INTRODUCTION
The first visible sign of tooth caries, the white-spot lesion (WSL), has been defined as “subsurface enamel porosity from carious demineralization” that is manifested clinically by a white-spot lesion (WSL), has been defined as “subsurface enamel porosity from carious demineralization” that is manifested clinically by a white-spot lesion (WSL). In the minimally invasive dentistry paradigm, incipient enamel carious lesions should be treated with non-invasive remineralization strategies wherever possible, instead of surgical intervention 2. For this purpose, topical gels, varnishes, mouthwashes, and dentifrices that contain fluoride are used by dentists for the treatment of WSLs. With the concentration of 1100-1400 ppm in dentifrices, while topical gels (=10 000 ppm F) and varnishes (=20 000 ppm F), fluoride is the best only remineralizing agent for chemical treatment of initial carious lesions.

Fluoride is a proven agent for caries prophylaxis but excessive use of fluoride may cause dental fluorosis if ingested by very young children, and the use of bactericides or antibacterial agents may cause various side-effects, such as vomiting, diarrhea, and tooth staining, with increased resistance to these chemicals. For financial reasons, in developing countries, there is also a need for alternative prevention and treatment options that are safe, effective, and economical. Thus, instead of using artificial antibiotics and bactericides, it has been proposed that various medicinal plant extracts that have effects on bacteria causing tooth decay be used.

Among natural food sources with antimicrobial activities, ginger (Zingiber officinale Roscoe, Zingiberaceae) and rosemary (Rosmarinus officinalis L., Lamiaceae) have been used as food spices and medicinal plants for centuries. Moreover, they are natural materials, showing no toxicity, and are considered ‘generally recognized as safe’ (GRAS) by the US Food and Drug Administration (FDA). In particular, their pungent oil components harbor a series of polyphenolic ketones with many pharmacological activities. Their antifungal and antimicrobial effects on oral cavity pathogens have been reported in many studies. However, there is no reported study about the effects of these herbal medicaments on remineralization of initial enamel caries.
inhibit the growth of pathogens because most thrive at pH 4.0-4.5. However, dilution of honey, for example by saliva, will increase the pH and reduce this effect. Dilution also results in a 2500-50,000 times increase in enzyme activity and the production of hydrogen peroxide, which is an oxidizing agent, will increase. In honey, hydrogen peroxide is present at a very low level, yet it is still an effective antibacterial agent and compatible with cellular preservation. There are a few reports about the antibacterial effect of honey on oral pathogens but none about the effect of honey on remineralization of initial enamel caries. According to the results of the research by Patel RV et al., a paste made by lending ginger and honey was found highly effective on inhibition of S. mutans, L.acidophilus, A.viscosus, Paeruginosa, V.valcaligines and S.aureus.

The fluorescence systems do measure the mineral loss and gain in the lesion area as mm². These systems compare the mineral differences of the lesion area from its base either sound surface on demineralized surface quantitatively. This way white spot lesions and any remineralizations in these lesions can be detected and recorded non destructively.

The aim of this study was to evaluate the remineralization potential of herbals: ginger, rosemary, and honey. Moreover, we reported the antibacterial effect of honey on oral pathogens but none about the effect of honey on remineralization of initial enamel caries. According to the results of the research by Patel RV et al., a paste made by lending ginger and honey was found highly effective on inhibition of S. mutans, L.acidophilus, A.viscosus, Paeruginosa, V.valcaligines and S.aureus.

MATERIALS AND METHODS

Study Design. This in situ investigation was a single-site, randomized, four-way crossover study involving 10 participants. The study design is shown in Figure 1. This study commenced following the approval from the ethical committee, and it was presented as the thesis of Dt. GÜlçin Bilgin.

Figure 1. Illustration of the study experimental design.

Surfaces of the specimens were polished with a 600-grit grinding disk and with a slurry of 0.05 µm gamma alumina polishing gel. Artificial subsurface carious lesions were formed on each enamel specimen by placing the specimens individually for 72 h at 37°C in 7.0 mL of a demineralizing solution containing 0.1 molar lactic acid and 0.2% Carbopol 907, 50% saturated with hydroxyapatite and adjusted to pH 5.0 using NaOH.

Intraoral Appliance. All specimens were mounted on a mandibular removable acrylic appliance. The appliance had two bilateral troughs with a window on the buccal surface to house enamel specimens. In each appliance, three enamel specimens were retained with temporary filling material (Clip, Voco, Germany; Figure 3).

Figure 3. Images of intraoral appliance.

The participants wore the appliance intraorally for 3 weeks continuously, except when eating, drinking water was permitted. With their appliances in place, they brushed their teeth with the same toothpaste twice per day (morning and evening), (Ipansa Kalsident, Procter & Gamble, Turkey) during each treatment period. They were careful not to touch the specimens’ surfaces during brushing. The test materials were applied immediately following brushing with fluoride toothpaste. ReminPro was applied as a pea-size amount by finger to the specimens. The chocolates were chewed in the molar
region and stayed there approximately 3 min. Then, the mate-
rials were distributed throughout the mouth with the tongue. 
Participants were not allowed to eat or drink for 30 min fol-
lowing the treatments. The appliance was kept fully hydrat-
ed by immersion in distilled water in a plastic box when not in use. After every week, participants were asked to visit 
to check their general health, compliance, and the fitting of the 
appliance and specimens. All subjects complied well with the 
protocol. No adverse reaction related to the appliance or treat-
ment materials was observed.

TreatmetMaterials. Ipana is a standard fluoridated dentifrice (NaF, 1450 ppm). ReminPro is a dental cream that contains hydroxyapatite, fluoride (1450 ppm), and xylitol. Ginger and rosemary were in powder form and mixed with honey (8 mg/mL). The mixtures then were covered with chocolate, which was bitter (15% sugar). The chocolate was specially produced by Bind Chocolate (Tatlı Çikolata San. ve Tic. A.Ş., Tekirdağ, Turkey).

Analysis of Remineralization. The mineral density of the spec-
imens was determined using a digital microhardness tester 
(Leco LM247AT, Leco Corp., MI, USA) with a load of 200 g for 15 s in four areas. Assessments of the mineral content 
of the demineralized area of each specimen were obtained before and after each test period using both the FluoreCam 
(Therametric Technologies, Inc., Noblesville, IN, USA) and QLF 
(Inspector Pro, Inspector Research Systems, Amsterdam, The 
Netherlands) systems. The parameters assessed included % 
fluorescence loss, area (mm²) and lesion volume (mm³). The 
fluorescence values (∆F and ∆Q) in all of the treatment groups 
were significantly different (p < 0.05). Moreover, no signif-
icant difference was observed in FluoreCam values among the groups using ∆F or ∆Q measurements (p > 0.05).

However, the surface microhardness increases (Table 1, Fig-
ure 4) showed significant differences between all supple-
ment-treated groups. The additional dental cream treatment, 
ReminPro, and the herbal mixture (ginger + honey + choco-
late) treatment showed greater remineralization and they were 
significantly different from each other (p < 0.05). The other 
herbal treatment (ginger + rosemary + honey + chocolate) 
was more effective than fluoride dentifrice alone and it was 
also significantly different (p < 0.05).

**DISCUSSION**

The major interactions of the enamel with the oral environ-
ment occur on the surface layer of the sample. The remin-
eralizing agents applied on the surface of enamel specimens 
were evaluated by fluorescence (QLF, FluoreCam) and surface 
microhardness test methods. These methods are simple, fast, 
and easy to measure as non-destructive methods and are ca-
pable of reflecting mineral changes that have occurred due to 
treatments. They also allow repeated measurements of the 
same specimen over a given period of time if required by the 
study design. Enamel fluorescence assessments using QLF 
have shown great promise as an early caries detection meth-
’od. Based on data available, it is suitable for in vivo monitor-
ing of mineral changes, as well as for caries prevention pro-
grames, although the cost of the equipment is significant and image analyses are time-consuming. Also, there are certain 
facets that may influence its success, such as dehydroxy-
lation and angulation. The FluoreCam instrument uses the same 
basic principles to assess enamel fluorescence as QLF. Howev-
er, the analyses of the suspected demineralization or reminer-
alization area are performed automatically by the FluoreCam 
software. Although we did not observe any statistical differ-
ences in fluorescence assessments induced by the different 
treatment regimens in this study, due to the large variances, 
the fluoride dentifrice (NaF) in the herbal treatment groups 
changed numerically, suggesting greater remineralization than 
was observed with only the fluoride dentifrice in QLF measure-
ments (Table 1).

ReminPro is a relatively new agent that is marketed for en-
hancing remineralization. In this in situ study, we found that 
ReminPro provided additional remineralization beyond that 
achieved with the fluoride dentifrice, as assessed by both 
microhardness and fluorescence assessment methods. Prob-
ably both ingredients in this treatment agent, fluoride and 
hydroxyapatite, enhanced the remineralization by infiltrating 
through the lesion body and increasing remineralization of the 
lesion.

Brushing the teeth with fluoride toothpaste is a must for an 
in situ study, for ethical reasons. Thus, all volunteers used NaF.
toothpaste in all test groups twice per day. Fluoride is retained in enamel lesions in the form of a fluorapatite, and on the enamel surfaces as CaF₂, which may act as a fluoride reservoir; thus, there was a significant amount of remineralization from the fluoride dentifrice in each study group in addition to the added remineralization effects of the test materials.

Recently, many phytochemicals, including antibacterial agents, have been identified from edible plants. There are also numerous reports on components of plants that have revealed antibacterial activities against Streptococcus mutans, which is widely known as a cause of dental caries. Ohara et al. 7 investigated the antibacterial activities of 81 edible plants on S. mutans in polarity-differing solvents (hexane and ethyl acetate) and ginger was found to be effective (MIC 23 mg/g and 8 mg/g). Moreover, after boiling for 10 min at 100°C or after storage for 1 week at 4°C, ginger retained its antibacterial activity 7. Gregorio et al. 24 found that in glycolic or hydrochloric solvents, ginger was effective against S. mutans at a MIC of 5 mg/mL.

Streptococcus mutans produces glucosyltransferases and synthesizes an adherent and water-insoluble glucan from sucrose, which allows the organism to adhere firmly to the tooth surface. Cacao beans, which are a main ingredient of chocolate, contain some polyphenols that exhibit anti-glucosyltransferase activity. Ooshima et al. 25 reported that cacao bean husks have an anticariogenic effect on S. mutans and S. sobrinus in rats, demonstrating that the extract could become a novel anti-caries substance, as a mild chemo-prophylactic agent. They found that the husk extract might be able to change a cariogenic flora into a non-cariogenic flora without destroying the ecological balance within the oral cavity, because it markedly reduced the growth rate of S. mutans, but did not affect other oral streptococci so strongly 25. Osawa et al. 26 demonstrated that a 50% ethanol extract of cacao bean husk was superior to the 30% ethanol extract they had used in their previous study. They found that the cacao bean husk had two types of cariostatic substances. Higher-molecular weight polyphenolic compounds and unsaturated free fatty acids, such as oleic and linoleic acids, were isolated from the cacao bean husk. The former showed anti-glucosyltransferase activities and the latter showed antibacterial activity against S. mutans. 26 Percival et al. 27 reported that cacao polyphenols can inhibit biofilm formation and acid production by S. mutans. In our study, we used chocolate as a carrier for the ginger and honey mixture, and also as a part of the treatment material. Honey has potent broad-spectrum antibacterial activity and studies have demonstrated that manuka honey has high antibacterial activity and is likely to be non-cariogenic. Patel et al. 28 reported that ginger and honey were more effective than gentamycin against S. mutans. They found the MIC of ginger to be 31.25 mg/mL, and that of the mixture of honey 1.2% (w/v) and ginger to be 15.63 mg/mL. Our study was consistent with these reports in demonstrating that ginger + honey (8 mg/mL) was a strong remineralizing agent. The high remineralization obtained was probably due to the antimicrobial properties and the high fluoride content (79 ppm) of ginger. Thermometric Co. laboratory results revealed that the pH of the ginger and honey mixture was essentially neutral (pH 6.35). Even though NaF toothpaste had much more fluoride (1450 ppm), the amount of remineralization increased further with the ginger + honey + chocolate treatment (Table 1). Under the conditions of the study, we could not distinguish whether a larger remineralization effect resulted from the ginger, but it seems that there might be some enhancing effect on dental lesions. However, honey with its low pH may activate the release of fluoride from ginger at the time of application, resulting in higher remineralization. Tsai et al. 28 demonstrated an inhibitory effect of rosemary on S. sobrinus. They found the minimum inhibitory concentrations of aqueous and methanolic rosemary extracts against S. sobrinus were 16 and 4 mg/mL. Dalirsani et al. 29 compared rosemary methanolic extract (30 g/100 mL) with chlorhexidine and found that rosemary has inhibitory effects on S. mutans. We found that the rosemary-containing treatment mixture was effective in enhancing the remineralization process of enamel but it was not as effective as the ginger + honey mixture alone. In this in situ investigation, all test materials enhanced remineralization as NaF toothpaste did as control group. QLF and FluoreCam systems showed remineralization in all groups. All groups were similar in remineralization including NaF toothpaste positive control group in both fluorescence systems. All groups showed remineralization in microhardness test method. Test groups showed higher microhardness values compared to NaF toothpaste positive control group. Herbal mixture (ginger+ honey+ chocolate) and ReminPro groups showed higher microhardness value in this study. Further research is still needed to check the accuracy of FluoreCam system.

The daily application of chocolate containing ginger and honey with or without rosemary resulted in enhanced remineralization beyond that provided by the daily use of a fluoride dentifrice. Because more natural products are preferred today, the herbs, ginger and rosemary, may be preferable for prevention purposes on initial remineralization of enamel lesions beyond the daily use of NaF toothpaste, at least under the conditions of this in situ study. With these promising findings, we suggest further investigations of the potential benefits of herbal treatments for dental health.

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