surface exposed and polished the teeth specimens by using a fin grit silicon carbide papers 400 and 600 grit to standardize the buccal surface for microhardness test machine [9], figure (2). Lastly, all samples were cleaned with deionized water and kept in a 0.1 % thymol till the beginning of the pH cycle (the erosion in specimens was done by immersing the teeth in Pepsi drink as a demineralizing agent- pH cycle).

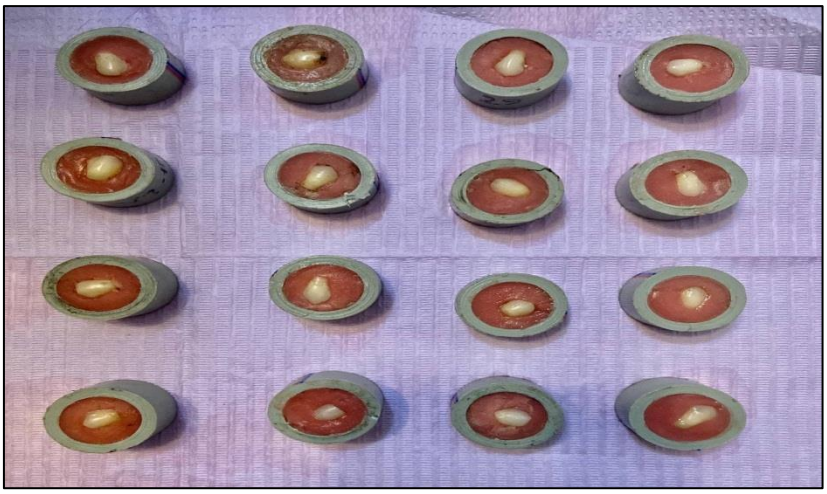

Figure (2): The crowns were mounted in cylindrical plastic tubes (16mm diameter ×14mm depth) with cold cure acrylic resin with the outer labial enamel surface exposed. (after demineralization).

Experimental Design of Study:

The total number of samples in the main study is (80) samples and randomly divided into 8 groups (10) specimens for each group, figure (3).

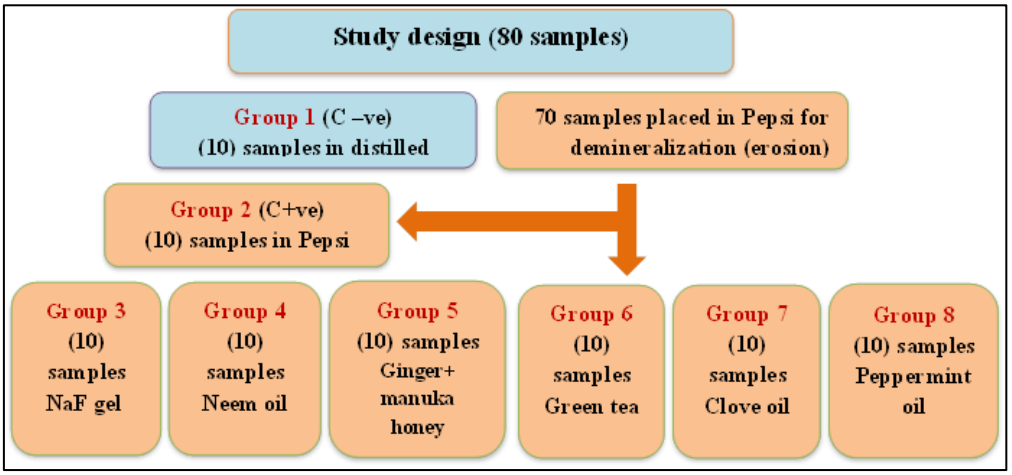

Figure (3): Experimental Design of Study

1. Negative control group C-ve (Baseline group): Ten samples were placed in distilled water at room temperature throughout the study.

The other specimens were immersed in a beaker filled with 200 ml Pepsi drink at room temperature, which is a demineralizing agent, for 5 minutes, 3 times daily for 6 days. Pepsi drink was changed every day and the specimens were kept in a closed container to complete the demineralization process, after which they were rinsed with distilled water.

2. Positive control group C +ve (Pepsi group): Ten samples were taken to represent the (C +ve) group after demineralization by Pepsi drink.

The remaining demineralized specimens were subdivided into six subgroups specimens each representing different remineralizing agents as follows:

3. NaF gel group: Ten samples were brushed with NaF gel for 5 minutes 3 times daily for 6 days by the cotton applicator.

4. Neem group: Ten samples brushed with Neem extract oil 10% for 5 minutes 3 times daily for 6 days

5. Ginger+Honey group: The Ginger powder was mixed with Manuka honey (MGO activity of 580) in a ratio of 8mg/ml (w/v) [17].

6. Green tea (Ahmad green tea classic teabag): To prepare green tea, a teabag, was placed in 200 ml of boiled distilled water and kept for 5 minutes. The drink was then left to cool until reaching room temperature. After which, the specimens were immersed in a beaker filled with the green tea drink for 5 minutes, 3 times daily

for 6 days. They were kept in distilled water between intervals of application.

7. Clove oil group: Oil was applied with an applicator for 5 minutes, 3 times daily for 6 days.

8. Peppermint oil group: Oil will be applied with an applicator, 5 minutes 3 times daily for 6 days.

All materials were prepared freshly at each application of remineralization materials. and washed with distilled water perfectly after the end of the time of remineralization cycle and restored in the artificial saliva bath.

Surface Microhardness Measurement:

The surface microhardness (SMH) of the specimens was determined using a Vickers microhardness testing machine (OLPERT, Germany) as shown in figure (4), with a Vickers diamond pyramid indenter, which has a square-based diamond indenter with a 136º angle and 600X magnification of the microscope. A load of 1 kg was applied to the surface of the specimens for 15 seconds [18].

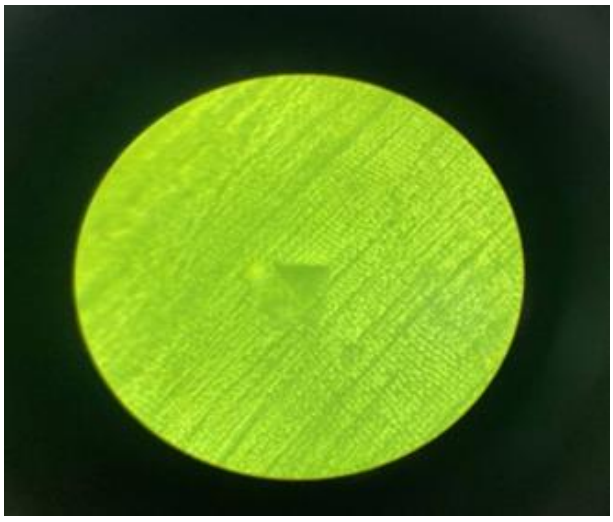

Figure (4): Light microscope images of well-shaped indentations in enamel.

Three indentations were equally placed over a circle of 1 mm diameter at the middle third of the specimens, then the average of three measurements was calculated and obtained as one reading. Indentation result can be seen at projector screen in the form of shadow shaping rhomb, the diagonal length of the indentations was measured by microscope in micron. The Vickers values were converted into microhardness values. SMH was obtained using the following equation:

- HV=1.854 P/d2
- where HV is a Vicker hardness in Kgf/mm2 (Mpa),
- P is the load in Kgf and
- d is the length of the diagonal in mm [19].

Enamel microhardness was measured for sound enamel, before and after the cycling regime in each tested group. This load and time were constant for all samples throughout the study. All readings were performed by the same examiner using the

same calibrated machine. The test was conducted at Technical Institute/Mosul**.**

RESULTS

According to the obtained measurements of this study, table (3) showed the descriptive statistics including means, standard deviations, minimum values, and maximum values in addition to the numbers of the samples of tested groups at baseline, after demineralization, and after remineralization.

Table (3): Descriptive Statistics of Microhardness Measurements.

Microhardness		Mean	N	Std. Deviation	Minimum	Maximum
Control -	Baseline	341.2000	10	13.57121	320	359
	after pepsi	-	-	-	-	-
	After treatment	343.1000	10	13.51090	322	362
Control +	Baseline	343.2000		14.61202	320	360
	after pepsi	255.4000		8.26236	240	268
	After treatment	255.4000	10	8.26236	340	368
Neem	Baseline	338.5000	10	9.78945	329	357
	after pepsi	258.9000	10	5.30094	251	268
	After treatment	313.9000	10	4.12176	308	320
Ginger+Manuka honey	Baseline	345.0000	10	18.93263	320	375
	after pepsi	258.4000	10	9.74337	240	275
	After treatment	323.4000	10	6.70323	315	338
Green Tea	Baseline	339.3000	10	9.40508	329	352
	after pepsi	253.1000	10	7.06242	241	262
	After treatment	288.2000	10	5.07280	280	295
NaF	Baseline	336.9000	10	10.78528	320	357
	after pepsi	260.3000	10	9.77582	240	277
	After treatment	309.3000	10	4.42342	300	315
Clove	Baseline	336.1000	10	9.17061	320	348
	after pepsi	259.2000	10	9.29516	245	278
	After treatment	272.0000	10	9.66092	260	289
Peppermint	Baseline	343.9000	10	10.85715	329	358
	after pepsi	251.9000	10	8.06157	240	264
	After treatment	270.9000	10	7.83794	260	288

Based on the means values for tested groups after remineralization, the Ginger and Manuka honey group had the maximum increase in the surface microhardness mean value and thus considered the most effective preventive agent when compared with other groups in the presence of the control group. Then followed by Neem oil group, Sodium fluoride gel, Green tea, Clove oil, and Peppermint oil groups respectively.

Table (4) ANOVA test explains that there was no significant difference at baseline and after demineralization at $p \leq 0.05$ but there are highly significant differences for the surface microhardness

readings that existed among the tested groups after treatment at $p \leq 0.05$.

As shown in table (5) of Duncana multiple analysis range test which was done to further explain that there was a highly significant difference among tested groups after remineralization existed at $p \leq 0.05$.

Table (4): Analysis of Variance (ANOVA) Test of Mean Microhardness Values for Comparison between the Eight Groups at Every Stage in the Study.

Microhardness		Sum of Squares	df	Mean Square	F	Sig.
Baseline	**Between Groups**	773.487	7	110.498	.702	.670
	Within Groups	11328.500	72	157.340		
	Total	12101.987	79			
After pepsi	**Between Groups**	646.171	6	107.695	1.544	.178
	Within Groups	4393.200	63	69.733		
	Total	5039.371	69			
After treatment	**Between Groups**	63732.750	7	9104.679	142.039	.000**
	Within Groups	4615.200	72	64.100		
	Total	68347.950	79			

df : degree of freedom.

** Highly significant difference existed at $p \leq 0.05$

Table (5): Duncana Multiple Analysis Range Test for Groups After Remineralization.

Groups	N	1	2	3	4	5	6
Control +ve	10	255.4000					
Peppermint	10		270.9000				
Clove	10		272.0000				
Green tea	10			288.2000			
NaF	10				309.3000		
Neem	10				313.9000		
Ginger+Manuka honey	10					323.4000	
Control -ve	10						343.1000
Sig.		1.000	.760	1.000	.203	1.000	1.000

Subset for alpha = 0.05

All groups were arranged in nonhomogeneous subsets of data representing the surface microhardness means values of each group after remineralization at which Ginger and Manuka honey had a highly significant resistance against microhardness loss and then Neem and NaF groups and there is no significant difference between Neem and NaF gel groups, then followed by Green tea then Clove oil and Peppermint oil groups with no significant difference between them. The least value of surface microhardness belonged to the Control +ve group where no treatment agent was applied .

DISCUSSION

Prevention of teeth demineralization focused on increasing the teeth resistance to acid attack by the multiple fluoride treatment, but the use of natural herbs products as an alternative to conventional treatment in healing and treatment of different diseases has been on the rise of the last few years.

According to the result of the current study in all tested groups, Pepsi reduced the microhardness of teeth enamel. Pepsi considers as one of the most commonly consumed acidic drinks thus it was used in our study and had a pH value of 2.5. Its erosive effects are related to acids phosphoric, citric acid, and/or citrates present in numerous soft beverages and can chelate calcium, reduce the buffering capacity of saliva and so increase the destruction of the tooth [20].

The result of the current study is in agreement with the results of the Brazil study, it was demonstrated that the effect of Pepsi drinks is the reduction of the tooth structure and hardness so results in erosion of tooth structure [21].

On the other hand, a previous review suggests that the acidity of the beverage, the method of sipping, pH, the duration of the beverage staying inside the mouth, and the duration of the beverage swished inside the mouth, all these factors affect the potential tooth damage [22].

Rajab *et al.,* (2018) [23] proved that when the PH decreases with the presence of intrinsic or extrinsic acids such as Pepsi the MMPs are activated. When they get activated they begin to hydrolyze the extracellular matrix components (ECM) of enamel. In this context, the existence of MMPs on eroded enamel would possibly increase the development of erosion that could be inhibited by the use of inhibitors of MMP [24].

Surface microhardness is an important property that can be related to tooth wear and abrasion resistance. As the Vickers hardness increases, the surface hardness as well increases. The microhardness tester was used in the current study, as it gave indications of the re- and de-mineralization during the experiments. Thus, the microhardness test has been used for the reason that it is a more correct and less cumbersome method than others [25, 26].

The dental Enamel microhardness was measured for sound enamel before demineralization, after demineralization, and after treatment with the chosen products. Statistically, a highly significant decrease was found in the microhardness of enamel surface after pH cycling as an indication of enamel demineralization and the beginning of initial eroded enamel lesion.

After treatment of enamel samples with the treatment agents, there was an increase in the microhardness value. This may be a sign of the integration of ions that decreases the porosity and raises the microhardness of demineralized enamel, this remark was not seen for samples treated with de-ionized water.

Due to the differences of the tested herbal product's many effects, which are tested as a remineralizing agent on the enamel surface hardness, is thought to be associated with the difference of the composition of each different agent, which can also be more than one component and each one with its method of remineralization [27].

So, according to the result, based on the means values, the preventive effect of ginger and honey was better than the sodium fluoride and other herbals and all tested components have a benefit to protect the microhardness of the enamel surface of permanent teeth in comparison with the control negative group.

after remineralization there was a highly significant difference between tested groups as further noticed at Duncan a Multiple Analysis Range Test for test groups after remineralization which illustrated that the ginger + honey group had a maximum increase in the microhardness mean value followed by the mean group and NaF group, then which had the minimum increase in the surface microhardness of enamel were green tea, mint and clove oil, so the ginger and honey will effectively protect the enamel of teeth more than sodium fluoride and this is in agreement with Bilgin *et al.,* (2016).[17]

Fluoride ions (F^+) seem to raise the enamel microhardness, so improving its resistance to acid dissolution affects re- and demineralization cycle and resulting in the precipitation of CaF_2-like material on eroded enamel surfaces [28].

People who are aware of the side effects of fluoride have begun to favor natural herbal products. Studies have focused on the therapeutic properties of plants in terms of dentistry and new oral care products have been developed [29].

In the present study, the variable herbal products used have the capability of protecting the eroded enamel via increasing the surface microhardness of eroded enamel.

The uses of ginger + manuka honey as herbal medicines established an inhibitory effect on demineralization and enhanced remineralization on enamel under the conditions of this in vitro study [30].

Bilgin *et al.* (2016) [17] concluded that herbals as (ginger, honey) have enhanced remineralization of initial enamel lesion, the achieved high remineralization is maybe as a result of antimicrobial properties of ginger which might be the result of the high amount of fluoride content (79 mg/kg fluoride in 8 mg). By adding honey, the content of fluoride has been reduced to 23.7 mg/kg. In addition, pH of ginger and honey content was quite high at 6.35 (Therametric Technologies, Inc., Indiana, USA) [31]. Although NaF toothpaste had much more fluoride (1 450 mg/kg), it has provided a less remineralization effect than ginger and honey. These results were consistent with the in situ studies done by Bilgin *et al.* (2016). [17]

Many studies concluded that ginger may be desirable for prevention purposes on initial remineralization of enamel lesions, as more natural products are preferred today [32, 33].

Neem group promoted enamel remineralization. Neem has a variety of therapeutic effects like antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant, analgesic, antipyretic and immuno-stimulant activity [34]. This result may be due to the composition of Neem, which contains the alkaloid margosine, fluoride, gum resins, sulfur, tannins, chloride, silica, oils, sterols, saponins, flavonoids, and calcium [35].

Also, the component of Neem may act as a mechanical barrier because tannins and resins theoretically have an astringent effect on the mucous membrane and they form a layer over the dental enamel, thus protecting demineralization [36], [as] in the study of Prashant, *et al*. (2007) [37] Because the dental enamel is protected from acids, abrasion, and attrition by the biofilm so this can help in the prevention of tooth wear. On the other hand, tannins, one of the main phytochemicals of neem, act as an astringent and give a

Protection of enamel from the adhesion and aggregation of bacteria by coating over the enamel [38]. Thus, calcium and fluoride in Neem are considered important minerals for remineralization of eroded lesions of dental enamel [39].

Moreover, raising in the surface hardness has been recorded with the application of Clove extract oil. Clove is rich in minerals like calcium, iron, sodium, phosphorus, potassium, hydrochloric acid, and vitamin A and vitamin C [40]. The increase in the microhardness of demineralized enamel surfaces may be due to their content of calcium and phosphorus ions which are the main constituents of apatite crystal[41].

Meanwhile, the results of the current study are varying from those of other previous studies which report that the presence of Clove extract might increase the microhardness of demineralized enamel surface that was attributed to their content of calcium and phosphorus ions which are the major constituents of hydroxyapatite [42].

As well clove contains iron, Martinhon *et al*. (2009) [43] in their study they explored the in situ decremental effect of the iron on the demineralization of bovine enamel, also on the composition of dental biofilm, the results showed that ferrous sulphate decreased the demineralization of enamel sample blocks and changed the ionic composition of the dental biofilm formed in situ [44].

Also, the group of green tea displayed an increase in hardness in comparison with the control group but it was less than the effect of fluoride and this agreed with Jaâfoura *et al.* (2014) [45] who showed that the anti-erosion effect of sugar-free green tea could be clarified by its high pH value. The PH of green tea is about 6.3. They as well found that alteration of green tea by the addition of calcium, phosphate, or fluoride ions could improve the anti-erosion effect.

Green tea contains around 5-7% minerals, mainly calcium potassium, magnesium, and phosphorus along with small quantities of zinc, copper, and manganese as well it contains vitamins, chlorophyll pigments and carotenoids [46].

The results of Bozorgi *et al*. (2018) [47] study exhibited that the intake of green tea can increase the microhardness and the resistance to demineralization of the deciduous teeth enamel increased also Barbosa *et al.* (2011) [48] study resolved that green tea has been suggested to supplement the carbonated soft drinks to decrease the erosive potential of these drinks.

In an Iraqi study it was found that after drinking carbonated drink saliva pH fall to 5.47 immediately, while, after herbal mouthwash of green tea, it re elevated directly to reach 6.79 which is higher than the baseline pH (6.65) that stay for a few minutes then it retains to approach baseline pH, it as well has a refreshing feeling with no bitter test or loss of sensation which seen with other chemical mouthwash [49].

Finally, tea, especially green tea, is rich in catechins like epigallocatechin gallate, epicatechin gallate, epigallocatechin and epicatechin [50]. Jose *et al*. (2016) [50] stated in their study, tea was increased the enamel microhardness through these constituents.

Then again, the Peppermint group promoted enamel remineralization but demonstrated one of the least percentage changes in microhardness compared to the other groups. The constituents of mint oils differ with plant maturity, geographical region, variety, and processing conditions[51]. There is information on the effects of mint products on oral bacterial biofilms [52] and S. mutans was inhibited by Peppermint oil [53]. Regarding the importance of peppermint in the curing of dental caries, it can be considered as one of the potent and highly safe medicines used for their treatment because of its effective agent against cariogenic bacteria. It has a good future in this field due to its great benefits and its safety for use in humans without any side effects or contraindications [54].

Additionally, many researchers believe that the oils inhibit plaque build-up, so prevent bacterial adherence to the walls of the mouth and teeth. The prevention of plaque buildup indirectly aids in tooth enamel remineralization. When the mouth is free of harmful bacteria, enamel has the chance to remineralize, meaning teeth are strengthened and protected. Plus its capability to enhance the production of saliva [55] and decrease halitosis [56] but there is no study concluded the effect of peppermint on enamel surface microhardness for this reason its effect on surface hardness has been included in the current study to evaluate its effect in remineralization of teeth enamel because it has many minerals and vitamins in its composition.

It contains minerals such as calcium, potassium, magnesium, iron, phosphorus, and manganese which are required for the formation and maintenance of teeth and

oral bone density. Phosphorus has a critical role in dental tissue health because it naturally protects and restores tooth enamel. In the same way, calcium and phosphorus have an important role in process of remineralization of the enamel surface and play an important role in the conflict between demineralization and remineralization processes [57].

Teeth with higher magnesium content are less susceptible to demineralization [58]. Kunin *et al.* (2014) [59] concluded that the application of a gel containing magnesium and calcium led to a high remineralizing effect which was achieved in patients with early stages of demineralization along with those with non-caries lesions.

CONCLUSIONS

The herbal products have the capability of protecting the eroded enamel via increasing the surface microhardness. So, the Ginger+Manuka honey was significantly better than other groups against demineralization and preserving enamel microhardness.

Declaration of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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