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The Synergetic Effect of Silver Diamine Fluoride with Potassium Iodide and Grape Seed Extract on Dentin Remineralization

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ABSTRACT

Purpose: This study was conducted to evaluate the synergetic effect of using silver diamine fluoride containing potassium iodide and grape seed extract on dentin microhardness. **Materials and Methods:** Sixty freshly extracted human deciduous second molars were enrolled in this study. Sixty dentin discs were cut from the crown and were randomly divided into four groups according to the applied treatment after dentin demineralization, (15 samples each). In the first group, samples were received no treatment, only stored in artificial saliva (positive control), in the second group, samples were treated with silver diamine fluoride containing potassium iodide only, in the second group, samples were treated with grape seed extract only (GSE), in the third group, samples were treated with silver diamine fluoride containing potassium iodide (SDF), whereas in the fourth group, samples were treated with combined therapy of grape seed extract followed with silver diamine fluoride containing potassium iodide (GSE+SDF). Microhardness measurements were taken for each dentin disc at baseline, after demineralization, and after treatment. **Results:** There was a statistically significant increase in dentin microhardness after each treatment as compared to the demineralized dentin microhardness. The mean percent change of dentin microhardness was statistically significant increased in GSE, SDF, and GSE+SDF groups when compared to the control group. **Conclusions:** Considering the limitations of this study, monotherapy of GSE, SDF solution, and their combination can be recommended to enhance the micro-hardness of demineralized dentin. SDF applications after GSE improved the micro-hardness of demineralized dentin when compared to the monotherapy of each treatment.

KEYWORDS

*Grape seed extract,
Silver diamine fluoride,
Synergistic effect,
Microhardness,*

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INTRODUCTION

Caries of dentin begins with dissolution of the inorganic substances, hydroxyapatite crystals $\text{Ca}_5(\text{PO}_4)_3$, by organic acids, mainly lactic acid⁽¹⁾. By progression of caries, the organic component of dentin is degraded by some bacterial toxins and intrinsic protease system^(2,3). All these alterations lead to a decrease in the microhardness value of dentin⁽⁴⁾. Dental caries is a dynamic process of demineralization and remineralization, so one of the most important approaches regarding preventive therapies for caries is to enhance the remineralization process.

Contemporary minimal invasive dentistry strategy is increasingly directed toward; removal of the infected and non remineralizable outer layer of carious dentin and remineralization of the affected carious one using either solution chemistry or more essential tissue regeneration strategies⁽⁴⁾.

Dentin is the main bulk of the tooth contains about 70% inorganic substance, 20% organic substance, and 10% fluid. 90% of the organic matrix is a fibrillar type I, whereas the remaining 10% consists of non-collagenous proteins like phosphoproteins and proteoglycans. The collagen of dentin acts as a scaffold for precipitations of minerals, so it was essential for preservation and maintaining the stability of collagen during the re-mineralization process⁽⁵⁾.

The deeper layer of caries or the caries-affected dentin (CAD) is a remineralizable layer and contains a few cariogenic bacteria. There is evidence reported that, mineral precipitation and distribution within dentin can be affected with the condition of collagen matrix^(6,7). So, dentin biomodification modalities should be directed at the remained bacteria, healing of collagen matrix, and substitution of dissolved minerals. Potential therapeutic agents that enhance collagen matrix modifications should be regarded to improve the firmness of collagenous component of dentin matrix. Cross-linking agents could enhance the mechanical properties of collagen matrix and

its resistance to progression of dentin caries⁽⁶⁻⁸⁾. Another modality to enhance collagen matrix firmness is inactivation of MMPs enzyme in carious dentin^(9,10).

One of the most common occurring natural products is proanthocyanidin (PA), which available in vegetables, fruits, flowers, and nuts. It enhances the synthesis of collagen and modulates the remineralization and demineralization process of dentin⁽¹¹⁾. Silver (Ag) has an anti-microbial and anti-cariogenic effect. Combined therapy of both silver and fluoride was recommended for their synergetic effects of being anti-cariogenic and potentially enhancing remineralization of dental caries⁽¹²⁾. On the other hand, the chemical stability of silver diamine fluoride (SDF) is better compared to the old compounds, silver fluoride⁽¹³⁾.

Silver diamine fluoride (SDF) improves the resistance of hard tooth tissue against caries by enhancement of remineralization, inhibit the progression of the demineralization process by acids⁽⁶⁾, also it was believed to reduce the adherence of cariogenic micro-organisms on tooth surfaces⁽¹³⁾. It was believed that, the potent anti-cariogenic of silver diamine fluoride (SDF) against cariogenic bacteria has been related to its contents of metallic silver ions⁽¹⁴⁾.

Silver diamine fluoride (SDF) is considered non-invasive, simple, and cost-effective strategy to inhibit the progression of the carious process^(15,16). However, it has many disadvantages including discoloration^(12,17), so manufacture directed toward addition of potassium iodide into silver diamine compounds to overcome this problem. However, literature is sparse in evaluating the synergetic effect of grape seed extract and silver diamine fluoride containing potassium iodide on dentin remineralization.

This study aimed to investigate whether a synergetic effect exists between GSE and SDF to enhance the micro-hardness of carious affected dentin. The null hypothesis ran as follows: monotherapy of

grape seed extract or silver diamine fluoride containing potassium iodide, and the combined therapy of these two agents together do not affect the micro-hardness of carious affected dentin.

MATERIAL AND METHODS

I- Materials

Grape seed powder which used in this study was purchased from Herb store USA.com. Wainut, CA91788, USA (909) 8956985. According to the data which was provided by the manufacturer, it was consisted of 95% proanthocyanidin (PA). Catechins were the main monomers available in proanthocyanidin (PA). In this study, 6.5% (w/v) solution in phosphate buffer (0.025 M KH₂PO₄, 0.025 MK₂HPO₄, pH 7.4) was used. Grape seed solution was prepared from the same lot of the extract. Fluoride concentration in grape seed solution was measured using a fluoride electrode (Thermo Scientific Orion Ion-selective Solid State Combination Electrodes, 960900, it was less than 0.01ppm.

Riva Star (Silver diamine fluoride) which was used in this study was purchased from SDI, Bayswater, Australia, 292792. It was consisted of two steps according to the data which was provided by the manufacturer; step (1); application of ;(Silver capsule): which contains 30-35% of silver fluoride and > 60% ammonia solution; step (2); application of (Green capsule): which contains saturated potassium iodide solution(KI).

II-Methods

Selection of teeth

60 sound, caries free, and freshly extracted human primary molars which were extracted for orthodontic reasons were enrolled in this study. Teeth which were have cracks or had any signs of hypoplasia were excluded from this study. The extracted molars were cleaned and stored in 0.1% thymol solution ⁽¹⁸⁾.

Preparation of dentin discs

A thickness of approximately 1mm dentin disc was obtained from the coronal dentin of each deciduous molar. The enamel of each deciduous molar was firstly removed using diamond disc mounted in cutting machine (Demco), then the disc was obtained by making a parallel cut which was perpendicular to the long axis of the tooth to deliver 1 mm disc thickness. Each disc was inspected carefully to ensure that they were free of coronal enamel or pulpal exposure ⁽¹⁹⁾. A rounded hollow plastic mold was used for embedment of the selected disc in self-cure acrylic resin to provide a method for holding the disc during experiment.

Preparation of artificial saliva.

Artificial saliva used in this study was prepared using 9g NaCl+0.24g CaCl₂+0.43g KCl + 0.2gNaHCO₃ all dissolved in 1 L of water .according to a previous study ⁽²⁰⁾.

Preparation of grape seed solution.

6.5 gr of GSE in the form of powder (Puritans Pride Inc, Oakdale, New York, United States) were dissolved in 100 mL of distilled water to prepare 6.5% GSE solution ⁽²¹⁾.

Preparation of artificial carious dentin.

Each sample was immersed in an individual sterile container containing demineralizing solution. It consisted of 2.2 mM CaCl₂, 0.05M lactic acid, and 0.5 ppm F adjusted to pH4.5 with 50%NaOH for 48 hrs ⁽²²⁾.

Microhardness measurements

Microhardness measurements were taken for each specimen before exposure to acid demineralization (baseline) and after demineralization. Surface microhardness of the specimen was determined using Digital Display Vickers micro-hardness tester with a vicker diamond indenter and 20X lens as represented by figure(1). The surface of each specimen was subjected to a load of 200g for 20sec.

Three indentations were made on the surface of each specimen. These indentations were equally placed over a circle and not closer more than 0.5mm to the adjacent indentation. A built in scaled microscope was used to measure the diagonals length of the indentations and Vicker values were converted into microhardness values.

For recording the Vickers hardness number (VHN), each specimen was applied at right angle to the long axis of the indenter in the micro-hardness testing machine (SCTMC, MHV-1000Z, PRC). Gradually, the test load was increased at a constant rate up to 25Kgs F/mm² in 20 sec. Three readings were recorded for each sample, and then the mean value was established as the Vickers hardness number (VHN).

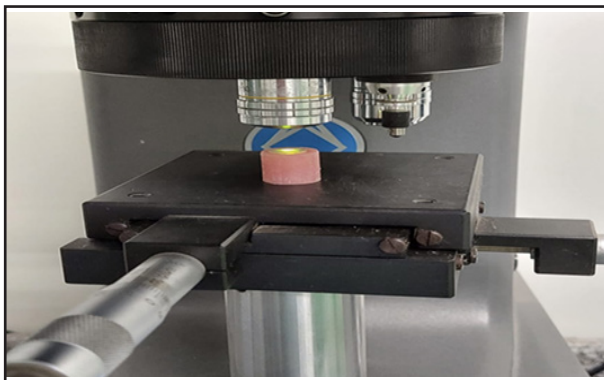


Figure (1): Measuring Vickers hardness number (VHN).

Exposure to the tested materials.

Sample grouping.

The demineralized dentin discs were rinsed thoroughly with deionized water, dried, and then randomly divided into four groups according to the applied treatment, (15 samples each). In the first group, samples were immersed in artificial saliva and received no treatment. In the second group, samples were immersed in 6.5% grape seed extract solution for 10 min⁽²¹⁾, in the third group, samples were treated with topical application of a 30% SDF solution for 3 min on the surface of dentin discs

according to the manufacture instructions, whereas in the fourth group, specimens were immersed in GSE solution firstly followed with topical application of SDF solution.

Storage of the specimens.

Each specimen was stored in a separate container containing artificial saliva at room temperature for 1 week⁽²³⁾. Artificial saliva was replenished every 24 hrs.⁽²⁴⁾. Three readings of micro hardness value of each specimen (MH) in all groups were recorded; the initial, demineralized, and final MH values. These readings were used to calculate the percentage of superficial remineralization (%MH) using the formula: %MH = (final MH - demineralized MH)/(initial MH – demineralized MH)×100⁽²⁴⁾.

Statistical analysis

Values were presented as mean, standard deviation (SD) and confidence intervals values. Kolmogorov-Smirnov test was used for exploring the normality of data. The results showed that the data were normally distributed, therefore, one way analysis of variance (ANOVA) test, followed with Tukey's post hoc test were used to compare dentin microhardness between all groups. Percent of superficial remineralization (MH%) of dentin was calculated according to a previous study⁽²⁴⁾.

$$(MH\%) = \frac{(\text{final MH} - \text{demineralized MH})}{(\text{Initial MH} - \text{demineralized MH})} \times 100$$

The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

RESULTS

I. Effect of each treatment on dentin micro-hardness (Intra group comparison)

Table (1) and figure (2) compare the absolute mean values of dentin microhardness at baseline, demineralization, and remineralization within each

group. Results showed that, there was a statistically significant increase in the mean values of dentin microhardness after treatment with GSE, SDF, and GSE+SDF, when compared to the demineralized dentin.

The mean value of dentin microhardness was statistically significant increased from 28.24±4.99 after demineralization to 52.93±4.51 after reminer-

alization by GSE, from 28.51±3.35 after demineralization to 51.13±4.63 after remineralization with SDF, and from 24.9±2.8 after demineralization to 56.62±4.87 after remineralization with GS+SDF. On the other hand, in the control group (artificial saliva), results showed that, there was no statistically significant increase in mean value of dentin microhardness from 29.43±5.24 after demineralization to 33.96±7.01 after treatment.

Table (1): Mean recorded values at baseline, after demineralization, and remineralization, standard deviations and results of One Way ANOVA test within each group.

Groups		Baseline	Demineralization	Remineralization	Within the same group	
					F	P
Saliva (control)		64.78 ^a ±3.8	29.43 ^b ±5.24	33.96 ^b ±7.01	48.85	0.00*
Grape seeds		66.72 ^a ±7.65	28.24 ^c ±4.99	52.93 ^b ±4.51	43.94	0.00*
SDF		65.7 ^a ±5.22	28.51 ^c ±3.35	51.13 ^b ±4.63	70.59	0.00*
GS+SDF		62.29 ^a ±6.39	24.9 ^b ±2.8	56.62 ^a ±4.87	67.67	0.00*
Between groups	F	0.411	0.877	14.16		
	P	0.748 ^{ns}	0.48 ^{ns}	0.00*		

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

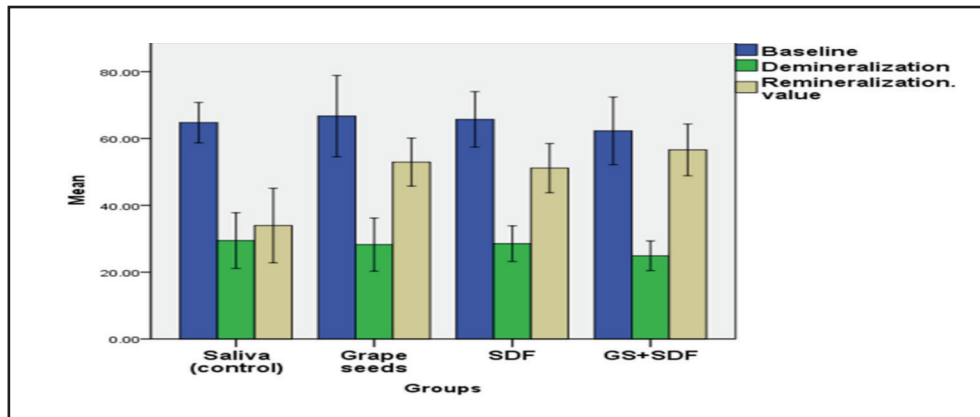


Figure (2) Bar chart illustrating mean recorded values at baseline, demineralization, and remineralization within each group

II- Interactions between different groups (Inter groups comparison)

Table (2) and figure (3) show the mean percent changes, standard deviation (SD) values and results of One Way ANOVA test for comparing mean values of dentin microhardness between different groups. Results showed, a statistical significant difference in the mean percent changes of mean dentin microhardness between the four groups, P value=0.000.

GSE+SDF group showed the highest statistically significantly increase in the mean percent changes of the mean value of dentin microhardness, this was followed by SDF and GSE groups, which showed lower a statistically significant increase in the mean percent changes of mean value of dentin microhardness, whereas the control group showed the highest statistically significant decrease in the mean percent changes of mean value of dentin microhardness.

Table (3) show the mean difference values and results of Tukey’s post hoc test for comparing the mean percent changes of the mean values of dentin microhardness between different groups. Tukey’s post hoc test revealed that, there was no a statistical significant difference between Grape seeds treated group and SDF treated group, whereas, there was a statistical significant differences between other paired groups.

Table (2): Mean, minimum, maximum, standard deviation (SD) values and results of one way ANOVA test for comparing the percent changes of the mean values of dentin microhardness test.

Groups	Mean	S.D	Min	Max	P
Saliva(control)	17.24 ^c	2.36	14.39	20.17	0.00*
GSE	64.61 ^b	6.66	58.32	72.47	
SDF	60.96 ^b	9.89	51.84	71.93	
GSE+SDF	85.03 ^a	5.52	77.87	90.13	

Significance level $p \leq 0.05$, *significant, sharing the same superscript letters are not significantly different

Table (3): The mean differences and results of Tukey’s post hoc test for comparing the mean percent changes of mean dentin microhardness between different groups.

Groups (I)	Groups (J)	Mean Difference (I-J)	Sig.
Saliva (control)	Grape seeds	47.37675*	0.000
	SDF	43.72050*	0.000
	GSE+SDF	67.78925*	0.000
Grape Seeds Extract (GSE)	Saliva (control)	47.37675*	0.000
	SDF	3.65625	0.864
	GSE+SDF	-20.41250*	0.005
Silver Diamine Fluoride (SDF)	Saliva (control)	43.72050*	0.00
	Grape seeds	3.65625	0.864
	GSE+SDF	24.06875*	0.001
Grape seeds Extract+ Silver Diamine Fluoride (GSE+SDF)	Saliva (control)	67.78925*	0.000
	Grape seeds	20.41250*	0.005
	SDF	24.06875*	0.001

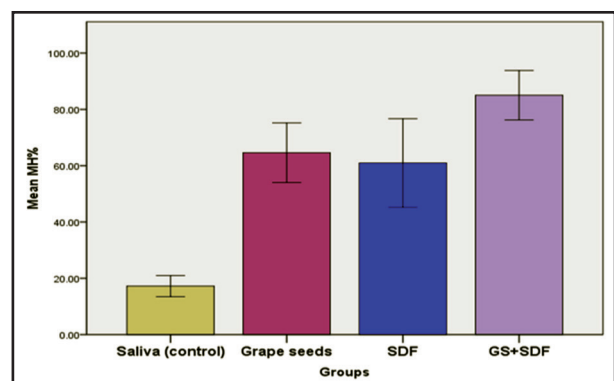


Figure (3) Bar chart illustrating mean value of MH% between different groups.

DISCUSSION

Caries lesions can be arrested by regulating the mineral balance favorably towards the remineralization. Dentin demonstrates great affinity towards the demineralization, this due to the little extent of dentinal crystallites and presence of dentinal fluid. After mineral disintegration of dentin during the demineralization cycle, the exposed organic matrices are additionally broken down by proteolytic enzymes, for example, bacterial derived collagenases and host derived matrix metalloproteinases⁽⁶⁻⁸⁾.

All these alterations drive the dentin deterioration to be an irreversible process and less well-suited to remineralization. Therefore, the management of dentin caries is much more challenging, highlighting an urgent need to seek novel and alternative strategies. Cross-linking agents could enhance the mechanical properties of collagen matrix and its resistance to caries progression⁽⁶⁻⁸⁾.

Grape seed extract (GSE) was used in this study because it contains proanthocyanidin (PA). Proanthocyanidin (PA) considered one of the most valuable natural cross linking agents that can enhance bonding to sound and carious affected dentin⁽²⁵⁾. It enhances the strength of collagen and inhibits its degradation with the activity of lytic enzymes.

The proposed interactions of PA with collagen may be one of the following mechanisms; covalent, ionic, hydrogen bonding, or hydrophobic interaction⁽²⁶⁾. Formation of calcium ion bridges between the telopeptide ends of collagen fibers and the dissolved hydroxyapatite crystals during demineralization cycle is one of the most accepted interactions. The second accepted interaction between PA and collagen fibers is hydrogen bonding between the amine carboxyl and the phenolic hydroxyl⁽²⁷⁾.

Silver diamine fluoride (SDF) was used in this study because it can arrest and prevent caries in both deciduous and permanent dentition in comparison to other intervention⁽²⁸⁾. Silver ions can completely infiltrate the demineralized dentin with further pen-

etration into the underlying mineralized dentin⁽²⁹⁾. It also, can inhibit enzymatic degradation of collagen fibers by MMPs activity⁽³⁰⁾. All these criteria make it useful in clinical trials for arresting caries.

Deciduous teeth were used in this study aiming to prevent early childhood caries due to difficulty and cost of treatment of such type of caries⁽³¹⁾. Demineralization of dentin surface in this study was performed by using demineralizing solution containing 2.2 mM CaCl₂, 0.05M lactic acid, and 0.5 ppm F adjusted to pH4.5 with 50%NaOH for 48 hrs, it was shown to be a reliable method for pretreatment of dental tissue to produce demineralization⁽²²⁾.

All samples were stored in artificial saliva which was renewed every 24 hrs to stimulate as possible, the conditions that are found in the oral cavity. It has been recorded that the artificial saliva could act as a chemical reservoir for calcium and phosphate ions promoting the remineralizing process⁽²³⁾.

Dentin constitutes the largest structure of the tooth and its mechanical properties considered a main determining factor in response of the tooth to the applied mechanical loading⁽³²⁾. To evaluate the bio-mechanical properties of dentin, micro-hardness measurements have been broadly used. Microhardness was utilized by previous study as an indirect method for detecting changes in mineral content that may reflect a decrease in mineral content⁽³³⁾.

Surface microhardness was assessed by recording the Vicker hardness number (VHN) of the specimens was at baseline, demineralization, and after application of the treatment. This assessment method provides relatively a simple, non-destructive and rapid method in evaluating the mechanical properties of dentin⁽⁹⁾. The null hypothesis of this study was rejected by its results. SDF, GSE, and SDF+GSE solutions were capable of enhancing the micro-hardness of demineralized dentin.

In this study, results showed that, the mean percent changes of mean dentin microhardness of GSE-treated carious dentin were statistically

significantly increased when compared to the control group. This may be contributed to the formation of insoluble complexes by GSE on dentin surface when blended with artificial saliva. This also may be explained by establishment of cross-links by GSE. These cross-links could improve the resistance of dentin surface to deformation by the indenter of microhardness tester.

GSE particles would link to the collagen, reinforcing the intertubular dentin to permit a more prominent remineralization process. These findings were confirmed with a previous study which evaluated the remineralizing effects of grape seed extract on artificial root caries⁽³⁴⁾, and recorded an increase in dentin hardness after application of GSE. Similar findings were recorded with previous study, which evaluated the remineralizing effect of grape seed on demineralized dentin. It proved that GSE successfully improved the mechanical properties of demineralized dentin and decreases its degradation⁽³⁵⁾.

The mean percent change of mean microhardness of SDF-treated carious dentin was statistically significantly increased compared to the control group. This can be explained by an increase in the mineral contents of dentin by extrafibrillar and intrafibrillar mineral formation. This was consistent with previous studies^(30,36), which recorded deposition of spherical grains by SEM investigations on demineralized dentin surface after treatment with SDF.

Those findings are confirmed with previous study⁽²⁹⁾, which recorded a complete infiltration of silver ions from SDF into the demineralized dentin with further penetration into the underlying sound dentin.

On the other hand, the greatest enhancement in the microhardness of demineralized dentin was recorded after applications of GSE followed with SDF solutions. This could be explained by the synergistic effect of both therapies. Furthermore, there is no other studies in literature has to our knowledge yet been investigated, which may agree with the current study.

However, the current findings are in contrary with previous study, which recorded that combined application of GSE followed with SDF solution showed the lowest statistically significant decrease of dentin hardness⁽²¹⁾.

These differences between the results of the current study and this previous study could be explained by two causes. The first explanation could be related to the chemical interactions of grape seed extract with fluoride⁽³⁷⁾. As complete interactions of SDF with hydroxyapatite of dentin could be interrupted by GSE which present on the surface of the specimens scavenge fluoride ions of SDF. The second explanation could be related to a rapid remineralizing process, in which ion-diffusion could be prevented into the deeper parts of the lesion by cross linked collagen matrix. This difference may be attributed to the different mechanisms used in these studies for remineralization.

CONCLUSIONS

Considering the limitations of this study, monotherapy of grape seed extract, silver diamine fluoride containing potassium iodide solution, and their combination can be recommended to enhance the micro-hardness of demineralized dentine. Silver diamine fluoride applications after grape seed extract (GSE) application improved the micro-hardness of demineralized dentine when compared to monotherapy of either treatment alone.

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