Original Article

In-Vitro Evaluation of the Effect of Herbal Antioxidants on Shear Bond Strength of Composite Resin to Bleached Enamel

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Abstract

Objectives: A reduction in bond strength of composite to bleached enamel has been reported immediately after bleaching treatment. Application of some antioxidant agents may decrease the adverse effects of whitening agents on bond strength and enhance composite bond to enamel. This study aimed to assess the effect of green tea, sodium ascorbate, sage and grape seed extract on bond strength of composite to bleached enamel.

Materials and Methods: In this in-vitro study, 90 human enamel surfaces were randomly divided into six groups as follows (n=15): G1, no bleaching; G2, bleaching with 40% hydrogen peroxide (HP); G3, HP+1000 µmol epigallocatechin gallate (EGCG) for 10 minutes; G4, HP+10% sodium ascorbate for 10 minutes; G5, HP+10% sage for 10 minutes and G6, HP+5% grape seed extract for 10 minutes. The specimens were bonded to composite in all groups. The shear bond strength of specimens was measured in Megapascals (MPa). Data were analyzed using one-way ANOVA and Tukey's HSD test (α=0.05).

Results: The highest and the lowest mean shear bond strength values were observed in group 1 (22.61±3.29MPa) and group 2 (5.87±1.80MPa), respectively. The reduction in bond strength in group 2 was greater than that in other groups ($P<0.001$). No significant difference was found among groups 1, 3, 4, 5 and 6 ($P>0.05$).

Conclusions: All the herbal antioxidants used in this study equally compensated for the reduced bond strength of composite to bleached enamel.

Keywords: Antioxidants; Tooth Bleaching; Composite Resins; Shear Strength

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INTRODUCTION

Demand for esthetic dental restorations has significantly increased. Patients pay more attention to smile esthetics and their appearance than before. Thus, treatment of tooth discolorations has become increasingly popular [1]. Bleaching is the most commonly used technique for treatment of discolored teeth. Bleaching agents often contain variable concentrations of carbamide peroxide or hydrogen peroxide (HP) [2,3].

Increased microleakage of composite restorations is one side effect of bleaching treatments, which occurs due to the presence of peroxide and free radicals of oxygen. These free radicals interfere with resin polymerization and inhibit the function of adhesive systems [4]. This inhibitory effect decreases over time. Researchers recommend postponing the composite restorations for one to four weeks after a bleaching treatment to achieve adequately high bond strength similar to baseline values before treatment [4-8]. One method to enhance the bond strength of composite to bleached enamel is application of antioxidant agents [9-11]. Antioxidants absorb the free oxygen radicals and increase the redox potential of the enamel surface [12,13]. Many studies have evaluated the effect of ascorbic acid and its salts at different concentrations and time durations on the bond strength of composite to bleached enamel [13-17]. Despite studies reporting the positive efficacy of sodium ascorbate for increasing the bond strength of composite to bleached enamel

[13-17], one study showed that sodium ascorbate had the potential of forming a three-dimensional, porous physical scaffold that would entrap the pathogenic microorganisms like Streptococcus mutans and this adverse property affected its efficacy for enhancing the bond strength and highlighted the need for other antioxidants [18].
Grape seed as an herbal antioxidant contains pro-antioxidant compounds and is capable of eliminating free radicals [3]. Studies have shown that polyphenols present in green tea are rapidly metabolized and show antioxidant activity [19,20]. Catechins present in green tea such as epigallocatechin gallate (EGCG) are antioxidant compounds that can eliminate free radicals as well [21]. Sage extract also has antioxidant capacity [22]. Previous studies have shown that these herbal antioxidants can reverse the decreased bond strength of composite to bleached enamel [17,22,23]. However, some studies have reported different efficacy values for herbal products in various application times [24-28]. Also, a large number of studies have compared only one or two herbal antioxidants with sodium ascorbate [23,26,27]. In order to better assess the efficacy of herbal and chemical antioxidants, this in-vitro study was carried out to assess the effect of sodium ascorbate, grape seed, sage and green tea extracts on the bond strength of composite to bleached enamel.

MATERIALS AND METHODS

In this in vitro experimental study, 90 maxillary anterior teeth extracted within the past three months were selected. The teeth were evaluated under a stereomicroscope at ×7 magnification to ensure absence of cracks, caries or restorations. The teeth were cleaned using a periodontal curette and immersed in 10% formalin solution (Shahid Ghazi Co., Tabriz, Iran) at room temperature for one week. The teeth were stored in distilled water (Shahid Ghazi Co., Tabriz, Iran) 24 hours prior to the experiment. To prepare the specimens, the roots were cut at the cementoenamel junction using a diamond bur (≠878-016M, SS White Inc., Lakewood, USA) and high-speed handpiece under water and air spray. The pulp tissue was removed by an excavator and the cavity was filled with composite resin (Filtek Z250, 3M ESPE, St. Paul, MN, USA). The labial surface of the teeth was polished with 400 and 600 grit silicon carbide papers, respectively to obtain a smooth, uniform enamel surface in all specimens. Then, the teeth were randomly divided into six groups as follows (n=15):
G1: No bleaching (control group),
G2: Bleaching with 40% HP gel,
G3: Bleaching with HP followed by 1000μmol EGCG solution for 10 minutes,
G4: Bleaching with HP followed by 10% sodium ascorbate solution for 10 minutes,
G5: Bleaching with HP followed by 10% sage solution for 10 minutes, and
G6: Bleaching with HP followed by 5% grape seed extract for 10 minutes.

***Preparation of antioxidants*:** The sage solution was prepared using the maceration technique; 50g of the dry plant material was weighed by a digital scale (Lib ROR AEU-210, Shimadzu, Kyoto, Japan), powdered and transferred to an Erlenmeyer flask. Next, 1500cc of ethanol solution (50% of 96% ethanol and 50% water) was added. The flask was covered with aluminum foil and placed in a shaker (Unimax 2010, Heidolph, Wetzlar, Germany) operating at 90 rpm for 48 hours. The homogenous mixture of plant and solvent was filtered using a 0.5mm filter paper (Whatman, Sigma Aldrich Co., St. Louis, USA). The solution was then transferred to a rotary evaporator (WD 2000, Heidolph, Wetzlar, Germany) to separate the solvent from the extract. Of 10mL extract obtained, 10% solution was prepared using the dilution technique [22]. To prepare grape seed extract, dry grape seed was ground and 5g of the powder was weighted by a digital scale (Lib ROR AEU-210, Shimadzu, Kyoto, Japan) and transferred to

an Erlenmeyer flask; 20mL of distilled water was added to dissolve the powder. The mixture was placed on a shaker (Unimax 2010, Heidolph, Wetzlar, Germany). The homogenous mixture was filtered using a 0.5mm filter paper (Whatman, Sigma Aldrich Co., St. Louis, USA). Distilled water was added to reach a volume of 100mL in order to obtain 5% grape seed solution [23]. To prepare 1000µmol solution of EGCG, 95% EGCG powder (Sigma Aldrich Co., St. Louis, USA) was dissolved in distilled water.

For preparation of 10% sodium ascorbate, 10mg of sodium ascorbate powder was dissolved in 50mL of distilled water and the volume of solution was reached to 100mL [17]. After preparation, all solutions were refrigerated until the experiment.

Bleaching protocol: In groups 2-6, 40% HP (Power Whitening YF White Smile GmbH, Birkenau, Germany) was applied three times to the enamel surfaces for 15 minutes according to the manufacturer's instructions. The gel was refreshed every 10 minutes. After completion, the specimens were rinsed with water and air spray and air-dried.

Application of antioxidants: Following bleaching, 1000µmol solution of EGCG, 10% sodium ascorbate, 10% sage and 5% grape seed extract were applied to the surface of specimens in groups 3, 4, 5 and 6, respectively for 10 minutes using a microbrush. To compensate for the evaporated solution, the solution applied to the surface was refreshed every one minute. Next, the surfaces were rinsed under running water and gently air-dried.

Composite resin bonding: To do a composite build-up, 37% phosphoric acid (Ultra etch, Ultra Dent, South Jordan, USA) was applied to the surface of specimens for 30 seconds, rinsed for 15 seconds and dried for 10 seconds. Etch and rinse bonding agent (Single Bond, 3M ESPE, St. Paul, MN, USA) was applied to the specimen surface in two coats with a five-second interval by a microbrush, air dried with air spray for five seconds and light-cured for 20 seconds from one-millimeter distance using a light curing unit (Hilux LED 550, Benlioglu Dental, Ankara, Turkey) with a light intensity of 450 mW/cm^2. Clear cylindrical molds (tubes) measuring 4mm in diameter and 5mm in length were placed on the adhesive-coated surfaces. Light-cure composite resin (Filtek Z250, 3M ESPE, St. Paul, MN, USA) was incrementally applied in one-millimeter layers. Each layer was cured for 20 seconds from both sides of the clear cylinder. Clear molds were removed by a scalpel and specimens were immersed in distilled water at room temperature for 24 hours to ensure complete polymerization. The shear bond strength was measured using a universal testing machine (Santam, Tehran, Iran) at a crosshead speed of 0.5 mm/minute. Following shear bond strength testing, the fracture surfaces were evaluated under a stereomicroscope (Zoom 2000, Leica Co., Vernon Hills, IL, USA) at x40 magnification. Mode of failure was classified as adhesive (failure at the enamel/composite interface), cohesive (failure in the enamel or composite) and mixed (combination of adhesive and cohesive failures). The data were analyzed with SPSS version 16 (SPSS Inc., IL, USA) using one-way ANOVA and Tukey's HSD test. Significance level was set at 95% (α=0.05).

RESULTS

The lowest mean bond strength was observed in group 2 (5.87±1.80MPa) and the highest in group 1 (22.61±3.29MPa).

Table 1: Descriptive statistics of bond strength values (MPa) in the six groups (n=15)

Groups	Mean (±SD) bond strength (MPa)	Minimum	Maximum
1	22.61±3.29	17.50	29.00
2	5.78±1.80	3.40	10.10
3	20.07±1.45	17.80	22.30
4	20.35±2.89	15.50	25.80
5	20.31±2.38	16.80	25.30
6	20.71±2.15	17.60	24.00

SD: Standard deviation

Table 1 shows the mean and standard deviation (SD) values of shear bond strength in the six groups studied. One-way ANOVA revealed significant differences in bond strength among the six groups (P<0.001; Table 2).

Table 2: Results of one-way ANOVA

	Sum of squares	df	Mean square	F	Sig.
Between groups	2855.792	5	571.158	98.729	.000
Within groups	485.952	84	5.785		
Total	3341.744	89			

For multiple pairwise comparisons of the groups, the bond strength between group 2 and other groups was significantly different (P<0.001); but no significant difference was found in bond strength between group 1 and groups 3, 4, 5 or 6 (P>0.05). Also, there was no significant difference between groups 3, 4, 5 and 6 (P>0.05; Table 3). The results of mode of failure in the groups using a stereomicroscope are shown in Table 4. Group 2 had the highest frequency of adhesive failure while the mode of failure in other groups was almost similar.

DISCUSSION

Treatment of bleached teeth is challenging for dentists because they cannot immediately perform a resin restoration on bleached teeth due to the presence of oxygen or peroxide residues on the surface, since they prevent complete polymerization of adhesive resin [6]. However, by postponing the composite restoration for two weeks following bleaching, no reduction in bond strength would occur [5,15,17]. But, sometimes it is not possible for the patient to wait that long. Therefore, use of antioxidants like ascorbic acid or sodium ascorbate is one method to immediately increase the bond strength of composite to bleached enamel [8,10]. It has been proven that application of sodium ascorbate can result in accumulation of Streptococcus mutants on bleached surfaces [18]. Also, pH of sodium ascorbate is 1.8, which has adverse effects on tooth structure in clinical application. Short shelf life of sodium ascorbate solution or gel is another disadvantage of using it [26,27]. It has been demonstrated that use of herbal antioxidants such as green tea and grape seed is an effective alternative strategy for this purpose.

Table 3: Multiple pairwise comparisons of the six groups

Group (I)	Group (J)	Mean difference (I-J)	Standard error	Significance	95% Confidence interval	
					Lower bound	Upper bound
1	2	16.74800*	.87827	.000	14.1865	19.3095
	3	2.54133	.87827	.053	-.0202	5.1028
	4	2.26800	.87827	.113	-.2935	4.8295
	5	2.30800	.87827	.102	-.2535	4.8695
	6	1.90133	.87827	.265	-.6602	4.4628
2	3	-14.20667*	.87827	.000	-16.7682	-11.6452
	4	-14.48000*	.87827	.000	-17.0415	-11.9185
	5	-14.44000*	.87827	.000	-17.0015	-11.8785
	6	-14.84667*	.87827	.000	-17.4082	-12.2852
3	4	-.27333	.87827	1.000	-2.8348	2.2882
	5	-.23333	.87827	1.000	-2.7948	2.3282
	6	-.64000	.87827	.978	-3.2015	1.9215
4	5	.04000	.87827	1.000	-2.5215	2.6015
	6	-.36667	.87827	.998	-2.9282	2.1948
5	6	-.40667	.87827	.997	-2.9682	2.1548

*The mean difference was significant at P<0.05.

Table 4: Fracture mode in the six groups

Group	Adhesive N(%)	Cohesive N(%)	Mixed N(%)
1	9(60)	2(13)	4(27)
2	14(93)	0(0)	1(7)
3	9(60)	3(20)	3(20)
4	10(57)	2(13)	3(30)
5	9(60)	2(13)	4(27)
6	9(60)	3(20)	3(20)

Several studies have indicated the anticariogenic, antibacterial and antioxidant effects of these herbal extracts [4,6,17]. In addition, herbal products are non-cytotoxic, easily available and affordable with long shelf life. Several studies have indicated that some of these herbal products have antioxidant properties 20 times greater than that of sodium ascorbate [4,29].

In the current study, 1000µmol EGCG, 10% sodium ascorbate, 10% sage and 5% grape seed extract were used because previous studies have demonstrated that these concentrations of the respective compounds can increase the bond strength of composite to bleached enamel to the level of unbleached samples [4,10,17,22,23]. Duration of 10 minutes for the application of antioxidants was selected in the current study according to Turkun and Kaya [6], Kaya and Turkun [8], Vidhya et al, [4] Khamverdi et al, [17] and Khamverdi and Safari [22].

Different bleaching agents are used for tooth bleaching depending on the technique. In the current study, 40% HP was used, which is commonly used for in-office bleaching. Some previous studies used other concentrations (10 to 30%) of HP [17,23,30].

The results of the current study showed the lowest bond strength in group 2. Also, significant differences were observed in bond strength between group 2 and other groups. It means that tooth bleaching results in a significant reduction in bond strength.

Reason of this reduction can be related to production of free oxygen radicals in the process of bleaching, which can decrease the bond strength of composite to enamel. Some researchers indicated a reduction in calcium content of the enamel and a reduction in the enamel surface microhardness, which decrease bond strength [13,18]; while other studies confirmed that oxygen residues create porosities in the enamel surface and negatively affect the bond strength [13,17,20,31]. In the current study, the results showed significant differences in bond strength between group 2 and groups 3-6. These results can be attributed to the antioxidant activity of green tea, sodium ascorbate, sage and grape seed. Lambert and Elias [9] concluded that EGCG had antioxidant activity. The antioxidant activity of EGCG is due to its chemical formulation and polyphenolic nature. Each molecule of this material includes 4 rings of A, B, C and D. Tri-hydroxyl and di-hydroxyl groups of B ring are believed to be responsible for the antioxidant property of this material. Polyphenols inhibit excess generation of reactive oxygen species, increase the degree of polymerization and enhance the bond strength of resin to bleached enamel [32]. Similarly, in our study, green tea increased the bond strength of composite to bleached enamel, which is in line with the results of Khamverdi et al, [17] and Lambert and Elias [9]. However, Ozelin et al, [26] indicated that green tea had positive antioxidant effect after 60 minutes; in shorter time periods such as 15 and 30 minutes, it did not increase the bond strength. Moreover, Berger et al, [25] evaluated the effect of green tea as an antioxidant on bond strength of composite to tooth structure after bleaching for one and six hours and reported that green tea improved the reduced bond strength. Proanthocyanidin is a plant metabolite containing monomers, oligomers and catechin polymers. It is commonly found in fruit seeds. Proanthocyanidin has strong antioxidant activity and is capable of eliminating free radicals. In medicine, it is used for its anti-allergic, antibacterial and anticariogenic properties [29]. Grape seed extract contains 98% proanthocyanidin, which is currently used as a

nutritional supplement [33]. Our study also showed the antioxidant activity of grape seed. Its possible mechanism is via the presence of donor sites on oligomeric proanthocyanidin complexes that enhance free radical scavenging and increase the antioxidative effect by esterification of polyphenols in proanthocyanidin complexes [28]. Therefore, it is capable of eliminating free radicals and can increase the bond strength of composite to bleached enamel. This finding was confirmed by Vidhya et al [4].

Subramonian et al, [24] reported that herbal products such as pine bark and grape seed extract can compensate for the reduced bond strength of composite to bleached enamel, and pine bark had a greater efficacy than grape seed; the results of our study demonstrated similar antioxidative efficacy of green tea and grape seed, which was in agreement with the results of Sharafeddin et al [23]. However, sage was not evaluated in their study. The current study showed that sage had antioxidant activity and increased the microshear bond strength of composite to bleached enamel. The mechanism of action of sage has yet to be fully understood; however, the antioxidant property of this material is due to its polyphenolic nature and chemical formulation. Polyphenols like caffeic acid, hispidulin, apigenin, rosmanol, carnosic acid, carnosol and ursolic acid are among the active ingredients of sage. Similar to other antioxidants, these polyphenols inhibit the formation of reactive oxygen species [22,34]. This finding was in accord with the results of Khamverdi and Safari [22]. Abraham et al, [27] compared the antioxidant property of sodium ascorbate and grape seed and concluded that application of grape seed had greater efficacy for reversal of compromised bond strength of composite to bleached enamel; whereas, Arumugam et al, [28] reported opposite results. It is stated that high molecular weight of proanthocyanidin is an important factor responsible for its less penetration into tooth structure and reducing oxygen free radicals. The findings of our study showed equal antioxidant efficacy of grape seed and sodium ascorbate, which is in accord with the results of Vidhya et al, [4] and Sharafeddin et al, [23]. Two in-vitro studies demonstrated that grape seed extract inhibited the demineralization and enhanced the remineralization of carious root lesions [4,33]. The results of microscopic analysis confirmed the findings of shear bond strength testing in our study, since group 2 with the lowest bond strength value had the highest frequency of adhesive failure. In the current study, antioxidants were applied in the form of solution. Use of antioxidants in the form of hydrogel is recommended in future studies. In the current study, antioxidants were used for 10 minutes. Other studies are required to assess the effect of higher concentrations of antioxidants with shorter application times. Since antioxidants have a short shelf life, future studies are recommended to focus on the storage methods of antioxidants. The current study had an in-vitro design and clinical studies are needed to further elucidate this issue.

CONCLUSION

Within the limitations of this study, tooth bleaching significantly decreased the bond strength of composite to enamel. All the herbal antioxidants used in this study equally reversed the reduced bond strength of composite to bleached enamel.

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