Original Article

Do Ginger Extract, Natural Honey and Bitter Chocolate Remineralize Enamel Surface as Fluoride Toothpastes? An *In-Vitro* Study

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ABSTRACT

Background: In recent literatures, much attention has been given to natural products for their health benefits. **Aims:** In this study, the objective was to measure the efficacy of the ginger-honey-chocolate mixture as the remineralization effect has been shown in the literature previously and to evaluate the individual contributions of this mixture; ginger, natural honey, bitter chocolate separately on remineralization of initial enamel caries lesion. **Materials and Methods:** All specimens were divided into eight groups as: Ginger (Arifoglu®, Turkey) in powder form, (n = 8); Ginger-Honey-Chocolate (n = 8); Natural honey (Balparmak Plateau Blossom Honey®, Turkey) (n = 9); Bitter chocolate (Nestlé®, Switzerland) (n = 8); MI Paste (GC, Japan) (n = 8); Paradontax (Sensodyne, Glaxosmithklein, USA) (n = 9); Pronamel (Sensodyne, Glaxosmithklein, USA) (n = 9); Control (n = 9) groups. Samples were carried out five pH cycles along 7 days at 37°C for each group. During pH cycling, blocks were put in a demineralization (6 h) and a remineralization solution (18 h). The treatment consisted of 1 min. interaction of enamel surfaces with agent/deionized slurries (1:3 w/w) on a daily basis. The surface microhardness (SMH) was determined before and after pH cycling with a Digital Micro-Vickers Hardness Tester (Wilson Wolpert; Europe BV, 401 MVD, Netherlands). Mineral changes were determined by using FluoreCam® and recovery values were calculated as SMHR% and FΔ%, respectively. **Results:** All groups showed an enhanced remineralization. There was no significant difference in terms of FΔ% ($F = 1.223$, $P = 0.304$) and SMHR% ($F = 0.709$, $P = 0.664$) between all groups. **Conclusion:** The herbals (ginger, honey, and bitter chocolate) examined in this study gave promising results with a high remineralization potential.

KEYWORDS: *Bitter chocolate, fluoride toothpaste, ginger, honey, remineralization*

INTRODUCTION

Tooth caries is a problem that affects both adults and children and is even one of the most common health problems in childhood. The importance of fluoride compounds in preventing dental caries has been proven and emphasized in the light of many studies.[1-4] The reduction in the incidence of caries in developed countries has been associated with tooth brushing habits and fluoride content of toothpastes.[5,6] While the use of fluoride have anti-erosive and remineralizing effects,[7] it also comes at a cost may be with adverse effect, such as fluorosis—a condition caused by fluoride intake in excessive amount leading to hypo-mineralization of enamel. Alternative non-fluoride toothpastes contain herbal products or enzymes with antiseptic and antimicrobial effects instead, to protect teeth against caries and opportunistic microorganisms in the oral cavity.[6,8]

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Potential non-fluoride active ingredients

Recently, much attention has been given to natural products for their health benefits. In this study, three novel agents—ginger, natural honey, bitter chocolate—that are thought to have a promising remineralizing potential, are investigated.

Ginger

Natural antimicrobial, antifungal, and antioxidant properties of plant sources, ginger rhizome (Zingiberofficinale Roscoe, Zingiberaceae) were investigated in previous studies, and significant effects were displayed, including the effects in oral cavity.[9-12] Ginger's remineralization potential has been studied, and it has been suggested that the non-invasive treatment with a ginger containing solution of artificially made early caries lesions showed an enhanced remineralization, and a potential to yield clinical benefit in the management of this disease.[13] Recognizing the potential benefits of ginger for oral health, there is now a toothpaste named *Gumgumix*© in the market, which contains honey and ginger as active ingredients.

Natural honey

Honey is a natural food product produced by honeybees (Apismellifera), consisting of the nectar secreted from flowers and substances secreted by insects. Due to its chemical composition, honey has been used for many years in the treatment of burns and infected wounds.[14,15] Honey has been found to have a promising potential as an alternative substance in the management of disease conditions such as dental caries and gingivitis following orthodontic treatment.[16]

Chocolate/bitter chocolate

Cocoa powder and chocolate might have caries-inhibitory potential as shown by a series of studies.[17-19] Pure cocoa powder inhibited dental caries in hamsters' diet and it has shown that zero fat cocoa exhibiting significant anti-caries effect.[18] This study and other similar studies suggest that when cocoa extract is consumed alone, it might display potential anti-caries efficacy.[20]

This in-vitro study investigated the potential efficacy of ginger, ginger-honey-chocolate mixture, natural honey, and bitter chocolate on enamel remineralization measured as surface microhardness recovery (SMHR%) and florescence methods.

Materials and Methods

A total of 68 human molar teeth were enrolled in the study. Sixty-eight enamel blocks (2 × 3 mm) were prepared by using diamond bur in high-speed handpiece and were kept in saline solution. All specimens were divided into eight groups as: Ginger (Arifoglu®, Turkey) in powder form, (n = 8); Ginger-Honey-Chocolate (n = 8); Natural honey (Balparmak Plateau Blossom Honey®, Turkey) (n = 9); Bitter chocolate (Nestlé ®, Switzerland) (n = 8); MI Paste (GC, Japan) (n = 8); Paradontax (Sensodyne, Glaxosmithklein, USA) (n = 9); Pronamel (Sensodyne, Glaxosmithklein, USA) (n = 9); Control (n = 9) groups shown in Table 1.

Demineralization-remineralization cycles and treatment regimens

Samples were carried out five pH cycles along 7 days at 37°C for each group.[21] During pH cycling, blocks were put in a demineralization solution [demineralization solution in 75 mmol/L acetate buffer, pH 4.7; 2.2 mL/mm^2; 2.0 mmol/L Ca $(NO_3)_2.H_2O$, 2.0 mmol/L $NaH_2PO_4.H_2O$and 0.04 µg F/mL (NaF)] for 6 hours and in a remineralization solution [remineralization solution, in 0.1 mol/L cacodylate buffer, 7.0 1.1 mL/mm^2; 1.5 mmol/L Ca $(NO_3)_2.H_2O$, 0.9 mmol/L $NaH_2PO_4.H_2O$, 150 mmol/L KCland 0.05 µg F/mL (NaF)] for 18 hours. The treatment consisted of insertion of enamel blocks into testing materials + deionized water slurries (1:3 w/w) on a daily basis. Samples were rinsed with deionized water was applied before prior all immersion steps. These procedures were applied to imitate daily routine intraoral pH change. Demineralization-remineralization solutions used in study did not form any lesions as these procedures aimed to observe the effect of study groups on teeth in daily oral hygiene routine.

Surface microhardness (SMH) measurements

The hardness of the enamel surface was determined before and after pH cycling with a Digital Micro-Vickers Hardness Tester (Wilson Wolpert; Europe BV, 401 MVD, Netherlands). It was fitted with a Vickers diamond tip loaded 25 grams and all three measurements were performed by 10 seconds intervals. SMH was determined at the baseline, after demineralization and remineralization with testing materials. The extent of remineralization was calculated as the percent SMH recovery (SMHR%), Fluorecam recovery (FΔ%) as shown:

$$SMHR\% = [(SMH^3-SMH^2)/(SMH^1-SMH^2)] \times 100$$

$$F\Delta\% = [(F\text{-}size^3-F\text{-}size^2)/(F\text{-}size^1-f\text{-}size^2)] \times 100$$

(SMH^1: baseline SMH, SMH^2: after 18 hours demineralization application, SMH^3: after pH-cycling; $F\text{-}size^1$: baseline F-size, $F\text{-}size^2$: after 18 hours demineralization application, $F\text{-}size^3$: after pH-cycling).[22]

Fluorecam measurements

Autofluorescence is the characteristic of dental tissues' natural fluorescence. It is the hydroxyapatite crystals that give the enamel its

fluorescence characteristic, while in dentin, it is the dentinal tubules.[23] Quantitative light source fluorescence (QLF) is a fluorescent device used in dentistry to distinguish between mineralized and demineralized areas of teeth. FluoreCam (Daraza Therametric Technologies, Noblesville, Indiana, USA), which operates on similar working principles as QLF, is used in clinical quantitative evaluation of enamel demineralization. The tooth surface exposed to a certain wavelength of light with FluoreCam emits fluorescent light at different wavelengths according to the demineralization of enamel. The advantage of this technology is that caries can be diagnosed early long before its progression. The size (percent loss of enamel) and intensity (intensity of fluorescence emitted) of caries are calculated as two separate parameters, and the combination of these two parameters yields the total impact score.[24] In a present study, fluorecam size and fluorecam intensity values were recorded.

Statistical analysis

Statistical analysis was performed by using the SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). Correlation between methods were analyzed using Pearson Correlation test. The differences between the groups and SMHR% and FΔ% were performed by ANOVA test.

Results

The SMHR% and FΔ% values were shown in Table 2.

There is a moderate and statistically significant positive correlation between SMHR% and FΔ% in the measurements made in all groups. (r = 0.377, P = 0.002).

There is no significant difference between the groups in terms of FluoreCam-size measurements for remineralization (F = 0.897, P = 0.515). Moreover, there is no significant difference between the groups in terms of FluoreCam-intensity values for remineralization (F = 0.531, P = 0.807).

In addition, all groups have shown enhanced remineralization after pH cycle regimens and there was no significant difference in terms of FΔ% (F = 1.223, P = 0.304) and SMHR% (F = 0.709, P = 0.664) between all groups including control group [Figure 1].

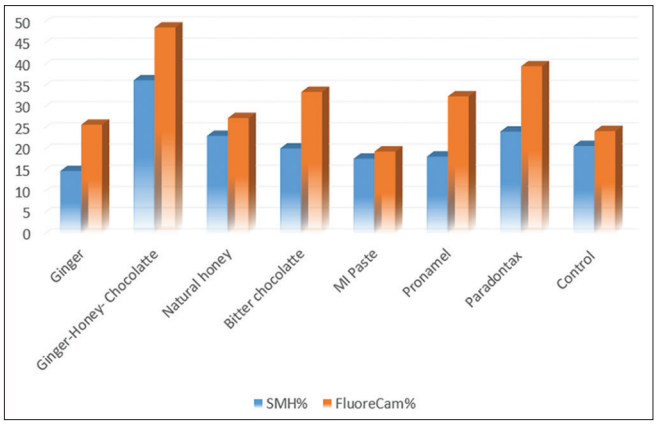

Figure 1: Remineralization percentages of enamel surfaces after ginger, natural honey, bitter chocolate, fluoride toothpastes, and MI Paste applied

Table 1: Study groups shown by their chemical contents and brands

Groups	*n*	Chemical content	Brand
Ginger extract	8	Powder form	Arifoglu®, Turkey
Natural honey	9	viscose cream form	Balparmak Plateau Blossom Honey®, Turkey
Ginger-Honey-Chocolate	9	viscose cream form	Arifoglu®, Turkey; Balparmak Plateau Blossom Honey®, Turkey; Nestlé®, Switzerland
Bitter chocolate	8	viscose cream form	Nestlé ®, Switzerland
MI Paste	8	Non-fluoride,	GC, Japan
Paradontax	9	1450 ppm F	Sensodyne, Glaxosmithklein, USA
Pronamel	9	1450 ppm F	Sensodyne, Glaxosmithklein, USA)
Control	9	deionized water	-

Table 2: SMH and FΔrecovery values of all groups

Groups	% SMHR	% FΔ
Ginger	14,91	25,82
Ginger-Honey-Chocolate	36,32	48,75
Natural honey	23,19	27,44
Bitter chocolate	20,22	33,54
MI Paste	17,82	19,53
Paradontax	24,22	39,62
Pronamel	18,35	32,52
Control	20,83	24,31

P>0,05, P>0,05

Discussion

The aim of this study was to compare the remineralization potential of the main ingredients used as active contents in toothpastes with fluoride and non-fluoride toothpastes. The main function of toothpastes is their therapeutic effect in preventing tooth decay and providing dental hygiene. What makes tooth brushing experience pleasant and supports people to solidify this habit is the taste, fragrance, post-brushing sensation, and foaming characteristics of a toothpaste.

Fluoride is the most potent and effective substance used as active ingredient yet developed for the prevention and treatment of caries. Fluoride at optimal level, decreases the incidence of dental caries but at the same time at higher levels it can cause adverse effects like dental or skeletal fluorosis. Therefore, there are many studies on non-fluoride ingredients that may enhance any remineralization of tooth surface as fluoride toothpastes.[5,6,8,13,16]

In this study after the samples were treated with demineralization and remineralization solutions in repetitive cycles for 7 days, enamel specimens were then investigated for their any mineral changes after application ginger extract, natural honey, and bitter chocolate and other commercially available toothpastes.

This study also compares the remineralization potential of three commercially available toothpastes using a pH cycle model. All samples in the study were subjected to a standard pH cycle of seven days.[21] The advantage of using such a model is that it greatly adapts the real-life conditions that led to the development of caries.[22] When the groups are examined separately, it was observed that all groups, including the control group, showed various degrees of remineralization [Table 2].

It is considered that the amount of fluoride (NaF) in the remineralization (0.05 μg F/mL) and demineralization (0.04 μg F/mL); solutions were effective on the remineralization observed in the control group.

When the commercially available remineralization agents MI Paste, Paradontax, and Pronamel were compared with each other, it was observed that the remineralization in MI Paste group was the lowest. Subsequently, the lowest remineralization (SMHR%) was observed in the Pronamel group (18.35%). Although significantly greater SMHR ($p < 0.0001$) was observed for Pronamel (Sensodyne®) in *in situ* studies,[25] polyvinylmethylether-maleic acid (PVM/MA) copolymer, sodium lactate at low pH may be the reason for the outcomes of our study. Since the anti-caries and anti-erosive effect of Pronamel occurs at low pH, it is thought that the fluorine release is lower compared to other groups (SMHR = 18.35%).

In addition, among the non-fluoride agents, the ginger-honey-chocolate group was the group that showed the highest surface hardening in both SMH (36,32%) and FluoreCam measurements (48,75%). Similarly, it has been shown that ginger and honey mixture has anti-caries significant synergistic effect of antimicrobial activity in preventing caries.[26] According to the results of our study, the same synergistic effect may be valid in terms of surface remineralization.

Bilgin G. *et al*. conducted two studies with herbal mixture of chocolate, ginger and honey, and this mixture was found to have a positive impact in the remineralization of early enamel lesions.[13] In both in vitro- and in situ-controlled studies, it was observed that when the alternative materials used, an increase in enamel lesion hardness of 50-80 microns were observed compared with the regular fluoride toothpaste used in that study. In another study, it was determined that a larger enamel lesion of 140 microns could be remineralized with ginger and honey-containing herbal toothpaste. Hypothesis of the first study was that ginger alone was an effective agent in the remineralization of the initial enamel caries lesion. Then, the aim of the second study was to evaluate the remineralization potential of ginger, chocolate, honey mixture of these three substances on human softened enamel surfaces.[13]

In terms of caries prevention, natural honey had shown no erosion on enamel surface in Scanning electron microscope (SEM).[27] In addition, Knoopmicrohardness test was used to determine any deterioration in the structure of enamel placed in a 4-fold diluted honey solution. This test did not show any erosion in the sample group treated with honey as seen in the confirming the findings of SEM.[25] Similarly, our study also determined that natural honey may remineralize enamel surface as fluoride toothpastes under the conditions of this research.

Bitter chocolate was another potential active ingredient due to its content “theobromine”. Many studies showed its remineralizing effect on tooth surface and its anti-caries properties.[20,28-30] The study conducted by Amaechi *et al*. (2012) also supports this study. It has been suggested that theobromine found in chocolate is better on tooth enamel remineralization than fluoride.[20] Our study also reveals enhanced remineralization and there was no significant difference between bitter chocolate group and fluoride toothpaste in terms of surface hardness and mineral loss/gain.

In addition to the SMH measurement method that was used in many *in vitro* studies as the standard method, fluorescence technology was also used in this study. It has been observed that demin-remin pH cycle, which imitates the oral environment, is a very suitable method to determine the amount of remineralization after pH cycle and to monitor the recovery in clinical conditions in a non-invasive manner.[23] Korkut *et al*. measured demineralization of teeth around orthodontic brackets with FluoreCam, suggested that FluoreCam is a clinically applicable, non-invasive and reliable method.[24] Similarly in our study there was a correlation between SMH recovery and FluoreCam recovery results.

New generation dentistry focuses on minimally invasive approaches and effective agents that do not cause side effects to the individuals. Ginger, natural honey, and dark chocolate may have some properties comparable to those of fluoridated toothpastes and MI Paste when used as a remineralizing agent, with their remineralization enhancing potential. They all each and together as the mixture promises to be an active ingredient that can be included in toothpastes as an active agent for remineralization such an alternative to fluoride.

Conclusion

Fluoride toothpastes have proven effect in terms of remineralization. Herbals and natural toothpastes are very common today and they show enhancing effects such as fluoride toothpastes do, including their remineralization potential with many features. Extracts of ginger, honey, bitter chocolate, and the mixture of these three agents examined in the study are given promising results for being active ingredients with a high remineralization potential.

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Conflicts of interest

There are no conflicts of interest.

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