

Effectiveness of Natural Remineralizing Agents on Initial Enamel Caries: In Vitro Study

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Abstract:

Purpose: The objective of this in vitro study is to evaluate the effectiveness of natural remineralizing agents (Ginger and Rosemary extracts) on initial enamel caries compared to sodium fluoride.

Materials and methods: Thirty human extracted intact maxillary central incisors were used in this study. The teeth were divided into 3 groups of 10 teeth according to the remineralizing protocol used. Two Intervention groups and control group. Group 1 (n=10) teeth were treated with Rosemary remineralizing agent, Group 2 (n=10) teeth were treated with Ginger remineralizing agent and Group 3 (n=10) teeth were treated with sodium fluoride varnish. Laser fluorescence testing was done to all samples using (DIAGNOdent). Then artificial initial enamel caries was created by subjecting all samples to demineralizing solution for 4 days without stirring. Laser fluorescence was tested again after creation of the initial enamel caries. Then each group of samples were treated with one of the remineralizing agents. The agents were brushed to the labial surface. In between treatments the samples were stored in artificial saliva at 37 ° to resemble the oral conditions. And finally, Laser fluorescence was assessed once again.

Results: The comparison of Diagnodent values between groups shows that the highest mean value was recorded in ginger group (7.51 ± 1.42). The comparison of difference and percent change in Diagnodent values between groups shows that the greatest increase was recorded in Ginger group (median =2.16), followed by Rosemary (median= 0.84), then sodium fluoride (median= -0.7). The difference between Ginger and Sodium fluoride groups was statistically significant ($p=0.014$), Regarding the percent change from baseline to D2, the greatest percent increase was recorded in Ginger group (median =61.85), followed by Rosemary (median= 19.3), then sodium fluoride (median= -14). The difference between Ginger and Sodium fluoride groups was statistically significant ($p=0.017$).

Conclusion: Fluoride varnish, ginger and rosemary can decrease early enamel lesions. The herbals ginger and rosemary may be preferable for prevention purposes on initial remineralization of enamel lesions as more natural products are preferred today.

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Introduction

Initial enamel caries is the first sign of tooth caries that is defined as “subsurface enamel porosity from carious demineralization” and is clinically manifested by a chalky white opacity. This subsurface porosity is caused by a biologically dynamic imbalance between de- and remineralization. In minimally invasive dentistry, the non-invasive remineralization strategies are the treatment of choice in incipient enamel carious lesions. So, topical gels, varnishes, mouth washes and dentifrices contain fluoride being used by dentists for the treatment of initial enamel caries ⁽¹⁾.

Fluoride is the gold standard for caries prophylaxis and remineralization; however, excess use of fluoride causes fluorosis. Moreover, this agent has several negative effects on gastrointestinal system with increased resistance to these chemicals. Being a developing country, we need a cost effective, biocompatible, and patient friendly preventive and remineralizing methods instead of using artificial products. Therefore, it has been proposed to use natural plant extracts which influence cariogenic bacterial responsible of tooth decay ⁽²⁾.

Ginger rhizome (*Zingiber officinale* Roscoe, Zingiberaceae) and rosemary (*Rosmarinus officinalis* L., Lamiaceae) are natural herbals that have an antimicrobial activity. Moreover, they do not show any toxicity approved by ‘generally recognized as safe’ in the Food and Drug Administration of the United States. These herbals contain Pungent oil which harbor some polyphenolic ketones with many pharmacological activities. Many studies reported their antifungal and antimicrobial effects on oral cavity pathogens ⁽³⁾. However, there is no sufficient studies in current literature about the effect of these herbal medicaments on remineralization of initial enamel caries.

Aim of the study

The aim of our study is to evaluate the effectiveness of natural remineralizing agents on initial enamel caries compared to sodium fluoride by Laser fluorescence of enamel caries using diagnodent.

Materials and methods:

Selection of teeth:

Thirty freshly extracted human anterior teeth were collected, cleaned, disinfected then stored in distilled water at room temperature and changed daily until time of use ⁽⁴⁾. The criteria for tooth selection included absence of cracks caused by extraction forceps, as well as absence of decay.

Preparation of specimen:

The tooth roots were set in acrylic resin molds, and the enamel surfaces were coated with two layers of colorless acid resistant nail varnish (Maybelline, USA) and were left to dry individually at room temperature on each specimen for 24 hours Leaving a 4 × 4 mm window on the labial surface without nail polish, and then they were soaked again in distilled water.

Laser fluorescence testing:

Laser fluorescence was tested three times, At baseline(T0) and after the creation of Initial enamel lesions(T1) and after 10 days of remineralizing surface treatments(T2) ⁽⁵⁾, respectively using Diode laser fluorescence device (DIAGNOdent). At each time interval in the study, the Laser fluorescence was assessed three times for each specimen and the mean value was calculated and recorded.

Preparation and application of demineralizing agents:

A demineralizing solution was prepared (2.2 mm calcium chloride, 2.2 mM monopotassium phosphate, 0.05 mM acetic acid having pH adjusted to 4.4 and 1M potassium hydroxide) and all the samples were immersed in this demineralizing solution for 4 days without stirring to create artificial initial enamel carious lesions the pH meter was used to detect the pH value daily ⁽⁵⁾. After the specified period, all

samples were removed from the solution and washed for 5 minutes under tap water, then washed again under distilled water for 30 s and dried with air spray free of oil to be visualized for their chalky white appearance ⁽⁶⁾.

Preparation of Remineralizing agents:

Solution of a 0.5% concentration of ginger and rosemary extract were prepared through 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 concentration 0.5% ⁽⁷⁾

Grouping of the test specimen:

After creating the initial enamel carious lesions, samples were randomly divided into three equal groups (n = 10). Two Intervention groups and one control group. Group 1 (n=10) teeth was treated with Rosemary remineralizing agent, Group 2 (n=10) teeth was treated with Ginger remineralizing agent and Group 3 (n=10) teeth was treated with sodium fluoride varnish.

Application of Remineralization agents:

The two natural remineralizing agent were applied 60 seconds two times per day for ten consecutive days, using a micro brush. A new micro brush was used for each specimen. ⁽⁴⁾ The aqueous solution of these herbals was used. Sodium Fluoride varnish was used as a control group according to manufacturer instructions. The herbal aqueous solutions were wiped without

rinsing, followed by immersing the specimens in a fresh artificial saliva solution. These specimens were placed in artificial saliva at 37 °C during the duration of study to stimulate oral condition. Solutions were refreshed daily. After the treatment application, all specimens were washed with distilled water, the nail varnish was carefully removed with colourless acetone, and then specimens were washed again with distilled water and prepared for Laser fluorescence testing.

Laser fluorescence assessment:

Based on principle of fluorescence, it uses a diode laser light source and a fiber optic cable that transmits light to a handheld probe. Light is absorbed induces infrared fluorescence by organic and inorganic materials. Emitted fluorescence is collected at probe tip, processed and presented on display as an integer between 0 and 99. Based on the previous study by Yazıcıoğlu and Ulukapi (2014), laser fluorescence examination results had been scored using the manufacturer's scoring system ⁽⁸⁾.

Score 1: Laser fluorescence score 0-4, no caries or white opaque lesions.

Score 2: Laser fluorescence score 5-10, enamel caries limited to the outer half of the enamel thickness

Score 3: Laser fluorescence score 11-20, enamel caries limited to the inner half of the enamel thickness without obvious spread in the dentin.

Score 4: Laser fluorescence score ≥ 21 , caries spread in the dentin.

Statistical analysis

Values were presented as mean, standard deviation (SD) values and confidence intervals. Results of 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 Bonferroni post hoc test for pairwise comparison. Paired t test was used to compare different observations times within the same group. Data of percentage of change were non-parametric and were compared between groups using Kruskal Wallis test. The percentage of change was calculated using the formula:

$$\frac{(\text{Value after}-\text{Value at baseline})}{\text{value at baseline}} \times 100$$

The difference was calculated using the formula

$$(\text{Value after}-\text{Value before})$$

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 diagnodont value was evaluated at 3 observations time in all groups. D0(T base line), D1(after enamel demineralization) and D2(after remineralization). The overall value in different observations is summarized in Table (1) and Fig. (1) Overall, The value of D1 increased significantly in D 1 compared to D 0. This was followed by a decrease at D 2, to a value which was still significantly higher than baseline (D 0). The difference between each 2 observation times was statistically significant ($p=0.000$).

I- Comparison of Diagnodont values between groups

At D 0, there was no significant difference between groups ($p=0.655$),

(Table 2, Fig.2) At D 1, the highest mean value was recorded in **Sodium Fluoride** group (18.13 ± 3.83). This value was significantly higher ($p=0.002$) than the values recorded in ginger group (14.25 ± 2.1) and Rosemary group (13.86 ± 0.002). Post hoc test revealed no significant difference between ginger and Rosemary groups, (**Table 2, Fig.2**)

At D2, the highest mean value was recorded in ginger group (7.51 ± 1.42). This value was significantly higher than rosemary group (6.09 ± 0.86). A significantly lower value was recorded in Sodium Fluoride group (4.41 ± 0.67). The difference between groups was statistically significant ($p=0.000$). Post hoc test revealed a significant difference between each 2 groups, (**Table 2, Fig.2**)

Table (1) Descriptive statistics and comparison of the overall Diagnodont values at different observations (repeated measures ANOVA test)

	Mean	Std. Dev	95% Confidence Interval for Mean		Min	Max	F	P value
			Lower Bound	Upper Bound				
D 0	4.98 ^c	1.30	.24	4.50	5.47	2.00	197.8	0.000*
D 1	15.41 ^a	3.27	.60	14.19	16.63	11.00		
D 2	6.00 ^b	1.63	.30	5.39	6.61	3.00		

Significance level $P\leq 0.05$, * significant

Post hoc test: means sharing the same superscript letter are not significantly different

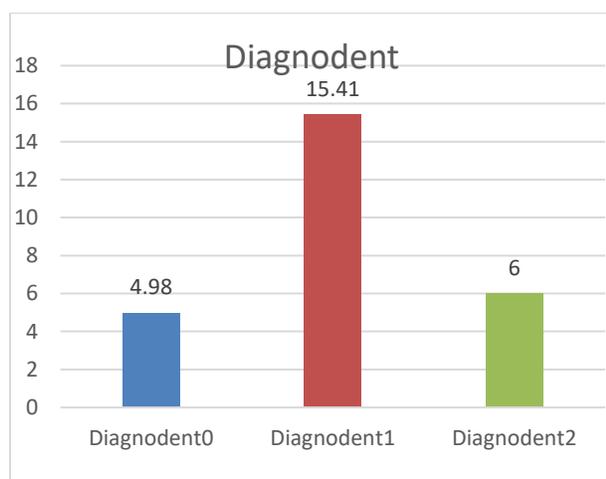


Fig. (1) Bar chart illustrating mean overall Diagnodent value at different observation times

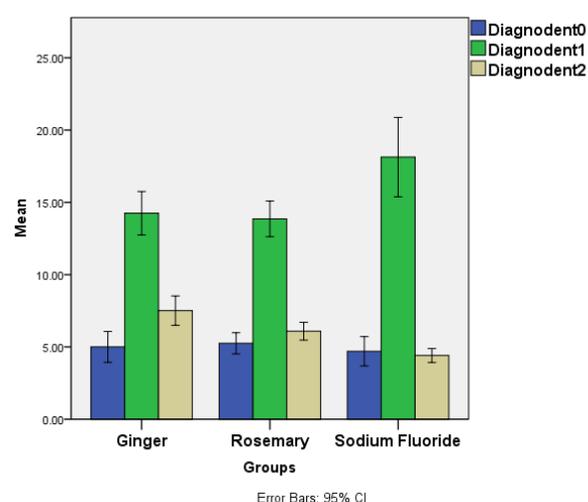


Fig. (2) Bar chart illustrating mean Diagnodent value in different groups

Table (2) Descriptive statistics and comparison of Diagnodent values between groups (ANOVA test)

		Mean	Std. Dev	95% Confidence Interval for Mean		Min	Max	F	P value
				Lower Bound	Upper Bound				
Diagnodent0	Ginger	5.00	1.49	3.93	6.07	2.00	7.00	.4	.655 ns
	Rosemary	5.25	1.03	4.52	5.98	4.00	7.00		
	Sodium Fluoride	4.70	1.42	3.69	5.71	3.00	7.00		
Diagnodent1	Ginger	14.25 ^b	2.10	12.74	15.76	11.20	17.60	7.7	.002*
	Rosemary	13.86 ^b	1.72	12.63	15.09	11.30	16.30		
	Sodium Fluoride	18.13 ^a	3.83	15.39	20.87	11.00	25.30		
Diagnodent2	Ginger	7.51 ^x	1.42	6.49	8.53	5.60	9.70	22.4	.000*
	Rosemary	6.09 ^y	.86	5.47	6.71	4.60	7.30		
	Sodium Fluoride	4.41 ^z	.67	3.93	4.89	3.00	5.30		

Significance level $P \leq 0.05$, * significant Post hoc test: Within the same comparison, means with different superscript letters are significantly different

II- Comparison of difference and percent change in Diagnodent values between groups

Regarding the difference (D1-D0), the greatest increase was recorded in sodium fluoride (median =14.1), followed by Ginger (median= 8.63), then Rosemary (median= 8.6). The difference between Sodium fluoride and the other 2 groups was statistically significant (p=0.031), Post hoc test revealed no significant difference between ginger and Rosemary groups, (Table 3, Fig.3), Regarding the difference (D2-D0), the greatest increase was recorded in Ginger group (median =2.16), followed by Rosemary (median= 0.84), then sodium fluoride (median= -0.7). The difference between Ginger and Sodium fluoride groups was statistically significant (p=0.014), Post hoc test revealed that Rosemary was not

significantly different from the other 2 groups, (Table 3, Fig.3)

Regarding the percent change from baseline to D1, the greatest percent increase was recorded in sodium fluoride (median =318.5), followed by Ginger (median= 205.45), then Rosemary (median= 156). However, the difference between groups was not statistically significant (p=0.138), (Table 3, Fig.4), Regarding the percent change from baseline to D2, the greatest percent increase was recorded in Ginger group (median =61.85), followed by Rosemary (median= 19.3), then sodium fluoride (median= -14). The difference between Ginger and Sodium fluoride groups was statistically significant (p=0.017), Post hoc test revealed that Rosemary was not significantly different from the other 2 groups, (Table3, Fig.4)

Table (3) Descriptive statistics and comparison of difference and percent change in Diagnodent values between groups (Kruskal Wallis test)

		Median	Mean	Std. Dev	95% Confidence Interval for Mean		Min	Max	P value
					Lower Bound	Upper Bound			
Diff. 1	Ginger	8.63	9.25 ^b	3.00	7.10	11.40	4.60	13.60	.031*
	Rosemary	8.60	8.60 ^b	1.92	7.23	9.97	5.30	11.60	
	Sodium Fluoride	14.10	13.43 ^a	5.00	9.85	17.01	4.00	21.30	
Diff. 2	Ginger	2.16	2.51 ^d	2.47	.75	4.28	-.70	6.70	.014*
	Rosemary	.84	.84 ^{d,e}	1.05	.08	1.59	-.70	2.60	
	Sodium Fluoride	-.70	-.29 ^e	1.60	-1.43	.85	-2.40	2.00	
% change1	Ginger	205.45	234.88	170.85	112.66	357.1	65.71	665.00	.138ns
	Rosemary	156.00	174.00	64.88	127.59	220.4	88.33	290.00	
	Sodium Fluoride	318.50	333.13	185.49	200.43	465.8	57.14	576.67	
% change2	Ginger	61.85	79.72 ^x	101.38	7.19	152.2	-10.00	335.00	.017*
	Rosemary	19.30	19.64 ^{x,y}	24.81	1.89	37.39	-10.00	65.00	
	Sodium Fluoride	-14.00	2.28 ^{y,z}	38.75	-25.44	30.00	-34.29	66.67	

Significance level $P \leq 0.05$, * significant, ns=non-significant

Post hoc test: Within the same comparison, means with different superscript letters are significantly different

Diff. 1= (Value1-Value0), Diff. 2= (Value2-Value0)

Percent change1= (Value1-Value0)/value0*100

Percent change2= (Value2-Value0)/value0*100

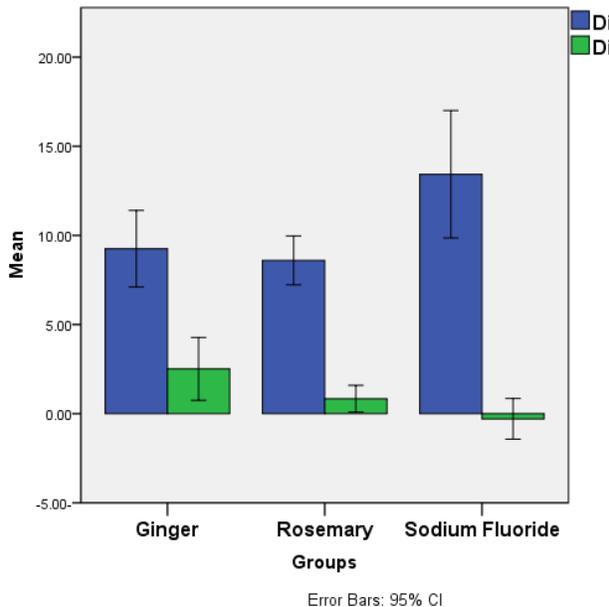


Fig. (3) Bar chart illustrating mean difference in Diagnodent value in different groups

III- Comparison of different observations within the same group

In Ginger group, the mean value recorded at D 1 and 2 was significantly higher than D 0, ($p=0.00$, $p=0.1$ respectively). Moreover, the value at 1 was significantly higher than 2 ($p=0.00$), (Table 4, Fig. 2),

In Rosemary group, the mean value recorded at D 1 was significantly higher than D 0, ($p=0.00$). Although the value recorded at D 2 was higher than D 0, the

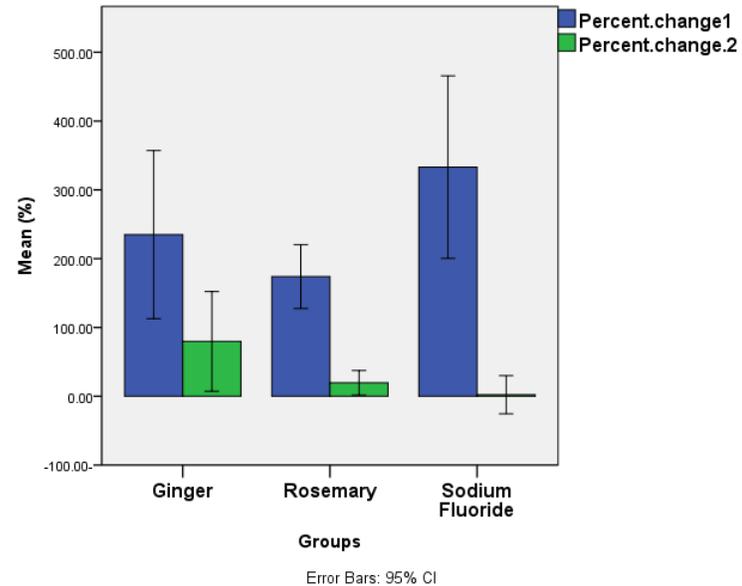


Fig. (4) Bar chart illustrating mean percent change in Diagnodent value in different groups

difference between D 0 and D 2 was not statistically significant ($p=0.093$). Moreover, the value at D 1 was significantly higher than D 2 ($p=0.00$), (Table 4, Fig. 2)

In sodium fluoride group, the mean value recorded at D 1 was significantly higher than D 0, ($p=0.00$). Although the value recorded at D 0 was higher than D 2, the difference between D 0 and D 2 was not statistically significant ($p=0.580$). Moreover, the value at D 1 was significantly higher than D 2 ($p=0.00$), (Table 4, Fig. 2)

Table (4) Descriptive statistics and comparison of Diagnodent values at different observation times within the same group (paired t test)

Groups		Mean	Std. Dev	t	P value
Ginger	Diagnodent0	5.00	1.49	-9.86	.000*
	Diagnodent1	14.25	2.10		
	Diagnodent0	5.00	1.49	-3.23	.010*
	Diagnodent2	7.51	1.42		
	Diagnodent1	14.25	2.10	14.79	.000*
	Diagnodent2	7.51	1.42		
Rosemary	Diagnodent0	5.25	1.03	-12.75	.000*
	Diagnodent1	13.86	1.72		
	Diagnodent0	5.25	1.03	-1.88	.093 ns
	Diagnodent2	6.09	.86		
	Diagnodent1	13.86	1.72	14.30	.000*
	Diagnodent2	6.09	.86		
Sodium Fluoride	Diagnodent0	4.70	1.42	-8.50	.000*
	Diagnodent1	18.13	3.83		
	Diagnodent0	4.70	1.42	.57	.580 ns
	Diagnodent2	4.41	.67		
	Diagnodent1	18.13	3.83	11.70	.000*
	Diagnodent2	4.41	.67		

Significance level $P \leq 0.05$, * significant, ns=non-significant

Discussion

Early enamel lesions are most seen in patients with fixed orthodontic appliances that induced a rapid increase in the volume of dental plaque. The composition of the bacterial flora of plaques shifts rapidly after introducing orthodontic appliances ⁽⁹⁾.

It was observed that there was a significant elevation in plaque and salivary levels of acidogenic bacteria, such as Streptococcus Mutans and lactobacilli in orthodontic

patients. The low pH of plaques adjacent to the orthodontic appliances and areas without optimal oral hygiene favors caries development; thus, decalcification of enamel and even carious cavity occurs. Moreover, because of low concentrations of calcium, phosphate, and fluoride in the saliva, natural remineralization after orthodontic treatment is difficult to achieve. Thus, intervention is needed to promote reversal of the caries process in the enamel lesions. The complete elimination of WSLs is unlikely, and some

WSLs last for up to 5–12 years. Therefore, it becomes necessary to apply remineralizing agents to repair the deeper parts of WSLs for better esthetics.

The commonly used agents for the treatment of WSLs are topical fluorides. The sodium fluoride varnish considered as control agent because it is the most popular used remineralizing agent in the treatment of white spot lesions. The fluoride has many roles in inhibiting the demineralization, enhancing the remineralization and having a strong bactericidal effect. Active free ions of Fluoride when it becomes available in the oral environmental fluids, formation of fluorapatite and calcium fluoride take place. Deposition of fluorapatite crystals dictates the presence of fluoride, calcium and phosphate ions in an adequate amount. While for the formation of calcium fluoride, presence of only calcium and fluoride ions is needed. Furthermore, the concentration of fluoride in the oral fluid and its pH are other deciding factors for formation of fluorapatite crystals. When it becomes lower than 50-ppm and pH is more than 4.5, the circumstance becomes favorable to Fluorapatite formation ⁽¹⁰⁾. On the other hand, ginger rhizome (*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). Their antifungal and antimicrobial effects on oral cavity pathogens have been reported in many

studies. The remineralization obtained was probably due to the antimicrobial properties and the high fluoride and calcium content of ginger. In addition, rosemary has inhibitory effects on *S. mutans*. It was found that the rosemary containing treatment mixture was effective in enhancing the remineralization process of enamel as discussed by Bilgin et al. in (2016) ⁽¹¹⁾.

Laser fluorescence (LF) is a method introduced for early diagnosis of the dental caries. It is useful for early detection of hidden caries in non-cavitated teeth through a non-invasive method. It emits infrared light (655 nm), that can be absorbed by organic and inorganic tooth materials, and the process of remitted fluorescence shows various scales between 0 and 99). In the presence of caries, light with a higher wavelength is reemitted, and the changes are registered in a digital number scale, with an audible sound indicating the fluorescence increase. ⁽¹²⁾.

The results of the current study revealed that the highest mean value was recorded in ginger group that was significantly higher than rosemary group and Sodium Fluoride group that is may be due to the antimicrobial properties and the high fluoride and calcium content of ginger.

However, Du et al. (2011) disagrees with the results of the current study. He discussed that the application of fluoride varnish associated with good oral hygiene were sufficient to promote remineralization. It has been found that fluoride increases the initial rate of remineralization and recommended

to use high doses of fluoride during and after orthodontic treatment for arresting areas of decalcification and preventing WSLs from progressing to carious lesions ⁽¹²⁾.

By the end of the discussion our null hypothesis was accepted that there is no significant difference between the remineralization potential of ginger, rosemary and fluoride varnish on caries early enamel lesions. According to the data obtained from this study it is possible to interpret that the ginger and rosemary can inhibit demineralization and/or promote remineralization of early enamel lesions and may serve as a promising alternative for the treatment of white spot lesion.

Conclusions

Within the limitations of this study the following conclusions could be drawn:

1. Fluoride varnish, ginger and rosemary can decrease early enamel lesions.
2. Ginger and rosemary may be preferable for prevention purposes on initial remineralization of enamel lesions, as more natural products are preferred today.

Recommendations

1. Further clinical trials with multiple applications of the ginger and rosemary gel and longer follow up periods.
2. Maintaining good oral hygiene is the most important prophylactic measure to prevent early enamel lesions.

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