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Effectiveness of some herbals on initial enamel caries lesion

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ABSTRACT

Objective: To evaluate the effectiveness of herbal medicaments such as ginger, rosemary and honey on remineralization of initial enamel lesion.**Methods:** Demineralized human enamel specimens were measured for baseline surface microhardness and fluorescence methods. Ten specimens in each of four groups were used in this *in vitro* recycling study with the following treatments which applied three times a day: 1) sodium fluoride toothpaste (Ipana, Procter & Gamble, Turkey), 2) ginger-honey (Arifoglu Herbals, Anzer Honey, Turkey), 3) ginger-honey-chocolate (Bind Chocolate, Turkey), 4) rosemary oil (Arifoglu Herbals, Turkey). Treatment regimens of demineralization and remineralization cycle were applied for 21 days. The post-treatment data were obtained by measurements of surface microhardness and fluorescence methods. Data were statistically analyzed by ANOVA test with Tukey's honest significant difference test.**Results:** Enhanced remineralization was observed with several of the treatment systems including ginger + honey and rosemary. Significant differences between treatments were observed by microhardness and FluoreCam fluorescence assessment, compared to the positive control group (NaF dentifrice). Significantly, greater remineralization was observed with the honey + ginger treatment regimen. No significant differences between groups were observed using the fluorescence assessment method, quantitative light-induced fluorescence.**Conclusions:** Herbals (ginger, honey and rosemary) have enhanced remineralization of initial enamel lesion.

1. Introduction

The first sign of tooth caries, opaque lesion defined as “subsurface enamel porosity from carious demineralization” is manifested clinically by a milky white opacity. This subsurface porosity is being caused by an imbalance between the dynamic biological processes of de- and remineralization. In minimally invasive dentistry paradigm, incipient enamel carious lesions

should be treated with non-invasive remineralization strategies. On this purpose, topical gels, varnishes, mouthwashes and dentifrices contain fluoride being used by dentists for the treatment of white spot lesions [1].

Fluoride is proved agent for caries prophylaxis, however, excess use of fluoride causes fluorosis, and hardening of cartilage. Moreover, the usage of bactericides or antibacterial agents has several negative effects on gastrointestinal system with increased resistance to these chemicals. Due to financial situation, developing countries need biocompatible and cost effective preventive methods. Therefore, instead of using artificial antibiotics and bactericides, it has been proposed to use medicinal plant extracts which have an effect on causative bacteria of tooth decay [2].

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Among natural food sources, ginger rhizome (*Zingiber officinale* Roscoe, Zingiberaceae) and rosemary (*Rosmarinus officinalis* L., Lamiaceae) are natural herbals with their antimicrobial activities. Additionally, they do not show any toxicity approved by 'generally recognized as safe' in the Food and Drug Administration of the United States. Pungent oil contents of these herbals harbor some polyphenolic ketones with many pharmacological activities. Their antifungal and antimicrobial effects on oral cavity pathogens have been reported in many studies [3–7]. However, there is no study in current literature about the effect of these herbal medicaments on remineralization of initial enamel caries.

Another regimen used in most ancient cultures for nutritional and/or medicinal aim is honey. It is believed that honey is a nutrient and can be used as a drug for a long time. Honey is a supersaturated sugar solution with low water activity that does not support the growth of bacteria [8]. The average pH value of honey is 3.9, and can show bacteriostatic effect on pathogens as most thrive at pH between 4.0 and 4.5 [9]. However, dilution of honey, for example by saliva will increase the pH and reduce this effect. On the other hand, dilution results in 2500–50000 times increase in enzyme activity and this glucose oxidase enzyme is the production of hydrogen peroxide, an oxidizing agent. Hydrogen peroxide is present in honey in small amounts, yet is still very effective antibacterial agent compatible with cellular preservation [10]. There are a few studies on efficacy of honey on oral pathogenic bacteria [11–13] and none is about effect of honey on remineralization of initial enamel caries.

Methylxanthines are plant-produced natural products. Most plants used for preparation of beverages on human consumption are enriched in methylxanthines [14]. The antioxidants of *Theobroma cacao* beans have psychoactive effects at high amounts because of methylxanthines [15]. Besides, theobromine could show protective effect on enamel surface of human molars as shown by a pilot study [16]. This protection was attributed to carbohydrate content of cacao which can be metabolized and a trap for bacteria to protect dental enamel from caries.

The purpose of this study is to evaluate remineralization potential of herbals (ginger, rosemary and also honey).

In addition to our main purpose, we investigated the efficiency of new detection device (FluoreCam), for demineralization and remineralization of human dental enamel by microhardness, also quantitative light-induced fluorescence (QLF) systems.

2. Materials and methods

2.1. Enamel specimens and preparation of subsurface lesions

A total of 40 human enamel specimens were used in this study. Extracted teeth obtained from oral surgeons were used; the teeth were stored in 0.10% thymol solution immediately after extraction and maintained in this solution prior to use. The sound enamel specimens required for this study were 3 mm in diameter and 1.6–2.0 mm thick from surface of enamel. These enamel cores were mounted on acrylic rods. Surfaces of specimens were polished by 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 lactic acid as 0.1 molar

amount and Carbopol 907 as 0.2%, 50% hydroxyapatite-saturated in volume and adjusted to pH 5.0 using NaOH [17]. This procedure resulted in lesions approximately 35–50 μm in depth.

2.2. Study design

The specimens were divided randomly to six groups (10 specimens/group) with the treatment materials. Treatment regimen was designed with approximate pH oral environment and modified by Dunipace *et al.* [18]. The demineralization and remineralization cycles showed episodes as observed in Table 1. Each cycle contained 3 h of demineralization to simulate the daily acid challenges in oral cavity. The samples were kept in laboratory produced saliva which consisted of 2.00 g/L methyl *p*-hydroxybenzoate, 10.0 g/L sodium carboxymethyl cellulose, 8.38 mmol/L KCl, 0.29 mmol/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.13 mmol/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 4.62 mmol/L KH_2PO_4 , 2.40 mmol/L K_2HPO_4 ; and adjusted pH was 7.0 using KOH and there was not any precipitation observed during the experiment [19]. Repeated treatment regimen lasted during 21 days. This saliva was changed each day and these treatment materials were freshly prepared in every application. All the time except applications, the samples were kept in artificial saliva that was mixed by a magnetic stirring machine (Multipoint HP15P, Variomax, USA).

2.3. Treatment materials

Ipana, NaF toothpaste consisted of 1450 mg/kg fluoride and was used as a positive control group. Based on the previous studies that have found the minimum inhibition concentration (MIC) of ginger (5–8 mg/mL) [4,20], we used ginger in powder form (Arifoglu Herbals, Turkey) and applied 8 mg into 1 mg honey (Anzer honey, Turkey). Chocolate (Sokella, Turkey) was added as 1 mg into the mixture of ginger + honey. All materials were mixed homogeneously and applied on the surfaces by smearing. Rosemary (Arifoglu Herbals, Turkey) oil was applied with an applicator. All pastes were prepared freshly at each application of remineralization materials.

2.4. Assessment of mineral content – FluoreCam & QLF

Before and after each test period, assessments of the mineral content of demineralized area of each specimen were obtained using both the FluoreCam (Daraza, Corporate Headquarters, Indiana, USA) and QLF (Inspektor Pro, Inspektor Research Systems, Amsterdam, Holland) systems. Using the FluoreCam instrument, the images were collected with and without

Table 1

The pH-cycling model in the experiment.

Time	Application
08:00–09:00	Lactic acid
09:00–09:01	Treatment materials
09:01–13:00	Artificial saliva
13:00–14:00	Lactic acid
14:00–14:01	Treatment materials
14:01–19:00	Artificial saliva
19:00–20:00	Lactic acid
20:00–20:01	Treatment materials
20:01–08:00	Artificial saliva

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