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Effectiveness of Ginger-Honey Mixture and Rosemary on Remineralization and Prevention of Enamel White Spot Lesions Measured by Color Change

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ABSTRACT

Purpose: To evaluate the effect of three natural materials (Ginger and honey mixture and rosemary oil) on remineralization and prevention of enamel white spot lesions measured by color change using vita easy shade. **Material and methods:** The study was carried out in two phases remineralization and prevention phase, sixty four anterior teeth were used for each phase. Teeth were prepared and randomized into four groups according to treatment material (n=8 samples). Group I: control group which receives no treatment. Group II: treated with 1.23% sodium fluoride gel. Group III: treated with ginger-honey mixture. Group IV: treated with rosemary oil. Treatment material was applied for 3 or 6 minutes. In phase (1) color was measured at baseline, after creation of white spot lesion, and after treatment. In phase (2) color was measured at baseline and after treatment. Data were obtained and the differences between groups were estimated by ANOVA test followed by Tukey's post hoc test. **Results:** for phase1: There was a statistically significant difference among different groups. The highest mean value of ΔE was for sodium fluoride gel group followed by ginger and honey group at 6 min. For phase2: group III had a better acid resistance effect followed by group II at 6 min. **Conclusion:** Natural materials can be used for remineralization and prevention of WSL as ginger and honey mixture enhance remineralization following sodium fluoride gel which was more effective. And for acid resistance phase, ginger and honey mixture was more effective.

KEYWORDS

Remineralization, Prevention, Color change.

- Paper extracted from Master thesis titled 'Effectiveness of Ginger-Honey Mixture and Rosemary on Remineralization and Prevention of Enamel White Spot Lesions Measured by Color Change'

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INTRODUCTION

Tooth caries leads to loss of phosphate, calcium, and other ions and dissolution of apatite crystals, which result in demineralization of the tooth structure. White spot lesion is considered the first visible sign of tooth caries, white spot lesion has been known as "enamel subsurface porosity due to carious demineralization" that is showed clinically as a white milky opacity[1]. Early carious lesions found in enamel must receive non-invasive remineralization treatment, rather than surgical treatment. For this reason, varnishes, mouthwashes, and topical gels containing fluoride are being used for treatment of WSLs. Fluoride is recognized as a proven agent used for protection from dental caries. But dental fluorosis may occur from excessive use of fluoride. Also, the use of antibacterial or bactericides agents can cause many side effects, such as tooth staining, diarrhea, and vomiting[2,3].

Thus, instead of using artificial bactericides and antibiotics, Various therapeutic plant extract that has effects on bacteria causing decay of tooth structure be used. Among natural materials with antimicrobial activities and showing no toxicity, Ginger rhizome, honey, and rosemary. Z. Officinale (Ginger) is one of the most widely used herbs. There are many studies that show the antibacterial effect of ginger against different microorganisms including S. mutans[4]. Another natural material that has an effect on dental caries is Honey. Honey's pH value is 3.9, which is acidic, so it can inhibit the growth of different pathogens, Honey has many antioxidant compounds and some bio compounds as amino acids, enzymes, organic acids, vitamins, carotenoids, and minerals[5]. Rosemary has an inhibitory effect on S. sobrinus and S. mutans[6]. The goal of the current study was to examine the effect of three natural materials (Ginger and honey mixture and rosemary oil) on the prevention and remineralization of enamel white spot lesions.

MATERIALS AND METHODS

Sample size:

Sixty-four anterior teeth were used for each phase. Teeth were prepared and randomized into four groups according to treatment material (8 samples each). Group I: control group which receives no treatment. Group II: treated with 1.23% sodium fluoride gel. Group III: treated with ginger-honey mixture. Group IV: treated with rosemary oil. Treatment material was applied for 3 or 6 minutes.

Teeth selection and tooth preparation:

This study was carried out in two phases (remineralization) and (acid resistance) phase. Sixty-four human freshly extracted anterior teeth were used for each phase. The selected teeth were free of caries and defects detected by fiber-optic transillumination. The teeth were cleaned, rinsed, and kept immediately in 0.10% thymol solution directly after extraction and preserved in the thymol solution at 4^{o}C until being used. All teeth were obtained from patients who have already signed a consent form approving using of their extracted teeth for research. Research ethics committee approval of Faculty of Oral and Dental Medicine for girls was obtained (REC-OP-21-03).

Treatment materials:

1.23% sodium fluoride gel applied by a micro applicator. Ginger powder (IMTENAN) and honey (clover flower honey IMTENAN) was mixed homogeneously with a ratio of 8 mg/ml (w/v)[7] and applied by a micro applicator. Rosemary oil (AYRA VEDA) is applied by a micro applicator (table1). The application followed manufacturer's instructions. Treatment material was applied for 3 or 6 minutes.

Table (1) *Materials used in the study, their composition and manufacturer.*

Material	Brand name	Composition	Manufacturer
Zingiber officinale	Ginger Imtenan	100% Organic Indian ginger Rhizomes powder	Imtenan Health shop Obour city, Egypt
Honey	Honey Imtenan	100% natural pure honey	Imtenan Health shop Obour city, Egypt
Rosemary oil	Rosemary oil (AYUR VEDA)	100% Pure Essential Rosemary oil	AYURVERDA Germany
Sodium fluoride gel	Maquira FLUOR GEL	Sodium fluoride 1,23%	MAQUIRA INDUSTRIA DE PROD. ODONTOLOGIES South Australia
Phosphoric acid etch	Meta Etchant	37% phosphoric acid gel	META BIOMED CO.LTD Germany

First phase (Remineralization phase)

Baseline color measurement:

The color was measured at baseline for each specimen using vita easy shade.

Creation of white spot lesion:

A piece of adhesive tape with 6mm diameter was attached to the labial surface of each specimen. Two layers of colorless acid resistance nail varnish were consequently painted and left to dry at room temperature. Each sample was first dried with an airway syringe. Then 37% phosphoric acid gel was put in the specific region for fifteen seconds, then the tooth surface was rinsed thoroughly using airway water spray for fifteen seconds and then dried with air from airway syringe (Fig. 1).

CIE L*a*b* color measurement

The color of enamel surface was measured after white spot lesion formation and after application of treatment material with a pH cycling model. For measurement of color vita easy shade was used. Color difference (ΔE*) was calculated by using the equation: $(\Delta E^*) = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$.

PH-cycling model

Teeth were exposed daily to a cycle treatment regimen which included exposing the teeth specimens to de- and remineralizing solutions. The demineralizing solution contained (0.9 mM KH_2PO_4, 1.5 mM $CaCl_2$, 50 mM acetate buffer, with pH 4.8). The remineralizing solution (0.9 mM KH_2PO_4, 1.5 mM$CaCl_2$, 20 mM HEPES (4-(2-hydroxyethyl)1-piperazineethanesulfonic acid), with pH(7.0)[8].

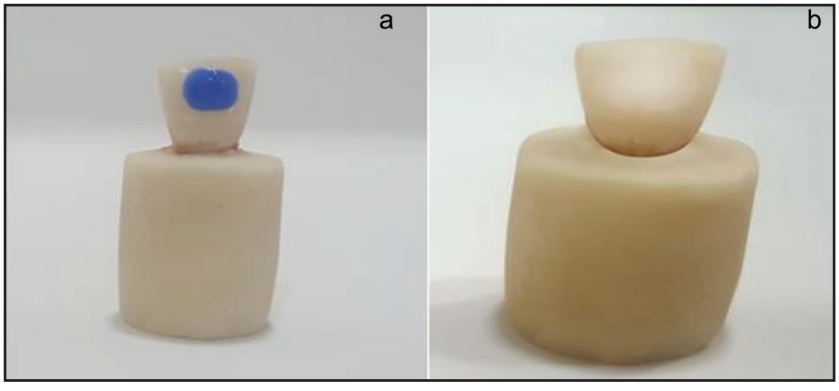

Figure (1): (a) Application of acid etchant, (b) Etched enamel surface

PH of the solutions was measured by using a PH meter that has a PH sensor at 37°C by putting the specimens into an incubator. Treatment material was applied to each specimen for a specific time (3 or 6 minutes) then washed with distilled, Then each specimen was put in 10 ml demineralizing solution for 3 hours at 37°C, then specimens were removed and then washed with distilled water, then tested material was applied for a specific time, then washed with distilled water and then immersed in10 ml of remineralizing solution for rest of the day (21h). The cycling model was carried out for 7 days. The demineralizing and remineralizing solutions were changed daily.

Second phase (acid resistance phase):

CIE L*a*b* color measurement

Color of enamel surface was measured at baseline and after application of treatment material with a pH cycling model. For measurement of color vita easy shade was used. Color difference (ΔE*) was calculated by using the equation:

$$(\Delta E^*) = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}.$$

PH-cycling regimen:

Teeth were exposed daily to a cycle treatment regimen which included exposing the specimen to de- and remineralizing solutions, The demineralizing solution (2.25 mM $CaCl_2$ $2H_2O$, 50 mM acetate, 130 mm KCl; 1.35 mM $KH_2PO_4$for pH=5.0), and the buffer solution (2.25 mM $CaCl_2 2H_2O$, 20 mM HEPES, $KH_2PO_4$130 mm KCl; 1.35 mM for a pH=7.0)[9,10]. The PH of the solutions was measured using a PH meter (PH 315\SET) that has a PH sensor at 37°C by putting the specimens into an incubator. Treatment material was applied to each specimen for a specific time (3 or 6 minutes) then washed with distilled, Then each specimen was immersed into a demineralizing solution for 3 hours at 37°C, then the specimens were removed and then washed with distilled water. then treatment materials applied for a specific time, then washed with distilled water, and then immersed in remineralizing solution for rest of the day (21 h). The cycling model was carried out for 5 days at 37°C and remained in the remineralizing solution for 2 days. The demineralizing and remineralizing solutions were changed daily.

At the end of the study: Teeth were disposed of in a medical waste container.

Statistical analysis

Data was gathered, coded, and analyzed using IBM® SPSS® Statistics Version 20 as the main data analysis tool. In each test, the mean and standard deviation values were computed for each group. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the data normality, and the results revealed a parametric (normal) distribution. To compare more than two groups in unrelated samples, a one-way ANOVA was performed, followed by a Tukey post hoc test. The effect of interaction between multiple variables was tested using a two-way ANOVA. P-value ≤ 0.05 was used as the significant level.

RESULTS

I. First (Remineralization) phase evaluation ΔE:

Application of materials for 3 minutes:

The analyzed data of the application of materials for 3 minutes in the first (Remineralization) phase evaluation ΔE showed a difference between the investigated four groups, these differences were significant, where (p=0.002). In more details, a significant (p=0.014) difference was found between group (I) and group (III), while insignificant differences were found between group (I) and both group (II) and group (IV) where p=0.812 and p=0.999 respectively. The analyzed data as well revealed a significant (p=0.002) difference between group (II) and group (III), while data showed an insignificant (p=0.884) difference between group (II) and group (IV). Finally, the analyzed data showed a significant (p=0.010) difference between group (III) and group (IV).

Application of materials for 6 minutes:

The analyzed data of the application of the material after 6 minutes in the first (Remineralization) phase evaluation ΔE showed a significant (p<0.001) difference between the investigated four groups. In more detail, a significant (p<0.001) difference was found between group (I) and both group (II) and group (III), whereas an insignificant (p=0.275) difference was found between group (I) and group (IV). The tested data as well showed a difference between group (II) and each of (group III) and group (IV), these differences were significant as *p values* =0.017 and p-value <0001 respectively. Moreover, a significant (p<0.001) difference was found between group (III) and group (IV) (Table2, Fig. 2).

Table (2) *The mean, standard deviation (SD) values of Remineralization phase ΔE (WSL-After treatment) evaluation along different time periods of different groups*.

Variables	Remineralization phase ΔE (WSL-After treatment)			
	B_1 (3 minutes)		B_2 (6 minutes)	
	Mean	SD	Mean	SD
A_0 (Control)	5.26 [a]	0.57	6.00 [c]	0.20
A_1 (Sodium fluoride gel)	5.67 [a]	0.95	13.26 [a]	1.84
A_2 (Ginger and Honey)	3.64 [b]	0.60	10.68 [b]	1.30
A_3 (Rosemary oil)	4.70 [a]	0.78	5.33 [c]	0.73
p-value	0.002 *		<0.001*	

Different superscript in the same column indicates significant difference between the values; significant (p≤0.05) ns; non-significant (p>0.05)*

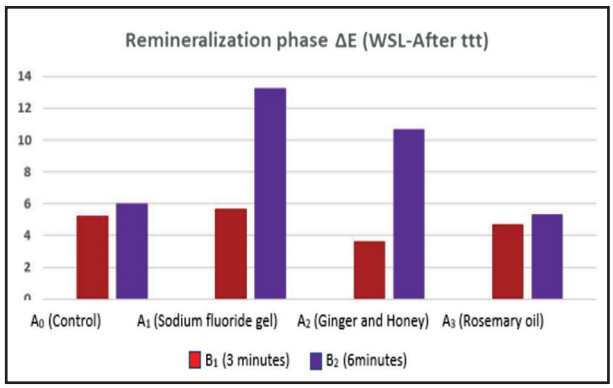

Figure (2) Bar chart representing Remineralization phase

II. Second phase (acid resistance) evaluation ΔE (Baseline-After treatment):

Application of materials for 3 minutes:

The tested data of the application of materials for 3 minutes in the second phase (acid resistance) evaluation ΔE (Baseline-After treatment) showed a significant (p < 0.001) difference between the four investigated groups. The analyzed data showed as well a significant difference between group (I) and each of group (II) and group (IV) groups where p values are less than 0.001) and p-value less than 0.028 respectively. While the analyzed data showed insignificant p=0.074difference between-group (I) and group (III). Additionally, insignificant (p = 0.096) difference between group (II) and group (III), while a significant (p<0.001) difference was found between group (II) and group (IV) groups. Finally, a significant (p<0.001) difference between group (III) and group (IV) was found.

Application of materials for 6 minutes:

The tested data of the application of materials for 6 minutes in the second phase (acid resistance) evaluation ΔE (Baseline-After treatment) revealed a significant difference between the investigated four groups where the p-value is less than 0.001. However, insignificant differences were found between group (I) and each of group (II) and group (IV) where (p=0.985) and (p=0.205) respectively. While a significant difference was found between group (I) and group (III) where the P-value equals 0.024.

The tested data showed as well insignificant differences between group (II) and group (IV) where the p-value equals 0.113, while the analyzed data showed a significant (p=0.048) difference between group (II) and group (III). Finally, the data output showed a significant difference between group (III) and group (IV) groups where the p value is less than 0.001 (Table 3, Figure 3).

Table (3) *The mean, standard deviation (SD) values of Second phase ΔE (Baseline-After Treatment) evaluation along different time periods of different groups.*

Variables	Second phase ΔE (Baseline-After treatment)			
	B_1 (3 minutes)		B_2 (6 minutes)	
	Mean	SD	Mean	SD
A_0 (Control)	6.02 ^{b}	0.59	4.44 ^{a}	1.02
A_1 (Sodium fluoride gel)	4.24 ^{c}	0.52	3.39 ^{a}	1.15
A_2 (Ginger and Honey)	5.73 ^{bc}	1.06	2.60 ^{b}	0.53
A_3 (Rosemary oil)	11.16 ^{a}	1.52	5.62 ^{a}	1.29
p-value	0.001*		<0.001*	

Different superscript in the same column indicates significant difference between the values

**; significant (p≤0.05) ns; non-significant (p>0.05)*

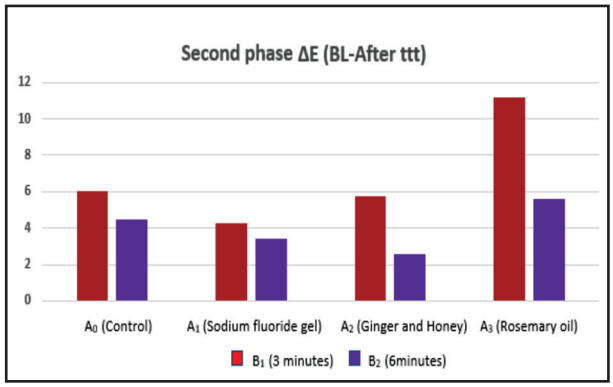

Figure (3): Bar chart representing Second phase

DISCUSSION

Tooth caries is one of the most found oral diseases worldwide. Dental caries is a process of tooth structure demineralization by acids which formed from bacterial fermentation of carbohydrates from dietary uptake [11]. The subsurface porosity result from demineralization gives the lesion a chalky white (milky) appearance that can be found on the surfaces of teeth. The first clinically visible sign of dental caries on the tooth is WSL, which is defined as "enamel subsurface porosity result due to demineralization" that is shown clinically as a white milky opacity[12]. Fluoride is a commonly known remineralizing agent, which interacts with oral fluids found on enamel subsurface and surface and combining with phosphate and calcium ions forming carbonate hydroxyapatite and fluoroapatite[13]. However, researches demonstrated that Fluoride is a proven agent for the prevention of dental caries but excessive use of fluoride may cause dental fluorosis, dental fluorosis affects formation of the enamel making it more porous [14].

Natural products are both safer to use, more accepted psychologically by the patient, and already present in our diet. Natural materials which are active against the virulent activity of cariogenic microorganisms have been known. Plant materials are known as a source of new antimicrobial agents[15]. This study examined three natural materials which are: Ginger, honey, and rosemary oil. Ginger is one of the most widely used herbs in medicine in India. Several studies have shown its several pharmacological activities, such as antibacterial, antioxidant, anti-inflammatory, antinociceptive, and antimutagenic. Also, Many studies are presenting the antibacterial effect of ginger against several micro-organisms including S. mutans[16,17].

Honey is a supersaturated sugar solution, which has low water action which does not support the growth of bacteria. Honey has an average pH value of about 3.9, so honey is acidic and it can decrease the growth of pathogens, honey is a compound mixture composed of proteins, sugars, amino and flavonoids, organic acids, polyphenols, Maillard reaction products [i.e. 5-hydroxymethyl(fural)], minerals, vitamins and water[18]. Rosemary oil has antioxidative, antibacterial, and antifungal activity. Studies showed that the hydroalcoholic extract of rosemary was effective against several oral bacteria (S. mutans, S. sanguinis, S. mitis, S. sobrinus and L casei), Also it has an anti-inflammatory and antioxidative effect[19]. Tsukatani et al. found that the R.officinalis ethanolic extract was able to remove S. mutans and P. gingivalis biofilm[20].

Vita easy shade was used in this study to measure color change. Optical characterization of the teeth was used in the recognition of dental carious lesions[21]. The improved scattering found in the porous enamel can act as a barrier for the light preventing it from reaching the underlying sound tooth tissues. Optical phenomena result from refraction and absorption of light in the affected porous area, due to the ultrastructural changes that happen in different parts of the lesion, but especially due to increase in mineral loss and porosity [22]. The CIE-Lab was used in this study because it can evaluate the lightness-darkness, red/green, and blue/yellow color measurements [23].

In first (remineralization) phase, on Comparing color changes (ΔE) value after the formation of WSL and after treatment with different treatment materials, results of this study revealed that 1.23% sodium fluoride gel had a potent remineralizing effect on WSL. Followed by ginger and honey mixture which show a high remineralization effect when applied for six minutes. While rosemary oil shows the least remineralizing effect.

In the second (acid resistance) phase, on comparing color changes (ΔE) value at baseline and after teeth subjected to PH cycle. Results showed that ginger and honey mixture had potent acid resistance effect for prevention of WSL formation when applied for 6 minutes. Followed by 1.23% sodium fluoride gel While rosemary oil shows the least acid resistance effect.

CONCLUSION

Natural materials as ginger and honey mixture enhance remineralization of WSL and can be used for acid resistance (preventive dentistry). While rosemary oil has a low remineralizing and preventive effect.

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RECOMMENDATIONS

Further clinical in-vivo study can be done to confirm the remineralization and acid resistance of natural materials used in this in-vitro study.

CONFLICT OF INTEREST: None declared.

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