

Original Article

Remineralization Potential of Grape Seed Extract Versus Fluoride Mouthwash in Management of Post-Orthodontic White Spot Lesions: A 6m Randomized Clinical Trial

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Abstract

Aim: This study compared the remineralization potential of grape seed extract vs sodium fluoride mouthwash in adult patients with postorthodontic white spot lesions assessed with DIAGNOdent after six months.

Materials and methods: A total of 14 participants with 40 lesions received mouthwashes randomly using either; grape seed extract 15% or sodium fluoride (1000 ppm), the participants were asked to use the mouthwash twice daily. The teeth were evaluated with DIAGNOdent after 1, 3 & 6 months. After 6 months, patient satisfaction was also recorded by using a binary questionnaire.

Results: Regarding DIAGNOdent scores, 6 months intergroup comparisons revealed statistically significant differences ($P=0.0198$) for grape seed extract, and a statistically significant difference between different follow-up periods was found in intragroup comparisons within grape seed extract or sodium fluoride ($P=0.0001$). Regarding patient satisfaction, after 6 months, there was no statistically significant difference between the two materials ($P = 0.5302$).

Conclusion: Both mouthwashes showed a potential remineralizing effect on post-orthodontic white spot lesions in the early testing periods, while grape seed extract mouthwash has a beneficial influence after 6 months.

Clinical significance: Using grape seed extract as a natural remineralizing agent to treat early enamel carious lesions has the potential to be a viable approach.

Keywords: Grape seed extract, Sodium Fluoride, DIAGNOdent, White spot lesions.

1.Introduction

One of the most widely spread diseases in the world is dental caries. During the early stages of enamel caries development, a chemical process known as enamel demineralization removes minerals (mostly calcium) from the enamel [1]. These lesions appear as white spots with an intact enamel surface layer. Teeth remineralization is one of the essential methods to arrest caries progression due to its dynamic nature; thus,

timing is crucial. Evidence showed that a partly demineralized subsurface lesion may be totally remineralized before cavitation if the enamel surfaces are intact, plaque-free and continually under sufficient salivary flow, particularly if frequently stimulated with sugar-free gum and treated topically with fluoride. [2]

One of the most common complications of orthodontic bracket removal is the appearance of white spot lesions (WSLs) which has a significant negative impact on the

aesthetic outcome. According to several studies, the prevalence of WSLs ranges from 2 percent to 96 percent, which has been related to permanent brackets and the longer amount of time that plaque lingers on teeth. [3] Enamel demineralization continues to be a postorthodontic challenge possibly prompting to carious lesions. Although primary prevention should be prioritized, there are two significant solutions for existing lesions after debonding: remineralization and masking. The first approach is based on using remineralizing materials such as topical fluoride, amorphous calcium phosphate, or self-assembling peptides. [4] The second is based on masking those lesions through bleaching or resin infiltration. [5]

Natural products have for been earlier utilized in conventional medication and now are promising therapeutic agents, particularly in the treatment of oral infections like dental caries. Herbs can be used in dentistry to help prevent and treat tooth decay. The primary benefits of using herbal substitutes are their ease of availability, low cost, longer shelf life, low toxicity, and lack of microbial resistance. [6]

As a strong antioxidant, grape seed extract (GSE) has been recognized for its promising caries-prevention capabilities. GSE has a variety of bioactive qualities, but its high

level of proanthocyanidins (PACs) is thought to contribute to its caries-prevention potential. PACs contain both hydrophobic and hydrophilic qualities, enhancing their capacity to attach to a wide range of substances, including minerals, proteins, and carbohydrates, in an irreversible manner. It has been shown that (GSE) could be a natural remineralizing agent [7] [8]

This clinical trial was conducted to test the null hypothesis in this research, which was that there would be no significant difference in the remineralization ability of grape seed extract mouthwash vs sodium fluoride mouthwash in post-orthodontic white spot lesions.

1. Materials and Methods

The study was conducted at Faculty of Dentistry - Cairo University and approved by the Ethics in Human Research Committee of the Faculty of Dentistry, Cairo University (#27/6/20), and the study protocol was registered at ClinicalTrials.gov (NCT04357093).

All materials used in this study as well as their active ingredients, lot number and manufacturer are listed in Table (1).

Table (1): Materials' specification, composition, manufacturer and lot number

Material	Composition	Active ingredient	Lot #	Manufacturer
1.1. Grape Seed Extract powder	Grape Seed Extract Powder	95% Proanthocyanidins	G3-075-1801107 Exp. Date: 12/2021	NuSci HerbStore., USA
1.2. Sodium fluoride powder	Minimum assay NF (NLT) 97%, Acidity as (HF) 0.1%, Chloride (Cl) 0.02%, sulphate (SO ₄) 0.05%, Silicate (SiO ₂) 0.5%, Iron (Fe) 0.005%, Heavy metals as (Pb) 0.005%	Sodium fluoride	F17M042 Exp. Date: 11/2022	Advent chembio pvt ltd, India
1.3. Signal toothpaste	Calcium Carbonate, Aqua (Water), Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, Sodium Monofluorophosphat	Sodium Monofluorophosphate 1450 ppm Fluoride	73 Exp. Date:20/02/2023	Made in Egypt Trademark: © Unilever England

	e, Aroma, Cellulose Gum, Potassium Citrate, Trisodium Phosphate, Sodium Saccharin, Phenylcarbinol, Limonene, CI 12490.			
1.4. Non-fluoridated prophylactic polishing paste		Xylitol	Expiry date: 1/3/2025	DHARMA QARTZ USA

2.1. Sample size calculation

Based on a study Andersson et al in 2007^[9], the predicted sample size was 16 subjects to be able to reject the null hypothesis (0.05) that the population means of the experimental and control groups were equal with probability (power) 0.8. Sample size was increased by 20 % to compensate for dropouts with a total of 40 subjects (i.e.20 per group).Sample size was calculated using PS version 3.1.6 for windows using T test for independent variables.

2.2. Study Design

Regarding the design, it was quadruple blinded (The participant, care provider, operator and outcome assessors); two parallel armed randomized clinical trial with an equal allocation ratio. This trial was reported following the Consolidated Standards of Reporting Trials (CONSORT) Statement (Schulz et al., 2010). Randomization has been done using simple randomization (www.randomization.com), by generating numbers from 1:40 into two columns according to interventions/Control assessment methods. The randomization list was kept secured to ensure no tampering with the random list. Each participant chose a random number from an opaque sealed envelope.

The study was conducted over a period of 6 months, with a total of four visits: first visit (baseline: T0), second visit (1 month: T1), third visit (3 months: T2), and final visit (6 months: T3).

2.3. Eligibility criteria

The patients included in this study was with age ranging from 13 to 30, and having at least two WSLs on the labial surfaces of six maxillary anterior teeth that were not present prior to orthodontic treatment with a DIAGNOdent score of (4-20). The excluded patients in this study whom have periodontal

disease that is severe or aggressive, undergone therapeutic irradiation to the head and neck, had participated in a clinical study six months prior to the start of this trial, unable to attend recall appointments, presence of abnormal oral, medical, or mental state, dentin caries, cavitated lesions or hypoplasia of the enamel of the maxillary anterior teeth and white spot lesion with (<4) or (>20) scoring.

2.4. Preparation of grape seed extract mouthwash formulations

The grape seed solution was prepared in National Research Centre (NRC) by measuring 10 grams of grape seed (GSE) powder with precise weighting balance. Then it was added to a solution of distilled water (66.6 v) for completing the volume to 100 ml to yield 15% GSE solution. The solution has been filtered through filter paper no 4. The formed solution has been kept in the refrigerator (at 8°C) for 24 hours before further use.^[7]

2.5. Preparation of sodium fluoride mouthwash formulations

The sodium fluoride mouthwash was prepared in NRC by measuring 1 gram of sodium fluoride powder with precise weighting balance. Then it was added to a solution of distilled water (1000 ml) for completing the volume to 1000 ml to yield 1000 ppm sodium fluoride solution. The solution has been filtered through filter paper no 4. Red food colouring liquid (Savory babaj) has been added to make the solution red, for blinding. The formed solution has been kept in the refrigerator (at 8°C) for 24 hours before further use.^[7] The mouthwash of intervention & control were put in unlabelled dark bottles to reassure more blinding.

2.6. Clinical procedure

2.6.1. Pre-operative examination

procedure:

Patients with WSLs after orthodontic treatment were selected. A bristle brush and non-fluoridated prophylaxis paste were used for dental prophylaxis and removal of any dental plaque deposits.

Each lesion was measured by the DIAGNOdent to assess the degree of demineralization. The probe tip was placed on the labial smooth surface of the lesion after it had been air dried and recorded a sound spot. The assessment was repeated three times to reduce measurement errors for each area then the mean of three consecutive measurements was taken.

2.6.2. For the intervention group (GSE mouthwash):

Patients were instructed to use the modified Bass technique to brush their teeth with a pea sized amount of the given Signal TM (1450 ppm Fluoride containing) toothpaste on a soft bristled toothbrush. After 30 minutes of brushing, the participants were instructed to take 10 mL of the provided mouthwash and rinse their mouth thoroughly for 30 seconds using the graduated bottle cover twice a day; after waking up and before going to bed. Also, they were instructed not to eat or drink for 30 minutes following application of mouthwash.

2.6.3. For the control group (Sodium fluoride mouthwash 1000 ppm)

The same instructions of the intervention GSE mouthwash were followed.

2.7. Outcomes

2.7.1. Primary outcome:

Quantitative changes of white spot lesion were evaluated before and after remineralization by using DIAGNOdent (Table 2).

2. Results

3.1. Demographic data

This study was conducted on 40 white spot lesions in 14 participants that were randomly allocated to the intervention and the control arms (20 lesions per group) (Figure 1),

Table (3): Gender distribution among groups

Gender	(Intervention)	(Comparator)	Total
Males	2 (28.6%)	0 (0%)	2 (14.3%)
Females	5 (71.4%)	7 (100%)	12 (85.7%)
Total	7	7	14

2). Also, DIAGNOdent Measurements were done at baseline, 1, 3, and 6 months. [10]

Table (2): Laser fluorescence scoring system

Score	Description
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

2.7.2. Secondary outcome:

The Patient satisfaction question with colour change (esthetics) (Yes/ No). [11]

2.8. Statistical Analysis

Medcalc software, version 19 for Windows, was used to evaluate the data (MedCalc Software Ltd, Ostend, Belgium). The Kolmogorov Smirnov and Shapiro Wilk tests were used to determine the normality of the data. Continuous data were distributed normally and were reported using the mean and standard deviation. The independent t test was used to compare continuous data across groups, while repeated measures ANOVA and two-way ANOVA were employed to test for variable interaction, followed by the Tukey post-hoc test. Binary data were expressed in terms of frequency and percentage, and comparisons between binary variables were done using the chi square test. Statistical significance was defined as a value less than or equal to 0.05, and all tests were two-tailed.

with at least three white spot lesions in each participant. After 6 months 14 participants completed the follow-up with 100% retention rate. As shown in (Table 3), there was no statistically significant difference between all groups regarding age (P = 0.909).

3.2. DIAGNOdent score:

Intergroup comparison between both materials have shown no statistically significant difference within follow up periods; baseline, 1 month and 3 months respectively (P = 0.8005, P = 0.1906 and P= 0.1331), while at

6 months intergroup comparison revealed statistically significant difference (P=0.0198). Intragroup comparison within Grapeseed or NaF have shown statistically significant difference between different follow-up periods (P < 0.0001). (Table 4)

Table (4): Mean and standard deviation of DIAGNOdent score of both materials at each follow-up

Intervention Follow-up	Grapeseed		NaF		P value
	Mean	SD	Mean	SD	
Baseline	12.048 ^a	3.943	12.333 ^a	3.307	0.8005
1 month	9.286 ^b	3.437	10.619 ^{ab}	3.041	0.1906
3 months	7.286 ^{bc}	2.795	8.571 ^{bc}	2.638	0.1331
6 months	5.333 ^c	2.331	7.048 ^c	2.247	0.0198*
P value	*0.0001>		<0.0001*		

Means that do not share a letter are significantly different, * corresponds to statistically significant difference

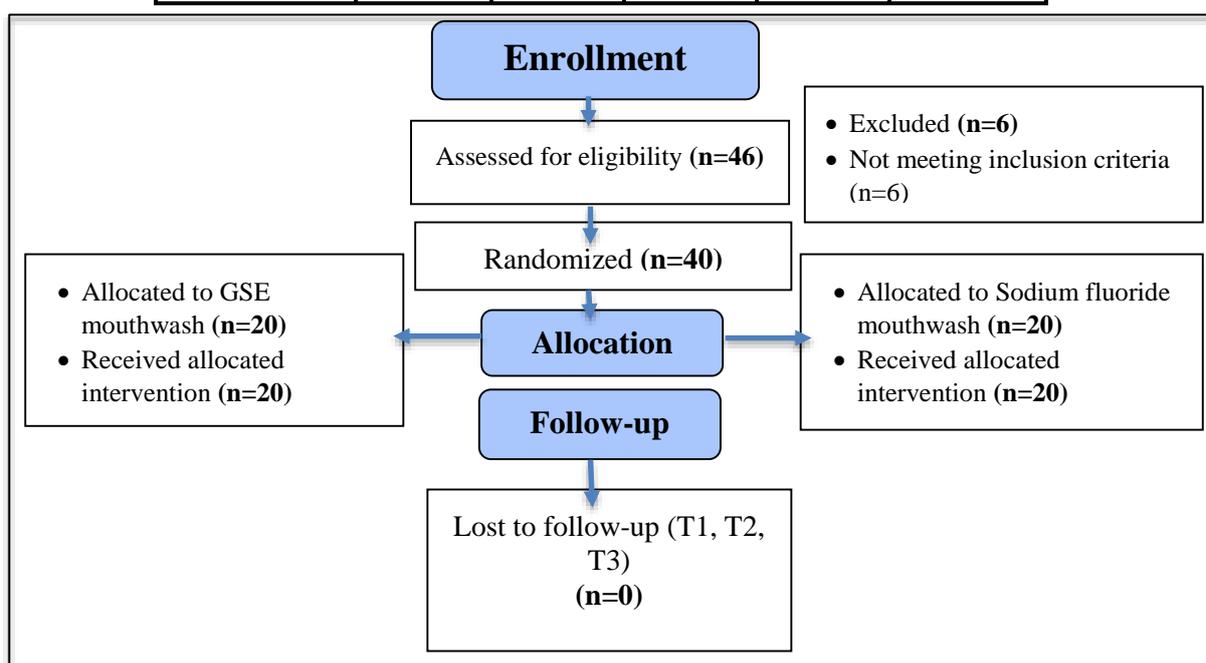
3.3. Patient satisfaction:

Intergroup comparison for patient satisfaction after 6 months revealed no

statistically significant difference between both materials (P = 0.5302). (Table 5)

Table (5): Frequency and percentage for patient satisfaction for both materials after 6 months

Material Follow up	Grapeseed		NaF		P value
	Yes	No	Yes	No	
6 months	6 (85.7%)	1 (14.3%)	5 (71.4%)	2 (28.6%)	0.5302



Discussion

White spot lesions (WSLs) are one of the most prevailing side effects of orthodontic treatment. The formation of WSLs is attributed to the fixed brackets' inhibition of proper oral hygiene and the prolonged period that plaque remains on the teeth, mainly at the gingival margins and at the bracket-adhesive enamel junction. Enamel demineralization remains an orthodontic challenge and the presence of WSLs jeopardizing the aesthetic outcome of orthodontic treatment and patient satisfaction. [12, 13, 14]

For early identification of caries lesions and monitoring preventative measures, DIAGNOdent® (KaVo), a laser fluorescence-based device with good to superb sensitivity, is promising according to **Lussi and Hibst (2004)**. [15] It can recognize a healthy tooth from a carious lesion based on fluorescing value and degree. Changes in mineral component properties such as reflection, refraction, transmission, and colour absorption are all influenced by demineralized teeth. [15]

Remineralization is defined as the process of gaining mineral content such as calcium and phosphate in the enamel crystal voids. Saliva serves as a source of calcium and phosphate ions, so it remineralizes tooth enamel naturally when the oral environment is at a neutral pH. However, under acidic conditions, saliva cannot overcome the acidity and demineralization because the rate of demineralization is much faster than the rate of remineralization. The goal of any remineralizing agent is to cause mineral deposition on the WSLs and to reduce them to acceptable levels for the patient. [16]

Fluoride beneficially affects the regression of WSLs, as per the scientific evidence of **Kalha et al. (2013)**. [17] WSLs may be less frequent and more severe if regular home use of fluoride mouth rinses and fluoride toothpaste is used consistently. It is important to make an attempt to avoid further deterioration of the teeth when prevention fails and WSLs develop. Fewer areas for plaque biofilms to accumulate and better oral self-cleaning are achieved when fixed appliances are removed. WSLs frequently regress

naturally in the first few months after removal of orthodontic brackets. [18]

Fluoride is incorporated into the enamel and forms fluorapatite particles, which are resistant to the acidity of bacterial metabolites and fermented carbohydrates, according to Faller and Casey (2011), who detailed the mechanism of action of sodium fluoride in remineralizing enamel lesions where remineralization is encouraged, while demineralization is prevented. [19]

The use of natural products are considered to be an alternative concept for remineralization of dental hard tissues as grape seed extract because it has antibacterial effect. [20, 21] GSE includes a high concentration of condensed tannins called proanthocyanidins (PACs), as well as flavan-3-ol polymers like catechin and epicatechin, both of which have been shown to have bioactive characteristics. Theoretically, it increases the value of caries prevention. Hydrophobic and hydrophilic properties allow them to bind to a broad range of substances, including minerals, proteins and carbohydrates, according to **Delimont and Carlson (2020)**. [8] According to the manufacturer, the GSE used in this study contained 95 % proanthocyanidins, and the GSE mouthwash concentration is 15%.

The primary component of GSE is gallic acid, which, according to **Xie and Bedran Russo (2008)** [20], was considered to enhance mineral deposition by interacting with calcium ions (Ca²⁺) in the surrounding medium (saliva) and producing insoluble calcium compounds (2008). In terms of remineralization, GSE seems to have a substantial impact. Remineralization begins by creating complexes with calcium, which aids the formation of mineral deposits on the lesion's surface. Second, GSE may stabilise the exposed collagen matrix by interacting with PA-collagen in the organic section of the enamel, which was discussed by **Rubel et al. (2016)**. [22]

In this clinical trial, adult patients with postorthodontic white spot lesions were evaluated six months after treatment with either a grape seed extract mouthwash or a sodium fluoride mouthwash to see which one would be more effective at remineralizing their teeth

using the laser fluorescence-based DIAGNOdent® device.

In this clinical trial, the assessment periods were baseline, one, three, and six months after intervention. This current study was quadruple blinded (The participant, care provider, operator and outcome assessors). The blinding was accomplished by using red food colouring liquid added to sodium fluoride mouthwash after being analyzed in the National Research Centre to avoid any confounders by having any minerals or ingredients affecting the remineralization process.

Before the baseline reading of the DIAGNOdent, scaling was done manually not by ultrasonic, as ultrasonic scalers can do cracks especially with WSLs. Polishing of teeth with non-fluoridated polishing paste has been used to avoid any variable or confounder that may affect the reading.

All the participants completed the trial till the end of the follow-up period. None of the subjects withdrew due to adverse effects or harms (e.g., allergies, gingival inflammation, enamel-staining, and accelerated plaque accumulation).

Improvement in DIAGNOdent scores for enamel lesions were seen in all treatment groups at 1 month, and these lesions continued to improve until the study's endpoint of 6 months. The significant increase in fluorescence, associated with a decrease in lesion area compared to baseline, suggests that improvement occurred gradually over time as remineralization occurred.

There was no statistically significant difference in the DIAGNOdent intergroup value between the two materials, At baseline, one month, and three months, which were consistent with the results of **Amin and Awad (2019)** ^[23], who stated that enamel surface which was exposed to the GSE showed high remineralizing effect like that in sodium fluoride treated group. This was explained by the precipitation of insoluble spherical particles of varying sizes within the artificial lesions on the enamel surface of the GSE treated group. Some particles clumped together to form complexes. Also, they found multiple spherical

deposits of calcific particles on the enamel surface after treatment with grape seed extract. This could be due to mineral precipitation, particularly in its interior, because most of its structure is made up of inorganic matter.

The DIAGNOdent intergroup comparison between GSE and sodium fluoride revealed statistically significant difference ($P=0.0198$) at 6 months, which agreed with the results of **Mirkarimi et al. (2013)**.

The DIAGNOdent values of the GSE group after six months when compared to its baseline, clearly revealed that significant remineralization had occurred. This finding agreed with the results of **Silva et al. (2015) and Rubel et al. (2016)**, who found that grape seed extract can increase the remineralization of carious enamel lesions. ^[24, 22] They proposed that the GSE's proanthocyanidins (PAs) and gallic acid are responsible for facilitating mineral deposition on enamel. In addition, PACs have a chelating mechanism with calcium ions, promoting mineral deposition on the surface and that it could be an effective natural agent for non-invasive dentistry.

Comparison of the mean DIAGNOdent value of sodium fluoride mouthwash after six months with its baseline showed that there were significant quantitative changes. This is consistent with a previous clinical remineralization trial of **Bhongsatiern et al. (2019)**, which showed that the combination of fluoride toothpaste and fluoride mouthwash improves the remineralization effect. ^[25]

The patient satisfaction results here showed no significant difference ($P = 0.5302$) between both groups after 6 months, where 85.7% of participants in grape seed extract group selected "Yes", while 14.3% selected "No". For NaF group, 71.4% selected "Yes", while 28.6% selected "No". This result showing the remineralization potential of GSE when it's compared to the gold standard sodium fluoride, which reduced the WSLs by gaining minerals to fill the enamel voids, decreasing the refraction & having low coefficient refractive indices.

According to the clinical findings and the results of this study, after 6 months of using GSE; it showed that it has potential

remineralization efficacy and this is a promising result which may lead to formation of more formulas and products containing the grape seed extract to be over the counter and can be used by population with reasonable cost and availability.

3. Conclusion

Under the limitations of this trial, the following conclusions could be mentioned: both prepared grape seed extract and sodium fluoride mouth washes had a potential remineralizing effect on the white spot lesions in post orthodontic patients in the early testing periods. Thus, using grape seed extract as a natural remineralizing agent to treat early enamel carious lesions has the potential to be a viable approach.

Conflicts of Interest and funding

The authors declare that there are no conflicts of interest regarding the publication of this paper. There is no funding in this research.

Ethical approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

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