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Evaluation of The Antioxidant Effect of Gallic Acid on Micro Tensile Bond Strength and Mode of Failure of Resin Composite to Bleached Enamel: An in - Vitro Study

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ABSTRACT:

Background: Antioxidants have been used in the literature to counteract the decreased bond strength that followed the immediate bonding to bleached enamel.

Objectives: To assess the effect of gallic acid pretreatment on the microtensile bond strength and mode of failure of resin composite bonded to bleached enamel.

Methods: Thirty-two extracted impacted third molars were sectioned into buccal and lingual halves. Each half was mounted on an acrylic block, then enamel surface was flattened. Samples were divided randomly into four main groups, an unbleached control group (G1), two-week delayed bonding group after bleaching (G2), sodium ascorbate group (G3) and gallic acid group (G4) (application of sodium ascorbate and gallic acid solutions, respectively, after bleaching). After bonding and resin composite application, samples were preserved in distilled water for 24 hours. Half of each group's samples were thermocycled followed by microtensile bond strength testing. A digital stereomicroscope was used to examine the failure modes.

Statistical analysis: Data were analyzed using two-way ANOVA, Bonferroni post hoc, Shapiro–Wilk, Levene, and Chi-square tests.

Results: Before thermocycling, the highest bond strength was recorded in control group with no significant differences between control, two-week delay groups. In contrast, the lowest value was recorded in gallic acid group with significant difference compared to control group. All groups showed significantly lower bond strength with thermocycling. Adhesive failures were the predominant failure mode.

Conclusions: Gallic acid pretreatment following bleaching resulted in bond strength statistically comparable to sodium ascorbate or 2-week delayed bonding but not to the level of unbleached teeth.

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INTRODUCTION:

Tooth bleaching has been reported to be a safe and common therapeutic approach for teeth discoloration. The active agent in bleaching agents, hydrogen peroxide, decomposes into highly reactive free radicals such as per-hydroxyl, superoxide anions, nascent oxygen, and hydroxyl radicals which interact with the tooth tissues' electron-rich pigments, and this causes large, pigmented molecules to decompose into smaller and less darker molecules. [1] Unfortunately, bleaching alters the enamel structure in three ways, as previously shown in the literature: mineral loss, morphological, and surface microhardness changes. These variations are concentration and time dependent. [2] More seriously, the presence of free radicals obstructs the polymerization of resin composite and the function of adhesive systems. [3] However, delaying bonding procedures from a one to four weeks could restore the bond's strength but it is clinically inconvinient. [4] Antioxidants have been suggested as a remedy that enables scavenging of leftover oxygen and immediate bonding. [5]

Sodium ascorbate is considered as the "gold-standard" when evaluating the efficacy of currently launched antioxidants used after bleaching to reverse the decline of bond strength. It is a neutral, biocompatible and potent antioxidant. [6] Gallic acid, a potent antioxidant with radical scavenging activity and also remineralizing potential, has been successfully used as an irrigant after sodium hypochlorite irrigation during endodontic treatment to remove liberated oxygen resulting from the use of sodium hypochlorite therefore enhancing polymerization and penetration of resin-based sealer into dentinal tubules. [7]

Therefore, this study was concerned with evaluating the antioxidant effect of gallic acid on micro tensile strength of resin composite bonded to bleached enamel. This study's null hypothesis claimed that there were no significant differences between the effect of gallic acid as antioxidant on micro tensile bond strength (μTBS) and failure mode of resin composite to bleached enamel in comparison to other tested groups.

MATERIALS AND METHODS:

This study is an in-vitro study which was carried out after being exempted from the Research Ethics Committee (no. 442/2022). Because T0 and T1 were separate specimens, the appropriate sample-size framework for μTBS is an independent 4 × 2 fixed-effects factorial ANOVA (G*Power: F tests, ANOVA: fixed effects, special, main effects and interactions). The μTBS dataset comprised 64 independent specimens: eight per treatment × thermocycling cell and 16 per treatment group. At α=0.05, N=64 provides 90% power to detect a large 3-df treatment or interaction effect of approximately f=0.49. Calculations were performed using G*Power version 3.1.9.6. [8–10]

Thirty-two intact, freshly extracted impacted third molars were used. Each tooth was sectioned into two halves to yield 64 samples, consistent with sample size calculations.[8–10] Teeth were sound, free of cracks, caries, hypomineralization, or developmental anomalies. [11] After cleaning and polishing, specimens were kept in saline containing 0.5% thymol for no more than three months until being used. [12] Surfaces were examined under digital stereomicroscope (×40) to exclude teeth with flaws. [13] All teeth were cut 2 mm beneath the cemento-enamel junction to separate the roots from the crowns then each crown was sectioned in mesiodistal direction to get buccal and lingual halves using an automatic water-cooling diamond saw (Isomet 4000, Buehler Ltd., Lake Bluff, USA). Both halves were used, and each half was considered as the study's statistical unit. Each half was embedded in acrylic blocks with enamel surfaces facing upwards and flattened using silicon carbide paper. [10]

Samples were randomly allocated into four groups (n=16 each): G1: Positive control (no bleaching), G2: Bleaching followed by two-week delay before bonding, G3: Bleaching followed by sodium ascorbate treatment and G4: Bleaching followed by gallic acid treatment. Furthermore, each group was divided into subgroups (n=8) without or with thermocycling (T0, T1).

Ten grams of sodium ascorbate powder were dissolved in 100 ml of distilled water to create a 10% sodium ascorbate solution. [14] Also, ten grams of gallic acid powder were dissolved in 100 ml of ethanol to create a 10% gallic acid solution. [15] Both were freshly prepared at the Faculty of Science.

Restoration of the no bleaching group (G1) was performed without any bleaching to the samples' enamel surfaces and therefore it acted as a positive control group. This group's bonding process was carried out in compliance with the manufacturer's instructions as follows: A 37% phosphoric acid etchant gel was used to etch the enamel surface for 30 seconds (Meta® Etchant, Meta Biomed Co, Ltd., Cheougja City, Chungbuk, Korea), then rinsed for 30 seconds and gently dried. The enamel surface was subsequently coated with two layers of the bonding agent (Adper Single Bond 2; 3 M ESPE, St. Paul, MN, USA), gently air dried, then light cured using (LED) curing light (Woodpecker iLED Max Light Cure, Woodpecker, Guilin, China) with an

intensity of 1000 mW/cm² for 20 seconds. After that, a teflon mold (5 mm × 4 mm × 6 mm height) was inserted in the central part of the enamel surface then filled with three layers of nanohybrid resin composite (Filtek Z250XT, 3M ESPE, St. Paul, MN, USA). Each 2 mm layer was incrementally inserted and light cured for 20 seconds then the mold was removed. [16]

Meanwhile, for the preparation of samples of the two-week delay group (G2) group, enamel surface was subjected to bleaching treatment utilizing bleaching gel with 40% hydrogen peroxide (power whitening; white smile, GmbH, Birkenau, Germany) following manufacturer's instructions as follows: a thick layer of the bleaching gel was administered for 15 minutes then removed by suction. This procedure was carried out three times. After that, it was washed away with distilled water, dried using a compressed air then stored for two weeks in distilled water.[14] Following that, bonding process and the build-up of resin composite were carried out as described for the positive control group.

The samples of groups (G3 & G4) were subjected to bleaching treatment as described before following manufacturer's instructions then sodium ascorbate and gallic acid solutions were applied, respectively, on the bleached samples using a syringe. Application of ten milliliters of each antioxidant solution was done to each sample's bleached enamel surface for ten minutes. A syringe was used to inject 1 ml of freshly prepared solution onto the sample every minute to maintain the enamel surface moist. Following that the surfaces were being rinsed then dried with air. [14] After that, the bonding process and build up of resin composite were performed immediately as described before and then all tested samples were kept at room temperature in distilled water for 24 hours. [10] All materials utilized are listed in [Table.1].

After storage of all prepared samples for 24 hours in distilled water, half of each group's samples were subjected to thermocycling in a water bath for 5000 cycles at 5°C, 55°C, and 20 seconds of dwell time at each temperature, with a 5-second break in between using a thermocycler (THE-1100SD Mechatronic thermocycler, Germany) to simulate six months of clinical service. [17]

Specimens were sectioned into stick-shaped beams containing resin composite, adhesive, enamel, and dentin and four central beams on average were obtained from each half.[18] Beams were mounted onto the universal testing machine using Geraldeli's jig with a load cell of 500 N. The beam was subjected to tensile stress at a cross-head speed of 0.5 mm/min until the bonding failure. By using a software (Bluehill Lite software, Instron, MA, USA), the microtensile bond strength (μTBS) was measured in MegaPascal, and averages per half-tooth were used for statistical analysis. [10,19]

Post-testing, beams were examined using digital stereomicroscope (×40) to detect the failure mode. It was described as adhesive when a failure existed at the enamel/resin composite interface, cohesive in enamel when the failure existed through the enamel, cohesive in resin composite if the failure existed through the resin composite material, and mixed. Later on, within each group, each failure mode's percentage was determined. [20]

Statistical analysis:

All data were tabulated and statistically analyzed using a two-way analysis of variance (ANOVA) for an independent 4 × 2 factorial design, with treatment group (G1, G2, G3, G4) and thermocycling condition (T0, T1) entered as between-specimen fixed factors. Model residual normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene tests, respectively. When appropriate, Bonferroni-adjusted comparisons were used to compare treatment-group estimated marginal means and to compare T0 versus T1 within each treatment group. To determine the significant correlation between categories of failure mode analysis, Chi square test was used. Statistical significance was set at $P \leq 0.05$. Analyses were performed using SPSS for Windows version 26.0 (IBM Corp., Armonk, NY).

RESULTS:

I-Micro tensile bond strength test results:

Data in [Table.2] show the microtensile bond strength results for the four restorative interventions under the two independent thermocycling conditions. The data were analyzed using a 4 × 2 two-way ANOVA. The two-way ANOVA showed a statistically significant main effect of treatment group on μTBS, $F(3,56)=6.848$, $P<0.001$, partial $\eta^2=0.268$, and a statistically significant main effect of thermocycling, $F(1,56)=46.460$, $P<0.001$, partial $\eta^2=0.453$. The treatment group × thermocycling interaction was not significant, $F(3,56)=0.009$, $P=0.999$, partial $\eta^2<0.001$, indicating that the magnitude of the thermocycling-related reduction was similar across the four treatment groups. Model assumptions were acceptable (Shapiro–Wilk test of residuals, $P=0.378$; Levene test, $P=0.879$).

Across thermocycling conditions, Bonferroni-adjusted comparisons of estimated marginal means showed that G1 had significantly higher μTBS than G3 (adjusted P=0.043) and G4 (adjusted P<0.001), whereas G1 versus G2, G2 versus G3, G2 versus G4, and G3 versus G4 were not significant after adjustment. The descriptive means retained the same rank order in both conditions. Within each treatment group, μTBS was significantly lower at T1 than at T0 after Bonferroni adjustment.

II-Failure mode results:

II. a. Failure mode results regardless of thermocycling:

Data in [Table.3] represent different failure mode results regardless of thermocycling recorded in all tested groups in the form of adhesive failure, cohesive failure in resin composite, cohesive failure in enamel and mixed failure. The results revealed that adhesive failures were prevalent in all tested groups, with the gallic acid group (G4) experiencing the highest percentage of adhesive failures. Meanwhile, the lowest percentage of adhesive failures was recorded for the no bleaching group (G1). At the same time, the no bleaching group (G1) recorded the highest percentage of cohesive and mixed failures. Also, no significant differences between all tested groups at each mode of failure were found. Meanwhile within each tested group, the various modes of failure showed significant differences.

II. b. Failure mode results regarding thermocycling:

Data in [Table.4] represents different failure mode results regarding thermocycling recorded in all tested groups. Generally, no statistically significant differences were found across the groups for the parameters measured, indicating similar performance levels among the different applied interventions.

DISCUSSION:

Bleaching compromises resin bonding due to residual oxygen, demineralization, reduced surface energy, and collagen denaturation. [21] Morphological changes in resin tags and hybrid layers have been observed with bleaching such as fewer, shorter, fractured resin tags and poorly defined hybrid layers. [22] These changes hinder adhesive infiltration and polymerization. Alternative strategies such as ethanol pretreatment, the use of an adhesive containing an organic solvent, or removal of bleached enamel have limited success. [23] Antioxidant therapy has shown encouraging effects in recent years in reversing the decline of bond strength. [5]

The μTBS test offers the advantages of consistent stress distribution, tooth tissue preservation, regional difference reduction, irregular bonding interface detection, and simplicity of microscope observation for failure modes following fracture. [24] Thus, in the current study, the μTBS test was employed. Also, the beams used for the current test were stick-like and measured approximately 0.9 mm width x 0.9 mm thickness ± 0.1. This is important because it reduces cohesive failures in tooth substrate or composite and enhances stress distribution at the adhesive junction. [25] Also, flattening of enamel surface guarantees consistent stress distribution, producing more accurate and repeatable outcomes. [5]

The no bleaching group had the highest recorded mean value of μTBS of all tested groups as expected since it employed neither bleaching nor antioxidants and possessed all the qualities necessary for successful bonding with resinous material. Regarding the two-week delay group, it exhibited not significantly lower bond strength than the no bleaching group as the remaining oxygen gradually dissipates with time, reducing oxidation so resins might reach their desired and inherent bond strengths to levels comparable to those of unbleached enamel. This aligns with Zaki et al, 2023 [6] while contradicts Mena-Serrano et al, 2023 who reported higher bond strength of the two-weeks delay group than the no bleaching group with no significant difference between them. [5] The fact that they employed bovine teeth rather than human teeth could be the cause of this inconsistency.

Sodium ascorbate is a common antioxidant, as it can reduce several oxidative substances including free radicals. Sodium ascorbate 's antioxidizing properties helped to counteract and reverse the bleaching agent's oxidizing effects. Consequently, the oxidized bonding substrate's changed redox potential is restored, and the adhesive's polymerization proceeds. [1] In this study, sodium ascorbate resulted in bond strength comparable to the two-week delayed bonding after bleaching but significantly different with the mean values of control group and this aligns with Attia, 2021 [26] and Kini et al, 2026. [27] Nevertheless, this finding contradicts those of Elawsya et al, 2021, who found that the mean values of sodium ascorbate group were higher than those of the control group (no bleaching- no antioxidant). [14] This contradiction could be related to utilization of different testing methods as this study used the μTBS test, whereas theirs used the micro shear bond strength test.

Gallic acid, a natural phenolic acid found in plants such as grape seeds, tea leaves, and oak bark, has a strong radical scavenging activity, due to its three hydroxyl groups, with antibacterial, anticarcinogenic, and cholesterol-lowering properties. [7] In the current study, gallic acid group revealed the lowest bond strength with significant difference with no bleaching group. This could be explained by the phenolic chemicals' ability to prevent free radical polymerization of methacrylate monomers as explained by Ahmet et al, 2019 when they studied the impact of adding gallic acid to self-adhesive resin cements and found that the tested materials' biaxial flexure strength and Vickers hardness values were considerably decreased. [28] But this inhibitory effect was less obvious in this study, as gallic acid solution was applied to enamel surfaces then rinsed off and not incorporated into the resinous material, which explains the no significant difference of the gallic acid group with the two-week delay group and sodium ascorbate groups.

Additionally, gallic acid was dissolved in ethanol, which may have contributed to reduced bond strength. Ethanol- solved antioxidants, such as alpha-tocopherol, have been shown to be less effective, possibly due to their non-aqueous nature interfering with polymerization as found by Moradian et al, 2022. [29]

Also, the current study's findings revealed that the mean values of gallic acid group were not significantly lower than two-weeks delay group and sodium ascorbate group and this could be explained by the antioxidant effect of gallic acid and its radical scavenging activity. [30] However, the results of this study contradict those of Christopher et al, 2016, who found that 10% gallic acid was better than 10% ascorbic acid in enhancing the penetration of resin- based sealer into dentinal tubules in root canal after irrigation with sodium hypochlorite and this dispute could be due to the use of ascorbic acid rather than sodium ascorbate and the different methodology employed in their study. [7]

Thermocycling reduced bond strength across all groups, maintaining the same order of values. This reduction was attributed to stresses at the bonded interface caused by mismatched thermal expansion coefficients between resin and tooth structure. These stresses produce cracks and flaws, weakening adhesion. [31] Failure mode analysis revealed adhesive failures predominated across all groups and this predominance reflects the small beam size used in μTBS testing, which favors interfacial failure and correctly reflected the bond strength as an advantage of micro tests. [25] The correlation between adhesive failure rates and bond strength was evident: gallic acid showed the highest adhesive failures and lowest μTBS, while the control group showed more cohesive and mixed failures with higher μTBS.

The null hypothesis was partially rejected as there was a significant difference between the control group and sodium ascorbate and gallic acid groups while, there were no significant differences between sodium ascorbate, gallic acid, and the two-week delay groups or between the control group and the two-week delay group.

The study's limitations include the study of the effect of various pre-treatments on enamel rather than enamel and dentin as shown in clinical cases. Additionally, the samples obtained only from third molar dentitions due to their availability. Also, there is disagreement among experts about how long to apply antioxidants to bleached enamel for best effects. Furthermore, the impact of gallic acid on bond strength following enamel bleaching has not been examined in any previous research. Additionally, the bond strength test is dependent on the test's actual state, the sample's geometric shape and surface area, as well as the loading device's positioning and geometry, which can alter the bond's strength by affecting how stress is distributed at the bonded interface.

The effects of varying gallic acid solution concentrations on reversing the deterioration in the bonding strength between bleached enamel and resin composite require further in vitro research. Also, future laboratory studies are needed to evaluate the remineralizing effect of gallic acid on bleached enamel. Furthermore, Additional clinical research is required to assess sodium ascorbate's and gallic acid's effects as antioxidants after bleaching in the oral environment.

CONCLUSIONS:

Given the constraints of the current research, it was concluded that gallic acid pretreatment following bleaching resulted in bond strength statistically comparable to sodium ascorbate or 2-week delayed bonding but not to the level of unbleached teeth. The thermocycling procedures resulted in reduced bond strength. Regarding failure mode analysis, adhesive failures were the most prevalent among all tested groups.

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Tables

Table 1: The materials' trade names, descriptions, compositions, manufacturers and batch numbers.

Materials' trade names	Description	Composition	Manufacturer	Batch number
L- Ascorbic acid sodium salt	Antioxidant	L-Ascorbic acid sodium salt, 99%	Alfa Aesar, Ward Hill, Massachusetts, USA	10143187
Gallic acid	Antioxidant	3,4,5-Trihydroxybenzoic acid	Sigma Aldrich, Burlington, Massachusetts, USA	S8385649 408

Meta Etchant	Etchant gel	37% phosphoric acid gel	Meta Biomed Co., Ltd., Cheougja City, Chungbuk, Korea	MET2112071
Adper™ Single Bond 2	Two-step etch-and-rinse dental adhesive	Bis-GMA*, HEMA[¶], photoinitiator, dimethacrylates, water, ethanol,methacrylate, 10% wt of 5-nm diameter spherical silica particles and functional copolymer of polyacrylic and poly (itaconic) acids.	3M ESPE, St. Paul, MN, USA	NC87572
Filtek Z250XT	Nanohybrid resin Composite	Matrix: Bis-GMA; UDMA[†]; Bis-EMA[‡]; PEGDMA[§] and TEGDMA[‖]. Fillers: .Nonagglomerated/ nonaggregated 20 nm surface-modified silica particles Surface modified zirconia/silica with a median particle size of approximately 3 µm or less.The filler loading is 82% by weight (68% by volume)	3M ESPE, St. Paul, MN, USA	NE54896
White smile bleaching kit®	Chemically activated bleaching gel	40% hydrogen peroxide	White smile GmbH; Birkenau, Germany	210496

*Bis-GMA, Bisphenol A-glycidyl methacrylate; [¶]HEMA, 2-hydroxyethyl methacrylate; [†]UDMA, urethane dimethacrylate; [‡]Bis-EMA, bisphenol A ethoxylated dimethacrylate; [§]PEGDMA, Polyethylene glycol dimethacrylate; [‖]TEGDMA, triethylene glycol dimethacrylate

Table 2: Mean and standard deviation (SD) values of micro tensile bond strength test of all tested groups.

Groups	No thermocycling		Thermocycling		% change	t value	P value
	Mean	SD	Mean	SD			
No bleaching group (G1)	24.82[a]	4.64	18.75[a]	3.78	-24.46	3.385	0.005*
Two-week delay group (G2)	22.63[ab]	4.09	16.32[ab]	2.92	-27.88	3.519	0.003*
Sodium ascorbate group(G3)	21.33[b]	3.85	15.17[b]	3.08	-28.88	3.435	0.004*
Gallic acid group(G4)	19.13[b]	2.91	13.22[b]	3.02	-30.89	3.293	0.007*

	*Bonferroni-adjusted T0 versus T1 comparison significant at P<0.01. Different superscript letters indicate Bonferroni-adjusted treatment-group differences; groups sharing a letter are not significantly different.

Table 3 : Failure mode results regardless of thermocycling

Type of failure	No bleaching group (G1)	Two-week delay group (G2)	Sodium ascorbate group (G3)	Gallic acid group (G4)	P value
Adhesive	28 (43.75%)	36 (56.25%)	42 (65.625%)	43 (67.1875%)	0.280 ns¶
Cohesive in composite	11 (17.1875%)	10 (15.625%)	9 (14.0625%)	6 (9.375%)	0.669 ns¶
Cohesive in enamel	15 (23.4375%)	9 (14.0625%)	6 (9.375%)	9 (14.0625%)	0.222 ns¶
Mixed	10 (15.625%)	9 (14.0625%)	7 (10.9375%)	6 (9.375%)	0.741 ns¶
Chi square	12.875	33.37	56.62	61.125	
P value	0.004*	<0.001*	<0.001*	<0.001*	
P value (overall)	0.2741				

*; mean significant at P<0.05 by column.

¶ns; no significant difference P>0.05.

Table 4: Failure mode results regarding thermocycling

Type of failure		Groups				P Value
		No bleaching group (G1)	Two-week delay group (G2)	Sodium ascorbate group (G3)	Gallic acid group (G4)	
Adhesive	No thermocycling	12 -37.50%	16 -50%	20 -62.50%	21 -65.625	0.4
	Thermocycling	16 -50%	20 -62.50%	22 -68.75%	22 - 68.75%	0.753

	P value	0.449	0.5	0.757	0.878	
Cohesive in composite	No thermocycling	8 -25%	6 -18.75%	5 -15.63%	3 -9.38%	0.5
	Thermocycling	3 -9.38%	4 -12.50%	4 -12.50%	3 -9.38%	0.962
	P value	0.1317	0.527	0.738	1	
Cohesive in enamel	No thermocycling	8 -25%	5 -15.63%	3 -9.38%	4 -12.50%	0.423
	Thermocycling	7 -21.88%	4 -12.50%	3 -9.38%	5 -15.625	0.605
	P value	0.796	0.738	1	0.738	
Mixed	No thermocycling	4 -12.50%	5 -15.63%	4 -12.50%	4 -12.50%	0.981
	Thermocycling	6 -18.75%	4 -12.50%	3 -9.38%	2 -6.25%	0.506
	P value	0.527	0.738	0.705	0.414	
No significant difference at P>0.05						