



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

International Journal of Current Research
Vol. 12, Issue, 01, pp.9900-9906, January, 2020

DOI: <https://doi.org/10.24941/ijcr.37756.01.2020>

INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

RESEARCH ARTICLE

THE COMPARATIVE EVALUATION OF EFFECT OF THREE NATURAL ANTIOXIDANTS ON THE BOND STRENGTH OF COMPOSITE RESIN TO BLEACHED ENAMEL – AN IN-VITRO STUDY

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ARTICLE INFO

Article History:

Received 24th October, 2019

Received in revised form

20th November, 2019

Accepted 19th December, 2019

Published online 30th January, 2020

Key Words:

Bleaching, Tulsi leaf extract, Olive Leaf Extract, Pine Bark extract, Shear Bond Strength, Universal Testing Machine.

ABSTRACT

Background: Tooth discolorations frequently affect people of various age groups and are multifactorial in nature. Increasing demand in aesthetic dentistry has resulted in the widespread practice of extra-coronal bleaching or vital bleaching. An important complication following bleaching is decreased bond strength of composite resin to bleached enamel when bonding immediately after the bleaching process. The use of antioxidants immediately after bleaching showed increased bond strength to bleached enamel. Hence, the main aim of the present study was to determine the effect of antioxidants, namely 10% Tulsi leaf extract, 10% Olive leaf extract & 10% Pine Bark extract on the shear bond strength of composite resin to bleached enamel. **Materials & method:** Ninety freshly extracted human anterior teeth were divided into five groups: Group I (n=18) – bleaching with 35% hydrogen peroxide gel (without antioxidant), Group II (n=18) – bleaching with application of 10% Tulsi leaf extract, Group III (n=18) – bleaching with application of 10% Olive leaf extract, Group IV (n=18) – bleaching with application of 10% Pine Bark extract, and Group V (n=18) – control group i.e., no bleaching. These groups were further subdivided into two subgroups based on whether composite build-up was done immediately (Subgroup A) or after a delay of 2 weeks (Subgroup B). Shear bond strength of the specimens were tested using universal testing machine. Data were statistically analysed using Two-way ANOVA test & Tukey's multiple posthoc test and independent t test. **Result:** There were statistically significant differences between shear bond strength of control groups & experimental groups and also among the experimental groups. **Conclusion:** The use of antioxidants effectively reversed the compromised bond strength of bleached enamel. Among the natural antioxidants, 10% pine bark extract application after bleaching showed better bond strength.

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Citation: Dr. Amulya Vittal Rai and Dr. Balaram Damodar Naik. 2020. "The comparative evaluation of effect of three natural antioxidants on the bond strength of composite resin to bleached enamel – an in-vitro study", *International Journal of Current Research*, 12, (01), 9900-9906.

INTRODUCTION

A frequent reason for patients to seek dental care is discoloured anterior teeth (Amarlal, 2006). Tooth discolouration is becoming a great concern as more people are conscious and aware of dental aesthetics. Discoloured, missing, or malformed anterior teeth can lead to important aesthetic problems for patients (Korkut, 2013). This often leads in an effort to avoid smiling. Correction of such dental problems may often produce drastic change, which results in enhancement of appearance, self-confidence, and quality of life (Korkut, 2013).

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Various treatment options for discolored anterior teeth include the removal of surface stains, bleaching, microabrasion, macroabrasion, veneering, and placement of porcelain crowns. But, increasing number of patients do not want their teeth to be cut down for crowns. Hence, conservative approaches such as bleaching and veneers, are being preferred over crowns as they preserve as much of the natural tooth structure as possible (Ritu Rana, 2019). Bleaching, which can be performed either at home or in office, is considered as an effective and conservative approach to lighten the discolored teeth. Currently, either hydrogen peroxide or carbamide peroxide is used for tooth bleaching (Bulut et al., 2005; Türkün et al., 2009). Despite excellent esthetic outcomes, the clinicians should be aware of the outcome of the bleaching treatment and the interactions with further dental treatments, especially additional adhesive esthetic interventions such as bonding of

composites or laminate veneers to restore the esthetic deficiencies (Ritu Rana, 2019). Reduction in enamel bond strength has become a major concern in esthetic dentistry with regard to clinical operative procedures that involve resin bonding (Gökçe, 2008). One of the mechanisms that may account for this reduction in enamel bond strength is due to the presence of residual peroxide liberating oxygen layer, which interferes with the resin tag formation and inhibits free-radical polymerization (Titley, 1991; Bittencourt, 2010). Various techniques were proposed to resolve this clinical problem, which are as follows: conditioning the bleached enamel with alcohol before restoration (Barghi, 1994), removing the surface layer of enamel (Cvitko, 1991) and employing adhesives containing organic solvents (Sung, 1999). Nonetheless, at present, the universal approach is to delay any bonding procedure for 4 days to 4 weeks (Cavalli, 2001). In order to avoid this delay, application of antioxidants such as sodium ascorbate, ascorbic acid, catalase, ethanol, acetone, glutathione, alpha-tocopherol, and sodium bicarbonate has shown reversal of bond strength on enamel surface after bleaching (Garcia, 2012). Sharafeddin F et al reported that grape seed extracts (natural antioxidant) neutralizes the harmful effects of bleaching agent on the bond strength of bleached enamel (Sharafeddin, 2013). There are very few studies available in the literature about the effect of natural antioxidants on shear bond strength of bleached enamel. Hence, the aim of the present study was to compare the effect of three different natural antioxidant treatment on the shear bond strength of composite resin to bleached enamel. This study was begun by considering null hypothesis stating that there is no significant difference between any groups & subgroups.

MATERIALS AND METHODS

Ethical clearance was taken from the Institutional Review Board of our institution (IRB. No. 2019/P/CONS/65). Ninety freshly extracted human maxillary anterior teeth were obtained from the Department of Oral and Maxillofacial Surgery, SDM College of Dental Sciences & Hospital. Selected teeth were non carious, unrestored and extracted for periodontal reasons. The teeth were cleaned, rinsed with water, and stored in saline.

Specimen Preparation

The roots were embedded in self-cure acrylic resin (DPI-RR Cold Cure) block till cemento-enamel junction (Figure 1). Labial surfaces were flattened using 600-grit silicon carbide paper. The samples were randomly divided into five groups of 18 samples each.

Group 1 – No Antioxidant application

Group 2 – Bleaching followed by application of Tulsi leaf extract

Group 3 - Bleaching followed by application of Olive leaf extract

Group 4 - Bleaching followed by application of Pine Bark extract

Group 5 – No Bleaching (Control)

Bleaching procedure: Except for the 18 teeth which served as the control (No Bleaching), the remaining 72 teeth were bleached using Pola Office kit (SDI Limited, Bayswater, Victoria, Australia), which contains 35% hydrogen peroxide for 24 mins, where the solution was washed away completely with water spray & replenished every 8 mins (Figure 2 & 3).

Preparation of antioxidant solution: 10% antioxidant solution was obtained by dissolving ten grams of plant extracts in the form of powder in 100 ml of distilled water and stirred for 1 min, and then, the solution was filtered. These groups were further subdivided into two subgroups based on the application of composite build-up.

Subgroup A - Composite build-up done immediately (n = 9)

Subgroup B – Composite build-up after a delay of 2 weeks (n = 9)

Subgroup B was stored at 37°C with 100% relative humidity for 2 weeks. The experimental groups 2, 3 & 4 were irrigated with respective antioxidant solutions for 10 min and washed thoroughly with water spray for 30 sec. Later the self etch bonding agent (Universal Bond, Tokuyama, Japan) was applied as per manufacturer's instructions to all the groups (Figure 4). After that, composite resin (Estelite posterior, Tokuyama, Japan) was built incrementally using split cylindrical shaped plastic matrix measuring 4 mm internal diameter & 3 mm height & were light cured (Figure 5). The same procedure was repeated for the samples of Subgroup B after 2 weeks, respectively.

Shear bond strength test: The bonded specimens were stored in deionized water for 24 hours with 100% relative humidity at 37°C. For shear bond strength testing, the specimens were secured in a holder placed on the platen of the testing machine and then sheared with a knife-edge blade on a universal testing machine (Lloyd LRX: Lloyd Instruments, Fareham, Hants, UK) at a crosshead speed of 0.5 mm/min. Shear bond strength in MPa was calculated by dividing the peak load at failure with the specimen surface area.

Statistical Analysis: The mean shear bond strength of specimens was statistically analysed using Two-way ANOVA test & Tukey's multiple posthoc test and independent t test.

RESULTS

The mean values and standard deviations of shear bond strengths are given in Table 1. Bond strength in Subgroup B was significantly greater than that of Subgroup A. Also, the bond strength of Group V followed by Group IV was significantly higher than other groups at both time intervals. When shear bond strength of the bleached enamel was compared between the groups & subgroups using Two way ANOVA test, it was found that there were significant differences with $P < 0.05$ (Table 2). Table 3 shows that there were significant differences with $P < 0.05$ between the groups, when Tukey's multiple posthoc test was applied between the groups. When shear bond strength of the bleached enamel was compared between the groups & subgroups using Tukey's multiple posthoc test, it was found that there were significant differences between the groups and subgroups such as - Group III with sub group B & Group I with sub group A; Group IV with sub group A & Group I with sub group A; Group IV with sub group B & Group I with sub group A & B, Group II with sub group A & B, Group III with sub group A; Group V with sub group A & B showed significant differences among Group I with sub group A & B, Group II with sub group A & B, Group III with sub group A & B (Table 4). When Comparison of sub group A and sub group B in each of five main groups with respect to bond strength by Independent t test was done, it was found that there were significant differences with $P < 0.05$ between the groups & subgroups except in Group V (Table 5).



Figure 1. Roots embedded in self-cure acrylic resin



Figure 2. Application of bleaching agent



Figure 3. Bleaching procedure activated with Light

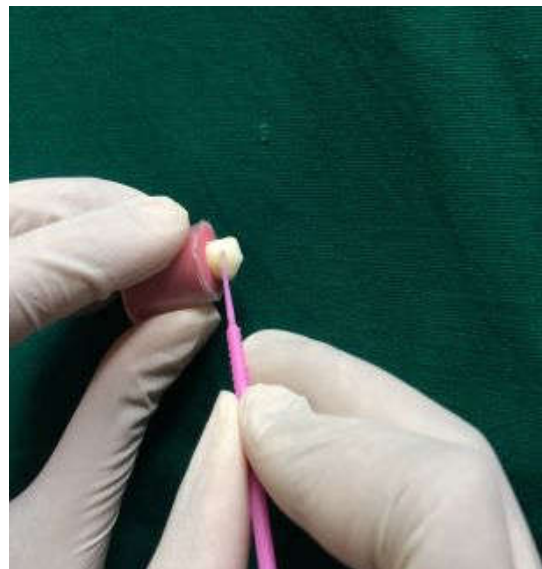


Figure 4. Application of bonding agent

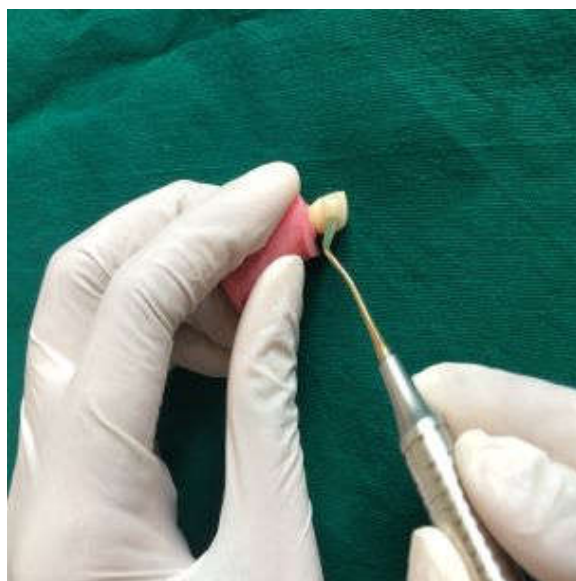


Figure 5. Composite material applied into plastic matrix

Table 1. Mean, SD and SE of bond strength in five main groups (I, II, III, IV and V) and two sub groups (A and B)

Groups and subgroups	N	Mean	SD	SE
Group I with sub group A	9	3.65	0.79	0.26
Group I with sub group B	9	4.36	0.42	0.14
Group II with sub group A	9	4.78	0.43	0.14
Group II with sub group B	9	6.21	1.26	0.42
Group III with sub group A	9	5.29	0.68	0.23
Group III with sub group B	9	6.33	1.23	0.41
Group IV with sub group A	9	6.96	1.68	0.56
Group IV with sub group B	9	8.78	1.50	0.50
Group V with sub group A	9	9.22	3.25	1.08
Group V with sub group B	9	9.22	3.25	1.08

Table 2. Comparison of five main groups (I, II, III, IV and V) and two sub groups (A and B) with respect to bond strength by two way ANOVA with interaction design

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Main effects					
Main groups	4	305.50	76.38	24.8018	0.0001*
Sub groups	1	22.36	22.36	7.2608	0.0086*
2-way interaction effects					
Main groups x Sub groups	4	8.71	2.18	0.7071	0.5894
Error	80	246.36	3.08		
Total	89	582.93			

*p<0.05

Table 3. Pair wise comparison of five main groups (I, II, III, IV and V) with respect to bond strength by Tukeys multiple posthoc procedures

Groups	Group I	Group II	Group III	Group IV	Group V
Mean	4.00	5.50	5.81	7.87	9.22
SD	0.71	1.17	1.10	1.81	3.15
Group I					
Group II	p=0.1044				
Group III	p=0.0282*	p=0.9851			
Group IV	p=0.0001*	p=0.0017*	p=0.0085*		
Group V	p=0.0001*	p=0.0001*	p=0.0001*	p=0.1753	

*p<0.05

DISCUSSION

Peroxide containing bleaching agents remove tooth discolorations through oxidation reaction. Hydrogen peroxide is a low molecular weight substance which decomposes into oxygen and perhydroxyl free radicals. The latter is associated with high permeability and high diffusibility into the tooth structure. Perhydroxyl radicals attack the long chained, dark-colored macromolecules of pigments and split them into smaller, less colored and more diffusible molecules which are removed from the tooth structure producing the bleaching effect (Tezel, 2012). Free radicals released from the hydrogen peroxide diffuse into enamel surface through interprismatic substances and may react not only with the pigmented organic molecules, but also with the organic enamel. Removal of this organic material results in morphological alterations and surface irregularities (Sasaki, 2009). In this study, 37.5% hydrogen peroxide gel (Pola Office Plus, SDI) was applied for three times of 8 min each. The reason for the gel to be refreshed is due to the fast degradation of hydrogen peroxide as significant amount of active ingredients will be available only for the first 15-20 minutes. In order to sustain the same degree of bleaching obtained, three applications of 8 min each for 24 mins were used for bleaching. A study by Reis et al, showed that hydrogen peroxide, used for tooth whitening has a pH around 7 immediately after application but when single application for longer time periods is used, the pH of the gel gets decreased to approximately 5, which may increase tooth sensitivity (Reis, 2011). The compromised bond strength following bleaching is due to the formation of residual oxygen layer which interferes with the resin infiltration into etched

enamel and inhibits the polymerization of resin (Kimyai, 2006; Khoroushi, 2010). During bleaching with hydrogen peroxide, peroxide apatite is formed as a result of the substitution of hydrogen radicals by peroxide ions. These structural changes caused by the incorporation of peroxide ions are eliminated after 2-3 weeks as the peroxide ions decompose and the substituted hydroxyl radicals re-enter the apatite lattice (Aksakalli, 2013). Despite the favourable results achieved with the bleaching agents, a study by Nour El-din AK et al. has reported surface alterations and decreased microhardness of enamel as well as reduced bond strength of composite resin to enamel following bleaching (Nour El-din, 2006). In this study, a statistically significant differences were observed between the immediate application group (Subgroup A) and delayed group (Subgroup B). The reduced bond strength in Group I when compared to Group V may be due to the residual oxygen layer left after the bleaching process which could have interfered with the resin infiltration into etched enamel and inhibited the polymerization of resin (Kimyai, 2006; Khoroushi, 2010). Group V showed significantly higher bond strength than compared to other groups, followed by Group IV. This could be attributed to the fact that the oligomeric proanthocyanidines are powerful antioxidants & are more potent than common antioxidants (Leigh, Jacena, 2003). This was in accordance with the study by Rajalekshmy Subramonian et al. (2015). Group III showed significantly higher bond strength followed by Group II, than compared to Group I. Sodium ascorbate, a potent synthetic antioxidant, which is a derivative of ascorbic acid was used since many years to reverse the bond strength of bleached enamel.

Table 4. Pair wise comparison of interaction effects of five main groups (I, II, III, IV and V) and two sub groups (A and B) with respect to bond strength by Tukeys multiple posthoc procedures

Groups and subgroups	Group 1 with sub group A	Group 1 with sub group B	Group 2 with sub group A	Group 2 with sub group B	Group 3 with sub group A	Group 3 with sub group B	Group 4 with sub group A	Group 4 with sub group B	Group 5 with sub group A	Group 5 with sub group B
Mean	3.65	4.36	4.78	6.21	5.29	6.33	6.96	8.78	9.22	9.22
SD	0.79	0.42	0.43	1.26	0.68	1.23	1.68	1.50	3.25	3.25
Group 1 with sub group A	-									
Group 1 with sub group B	p=0.9974	-								
Group 2 with sub group A	p=0.9316	P=1.0000	-							
Group 2 with sub group B	p=0.0760	p=0.4389	p=0.7798	-						
Group 3 with sub group A	p=0.6089	p=0.9796	p=0.9998	p=0.9827	-					
Group 3 with sub group B	p=0.0500*	p=0.3493	p=0.6916	P=1.0000	p=0.9614	-				
Group 4 with sub group A	p=0.0052*	p=0.0667	p=0.2220	p=0.9959	p=0.5925	p=0.9989	-			
Group 4 with sub group B	p=0.0002*	p=0.0002*	p=0.0004*	p=0.0500*	p=0.0026*	p=0.1065	p=0.4672	-		
Group 5 with sub group A	p=0.0002*	p=0.0002*	p=0.0002*	p=0.0167*	p=0.0005*	p=0.0257*	p=0.1801	p=0.9999	--	
Group 5 with sub group B	p=0.0002*	p=0.0002*	p=0.0002*	p=0.0167*	p=0.0005*	p=0.0257*	p=0.1801	p=0.9999	P=1.0000	

Table 5. Comparison of sub group A and sub group B in each of five main groups with respect to bond strength by independent t test

Groups	Sub groups	Mean	SD	SE	t-value	P-value
Group I	Sub group A	3.65	0.79	0.26	-2.3730	0.0305*
	Sub group B	4.36	0.42	0.14		
Group II	Sub group A	4.78	0.43	0.14	-3.2065	0.0055*
	Sub group B	6.21	1.26	0.42		
Group III	Sub group A	5.29	0.68	0.23	-2.2070	0.0423*
	Sub group B	6.33	1.23	0.41		
Group IV	Sub group A	6.96	1.68	0.56	-2.4168	0.0280*
	Sub group B	8.78	1.50	0.50		
Group V	Sub group A	9.22	3.25	1.08	0.0000	1.0000
	Sub group B	9.22	3.25	1.08		

*p<0.05

But, according to the material safety analyses, the level of health hazard for sodium ascorbate is higher than that of oligomeric proanthocyanidins. Moreover, sodium ascorbate has been found to be mutagenic for mammalian somatic cells, while oligomeric proanthocyanidins had no mutagenic effect when their material safety data were examined. Hence, the utilization of plant extracts as a viable alternative to chemical and synthetic antioxidants have been encouraging (Perumalla, 2011). However, there are very few studies available in the literature regarding the effect of natural antioxidants on shear bond strength of bleached enamel. So far, no study has been done to compare Tulsi & olive leaf extracts with other antioxidants on the reversal of reduced bond strength of bleached enamel. Hence, the findings of this study could not be corroborated or contradicted. Eshrat Halim et al. (2001) studied the antioxidant property of Tulsi & they found that there was increase in the level of not only the antioxidant compound reduced glutathione but also the activity of antioxidant enzymes such as glutathione peroxidase, glutathione S-transferase, superoxide dismutase & catalase. Patricia Goldschmidt Lins et al. (2018) studied the antioxidant property of Olive leaf & they found that Olive leaf has phenolic compounds & flavonoids, which display effective antioxidant property. Free-radical scavengers or antioxidants provide protection of cells against oxidative damage. They also show scavenging activity against reactive oxygen & nitrogen species. Oligomeric Proanthocyanidines, which is present in Pine Bark, have molecular weight of 500– 3000 g/mol, and it is a highly hydroxylated structure that can form insoluble complexes with proteins and carbohydrates hindering its action on the residual oxygen (2018).

Conclusion

Within the limitations of the study, it was observed that among the different natural antioxidants used, 10% pine bark extract was superior in reversing the reduced bond strength of composite resin to bleached enamel. Subgroup B showed significantly higher bond strength than Subgroup A. Hence null hypothesis is rejected. Therefore, with the use of antioxidants, composite restorations can be done immediately following bleaching.

Conflict of Interest: None

Source of Funding: None

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