



## Comparative Evaluation of Reversal of Bond Strength in Bleached Teeth with Different Antioxidants - In Vitro Study

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### Abstract

**Aim:** The aim of this in vitro study was to compare four different antioxidant solutions on bleached teeth and to evaluate the reversal of bond strength between composite resin and enamel immediately after 10 minutes of bleaching. **Materials and methods:** Sixty human permanent maxillary incisors were selected in this in vitro study. The selected teeth were randomly divided into six equal groups (n = 10). Except group VI, the labial enamel surface of all teeth in other groups was bleached with 37.5% hydrogen peroxide for 10 minutes. Immediately after bleaching the specimens of group I, group II, group III, group IV were treated with 10% sodium ascorbate, 10%  $\alpha$ -tocopherol, 10% guava seed extract and 10% amla seed extract antioxidant solutions for 10 minutes respectively. Group V bleached specimens were not treated with any antioxidant solution which serves as a negative control. Group VI unbleached specimen were positive control. All specimens were then bonded with composite resin. Specimens were stored in distilled water for 24 hours. Shear bond strength test was evaluated in universal testing machine. The data were analyzed statistically using repeated measures of ANOVA ( $p < 0.005$ ). **Results:** After application of respective antioxidants for 10 minutes, the mean Shear bond strength of the Group IV was the highest among all the other antioxidant groups followed by Group I, Group III, & Group II respectively. **Conclusion:** Application of antioxidants immediately after bleaching significantly increased the bond strength. Among the antioxidants tested in this study, Amla seed extract was the most effective antioxidant in reversing the bond strength. Hence, it may be an innovative option for esthetic treatment after bleaching. **Clinical significance:** Using antioxidant agents immediately after bleaching leads to absolute reversal of shear bond strength of composite restoration, thus enabling composite restoration to be done immediately after bleaching.

**Keywords:** Bleaching, Antioxidant, Sodium ascorbate, Alpha-tocopherol, Guava seed extract, Amla seed extract, Reversal of bond strength, Shear bond strength.

## INTRODUCTION

Bleaching is the first line of treatment for discolored teeth. Enamel has negative charge which allows chromogen or pre-chromogen to interact via ion exchange mechanism and generates stains due to presence of conjugated double bonds. Bleaching is defined as the chemical degradation of chromogens where the bleaching agents break the conjugated double bond to single bond which oxidize the chromogens to become light color compound<sup>[1]</sup>.

Hydrogen peroxide is an oxidizing agent which diffuses into tooth, dissociates to produce unstable free radicals like hydroxyl radicals [OH-], perhydroxyl radicals [HOO-], perhydroxyl anions [Hoo-] and superoxide anions [oo-]. These free radicals attack organic pigmented molecules by attacking the double bond of chromophore molecules within tooth tissues and break them into smaller less pigmented constituents thereby causing a shift in the absorption spectrum of chromophore molecules which changes the optical property and thereby improves enamel translucency<sup>[2]</sup>.

For stains associated with surface irregularities, which require immediate esthetic restoration followed by bleaching, the restoration procedure is complicated due to compromised bond strength of the restoration because of the presence of these free radicals<sup>[3]</sup>. Application of antioxidant agents which neutralize the free radicals by donating their electrons immediately after bleaching leads to absolute reversal of shear bond strength of composite restoration<sup>[4,5]</sup>.

Hence the rationale of this in vitro study was to compare and evaluate the effect and efficacy of 10% sodium ascorbate solution, 10%  $\alpha$  – tocopherol solution, 10% guava seed extract solution and 10% amla seed extract solution on the bond strength between composite resin and enamel immediately after 10minutes of bleaching<sup>[6,7]</sup>.

## MATERIALS AND METHODS

Sixty human permanent maxillary incisors extracted for periodontal reasons were collected and stored in distilled water. Labial surfaces of these teeth were flattened with 600 grit silicon carbide paper. All the specimens were tied with threads at CEJ and stuck to the walls of the glass beaker to standardize the bleaching procedure. 35% H<sub>2</sub>O<sub>2</sub> was taken in the beaker till the crown portion of the teeth was immersed in the solution (Figure 1). Fifty maxillary incisors were immersed in 35% hydrogen peroxide solution in a beaker at the level of CEJ for 10minutes and then rinsed off with water. Remaining ten samples kept as negative control were not subjected to bleaching process.



**Figure-1: Samples immersed in 35% Hydrogen peroxide solution for 10min**

To nullify the effects of free radicals, the 50 samples were divided into 5 groups of 10 each and Group I, II, III, IV were immersed in respective antioxidant solutions (Figure 2). The remaining 10 samples were not subjected to antioxidant solution and served as positive control. Groups were divided as follows:

- Group I (n=10) treated with 10% sodium ascorbate solution for 10 minutes.
- Group II (n=10) treated with 10%  $\alpha$  – tocopherol solution for 10 minutes
- Group III (n=10) treated with 10% guava seed extract solution for 10 minutes
- Group IV (n=10) treated with 10% amla seed extract solution for 10 minutes
- Group V (n=10) Bleached and not treated with antioxidant solutions
- Group VI (n=10) control [unbleached specimens]

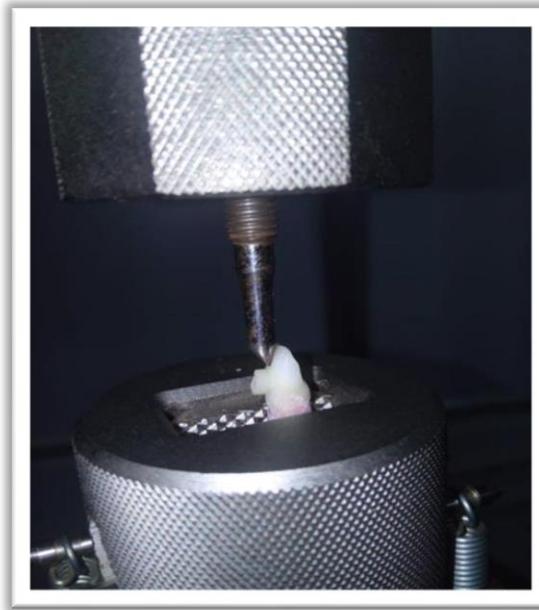
For Group I & Group II 10gm of sodium ascorbate and 10 ml of  $\alpha$  – tocopherol were taken and dissolved in 100ml of distilled water and 100ml of ethyl alcohol respectively. The  $\alpha$  – tocopherol dissolved only in ethyl alcohol due to nonpolar attractive interactions between ethanol and fat soluble vitamin molecules which facilitates increased solubility. For Group III & Group IV, guava and amla seeds were separated from guava & amla fruits. They were then rinsed in distilled water, dried using absorbent papers and then were ground into a fine powder. 10 gm of guava seed powder and 10gm of amla seed powder were taken and dissolved in 100ml of distilled water respectively.

After bleaching and rinsing, the specimens were immersed in beaker containing the respective antioxidant solutions for 10minutes for all four Groups. Threads were removed from the specimens and rinsed off with water and dried. In Group V after bleaching the samples were rinsed with water and dried which served as a negative control and in Group VI unbleached specimens were kept as positive control.



**Figure-2: Group I – Group IV specimens were treated with 10% Antioxidant solutions for 10 minutes**

Labial surfaces of all the specimens were etched with 37% ortho phosphoric acid for 15 seconds and were rinsed off with water and dried. Two coats of bonding agent [3M ESPE Scotchbond™ Universal Adhesive] were applied with microbrush and cured for 40seconds. To standardize the restorations, cylindrical moulds of 4mm diameter & 4mm height was placed on the labial surface and composite of A1 shade [ SwissTEC ] was added in increments and cured with Light cure unit for 40sec[LEDition].



**Figure-3: Shear bond strength done with universal testing machine at across head speed of 1mm/min**

The roots were embedded in acrylic resin block up to the level of CEJ and were stored in distilled water for 24 hours. Shear bond strength testing was done with universal testing machine at cross head speed of 1mm/min (Figure 3). Data were tabulated and analysed statistically.

## RESULTS

A repeated measures of ANOVA with a greenhouse – geisser correction determined that the mean SBS of Group VI (mean = 8.679 Mpa) was statistically significant when compared to all the other groups (Table 1). It was also observed that immediate bonding to bleached enamel resulted in statistically significant ( $P < 0.005$ ) decrease in the SBS of Group V (mean = 1.83 Mpa) . After application of respective antioxidants for 10 min, the mean SBS of Group IV (7.979Mpa) was the highest among all the other antioxidant groups followed by Group I (6.975Mpa), Group III (6.694Mpa), & Group II (2.424Mpa) respectively.

Group	Mean	Std. Deviation
Sodium ascarbate	6.97550	2.433575
Alpha tocopherol	2.42440	0.729300
Guava extract	6.6940	2.44072
Amla extract	7.97900	2.814877
Bleached	1.83020	1.454691
Control group	8.67950	2.557310

**Table-1: Descriptive statistics (MPa) of the different groups**

## DISCUSSION

All the four antioxidants were capable of reversing the reduced bond strength following bleaching<sup>[8]</sup>. Since the antioxidant preparations were in solution form, individual beaker containing solutions were prepared for adequate contact of antioxidants with the labial surface of the specimens. In clinical scenario, beaker can be substituted with customized trays that can be worn by the patient for an appropriate time<sup>[9]</sup>.

Gokce et al., reported that 10 minutes application of sodium ascorbate is effective in reversing the decreased bond strength<sup>[10-17]</sup>. Kaya et al., demonstrated that increasing the period of antioxidant application increased the bond strength of composite to enamel which occurred till 120 minutes<sup>11</sup>. To determine the exact time for reversing the reduced bond strength and have better clinical correlation, 10 minutes application period was chosen for the current study<sup>[7]</sup>.

In the present study, the mean SBS of Group VI was found to be statistically significant when compared to all the other groups. These findings indicate bleaching deteriorates the SBS of composite to tooth. It was also observed that immediate bonding to bleached enamel resulted in statistically significant ( $P < 0.005$ ) decrease in the SBS. These findings are in accordance with the results of Borges *et al.*<sup>[12]</sup>. This reduction in bond strength on immediate composite placement postbleaching may be due to delayed release of nascent oxygen, which in turn could either interfere with resin infiltration into the etched enamel or hinder resin polymerization<sup>[13]</sup>.

Sodium ascorbate and alpha-tocopherol allow free-radical polymerization of adhesive resin without premature termination by restoring the altered redox potential of the oxidized bonding substrate, thus improving the compromised bonding<sup>[14]</sup>. Furthermore, the presence of alcohol in the composition of the 10% alpha-tocopherol solution formulated for this study may have contributed in improving the compromised bond strength of bleached enamel since 10% alpha-tocopherol was immiscible in water solution. Thus, the phenomenon observed may be system-specific, not only due to antioxidant agent but also due to the presence of alcohol<sup>[15]</sup>. Furthermore, a lower mean SBS of Group II was observed when compared to that of control group. This indicates that application of 10% alpha-tocopherol for 10 minutes did not restore the reduced bond strength to baseline levels which is in accordance with the findings of Thapa *et al.*<sup>[16]</sup>.

After application of respective antioxidants for 10 minutes, the mean SBS of the Group IV was the highest among all the other antioxidant groups. High antioxidant activity of amla might be responsible for almost complete reversal of reduced bond strength<sup>[18]</sup>. Leong and Shui et al., investigated the antioxidant capacity of fruits and ranked amla and guava as the highest antioxidant contents<sup>[19]</sup>. M. Ayub Ali et al., had stated among the various fruits employed in the investigation, the highest antioxidant activity was observed in Amla followed by guava, pomegranate, sweet lime, passion fruit, tamarind, star fruit, orange, papaya, mango, butter fruit, pummelo, lemon, pears, pineapple, apple, grapes, banana, watermelon and coconut water.<sup>[20]</sup> Amla is an important dietary source of vitamin C (about 2%), minerals and amino acids and also contains phenolic compounds, tannins, phyllembelic acid, phyllembelin, rutin, curcuminoides and emblicol. Thus, the highest antioxidant activity observed for Amla was due to high content of vitamin C, polyphenols and non-phenolic compounds which acts as a free radical scavenger on bleached enamel<sup>20</sup>. So far, no study has been done to compare amla seed extract with other antioxidants on the reversal of reduced bond strength of bleached enamel.

## CONCLUSION

Bleaching significantly decreases the shear bond strength of composite resin to enamel. Application of antioxidants after bleaching showed significantly increased bond strength. Among the antioxidants tested in this study, Amla seed extract was the most effective antioxidant in reversing the bond strength followed by Sodium ascorbate, Guava seed extract and Alpha tocopherol.

### Clinical Significance

Using Amla seed extract antioxidant agent immediately after bleaching leads to absolute reversal of shear bond strength of composite restoration. It could be done without complexity in esthetic restoration immediately followed by bleaching.

### Limitation of the Study

The present study has certain limitations that should be considered. This in vitro study was carried out on extracted teeth. However, laboratory conditions are not exactly similar to that of clinical conditions, and results obtained by in vivo studies may differ.

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