EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 69, 715:722, JANUARY, 2023
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 14-10-2022 • Accept Date : 19-11-2022 • Available online: 1-1-2023 • DOI : 10.21608/edj.2022.166064.2275

SPECTROPHOTOMETRIC EVALUATION OF WHITE SPOT LESIONS TREATMENT USING GINGER AND ROSEMARY EXTRACTS: AN IN VITRO STUDY

Mona Eliwa*

ABSTRACT

Purpose: The current study was carried out to determine the effect of two natural herbal extracts (Ginger and Rosemary) on the color of artificially induced white spot lesions (WSLs) compared to sodium fluoride.

Materials and methods: Thirty maxillary central incisors were divided into 3 groups; 10 teeth were in each group according to the treatment used. Two experimental groups, one positive control. Group A (n=10) WSLs treated with Ginger extract, Group B (n=10) WSLs treated with rosemary extract, and Group C (positive control) (n=10) WSLs treated with sodium fluoride. The labial surfaces were coated with nail polish except for a window of 4x4mm. The samples were then subjected to color measurement using Spectrophotometer. Afterward, artificial white spot lesions were created, by subjecting all the teeth to demineralizing solution. Then the color assessment was repeated. Each group was treated with one of the three agents, according to the specified group. The treatment was done by brushing the agents to the labial surface for 60 seconds twice daily for 10 consecutive days. In between treatments the teeth were stored in artificial saliva. Then color was assessed once again after the completion of treatment.

Results: Significant decrease in the ΔE values was recorded after treatment with different agents. The difference between both observation times was statistically significant (p=0.0001). The highest mean value was recorded for the ginger group (13.6±2.23). This value was significantly higher than the rosemary group (6.81±1.01) and the Sodium Fluoride group (6.2±1.28). The difference between groups was statistically significant (p=0.000). Post hoc test revealed no significant difference between Rosemary and sodium fluoride groups.

Conclusion: Natural agent extracts could be effective in remineralizing WSLs with incomplete masking to the whitish appearance.

KEYWORDS: White spot lesions, Rosemary extract, Ginger extract, Sodium fluoride, Spectrophotometer

* Assistant Professor, Operative Department, Faculty of Dentistry, Ahram Canadian University

INTRODUCTION

One of the most prevalent oral disorders discovered globally is dental caries. During the caries process, the cariogenic bacteria ferment the dietary carbohydrates producing acids, which in turn demineralize the hard tooth structure. The first visible evidence of enamel caries is the milky white opaque appearance; known as a white spot lesion (WSL).[1] White spot lesion (WSL) is caused by the demineralization of enamel leading to subsurface porosity. This subsurface porosity occurs mainly due to a disturbance in the balance between the demineralization and the remineralization processes.[2] Demineralization alters the physiological enamel reflectivity, and the difference in the refractive index (RI) between the healthy and demineralized enamel leads to this milky white opaque appearance.[3]

The preliminary choice for the elimination of WSLs is remineralization.[1] This can be done through minerals' replacement in demineralized enamel or through the production of amorphous mineral deposits in the interrod and inter-crystal spaces.[4] At the atomic level, arresting the caries process at that white spot lesion stage with a non-invasive technique is the ultimate goal. With the progress of the lesion, cavitation will occur, which necessitates an operative intervention. Early intervention in the management of caries by preventive strategy is preferred to a curative approach.[2]

For white spot lesions' remineralization, sodium fluoride has always been considered the gold standard; however, it has many drawbacks; one of which is that a high concentration of fluoride application to WSLs is usually preferred. Unfortunately, this high fluoride concentration leads to hypermineralization of the surface layer of WSLs. Therefore, the penetration of calcium and phosphorus into the body of the lesion becomes blocked and may have some undesirable aesthetics.[1]

In recent years, natural products have gained much attention for their health benefits.[5] Natural products have been used in traditional medicines for thousands of years. Recently, most researchers are focusing on natural products for treating diseases[6]; as they have fewer side effects, are more tolerated by patients, are relatively less expensive, and are widely accepted, and are renewable in nature.[1]

Ginger rhizome (Zingiber officinale Roscoe, Zingiberaceae) and rosemary (Rosmarinus officinalis L., Lamiaceae) are natural products known for having antimicrobial properties. They are characterized by being non-toxic and are considered 'generally recognized as safe' (GRAS) by the Food and Drug Administration of the United States. These herbals are known to have many pharmacological activities, arising from the polyphenolic ketones of their pungent oil contents.[7] Moreover, ginger is a very effective antioxidant, anti-inflammatory, antibacterial and antifungal agent. It can boost immunity and sedate dental pain. Also, rosemary has antibacterial, antifungal, antioxidant, and anti-inflammatory properties. In addition, it is used in relieving toothache.[1] Both natural products have been proven for being able to remineralize the white spot lesion effectively when compared to sodium fluoride.[1] So, the aim of this study was to determine the effect of these natural products on the color change of white spot lesions.

The null hypothesis tested in this study, is that there is no difference between sodium fluoride varnish and the natural herbs ginger and rosemary on color change of early white spot lesions.

MATERIALS AND METHODS

Materials

0.5% Rosemary Extract Solution.

0.5% Ginger Extract Solution.

5 % Sodium Fluoride Varnish (Polimo, IMICRYL, Turkey)

Methods

Study Design

The study proposal was reviewed by the Research Ethics Committee (REC) of the Faculty of Dentistry, Cairo University, Egypt, with approval number 28322. Thirty intact maxillary central incisors were divided into 3 groups of 10 teeth according to the treatment used. Two experimental groups, and one positive control. Group A (n=10) WSLs treated with ginger extract, Group B (n=10) WSLs treated with rosemary extract and Group C (n=10) (positive control) WSLs treated with sodium fluoride. The labial surface of all teeth (experimental and positive control) was coated with a colorless nail polish except for a window of 4x4mm. Artificial WSLs were then induced by subjecting the teeth to demineralizing solution. Afterward, the samples were treated with one of the three agents according to the assigned group. Color was assessed three times, at baseline, after the creation of the WSLs, and finally after the treatment.

Sample Size Calculation

To Evaluate the effect of three different materials on ΔE; ANOVA test or an equivalent non-parametric test will be used for comparison between the three groups. According to a previous study by Shaker et al (2022) [8], ΔE of WSLs after treatment ranged from 10.68±1.3 and 5.33±0.73

Based on Shaker at al (2022) and Using G power statistical power Analysis program (version 3.1.9.4) for sample size determination[9], A Total sample size (n=30, divided to n= 10) in each group will be sufficient to detect a large effect size (f) = 1.06, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test.

Teeth Selection and Sample Preparation

Thirty permanent maxillary central incisors were included in the study. All teeth with carious or non-carious lesions, previously restored, endodontically treated, having developmental anomalies, or have been previously bleached were excluded from the study. [10,11] Teeth were immersed in 0.1% thymol solution for 48 hours,[4] then cleaned using a fluoride-free polishing paste. [11] All samples were then soaked in distilled water [4] at room temperature until the time of use, where the water was changed daily. [11] Two coats of colorless acid-resistant nail varnish (Maybelline, USA) were applied on the labial surface of the teeth, leaving a 4×4 mm window without nail polish. [4,11] All the teeth were left to dry at room temperature for 24 hours.[11] All teeth were subjected to color assessment (baseline). All teeth were then stored again in distilled water. [4]

Preparation of 0.5% Ginger and Rosemary Extract solutions

Ginger and rosemary extracts were prepared, and a solution for each extract was obtained. The ginger and rosemary used in this research were purchased from the local market. The fresh ginger and rosemary (250g each) were washed with tap water, chopped into 1-2mm size pieces, and macerated in ethyl alcohol for 12 hours at 40 ˚C. The combined Ethanolic extract was evaporated to dryness under reduced pressure at a temperature not exceeding 40˚C using a rotatory evaporator (Heidolph, Germany). Solution of a 0.5% concentration of ginger and rosemary extract was prepared by dissolving 50 mg of the dried powdered extra in aqueous ethanol (20% v/v) and completing the volume to 10 ml to yield a solution of the desired concentration. The formed solutions were kept at 8˚C till further use. [1]

Artificial White Spot Lesions Formation

A demineralizing solution was prepared at the faculty of pharmacy, Cairo University. The composition of the demineralizing solution was 2.2 mM calcium chloride, 2.2 mM monopotassium phosphate, 0.05 mM acetic acid, and 1M potassium hydroxide. The pH was adjusted to 4.4. To induce

artificial white spot lesions (WSLs), immersion of all the teeth in the demineralizing solution for 4 days [10] without stirring was done. A pH meter was used to detect the pH value daily [2]. After the specified period, all samples were washed for 5 minutes under tap water, then washed again under distilled water for 30 s. The samples were then dried using oil-free air spray to visualize their chalky white appearance. The color assessment was repeated after the white spot lesions have been created.

Treatment Protocol

After inducing the artificial white spot lesions, random division of the samples into three equal groups (n = 10)**.** Two groups were experimental groups (Ginger group, rosemary group) and one group for the positive control group (sodium fluoride group). [2] Therapeutic agents were brushed on the labial surface of the teeth for 60 seconds twice daily for ten consecutive days, using a micro brush. A new micro brush was used for each specimen. Nearly, an equal amount of 2 ml^3 of each agent was used for the treatment of each specimen. [11] After application, the sodium fluoride, and herbal solutions were removed by wiping them off, no rinsing was used. Then the specimens were immersed in freshly prepared artificial saliva. [4] Preparation of artificial saliva was at the faculty of pharmacy, Cairo University. It was composed of 4200mg/L $NaHCO_3$, 500mg/L NaCl and 200mg/L KCL, and the pH was adjusted to 7.4. [12] Throughout the study period, all specimens were kept in the artificial saliva in order to mimic the oral condition. The solution was kept at room temperature and was refreshed daily. [6] At the end of the treatment period, all specimens were washed with distilled water and a final color measurement was done. [11]

Color Assessment

Color assessment of all specimens was done at baseline (before the induction of the artificial white spot lesions), after the induction of WSLs, and after treatment with different solutions. A Reflective spectrophotometer (X-Rite, model RM200QC, Neu-Isenburg, Germany) was used to measure the color of the specimens. The specimens were aligned exactly with the device where the aperture size was set to 4 mm. A white background was selected, and measurements were made according to the CIE L*a*b* color space relative to the CIE standard illuminant D65. The following formula was used to evaluate the color changes (ΔE) of the specimens:

$$\Delta ECIELAB = (\Delta L^*2 + \Delta a^*2 + \Delta b^*2)\ ½$$

Where: L* = lightness (0-100), a* = (change the color of the axis red/green) and b* = (color variation axis yellow/blue)

Statistical Analysis

Values were presented as mean, standard deviation (SD) values, and confidence intervals. Results of the Kolmogorov-Smirnov test indicated that data were normally distributed (parametric data), therefore, one-way analysis of variance (ANOVA) test was used for intergroup comparisons. This was followed by the Bonferroni post hoc test for pairwise comparison. Paired t-test was used to compare different observation times within the same group. The significance level was set at $p \leq 0.05$. Statistical analysis was performed using a commercially available software program (SPSS 18.0-Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows

RESULTS

The ΔE value was evaluated after the white spot lesion creation and after treatment in all groups. The overall value of both observations is summarized in Table (1) and Fig. (1)

Overall, the value of ΔE decreased significantly after treatment compared to after white spot lesion creation. The difference between both observation times was statistically significant (p=0.0001).

Comparison of ΔE After Treatment Between Groups

The highest mean value was recorded in the ginger group (13.6±2.23). This value was significantly higher than the rosemary group (6.81±1.01) and the Sodium Fluoride group (6.2±1.28). The difference between groups was statistically significant (p=0.000). Post hoc test revealed no significant difference between Rosemary and sodium fluoride groups, (Table 2, Fig.2).

TABLE (1) Descriptive statistics and comparison of the overall ΔE values after white spot lesion and after treatment (t-test)

	Mean	Std. Dev	Min	Max	t	P
White spot lesion	16.02	3.56	10.14	22.83	7.58	0.0001
After treatment	8.87	3.74	4.97	16.51		

Significance level P≤0.05, * significant

TABLE (2) Descriptive statistics and comparison of ΔE after treatment between groups (ANOVA test)

	Groups	Mean	Std. Dev	95% Confidence Interval for Mean		Min	Max	F	P value
				Lower Bound	Upper Bound				
ΔE	Ginger	13.60[a]	2.23	12.00	15.20	10.58	16.51	66.293	.000*
	Rosemary	6.81[b]	1.01	6.08	7.53	4.97	8.12		
	Sodium Fluoride	6.20[b]	1.28	5.29	7.12	5.18	8.52		

Significance level P≤0.05, * significant

Post hoc test: means with different superscript letters are significantly different

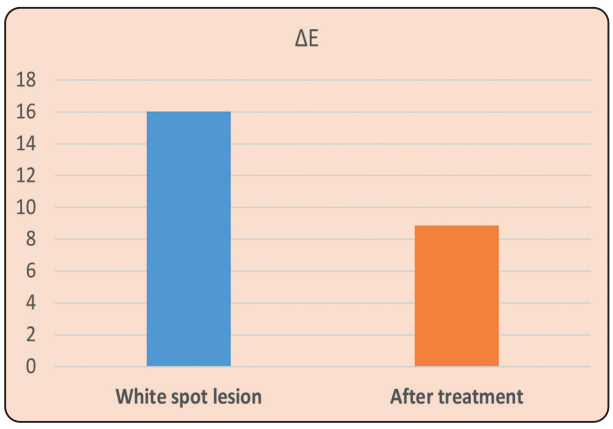

Fig. (1) Bar chart illustrating mean overall ΔE at different observation times

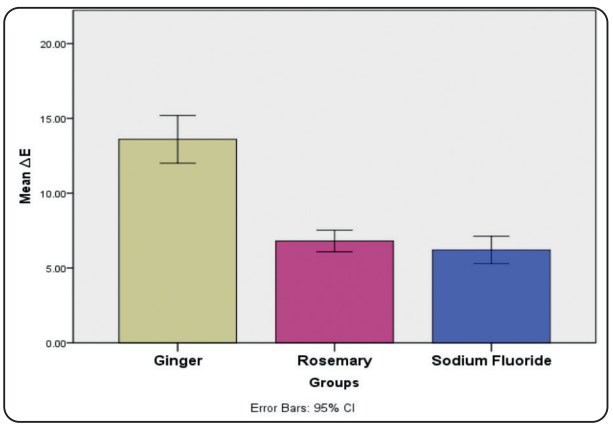

Fig. (2) Bar chart illustrating mean ΔE in different groups after treatment

DISCUSSION

White spot lesions are opacities that occur due to carious demineralization, which causes the formation of subsurface enamel porosity. The WSLs when present on smooth surfaces appear as a milky white opacity. When bacterial flora remains on the enamel surface for a long time, decalcification occurs. The bacteria produce organic acids that enter interprismatic spaces in tooth enamel, resulting in white lesions. The white lesions are the result of the dissolution of apatite crystals, calcium and phosphate ions, and demineralization. [13] The difference in refractive indices (RIs) between defective and sound enamel is the main cause of the whitish appearance of the demineralized enamel surface. This difference is the result of the presence of micro-porosities in affected enamel lesions. These micro-porosities are filled with either water (RI=1.33) or air (RI=1.0), unlike sound enamel, which has a RI of 1.62. When the micro-porosities are filled with water, the lesions appear opaque compared to sound tissue, while when they are dried, they become filled with air and the lesion appears more obviously. Thus, the difference in color is believed to be due to the difference in RIs between enamel crystals and the medium inside the porosities, which causes light to scatter, resulting in a whitish opaque appearance of those lesions, especially when they are desiccated. [11]

The complete elimination of WSLs is unlikely. Therefore, for better esthetics, it became mandatory to repair the deeper parts of the lesion using remineralizing agents. According to Bilgin et al, 2016. [14] WSL can be reversed by re-establishing a balance between demineralization and remineralization. Several remineralizing agents have demonstrated a positive effect on WSL including sodium fluoride, CPP-ACP, and natural products like grape seed extract, ginger, honey, and rosemary. [1] The plant-based phytochemicals and bioflavonoids will have more public acceptance compared to the chemical derivatives and fluoride-based agents for remineralizing white spot lesions. Also, most of these are natural food substances, which are non-toxic and safe. So much research has been carried out to evaluate the remineralizing effect of various natural herbal extracts and their effect on WSLs. It was proven that natural extracts may have a prime role in WSLs remineralization and treatment. [15]

The present in vitro study was done to compare and evaluate the esthetic improvement of WSLs treated with NaF, Ginger extract, and Rosemary extract, which have shown to have good remineralizing properties in artificially created enamel lesions. Where the agents were brushed on the labial surface of the teeth for 60 seconds twice daily for 10 consecutive days, and artificial saliva was used to mimic the oral conditions. [10]

The color assessment was done using Spectrophotometer. The instrumental color analysis offers a potential advantage over visual color determination because instrumental readings are objective, can be quantified, and are more rapidly obtained. Spectrophotometers are among the most accurate, useful, and flexible instruments for color matching. A spectrophotometer functions by measuring the spectral reflectance or transmittance curve of a specimen. They are useful in the measurement of surface color.[10]

The sodium fluoride varnish is considered a control agent because it is the most popular used remineralizing agent in the treatment of white spot lesions. Fluoride has many roles in inhibiting demineralization, enhancing remineralization, and having a strong bactericidal effect. When active free ions of Fluoride ions become available in the oral environmental fluids, the formation of fluorapatite and calcium fluoride takes place. [1]

On the other hand, ginger rhizome (Zingiber officinale Roscoe, Zingiberacae) and rosemary (Rosmarinus officinalis L., Lamiaceae) have been

used as spices and medicinal plants for a long time. Moreover, they are natural materials, with no evidence of toxicity. Additionally, many studies reported their antifungal and antimicrobial effects on oral cavity pathogens. The remineralization obtained was probably due to the antimicrobial properties and the high fluoride and calcium content of ginger. In addition, rosemary has been reported to have inhibitory effects on Streptococcus mutans. Rosemary-containing treatment mixture was able to enhance the enamel remineralization process as discussed by Bilgin et al. in 2016. [14] The reason behind using (0.5%) ginger concentration is that there is a maximum amount of free calcium ions that can be deposited on the tooth surface, but above this concentration, the amount of free calcium may be decreased due to the increase in the concentrations of other elements as ginger is also rich in iron, magnesium, phosphorus, potassium. So, the increase in the concentrations of the extract may increase the concentrations of the other elements as well, which in turn may substitute calcium ions from hydroxyapatite crystals as mentioned by Namir and wesal 2011. [16]

In the current study, comparing the ability of NaF and the two experimental extracts to mask the color of the induced WSLs, ΔE values after treatment were decreased for all groups but this decrease was not clinically accepted in improving the whitish appearance of the WSLs. An average of 3.3 ΔE was reported to be esthetically acceptable, and any difference above this limit can be easily percepted and is not accepted clinically.[11]

The highest mean value of ΔE was recorded in the ginger group. This value was significantly higher than the rosemary group and the Sodium Fluoride group. The NaF group showed the lowest ΔE, followed by the rosemary group. Both groups were not statistically different. Both groups showed better masking ability of WSLs compared to the ginger group, but still clinically unacceptable.

The reason for the above findings may be because remineralization of WSLs by the artificial saliva for the NaF, and the herbal extracts was a slow process, and need a longer time to reach the clinically acceptable color improvement [8] because it depends on calcium ion deposition. Additionally, a high concentration of fluoride may cause superficial remineralization of the WSLs. This could prevent the penetration of both calcium and phosphate into the deeper enamel layers, thus preventing deeper remineralization and limiting the esthetic WSLs' improvement. [1] This was in agreement with Huang et al 2013.[17]

This deposit on the demineralized surface leads to the formation of a new homogenous apatite surface layer, which can defend the underlying demineralized surface from further demineralization and enhance remineralization, through the inhibition of acid diffusion into deeper regions of enamel. Meanwhile, this new highly mineralized surface layer could hinder the mineral ions' diffusion into the body of the lesion, thus constraining the enamel recrystallization to the subsurface zone. This may explain why complete remineralization, and hence a complete color improvement of WSLs was not achieved. [11]

CONCLUSION:

Under the limitation of the present study, although sodium fluoride, ginger, and rosemary extracts have shown a promising remineralizing effect for white spot lesions as proved by various studies, still the three agents were not able to mask the whitish color of the WSLs and thus cannot be used in the esthetic zone for the treatment or elimination of the WSLS. These agents may be beneficial in remineralizing WSLs and initial carious lesions in the posterior region where esthetics is not of prime concern.

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