EGYPTIAN DENTAL JOURNAL

VOL.67, 2645:2654, JULY, 2021

PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

CONSERVATIVE DENTISTRY AND ENDODONTICS

www.eda-egypt.org • Codex : 21/21.07 • DOI : 10.21608/edj.2021.60472.1476

REMINERALIZATION POTENTIAL OF GRAPE SEEDS EXTRACT GEL VERSUS CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE IN WHITE SPOT LESIONS IN POST ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Aims: To determine the effectiveness of grape seed extract (GSE) gel compared to Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) application in managing white spot lesions (WSLs) in post orthodontic patients.

Statistical analysis used: All statistical calculations were done using computer program IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 25 (SPSS Inc., Chicago, IL).

Methods and Material: This study was a single-blinded randomized clinical trial where 24 participants with white spot lesions in at least two teeth were included and randomized into two groups (12 patients each) to receive either GSE gel or CPP-ACP paste application. GSE gel was used on WSLs after orthodontic treatment every week, while CPP-ACP paste was applied twice daily by the patients themselves. Baseline and post-intervention measurements of quantitative changes of WSLs were measured by KaVo DIAGNOdent at 1, 3 and 6 months. Data for quantitative changes of WSLs were recorded and analyzed for each group.

Results: There was no statistically significant difference in overall DIAGNOdent mean readings between GSE gel and CPP-ACP paste groups at P=0.360.

Conclusions: Based on the data obtained in this study, it is suggested that GSE promotes remineralization of WSLs post orthodontic treatment and, thus, might be considered an effective natural agent for treatment of early enamel carious lesions.

Key-words: Grape seed extract, Casein Phosphopeptide-Amorphous Calcium Phosphate, White spot lesions, Demineralization, Remineralization, Post orthodontic treatment.

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INTRODUCTION

Fixed orthodontic appliances have gained wide acceptance all over the world nowadays to treat different cases of malocclusions. Problems with the oral function due to malocclusion, as temporomandibular joint dysfunction, mastication, swallowing and phonetic difficulties. More liability to trauma, periodontal disease and tooth decay are also linked to malocclusions [1]. However, the first goal for most patients looking for orthodontic treatment is to accomplish a perceivable improvement in their dentofacial appearance while their optional objective of treatment is an oral health benefit. Unfortunately, this fixed type of orthodontic treatment can cause adverse effects, including white spot lesions (WSLs), which are prominent lesions that have a negative effect on the esthetics outcome of orthodontic treatment [2]. WSLs are defined as the "subsurface enamel porosity from carious demineralization which can be seen as milky opacity when located on smooth surface" [3]. The overall incidence of WSL in orthodontic patients was accounted for somewhere in the range of 2% and 97%. The most noteworthy frequency happens on the maxillary lateral incisors followed by the maxillary canine, premolar, and central incisors, respectively [4].WSLs are the consequence of sustained gathering of bacterial plaque on the enamel surface neighbouring to the fixed appliances, surface irregularities of orthodontic brackets, bands, ligature wires, and other attachments. Despite the compensatory action of the naturally occurring self-cleansing mechanism of the oral musculature and the saliva, the frequent intake of carbohydrates causes disruption of the normal balance between demineralization and remineralization, the former gaining the upper hand. This imbalance results in a net loss of minerals and a resultant highly porous, rough, dull enamel surface [5]. The treatment of WSLs has three main modules: prevention, control and atraumatic care of existing WSLs. Primary prevention of WSLs can be done adjacent to fixed orthodontic appliances, while secondary prevention of existing WSLs after orthodontic treatment can be divided into 3 distinct methodologies: (1) Strategy utilizing fluorides and phosphate-based remineralizing agents, (2) Strategy focusing on aesthetic enhancement of the lesions through infiltration by resins, and (3) invasive strategies such as microabrasion, bleaching or preparation and restoration [6]. As part of the minimally invasive approach, numerous remineralizing agents were presented all through the most recent decade. The Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) innovation has been created by Professor E. Reynolds and his group at the University of Melbourne Dental School. Casein Phosphopeptide (CPP) is a milk-derived protein casein has been reported to decrease demineralization of the tooth structure and improve remineralization. [7]. In recent years, much attention has been focused on natural plant-derived antimicrobials with their potential use as alternatives to chemicals compounds used for controlling dental plaque and dental diseases [8]. Grape seed extract (GSE), derived from the seeds of Vitis Vinifera, is one of the natural products used to treat WSLs [9]. Evidence supporting the reversal (demineralization) of early WSLs using natural products, as well diagnosed by accurate reproducible method has come from in-vitro and in-situ studies and to a lesser extent from clinical trials, which are still not enough to rely on. Thus, this study was intended to compare the ability of two remineralizing agents: casein phosphopeptide and GSE to remineralize WSLs in recently debonded orthodontic patients.

SUBJECTS AND METHODS

Trial design and settings

Regarding the design, it is a single Centered, single blinded (assessor); two parallel armed randomized control clinical trial with an equal allocation ratio. This trial was conducted following

the Consolidated Standards of Reporting Trials (CONSORT) Statement ***(Schulz et al., 2010)***. The CONSORT statement is an international consensus guide and checklist to improve reports on randomized controlled trials (RCT) reference. 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 (#19619), and the study protocol was registered at ClinicalTrials.gov (NCT03966378).

Participants Eligibility criteria

Participants were recruited from the clinic of Orthodontic Dentistry Department at the Faculty of Dentistry – Cairo University. Participants with good oral hygiene, of both genders and aged 16 to 35 years, having at least two WSLs on the labial surfaces of the six maxillary and mandibular anterior teeth that were not present before orthodontic therapy with a score of 1 or 2 on International Caries Detection and Assessment System 2005 (ICDAS II) were included in this study. Subjects had participated in a clinical trial within 6 months before commencement of this trial. Subjects with their teeth scoring higher than 2 according to ICDAS II and subjects with severe or active periodontal disease or allergic to any materials used in the study were excluded.

Preparation of GSE hydrogel formulations

The grape seed solution was prepared by measuring 10 grams of grape seed (GSE) powder (NuSci manufacturer, HerbStore USA) with a precise weighing balance. The powder was then added to a solution of distilled water/ethanol (20/80, v/v), which was filled up to a volume of 100 ml to yield 10% GSE solution. The pH of the solution was adjusted by utilizing a few drops of NaOH and measured by a pH meter. The solution was filtered through filter paper no 4. The GSE hydrogel was prepared by adding the gelling agent, sodium carboxymethyl cellulose, into the solution and continually stirring it at 1000 rpm using a magnetic stirrer. The formed hydrogel was kept in the refrigerator (at 8°C) for 24 hours before further use. This preparation was done after ***(Yassen and Safy)*** [10]***.***

Interventions

Interventions used in the study were either GSE gel (NuSci manufacturer, HerbStore USA) or CPP-ACP (GC tooth mousse, USA). Intervention steps were carried out by a single operator. After isolation, cleaning and drying of the tooth surface, a generous layer of GSE gel extruded into the custom-fitted tray (Every week). The custom-fitted tray was put on the affected arch and left undisturbed in the mouth for a minimum of 4 minutes before it was removed. After the tray removal, the participants were instructed to use their tongue to spread the remaining GSE gel throughout the mouth. For the second group, the participants were instructed to brush their teeth with standard fluoride toothpaste (like Colgate Total, 1.450 ppm F) twice a day before CPP-ACP applications. After brushing their teeth, they used a clean dry finger or cotton tip to apply sufficient crème (a pea sized amount of a CPP-ACP paste to the teeth twice daily after breakfast and before bed time) to coat all anterior teeth of the affected arch. They used their tongue to spread the remaining crème throughout the mouth and then left the crème on the teeth undisturbed for a minimum of 3 minutes. All participants were instructed to avoid eating or drinking for at least 30 minutes after applying the intervention.

Outcomes

Quantitative changes due to white spot lesions were determined using DIAGNOdent. Dental plaque was removed before the assessment. Dental prophylaxis was performed with a bristle brush and non-fluoridated prophylaxis paste. Controlled dry air was applied on the anterior teeth to identify location of WSLs (using a dental syringe directed perpendicular at a distance 1-3 mm from the enamel).

Assessor pointed out the degree of demineralization as follows:

Initially, a non-carious portion of each tooth was selected in order to provide a baseline measurement. Under cotton roll isolation and after air drying with an air syringe, the probe tip of DIAGNOdent was held gently against the tooth at right angle to the labial surface while gently maintaining squeeze pressure on the ring of the handpiece. The set zero appeared on the display confirmed by two audible beeps indicating that the zero-base line has been established for the patient. The probe tip has been positioned on this spot and rotated around the vertical axis until the highest value have been found and a peak reading recorded .The results of the three measurements (which has been performed in triplicate positions) have been added together and divided by three. Laser fluorescence examination results have been scored using the manufacturer's scoring system [11] Table (1).

TABLE (1): 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

DIAGNOdent measurements were carried out at baseline before application of intervention and at 1, 3, and 6 month follow-up periods. Participants were recalled by telephone on each follow-up visit to repeat the DIAGNOdent measurements.

Sample size calculation

Sample size calculation was achieved using PS: Power and Sample size calculation software version 3.1.2 for windows using T test (Franz Faul, Universitat Kiel, Germany), based on the previous paper [12]. The response within each subject group was normally distributed with standard deviation 0.72. Using power 80% and 5% significance level, we needed to study 9 subjects in each group. This number was increased to 12 to compensate for possible losses during follow-up.

Allocation sequence generation and concealment

Simple randomization was done by generating numbers from 1:24 using Random Sequence Generator, Randomness and Integrity Services Ltd ***(https://www.random.org/) 2019.*** Regarding the allocation concealment the randomization list was kept securely away from the operator and the participants to ensure no tampering with the random list**.** Each participant has chosen a sequentially numbered opaque sealed envelope **.**Participants choose the envelope after signing the written informed consent**.** After the participant has chosen an envelope, it was signed by the patient and the supervisor. The number on the envelope was recorded in the patient chart to ensure that the patient assigned to the randomized group. To improve the retention of the volunteer in the study, their information and telephone numbers of two easily reachable relatives or friends were recorded. Participants were rewarded by being provided treatment for their other dental necessities in between visits and financial reimbursement for those taking a vacation day from work or coming from country regions to attend the subsequent visit.

Statistical methods

Data were statistically described in terms of mean (±), standard deviation (SD), frequencies (n) and percentages. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess data normality of continuous data. Two-way ANOVA

was conducted to investigate the effect of study variables and their interaction on remineralization of white spot lesions. Comparison between the study groups was done using Mann Whitney *U* test for independent samples and Kruskal-Wallis test for multiple comparisons. Chi-square test was used to compare frequency distribution of categorical data. A p-value less than or equal to 0.05 was considered statistically significant and all tests will be two tailed. All statistical calculations were done using computer program IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 25 (SPSS Inc., Chicago, IL).

RESULTS

24 participants were randomized into two groups of 12 each. Three participants were lost to follow-up in the GSE group (one after 3 months and two after 6 months). Two participants were lost to follow-up in the CPP-ACP group (both after 3 months).

Effect of the remineralizing agent (overall effect)

Mean and SD values of DIAGNOdent readings for different interventions for quantitative changes of WSLs were presented in Table (2). They showed that there was no statistically significant difference between the mean and SD of the GSE gel group and the mean and SD of CPP-ACP group at (P=0.360).

TABLE (2): Mean and SD of of DIAGNOdent readings for different interventions for quantitative changes of WSLs.

Intervention Mean±SD		P-value
GSE	CPP-ACP	
9.4±3.4	9.04±4.1	**0.360ns**

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

Effect of Follow-up periods:

There was a statistically significant difference in overall DIAGNOdent mean and SD readings between follow-up intervals groups at P<0.001 Table (3). Baseline yielded the significant highest overall readings, followed by 1 month, then 3 and 6 months, which were statistically similar.

TABLE (3): Mean±SD and P-value for the comparison of overall DIAGNOdent readings between follow-up intervals groups.

Baseline	1 month	3 months	6 months	P-value
11.6±3.5[a]	9.7±3.2[b]	8.1±3.3[c]	6.9±3.4[c]	<0.001*

Different superscript letters within the same vertical column indicate a statistically significant difference*; significant (p ≤ 0.05) ns; non-significant (p>0.05)

Effect of Intervention within each Follow-up Interval

Mean and SD values of DIAGNOdent readings for different interventions and follow-up intervals are presented in Table (4). Regarding the baseline records, there was statistically significant difference between the two groups (P=0.010).

There was no statistically significant difference between them during successive follow-up intervals (P=0.414), (P=0.593), (P=0.360).

TABLE (4): Mean±SD and P-value for the comparison of DIAGNOdent readings between remineralizing agents at each follow-up intervals.

Follow-up Intervals	Intervention Mean±SD		P-value
	GSE	CPP-ACP	
Baseline	12.7±2.2	10.3±4.4	**0.010***
1 month	9.9±2.6	9.4±3.9	**0.414ns**
3 months	8.1±2.7	8.2±4.0	**0.593ns**
6 months	6.4±2.7	7.6±4.0	**0.360ns**

; significant (p ≤ 0.05) ns; non-significant (p>0.05) range; difference between highest and lowes

Effect of follow-up intervals within each intervention

Mean and SD values of DIAGNOdent readings for different interventions and follow-up intervals are presented in Table (5) for each test and time point.

Regarding the baseline records, the GSE gel group, there was a significant decrease of mean and SD value during successive follow-up intervals (P<0.001).

Regarding CPP-ACP group, the same results were found. There was a significant decrease of mean and SD value during successive follow-up intervals (P=0.019).

Distribution of laser fluorescence score system

Chi-square presented in Table (6) showed that there was a statistically significant difference in score distribution between the GSE gel group and CPP-ACP group at baseline (P=0.002) and 1 month (P=0.018). While there was no statistically significant difference in score distribution between both groups at 3 months (P=0.069) and 6 months (P=0.142).

TABLE (5): Mean±SD and P-value for the comparison of DIAGNOdent readings within each remineralizing agent at different follow-up intervals.

Follow-up Intervals	Intervention Mean±SD	
	GSE	**CPP-ACP**
Baseline	12.7±2.2[a]	10.3±4.4[a]
1 month	9.9±2.6[b]	9.4±3.9[ab]
3 months	8.1±2.7[bc]	8.2±4.0[bc]
6 months	6.4±2.7[c]	7.6±4.0[b]
P-value	**<0.001***	**0.019***

Different superscript letters within the same vertical column indicate a statistically significant difference*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

TABLE (6): Distribution of laser fluorescence score system within each remineralizing agent at different follow-up intervals.

Follow-up Intervals	Intervention	Scoring system (n/%)			P-value
		Score 1	**Score 2**	**Score 3**	
Baseline	**GSE**	4 (12.1%)	13 (39.3%)	16 (48.4%)	**0.002***
	CPP-ACP	0 (0%)	6 (15.3%)	33 (84.6%)	
1 month	**GSE**	7 (21.2%)	12 (36.3%)	14 (42.4%)	**0.018***
	CPP-ACP	1 (2.5%)	24 (61.5%)	14 (35.9%)	
3 months	**GSE**	9 (34.6%)	10 (38.4%)	7 (26.9%)	**0.069**
	CPP-ACP	6 (16.2%)	25 (67.5%)	6 (16.2%)	
6 months	**GSE**	13(50%)	6 (23.0%)	7 (26.9%)	**0.142**
	CPP-ACP	16(50%)	13 (40.6%)	3 (9.3%)	

; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

DISCUSSION

The treatment of these WSL is of clinical significance, also for aesthetic matter. The way in to the avoidance and the treatment of WSL is finding an observing strategy for WSL initiation and progression. Lesion assessment is defined as the process for deciding if dental caries is occur or not and to describe or screen a decay whenever it has been distinguished. The laser fluorescence-based device DIAGNOdent® (KaVo) is a potential device for identifying early tooth decay and observing preventive interventions, and it has good to excellent sensitivity [13]. It can detected the distinction in the fluorescing limit of the sound tooth and the dental caries. Variations in certain properties of mineral composition, for example, reflection, transmission, and color absorption in demineralized teeth, influence laser fluorescence-based device DIAGNOdent® (KaVo) interpretation and so the measure is different from sound teeth, which aids in identification of dental caries [14]. At the point when the preventive measures has not succeeded, and WSLs have developed, efforts must be done to avoid additional caries progression. Debonding the fixed appliances brings about less stagnation zones for plaque biofilm build-up and results in improve self-cleaning by the oral musculature and saliva. The first few months after removing the fixed appliance, a natural reversion of WSLs frequently happens. A lot of remineralization agents can be utilized to encourage remineralization by ionic exchange mechanism without obstructive the surface layer. CPP-ACP-containing products are thought to be effective in preventing and treating WSLs [15]. A newer concept for the remineralization of dental hard tissues is the utilization of natural derived or nutraceuticals as GSE due to its antimicrobial effects on oral cavity pathogens, antioxidant and free radical scavenging properties [16, 17]. The GSE used in this trial comprised of 95 % proanthocyanidin. One of the main ingredients of GSE is gallic acid, which was supposed to aid mineral deposition, mainly on the surface layer by uniting with Ca^{2+} from the contiguous media and furthermore by forming insoluble compounds with Ca^{2+} [17, 18].

In this study, all the steps were performed according to the guidelines for the design and conduct of clinical trials on WSLs [12] The randomization was done by assignment of patients into either the intervention or the comparator group before the treatment, using from random numbers prepared, and using specialized software to avoid any human involvement. The allocation system was set up so that the person enrolling participants did not know in advance which treatment the patient would get. The process is termed allocation concealment. Allocation concealment was the process that prevented any study participant from knowing in advance the treatment to which the participants would be assigned. It is important that the decision to enroll a participant was made with their lack of knowledge of the treatment to which they were assigned, as this knowledge might have influenced their decision as to whether or not to enroll. In the current study, blinding was single blind (Assessor). The patient knew what intervention she/he was taking. The operator could not be blinded to the intervention given, as the the application method of the intervention was completely different from that of the comparator. While the ideal outcome of any remineralizing agent or intervention is to eliminate the WSLs, a more realistic goal would be to reduce WSLs to levels that are acceptable to the patient. Regarding the assessment periods, the trial assessment periods should be enough to allow expression of the maximum effectiveness of the intervention used. In this study, the evaluation periods were at the baseline and after 1, 3 and 6 months of the intervention.

The results of this study showed that the baseline data using DIAGNOdent had the highest mean and SD for GSE (Intervention A; 12.7±2.2), and CPP-ACP (Intervention B;10.3±4.4) were consistent with the results of Mendes et al. and Silva et al. [19, 20]. They attributed this increase in values

to enhanced porosity in enamel surface due to a decrease in overall mineral content. Furthermore, was in agreement with the results of Mohanty et al. [21], which indicate a significant loss of Ca, P, and F ions, during the demineralizing procedure. In all treatment groups, improvements in DIAGNOdent scores for enamel lesions were seen at 1 month and these lesions continued to improve throughout the 6 months, which was the end point of the study. The significant gain in fluorescence, accompanied by a decreased lesion area compared with the baseline, indicates that improvement occurred progressively over time as remineralization occurred.

On comparing the DIAGNOdent value of GSE group after one month to its baseline DIAGNOdent value, it was evident that a significant amount of remineralization had occurred. These results were in agreement with the results of[8, 17, 22], which showed that GSE can increase the remineralization of carious enamel lesions. They suggested that proanthocyanidins (PAs) and gallic acid present in the GSE are responsible for facilitating mineral deposition on enamel, for it could be an effective natural agent for non-invasive dentistry.

Bedran-Russo et al. [16] demonstrated that GSE, as a collagen cross-linker, increased the stiffness of demineralized dentin in a study on changes in stiffness of demineralized dentin following the application of collagen cross-linkers. Furthermore, GSE has been shown to improve the ultimate tensile strength of demineralized dentin. Acil et al. [23] showed in his study done on enamel matrix degeneration that this is not the case and that Type I collagen is found in enamel. However, the concentration of collagen in enamel was considerably lower as compared to that in dentin. Furthermore,[24] found that Type X collagen is one of the candidate molecules present in the enamel matrix, which might be involved in mineralization of enamel in his study, Type X collagen in human enamel development: A possible role in mineralization.

Comparison of the mean DIAGNOdent value of CPP-ACP group after 1 month with its baseline DIAGNOdent value showed that there were quantitative changes. This is consistent with a previous in vitro remineralization study of Wu et al. [25], which showed that the combination of fluoride toothpaste and CPP-ACP (Tooth Mousse) improves the remineralization effect. The reason could be attributed to the fact that CPPs have the ability to stabilize calcium phosphate on tooth surface, maintaining thereby high concentration gradients of calcium and phosphate ions and, thus, promoting remineralization of hard tissues. Kumar et al. [26] indicated that CPP-ACP remineralized initial enamel lesions and showed a higher remineralizing potential when applied as a topical coating after the use of fluoridated toothpaste.

The current results agreed with previous research of Thepyou et al. [27] that recommended multiple applications of MI paste with relatively long period of remineralization regime that may reach 4 weeks to obtain significant remineralization of demineralized enamel. In contrast to the findings of this study, Beerens et al. and Huang et al. [28, 29] reported that the application of a cream containing CPP-ACP or CPP-ACPF was not superior to brushing with a fluoride tooth paste for regression of WSLs.

For the first 3 and 6 months, no significant difference was found between the treatment groups and all showed parallel improvement. The change between 3 and 6 months was greater in the GSE group, which started with the higher mean and SD, than in the CPP-ACP group. On comparing values of DIAGNOdent readings for different interventions for quantitative changes of WSLs, there was no statistically significant difference between the mean and SD of GSE and the CPP-ACP groups. This could be explained by the fact that the DIAGNOdent does not directly show the exact amount of mineral content or quantify the degree of mineralization. These results were in line with those of other authors, namely [14, 19, 30], who found that the use of DIAGNOdent was not useful for assessment of remineralization. But it was contrary to Spiguel

et al. and Celiberti et al.[31, 32]. DIAGNOdent had some disadvantages, such as the fact that readings could also be affected by stains, calculus, and plaque and were based on bacterial metabolites [33]. Athough the DIAGNOdent readings should thus be regarded as an indirect measure of demineralization, the present results were in agreement with recent studies suggesting that DIAGNOdent can be used longitudinally to monitor changes of lesions. By the end of the discussion our null hypothesis was accepted that there is no significant difference between the reminerlization potential of GSE gel and CPP-ACP on caries white spot lesions. According to the data obtained in this study, after 6 month of application of GSE (Intervention A) and CPP-ACP (Intervention B) onto the tooth surface, it is possible to interpret that the GSE can decrease demineralization and/or encourage remineralization of WSLs in the enamel.

CONCLUSIONS

Under the limitations of this trial, the following conclusions could be mentioned:

- Both GSE and MI paste showed potential remineralization of WSLs in post orthodontic patients.
- The use of GSE within therapeutic agents may promote enamel remineralization of WSLs after orthodontic treatment.

As this clinical trial found that GSE was similar effective as CPP-ACP at remineralizing WSLs, high-quality, well-designed clinical protocols are required to allow for definitive recommendations and consensus.

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