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Original Article

Dentistry Section

# Effect of 3% Grape Seed Extract on the Bond Strength of Bleached Enamel with Natural and Commercially Available Bleaching Agents: An In-vitro Study

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## ABSTRACT

**Introduction:** Following bleaching with natural and commercial bleaching agents, to reverse the bond strength of enamel various antioxidants are used. The 3% Grape Seed Extract (GSE) has been more potent to be used as an antioxidant and has shown the immediate improvement in the Shear Bond Strength (SBS) values.

**Aim:** To evaluate the effect of 3% GSE as an antioxidant on the bond strength of bleached enamel with natural {Sweet Potato Extract (SPE)}, commercial {35% Hydrogen Peroxide ($H_2O_2$)} bleaching agents.

**Materials and Methods:** This experimental in-vitro study was conducted at Sibar Institute of Dental Sciences, Guntur, Andhra Pradesh, India, over a period of one week in January 2021. According to the bleaching agent used, the labial surfaces of 40 extracted maxillary central incisors have been divided into two groups. Group I (n=20) include teeth bleached by SPE (natural) for about 30 minutes. Group II (n=20) included teeth bleached using 35% $H_2O_2$ (Pola office) for about eight minutes. Each group were again divided into two subgroups (n=10) based on the application of 3% GSE. After bleaching procedures, composite resin cylinders were bonded incrementally on the labial surface and stored in artificial saliva at 37°C for 24 hours. The samples were subjected to SBS under Universal Testing Machine. Data obtained were analysed using One-way Analysis Of Variance (ANOVA) and Tukey's post-hoc test.

**Results:** On comparison of mean SBS values, statistically significant difference (p-value <0.05) was observed in all groups. The SBS values observed in 35% $H_2O_2$ bleached teeth were highly significant than the teeth bleached using 3% GSE application.

**Conclusion:** Application of 3% GSE immediately after bleaching the enamel surfaces, neutralises the deleterious effects of bleaching and increases SBS significantly.

**Keywords:** Antioxidant, Hydrogen peroxide, Shear bond strength, Tooth bleaching

## INTRODUCTION

In restorative dentistry, anterior teeth discoloration (intrinsic or extrinsic) is a major aesthetic concern, requiring an effective treatment [1]. Vital bleaching is said to be a safer, popular, conservative and well-accepted treatment modality of treatment for discoloured teeth [2]. For achieving rapid aesthetic results, bleaching agents in varying concentrations have been used. Various concentrations of $H_2O_2$ (Hydrogen Peroxide) from 5% to 35% and carbamide peroxide 10% are used to achieve lighter and more desirable tooth colour [3]. The 35% $H_2O_2$ when applied on the tooth surface causes dispersal of peroxide into the organic matter of tooth structure. This depends on the factors like duration of application, diffusion coefficient and concentration of active bleaching agent [4].

Sweet Potato Extract (SPE) was used as a bleaching agent in this present in-vitro study. Gopinath S et al., formulated a bleaching agent obtained from plant tubers containing vegetative enzyme extract (sweet potato). They concluded that addition of SPE to $H_2O_2$ resulted in the retrieval of natural tooth colour and decreased the effects of bleaching on the enamel morphology, when compared to using $H_2O_2$ alone [4].

Sweet potato extract contains antioxidant molecules present either in enzymatic or non enzymatic in nature, such as Superoxide Dismutase (SOD), Catalase (CAT), peroxidase dismutase, polyphenol dismutase and ascorbic acid carotenoids, a phenolic compound [5]. Teow CC et al., have observed that these phytochemicals have free radical scavenging activity [6]. Nair M et al., showed that addition of 2% SPE in bleaching agent has reduced harmful effects on enamel and increases the bonding effect of composites to bleached enamel. Grape Seed Extract (GSE) with SPE might show synergistic effect in scavenging of free radicals [7].

Postoperative sensitivity, pulpal irritation, tooth structure alterations and microleakage are few complication of bleaching. One of the important complications of bleaching is decrease in the bond strength of composite to enamel [8]. Vidhya S et al., and Lai SC et al., have suggested that, if bonding is undertaken immediately after bleaching, it adversely affects the SBS of resin composite to acid-etched enamel [2,9].

The classic method of avoiding this is that the bonding procedure to be delayed for about 2-3 weeks after bleaching as the peroxide ions decompose and decreased Shear Bond Strength (SBS) is restored [10]. To restore the decreased bond strength; various methods have been proposed, such as using alcohol treatment before the restoration to the bleached enamel, removal of outermost enamel layer and using adhesives containing organic solvents, such as antioxidants [11]. Among all the above mentioned methods, usage of antioxidants has shown immediate improvement in SBS values [2,9].

Naturally occurring antioxidants, like GSE, contains Oligomeric Proanthocyanidin Complexes (OPCs). These OPCs present in the natural antioxidants have the free radical scavenging activity. Vidhya S et al., and Lai SC et al., have suggested that the decreased bond strength can be reversed by the application of antioxidants such as proanthocyanidin and sodium ascorbate [2,9]. The 3% GSE has been shown to be more potent and safe to be used in various clinical applications [11].

Hence, this in-vitro study was conducted to evaluate the effect of 3% GSE as an antioxidant on the bond strength of bleached enamel with natural (SPE) and commercial (35% $H_2O_2$) bleaching agents.

## MATERIALS AND METHODS

This in-vitro study was performed at the Sibar Institute of Dental Sciences, Guntur, Andhra Pradesh, India for duration of one week in January 2021. The study was undertaken after obtaining clearance from Ethical Committee of the Institutional Review Board (Protocol number 44/IEC-SIBAR/2021).

**Inclusion and Exclusion criteria:** Forty recently extracted human maxillary central incisors (extracted for periodontal reasons) were included in the study. The teeth with decay, fractures, cracks, severe attrition, erosion, dried teeth with developmental defects were excluded from the study.

### Preparation of Solution

**Sweet potato extract preparation:** About 200 grams of purple colour sweet potatoes were washed, peeled and cut into cubes. A 25 mL of deionised water was used to smash the cubes in a blender and was filtered. A 100 mL of the filtered SPE was centrifuged at 2000 rpm for two minutes at 4°C. Thus, the clear liquid obtained is the SPE. Until use, the extract has been refrigerated at 4°C [4].

**Grape seed extract preparation:** Three grams of GSE powder (Ambe Phyto Extract Rx Ltd., Delhi) was dissolved in 100 mL of distilled water to make a 3% grape seed extract solution [1,8].

**Specimen preparation:** All the samples were embedded in plastic moulds with self-cure acrylic resin, till the cementoenamel junction and the labial surfaces were cleaned with pumice slurry using slow speed hand piece. Until use, the samples were stored in 0.1% thymol.

Samples were divided into two groups (n=20), depending upon bleaching agents used.

**Group 1 (n=20):** A 30 mL of SPE solution was taken for the study. Gauze piece was saturated with 3 mL of SPE solution and placed on the labial surface of the sample for a period of 30 minutes with the SPE solution being changed for every 10 minutes [4]. All the samples were washed under water for about one minute.

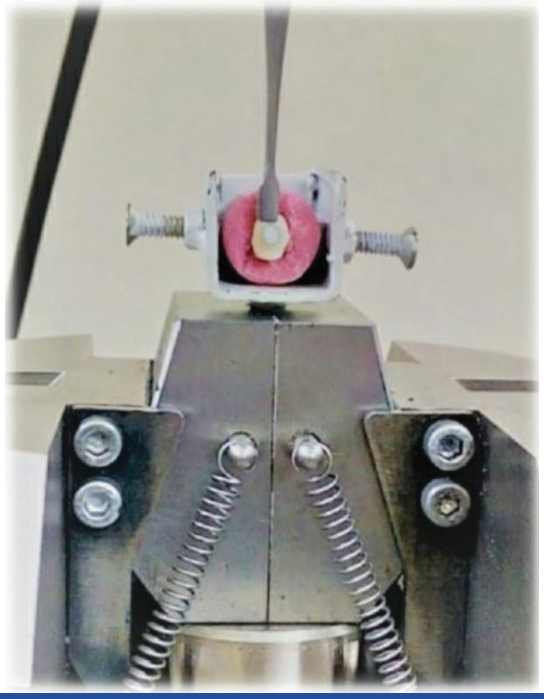
**Group 2 (n=20):** Labial surfaces of all the samples were treated with a 2 mm thick layer of 35% $H_2O_2$ gel (Pola office, SDI, Australia) at three intervals for eight minutes each, with a waiting period of three minutes between each session, as per manufacturer's instructions. Using suction tip, the gel was removed and were washed under running water for about one minute.

Both the groups, were again divided into two subgroups, group 1A, 1B and group 2A, 2B (n=10) [Table/Fig-1], based on the application of an antioxidant agent, i.e., 3% GSE. Gauze piece was saturated with 3 mL of SPE solution and placed on the labial surface of the samples for a period of 10 minutes and rinsed [1].

| Groups | Bleaching agent | Antioxidant used | Composite build-up |
|---|---|---|---|
| Group 1A (n=10) | Sweet potato extract | None | Done immediately |
| Group 1B (n=10) | Sweet potato extract | 3% GSE | Done immediately |
| Group 2A (n=10) | 35% $H_2O_2$ | None | Done immediately |
| Group 2B (n=10) | 35% $H_2O_2$ | 3% GSE | Done immediately |

**[Table/Fig-1]:** Distribution of specimens and study groups.

**Testing procedure:** Immediately after the bleaching procedure, the samples were etched with 37% phosphoric acid (Ivoclar Vivadent, Schaan) for 15 secs and rinsed. Adper Single Bond (Dental Products, 3M ESPE, St Paul, MN, USA) was used for bonding the etched surface and cured for 20 seconds. Finally, composite (spectrum, microhybrid composites, Dentsply, Germany) build-up was done incrementally with teflon mould of 2 mm height and 2 mm diameter and cured for 30 seconds. Sample storage was done in distilled water for 24 hours. The SBS values were analysed under Universal Testing Machine (Instron Machine) [Table/Fig-2]. The knife edge was targeted at interface between enamel surface and composite resin at a crosshead speed of 0.5 mm/min, till the dislodgement of the composite resin.



**[Table/Fig-2]:** Shear bond strength test.

## STATISTICAL ANALYSIS

The values were statistically analysed using software Statistical Package for Social Sciences (SPSS) version 16.0. One-way Analysis Of Variance (ANOVA) followed by the Mann Tukey's post-hoc test were used to analyse the data. Statistical significance level was established at p-value <0.05.

## RESULTS

Total 40 maxillary central incisors were included in the study. [Table/Fig-3] shows the mean of SBS (MPa) of all the groups. Among the experimental groups, mean highest SBS was observed in group 2B, group 1B i.e., 14.77 MPa and 12.40 MPa respectively where 3% GSE has been used. Group 1A (p-value <0.05) have the lowest mean SBS value of 6.54 MPa than the group 2A. Group 1B displayed a significantly higher SBS of 12.40 MPa than the group 2A.

| Groups | Shear bond strength (MPa) | | | 95% Confidence interval for mean | | p-value (ANOVA) |
|---|---|---|---|---|---|---|
| | Mean (MPA) | Standard deviation | Standard error | Lower bound | Upper bound | |
| Group 1A | 6.54 | 5.38 | 1.70 | 2.68 | 10.39 | <0.05 |
| Group 1B | 12.40 | 3.82 | 1.21 | 9.66 | 15.14 | |
| Group 2A | 8.83 | 4.00 | 1.26 | 5.96 | 11.70 | |
| Group 2B | 14.77 | 4.04 | 1.28 | 11.87 | 17.67 | |
| Total | 10.63 | 5.28 | .83 | 8.94 | 12.32 | |

**[Table/Fig-3]:** Comparison of mean SBS values of four different groups after various bleaching protocols using One-way Analysis of Variance (ANOVA).

[Table/Fig-4] shows intergroup comparison of mean SBS values (MPa) of all groups. The mean SBS value of group 1A was statistically significant with that of group 1B (p-value <0.024) and group 2B (p-value <0.001). The mean SBS value of group 1B was statistically significant with that of group 1A (p-value <0.024). The mean SBS value of group 2A was statistically significant with that of group 2B (p-value <0.021). The mean SBS value of group 2B was statistically significant with that of group 1A (p-value <0.021) and group 2A (p-value <0.05).

| Intergroup comparison | | | | | | |
|---|---|---|---|---|---|---|
| (I) Groups | (J) Groups | Mean Difference (I-J) | Standard error | p-value | 95% Confidence interval | |
| | | | | | Lower bound | Upper bound |
| Group 1A | 1B | -5.8650000* | 1.9510832 | 0.024 | -11.119710 | -0.610290 |
| | 2A | -2.2920000 | 1.9510832 | 0.646 | -7.546710 | 2.962710 |
| | 2B | -8.2360000* | 1.9510832 | 0.001 | -13.490710 | -2.981290 |
| Group 1B | 1A | 5.8650000* | 1.9510832 | 0.024 | 0.610290 | 11.119710 |
| | 2A | 3.5730000 | 1.9510832 | 0.276 | -1.681710 | 8.827710 |
| | 2B | -2.3710000 | 1.9510832 | 0.621 | -7.625710 | 2.883710 |
| Group 2A | 1A | 2.2920000 | 1.9510832 | 0.646 | -2.962710 | 7.546710 |
| | 1B | -3.5730000 | 1.9510832 | 0.276 | -8.827710 | 1.681710 |
| | 2B | -5.9440000* | 1.9510832 | 0.021 | -11.198710 | -0.689290 |
| Group 2B | 1A | 8.2360000* | 1.9510832 | 0.001 | 2.981290 | 13.490710 |
| | 1B | 2.3710000 | 1.9510832 | 0.621 | -2.883710 | 7.625710 |
| | 2A | 5.9440000* | 1.9510832 | 0.021 | 0.689290 | 11.198710 |

**[Table/Fig-4]:** Intergroup comparison of mean SBS among four groups using Tukey's post-hoc test.

## DISCUSSION

Tooth discolouration has always been an important factor in the recent times, due to growing concentration on the aesthetics. This growing awareness towards aesthetics has showed great demand to different treatment options in treating tooth discoloration. Of all these treatment modalities, most commonly used effective and conservative treatment options with minimal expense is vital tooth bleaching [8].

The SPE is a naturally obtained bleaching agent which contains a plurality of antioxidants, which act as scavengers [11]. Vidhya S et al., have shown that proanthocyanidin application as a separate step, post bleaching resulted in immediate reversal of SBS of bleached tooth [2]. In present study, 35% $H_2O_2$ (Pola office, SDI, Australia) was used thrice; each application was done for eight minutes. It has to be refreshed as the active ingredients of $H_2O_2$ are available for the first 15-20 minutes and has fast degradation action. It was applied thrice to attain same amount of bleaching obtained in the initial 15 minutes. The $H_2O_2$ being an oxidising agent, diffuses and dissociates into tooth structure to produce unstable free radicals such as hydroxyl ($HO^-$), per hydroxyl ($HOO^-$) and superoxide anions ($OO^-$). These attack the organic pigmented molecules present between the inorganic salts of enamel and double bonds of chromophore molecules present in the tooth structure [12,13].

Studies have stated that there will be less microhardness, surface alterations and reduced SBS of composite to enamel [13,14]. Upon storage, for 24 hours to three weeks, changes in structure caused by peroxide ions incorporation are eliminated, as peroxide ions decompose and replaced hydroxyl radicals re-enter the hydroxyapatite lattice [12]. The reduced SBS in group 1A and 2A, compared to group 1B and 2B might be due to the following bleaching process residual oxygen layer left behind could have inhibited polymerisation of resin composite and interfered with infiltration of the resin into etched enamel. Lai SC et al., showed that by use of an antioxidant, i.e., 3% GSE, the presence of peroxide ions might be reversed [9].

The results in the present study showed that treatment with 3% GSE; group 1B and 2B increased bond strength significantly compared with group 1A and 2A. These observations were in accordance with study done by Subramonian R et al., who stated that this could be due to presence of OPCs in the antioxidant i.e., 3% GSE. OPCs have multiple donor sites which trap the superoxide radicals and epicatechin esterification by gallic acid enhances the free radical scavenging activity [15]. Vidhya S et al., have shown that proanthocyanidin application as a separate step after bleaching resulted in immediate reversal of SBS of bleached enamel [2]. [Table/Fig-5] shows comparison of present study with previous studies [7,15].

In recent years, it has been reported that utilisation of plant extracts i.e., natural antioxidants as a viable alternative to synthetic and chemical antioxidants. The GSE was chosen for this study since it has a greater degree of oxygen free radical scavenging potential and also yields a 10% higher concentration of proanthocyanidins. GSE contains oligomeric proanthocyanidins and free flavanol monomers. High molecular weight polymers, OPCs consist of (+)-catechin, monomeric flavan-3-ol and (–)-epicatechin [8]. Other in vitro studies have shown that OPCs are 50 and 20 times more effective and stronger than vitamin E and C, respectively [8,16]. In order to reverse the reduced SBS of composite to the bleached enamel, 3% GSE was administered as an antioxidant right after the bleaching treatment.

According to Subramonian R et al., applying 10% pine bark extract after bleaching with 37.5% $H_2O_2$ showed better bond strength, whereas 37.5% $H_2O_2$ bleaching followed by treatment with 10% grape seed extract solution has shown better bond strength values compared to 37.5% $H_2O_2$ bleaching followed by treatment with 10% sodium ascorbate solution. It was determined from the results of the current investigation that, antioxidant use effectively restored the bond strength of bleached enamel [15].

| Author's name and year | Place of study | Number of samples | Materials used | Parameters assessed | Conclusion |
|---|---|---|---|---|---|
| Nair M et al., (2016) [7] | Chennai, India | 90 | 30% $H_2O_2$, 2% SPE, 5% GSE, Composite resin | Effect of antioxidants, SBS | The addition of 2% SP to 30% $H_2O_2$ enhances bond strength, 5% GSE was better when used separately. |
| Subramonian R et al., (2015) [15] | Tamil Nadu, India | 90 | 37.5% $H_2O_2$, 10% Sodium ascorbate, 10% GSE, 10% pine bark extract, Composite resin | Effect of single and two-step application of antioxidants, SBS | It was concluded that the use of antioxidants effectively reversed the compromised bond strength of bleached enamel. |
| Present study; 2022 | Andhra Pradesh, India | 40 | 35% $H_2O_2$ 2% SPE, 3% GSE, Composite resin | Effect of antioxidants, SBS | The use of 3% GSE immediately after bleaching the enamel surface completely neutralises the deleterious effects of bleaching and increases SBS significantly. |

**[Table/Fig-5]:** Comparison of present study with previous studies [7,15].

In comparison to using hydrogen peroxide alone, Gopinath S et al., demonstrated that using 35% and 10% $H_2O_2$ containing SPE as bleaching agents not only produced better bleach, but also lessened the effects of bleaching on enamel morphology. These effects were more pronounced when using a higher concentration of $H_2O_2$. This excluded the additional step of applying antioxidants [4]. Nair M et al., reported that compared to $H_2O_2$ and 5% GSE, usage of 2% SPE had shown significant bond strength. When applied after bleaching as a separate step, the effectiveness of 5% GSE was appreciated [7].

The molecular weight of the bleaching solutions employed in the present study appears to have high significance. Since, $H_2O_2$ which has a low molecular weight, permeates the dental hard tissues and decomposes into free radicals [17], any antioxidant surface treatment used to get rid of these free radicals should also have a low molecular weight for effective scavenging action.

### Limitation(s)

The in-vivo environment cannot be replicated by the present study because it was conducted in-vitro. The restoration-tooth interface is subjected to a variety of stresses acting simultaneously in the oral cavity. A restoration is subjected to cyclic loading over the course of its existence; although no single stress is sufficient to cause a breakdown, over time it may result in the loss of the restoration and marginal deterioration. Thus, it is anticipated that fatigue testing of dental adhesive, would enable better in-vivo performance prediction.

## CONCLUSION(S)

The 3% GSE as an antioxidant increased SBS of bleached enamel with natural (sweet potato), commercial (35% hydrogen peroxide) bleaching agents. 35% $H_2O_2$ bleaching, followed by the application of 3% GSE as an antioxidant, has shown significant bond strength values. Treatment of bleached enamel surface with 3% GSE has reversed, the reduced SBS of composite resin. Additional studies are necessary to test higher concentrations of antioxidant agents, reducing time required to an efficient neutralising action and to improve SBS of composite resin.

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**PLAGIARISM CHECKING METHODS:** [Jain H et al.]
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