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EFFECTIVENESS OF NATURAL REMINERALIZING AGENTS ON MICROHARDNESS OF WHITE SPOT LESIONS: IN VITRO STUDY

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ABSTRACT

Purpose: This in vitro study is conducted to compare the effect of extracts of Ginger and Rosemary on the microhardness of white spot lesions to sodium fluoride varnish.

Materials and methods: The current study used thirty extracted incisors. Ten teeth(n = 10) in each group had a remineralizing treatment: group 1:rosemary, group 2: ginger, and group 3: sodium fluoride varnish. A nail polish was applied on labial surfaces except to a window of 4x4mm and the microhardness test was done. Then all samples were immersed in demineralizing solution for four days to produce white spot lesions. Then testing microhardness was conducted. The remineralizing agents were applied to each group for ten days. The samples were immersed in artificial saliva between treatments. Lastly, microhardness test was conducted.

Results: ANOVA test was used to Compare between groups. Comparison within the same group was performed using a paired t-test. The Fluoride group was not significantly different than the other 2 groups after remineralization, The mean value was 63.04±3.79 in the fluoride group, in comparison to 64.01±3.85 in the Ginger group and 62.20±3.91 in the Rosemary group. The mean value recorded in the rosemary group was significantly higher than ginger group (p=0.047). Considering the percent of change from Demineralization to remineralization the highest mean value of percentage increase occurred in the ginger group (34.50±18; median 30.98), succeeded by fluoride (30.49±15.54; median 32.53).

Conclusions: Rosemary, ginger, and fluoride varnish reduce white spot lesions and are more favorable for prevention and remineralization of early enamel lesions.

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

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INTRODUCTION

One of the main concerns for parents, patients, and orthodontists is enamel demineralization following orthodontic treatment. The irregular surfaces of orthodontic brackets and other equipment are the primary reason for the increased incidence of these lesions after receiving fixed orthodontic therapy, which increases the plaque accumulation on tooth surfaces. Additionally, placing appliances on tooth surfaces reduces teeth's ability for self-cleaning through saliva flow and oral muscle motions, complicating oral hygiene [(1)]. The literature indicates that the incidence of white spot carious lesions after orthodontic treatment ranges from 2% to 96%. Despite numerous research on their prevention, WSLs continue to be an issue after orthodontic treatment [(2)]. Numerous approaches have been proposed to treat these lesions. Good oral hygiene is the first line of prevention and treatment for orthodontic patients. Another therapy strategy involves the application of products containing fluoride, such as mouthwashes, toothpaste, gels, varnishes, and fluoride-releasing cement [(3)].

Recent years have seen a significant increase in the use of fluoride to prevent and treat early caries lesions, and regulatory and scientific agencies strongly advise against its usage. Although fluoride has many benefits, it can also be harmful, leading to birth abnormalities, hypersensitivity reactions, hypersalivation, stomach discomfort, and dental and skeletal fluorosis. To prevent its harmful effects, fluoride use is limited in children under the age of six, and substitute remineralizing agents have been investigated [(4)].

Therefore, safe, efficient, and affordable alternatives to current preventative and treatment methods are required. Therefore, it has been proposed that rather than using fluorides, synthetic antibiotics, and bactericides a range of medicinal plant extracts that affect the bacteria that cause tooth decay should be used.

For ages, people have employed ginger rhizome (Zingiber officinale Roscoe, Zingiberacae), and rosemary (Rosmarinus officinalis L., Lamiaceae), as therapeutic herbs. These organic sources of food are known to have antibacterial properties. Furthermore, the US Food and Drug Administration (FDA) classifies them as "generally recognized as safe" (GRAS) since they are natural food ingredients and do not exhibit any toxicity. Specifically, a range of polyphenolic ketones with numerous pharmacological properties are contained in their strong oil constituents [(5)].

The general population may be more receptive to natural anticariogenic and remineralizing agents than to fluoride-based systems. Many studies have documented their antibacterial properties on oral cavity pathogens. On the other hand, there is either no research available or very little information regarding the impact of these therapeutic herbs on the remineralization of early enamel caries. Therefore, the goal of the current study is to examine the ability of herbal extract to remineralize artificially generated early carious lesions.

MATERIALS AND METHODS

First, The Research Ethics Committee Faculty of Dentistry, Cairo University (CREC) evaluated the study proposal, with approval Research number: 22-3-26. Thirty non-carious human anterior teeth that have been extracted because of physiological movement were collected. Teeth with caries, hypoplastic lesions, white spot lesions, enamel fractures or cracks, developmental anomalies, and teeth undergoing pulp therapy have been excluded from the study.

The sample size required for a one-way analysis of variance (ANOVA) test to compare the three groups was calculated using the G*Power software. The results showed that eight participants in each group, or a total sample size of n = 24, were needed to obtain a power of 0.80 with an alpha error of

0.05. Each group received two extra samples, for a total sample size of n = 30 with 10 in each group, to prevent sampling errors. Teeth then were rinsed, disinfected, and immersed in distilled water at room temperature that was changed every day until it was used to avoid dehydration [6].

The roots of the teeth were placed in molds of acrylic resin, and the enamel surfaces were covered in two coats of colorless, acid-resistant nail polish (Maybelline, USA) with a 4 x 4 mm window left unpolished, defining the area to be investigated by applying plaster to the exposed tooth surface, then letting it dry at room temperature (fig. 1). Finally, the specimens will be immersed in distilled water once more [7]. Then, the baseline Vickers hardness test was done.

To assess Surface Micro-hardness, samples were set up on a Digital Display Vickers Micro-hardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd., China) equipped with a 20X objective lens and a Vickers diamond indenter. The specimens' surface was subjected to a 100g load for 15 seconds. Each specimen had three indentations made on its surface, evenly spaced around a circle and separated by no more than 0.5 mm from one another. A built-in scaled microscope was used to quantify the indentations' diagonal length, and Vickers values were converted into micro-hardness values. The following formula, where HV is Vickers hardness in Kgf/mm2, P is the load in Kgf, and d is the diagonal length in mm, was used to determine micro-hardness.

$$HV=1.854\ P/d2$$

Following the baseline assessment of microhardness, the demineralization procedure was applied to the samples. to create artificially early carious lesions. A demineralizing solution was prepared (2.2 mMcalcium chloride, 2.2 mM monopotassium phosphate, 0.05 mM acetic acid having pH adjusted to 4.4 and 1M potassium hydroxide) After immersing in this demineralizing

Fig. (1) The roots of the teeth were placed in molds of acrylic resin

solution for four days without stirring, the pH of the solution was measured daily using a pH meter [8]. Following the designated time frame, after removing all samples from the solution, they were all provided a five-minute rinse under tap water. Subsequently, they were rinsed under distilled water for thirty seconds and dried using an oil-free air spray to achieve their characteristic chalky white look and evaluated for microhardness [9].

Afterward, specimens were randomly divided into three groups of 10 teeth and subjected to the allocated remineralizing protocols. Two natural remineralizing agents were used in this study (%0.5 Ginger and Rosemary extracts) and 5 % Sodium Fluoride Varnish (Polimo, IMICRYL, Turkey) was used as a positive control. To prepare the Rosemary extracts the dried ground leaves of rosemary were extracted with methanol (5 ml/g), using a magnetic mixer at room temperature for 3 h. After extraction, the mixture was filtered, and the residue was reextracted with fresh methanol (5 ml/g) overnight. The combined methanolic solution was centrifuged at 12,000 rpm for 10 min and evaporated on a rotary evaporator (10). A Solution of a %0.5 concentration of ginger extract was prepared by dissolving 50 mg of the dried powdered extract in aqueous ethanol (%20 v/v) and completing the volume to 10 ml to yield a solution of a concentration of 11) %0.5).

Group (1) specimens were treated with NaF varnish %5 according to manufacturer instructions. After three minutes, they were cleaned with a cotton roll and placed in different containers with artificial saliva. The artificial saliva was prepared at the faculty of pharmacy, at Ahram Canadian university. It was composed of 4200mg/L NaHCO500 , 3mg/L NaCl and 200mg/L KCL, and the pH was adjusted to 12) 7.4). The samples were brushed every day with a soft toothbrush and incubated for ten days at °37C (13). Artificial saliva was refreshed daily. In Group (3&2) the aqueous solutions of the two herbals' natural remineralizing agents were applied for 60 seconds two times per day for ten consecutive days and refreshed daily using a micro brush that was changed for each specimen. [11]. The samples were then immersed in a fresh artificial saliva solution at 37 °C in an incubator after the aqueous solutions were wiped without being rinsed. For the duration of the investigation, all specimens were kept in an incubator at 37 °C to stimulate oral condition, except times during solutions were changed and microhardness was evaluated. All specimens were rinsed with distilled water following the application of the treatment, the nail polish was carefully removed with colorless acetone, and the specimens were rinsed once more with distilled water and prepared for microhardness testing [14].

Statistical analysis

A version of the Statistical Package for Social Sciences (SPSS) was used for data management and statistical analysis. The measures of mean, standard deviation, median, and range were used to summarize numerical data. By examining the distribution of the data and applying the Kolmogorov-Smirnov and Shapiro-Wilk tests, the normality of the data was explored. ANOVA test was used in Comparisons between groups concerning normally distributed numeric (VH), followed by the Bonferroni post hoc test for pairwise comparisons. Comparison between observations within the same group was performed using a paired t-test. The Kruskal-Wallis's test was performed to Compare groups concerning non-parametric numeric variables (percent change in microhardness and Comparison between different observations using the Friedman test. The percent change was calculated by the formula:

(value after-value before) / value before X100

RESULTS

The comparison between the microhardness values at baseline after the white spot lesion creation and after remineralization in all groups was summarized in Table (1) and Fig. (2) The difference between groups was not statistically significant at baseline and after white spot lesion creation, with (p=0.097)and(p=0.277) respectively. The mean value was 78.73±3.69 in the fluoride group, in comparison to 76.57±4.12 in the Ginger group and 77.71±3.84 in the Rosemary group at baseline and 50.08±4.74 in the fluoride group, in comparison to 52.34±4.90 in Ginger group and 50.11±4.93 in Rosemary group after demineralization. The Fluoride group was not significantly different than the other two groups after remineralization, The mean value was 63.04±3.79 in the fluoride group, in comparison to 64.01±3.85 in the Ginger group and 62.20±3.91 in the Rosemary group. The mean value recorded in the rosemary group was significantly higher than ginger group (p=0.047). among the same group, all groups the mean value recorded at baseline was significantly higher than that after demineralization (p=0.000) and after remineralization (p=0.000).

The comparison between groups regarding the percentage change in each interval. The Results are summarized in Table (2) and Fig (3). From Baseline to demineralization the mean value of percentage decrease was (-37.86±7.11; median -37.31) in the fluoride group, in comparison to (-36.97±6.92; median -37.72) in the ginger group and (-35.37 ±7.17; median -35.99) in Rosemary

group. The groups' differences were not statistically significant. (p=0.375). From Demineralization to remineralization the greatest mean value of percentage increase was recorded in the ginger group (34.50±18; median 30.98), followed by fluoride (30.49±15.54; median 32.53). The mean and median percent increase values recorded in these 2 groups was significantly greater than the lowest mean value recorded in Rosemary (25.49±16; median 26.47), (p=0.048). From baseline to Remineralization, The greatest mean value of percentage decrease was noted in the Rosemary group (-19.77±6.48; median -20.93), followed by fluoride (-19.69 ±7.04; median -20.69). The mean and median percent decrease values recorded in these 2 groups was significantly greater than the lowest mean value recorded in ginger group (-16.09±7.75; median -17.65), (p=0.043).

TABLE (1) Descriptive statistics and comparison of Vickers Micro-hardness (HV) between groups (ANOVA test)

		Mean	Std. Dev	Median	95% Confidence Interval for Mean		Min	Max	F value	P value
					Lower Bound	Upper Bound				
Baseline	Fluoride	78.73	3.69	79.86	77.38	80.08	69.56	84.09	2.400	.097ns
	Ginger	76.57	4.12	76.66	75.06	78.08	66.19	82.90		
	Rosemary	77.71	3.84	77.71	76.30	79.12	69.56	83.92		
Demineralization	Fluoride	50.08	4.74	49.30	47.05	50.52	39.68	58.08	1.303	.277ns
	Ginger	52.34	4.90	48.72	46.36	49.95	40.69	57.58		
	Rosemary	50.11	4.93	49.73	48.30	51.91	40.11	58.89		
Remineralization	Fluoride	63.04[ab]	3.79	63.15	61.64	64.43	54.37	71.25	1.711	.047*
	Ginger	64.01[a]	3.85	63.82	62.60	65.42	55.04	70.07		
	Rosemary	62.20[b]	3.91	62.81	60.77	63.64	52.68	71.25		

Significance level p≤0.05, ns=non-significant Post hoc test: means with different superscript letters are significantly different

TABLE (2) Descriptive statistics and comparison between groups regarding percentage change in Vickers Micro-hardness (HV) (Kruskal Wallis test)

		Mean	Std. Dev	Median	95% Confidence Interval for Mean		Min	Max	P value
					Lower Bound	Upper Bound			
From Baseline To demin	Fluoride	-37.86	7.11	-37.31	-40.47	-35.25	-52	-17	.446 ns
	Ginger	-36.97	6.92	-37.72	-39.51	-34.43	-48	-22	
	Rosemary	-35.37	7.17	-35.99	-38.00	-32.74	-48	-21	
From Demin to remin	Fluoride	30.49[a]	15.54	32.53	24.78	36.19	4.11	60.00	.048*
	Ginger	34.50[a]	18.00	30.98	27.89	41.10	4.69	71.14	
	Rosemary	25.49[b]	16.00	26.47	19.62	31.36	-.88	59.12	
From Baseline To remin	Fluoride	-19.69[a]	7.04	-20.69	-22.27	-17.11	-32	-2	.043*
	Ginger	-16.09[b]	7.75	-17.65	-18.93	-13.24	-31	2	
	Rosemary	-19.77[a]	6.48	-20.93	-22.15	-17.40	-32	-2	

Significance level p≤0.05, * significant, ns=non-significant Post hoc test: means with different superscript letters are significantly different

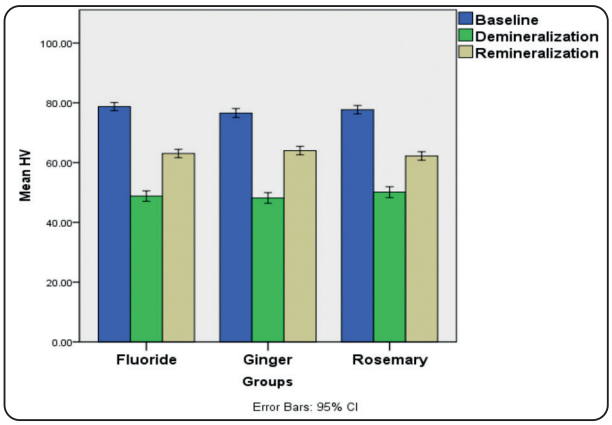

Fig. (2) Bar chart illustrating the mean value of Vickers Micro-hardness (HV) in different groups.

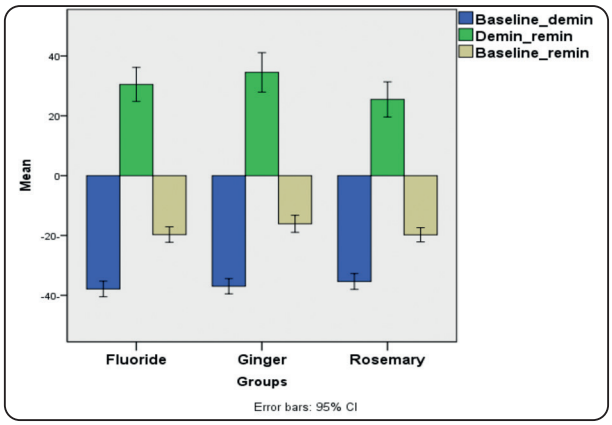

Fig. (3) Bar chart illustrating the mean value of percentage change in Vickers Micro-hardness (HV) in different groups

DISCUSSION

Enamel demineralization following orthodontic treatment is among the most prevalent aesthetic disorders. The imbalance in the cycles of demineralization and remineralization is the main cause. Both the patient's self-esteem and dental health are negatively impacted by this issue. The goal of remineralization treatments is to restore the depleted content of minerals by reintroducing calcium and phosphate, either from synthetic sources or saliva, inside the demineralized pores. Fluoride has a well-established reputation for promoting remineralization and preventing demineralization. [16]. When Fluoride is applied to the enamel's surface, hydroxide ions are exchanged into Fluoride ions, creating a crystal structure of fluorapatite that is less soluble and more resistant to acid when bacterial acids demineralize it. However, one of the main disadvantages of Fluoride is that its ability to encourage remineralization is constrained by the presence of Ca and phosphate ions [17]. Moreover, Fluoride has an insignificant impact on pit and fissure caries but an effective remineralization ability on smooth surface caries. Due to Fluoride's limitations, we are currently searching for natural remineralization alternatives that aren't fluoridated [18]. Therefore, evaluating the potential of natural compounds to remineralize the artificially created white spot lesions in permeant teeth was the objective of the current in vitro study.

In the present study, early carious lesions were induced using a demineralizing solution with a pH of 4.4 [8]. Depending on the required pH and exposure time for the formation of lesions, the main ingredients of demineralizing agents frequently consist of calcium, phosphorus, and either acetic acid or lactic acid. Previous research indicates that demineralizing solutions ranged in pH from 3.5 to 5, and that immersion times varied from two hours to 21 days [19]. The samples in this investigation were immersed for four days in a remineralizing solution with a pH of 4.4 in order to induce early carious lesions.

Various techniques have been employed to assess the degree of remineralization. Using a Vickers microhardness tester to measure changes in surface microhardness is one of the most common and reliable techniques for this [20]. Microhardness values were assessed in the current study following the demineralization and remineralization of samples. After treatment statistical analysis showed that the maximum increasing microhardness occurred in the ginger group followed by fluoride and both groups were significantly greater than Rosemary. Comparable outcomes were attained by

Gocmen GB et al in their study. The antibacterial qualities of ginger, which may be the consequence of a high fluoride level, are most likely accountable for the high remineralization that was obtained. Ginger rhizome (Zingiber officinale) has been shown to have antibacterial properties as a natural herb supplement.. The bioactive components of the oleoresin from the ginger rhizome include [6]-gingerol (1-[4′-hydroxy-3′-methoxyphenyl]-5-hydroxy-3-deconone), is the main component thought to have a variety of physiological and pharmacological effects. Gingerol and other bioactive substances obtained from the ethonolic extracts of ginger have antibacterial and antifungal properties. Ginger's high fluoride concentration and antibacterial qualities are responsible for its remineralization ability [21].

Although the percent of change in remineralization is less in the sodium fluoride group may be due to the fact that the superficial portion of early carious lesions is where fluoride remineralization primarily happens. The aforementioned superficial layer may have the ability to prevent the penetration of calcium and phosphate into the deeper layers of the enamel, thereby inhibiting deeper remineralization. Therefore, there is ongoing controversy on the optimal fluoride concentrations and delivery systems [22].

CONCLUSIONS

Fluoride varnish, Rosemary, and ginger extracts may reduce white spot lesions. Since more natural products are preferred these days, the herbal extract of ginger and rosemary might be more favorable for preventative purposes and remineralization of early enamel lesions.

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