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Original Article

Dentistry Section

An In-vitro Comparative Study of Shear Bond Strength of Composite Resin to Bleached Enamel using three Herbal Antioxidants

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ABSTRACT

Introduction: If adhesive restorations are carried out immediately, the Shear Bond Strength (SBS) to bleached enamel is reduced. The use of antioxidants can be highlighted since it seems to be a more effective alternative to reduce or remove the residual oxygen and immediate improvement in SBS.

Aim: The purpose of this in-vitro study was an attempt to regain the lost bond strength, for which, the comparison of SBS of composite resin to bleached enamel was carried out using various antioxidants i.e., 5% grape seed extract, 5% pine bark extract, 5% pomegranate peel extract.

Materials and Methods: Labial enamel surfaces of 50 extracted human maxillary central incisors were randomly divided into 5 groups based on the antioxidant used as follows: Group I (n=10): Bleaching with 40% hydrogen peroxide gel for 10 minutes, without the use of an antioxidant; Group II (n=10): Bleaching followed by the use of 5% grape seed extract solution; Group III (n=10): Bleaching followed by the use of 5% pine bark extract solution Group IV (n=10): bleaching followed by the use of 5% pomegranate extract solution; and Group V (n=10): Control group in which no Bleaching was done. SBS of the specimens was tested using universal testing machine. The data were tabulated and statistically analyzed using computer software Statistical Package for Social Sciences (SPSS) version 16.0. One-way analysis of variance (ANOVA) followed by Mann Tukey Post hoc test.

Results: Mean SBS values were compared in all the groups and there was a statistically significant difference present among the groups ($p<0.05$). The SBS values were observed to be significantly higher in unbleached teeth (control group) followed by 5% pine bark extract, 5% grape seed extract, 5% pomegranate extract and the group in which teeth bleached with no antioxidant used.

Conclusion: The use of antioxidant immediately after bleaching the enamel surface, completely neutralizes the deleterious effects of bleaching and increases the SBS significantly. Among the antioxidants, 5% pine bark extract application after bleaching showed better bond strength.

Keywords: Bleaching, Grape seed extract, Pine bark extract, Pomegranate extract

INTRODUCTION

Aesthetic includes the appreciation and response to the beautiful in art and nature. Aesthetic dentistry not only relates to disfigured teeth, it also involves the need for a normal appearing individual who wishes to look younger, healthier and confident [1]. Discoloration of anterior teeth (extrinsic or intrinsic) is an aesthetic problem in restorative dentistry and it requires effective treatment [2]. The treatment of choice is dependent on the diagnosis. In many cases of discoloration, treatments of choice are Micro abrasion, Bleaching, Composite Resin Restorations, and Porcelain Veneers [3].

Among the several aaesthetic dental treatments, bleaching is a non-invasive, relatively simple procedure to be performed. Increase in the demand for aesthetic dentistry has resulted in widespread practice of vital bleaching [4,5]. Vital tooth bleaching is considered as a safe, popular, conservative and well accepted treatment option for discoloured teeth [5].

Bleaching agents in varying concentrations (Carbamide peroxide 35% to 37% or Hydrogen peroxide 30% to 40%) have been used to achieve rapid aesthetic results [2,5,6]. When applied on tooth surface, hydrogen peroxide undergoes ionic dissociation and gives rise to the formation of free radicals such as hydroxyl radical, per-hydroxyl, nascent oxygen, and superoxide anions, which are the most potent free radicals. These are extremely reactive and therefore react with the electron-rich regions of pigment within the tooth leading to dissociation of the larger pigmented molecules into smaller and less pigmented molecules [5,6]

Studies have shown that the Shear Bond Strength (SBS) of composite resin bonded to the unbleached tooth surface is significantly higher than that of tooth surface immediately after bleaching due to the absence of free residual oxygen layer [3,7]. To overcome this post bleaching effect on SBS, the bonding procedure should be delayed by a period varying from 24 hours to three weeks [3,7]. There are a number of methods that have been projected to overturn the reduced SBS which occurs due to bleaching, for example alcohol treatment of the bleached enamel surface before restoration, utilisation of organic solvent that contain adhesives, removal of the outermost layer of enamel, and the utilisation of antioxidants. Among all the methods, the antioxidant treatment has shown immediate improvement in SBS values, whereas other methods showed opposing results in regaining the bond strength values [7,8].

Antioxidants like 10% sodium ascorbate have also been used to reverse the reduced SBS of bleached enamel [4,5]. Since, there is a limited information available on the use of the newer natural antioxidant agents like Oligomeric Proanthocyanidin Complexes (OPCs) and pomegranate peel extract they increases the bond strength of composite resin to bleached enamel. OPCs present in natural antioxidants like grape seed extract and pine bark extract have free radical scavenging activity [4,9]. Pomegranate peel

extract contains phenolic compounds which have antioxidants property [10]. But the effect of pomegranate on bleached enamel has not been investigated so far. Hence, the aim of this in-vitro study was to evaluate and compare the effect of 5% grape seed extract, 5% pine bark extract, and 5% pomegranate extract on the SBS of composite resin to bleached enamel.

MATERIALS AND METHODS

The present in vitro study was conducted at the Ministry of Micro, Small and Medium Enterprises (MSME) at Hyderabad, Telangana State, India.

Preparation of solutions

Three solutions were prepared for this study:

1) 5g of Grape seed extract powder 5g (Ambe Phyto Extract Rx, Ltd., Delhi.) was dissolved in 100ml of distilled water to make 5% grape seed solution.

2) 5g of Pine bark extract powder 5g (Ambe Phyto Extract Rx, Ltd., Delhi) was dissolved in 100ml of distilled water to make 5% pine bark solution.

3) 5g of Pomegranate extract powder 5g (Ambe Phyto Extract Rx, Ltd., Delhi) was dissolved in 100ml of distilled water to make 5% pomegranate solution.

Specimen Preparation: Fifty recently extracted human maxillary central incisors were collected.

Inclusion Criteria: Freshly extracted human maxillary central incisor teeth, extracted for periodontal reasons.

Exclusion Criteria: Teeth with severe attrition, erosion, decay, fractures, cracks, dried teeth with developmental defects were excluded.

Roots were embedded in acrylic resin block with coronal portion being exposed. The labial enamel surfaces were flattened with 600 grit silicon carbide paper (P 600 3M) and later enamel surfaces of 40 teeth were bleached with Opalescence Boost (40% Hydrogen Peroxide Gel, Ultradent, USA) for 10 minutes according to manufacturer's instructions. Among bleached teeth, 10 teeth (Group I) received composite restoration immediately. Remaining 30 teeth were randomly divided into three groups of 10 teeth each (Group II, III and IV), depending on the type of antioxidant used. Ten teeth served as controls (Group V) and did not receive any bleaching treatment. The distribution of specimens and the study groups are listed in [Table/Fig-1].

Group I (n=10): Labial surface of specimens was etched with 37% phosphoric acid (Total Etch Etching Gel, Ivoclar Vivadent.) for a period of 15 seconds immediately after bleaching and rinsing. This was followed by rinsing with water for 20 seconds, and bonding with Adper Single Bond (3M ESPE, Dental Products). Finally composite build-up was done using incremental technique to obtain 5mm height and 3mm diameter (Filtek Z350 XT, 3MESPE, Dental Products).

Group II (n=10): Immediately after bleaching and rinsing, the labial surfaces of the specimens were treated with 5% grape seed solution for 10 minutes and rinsed. The surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond. This was followed by composite build-up in increment technique.

Group III (n=10): Immediately after bleaching and rinsing, the labial surfaces of the specimens were treated with 5% pine bark solution for 10 minutes and rinsed. The surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond. This was followed by composite build-up in increment technique.

Group IV (n=10): Immediately after bleaching and rinsing, the labial surfaces of the specimens were treated with 5% pomegranate extract for 10 minutes and rinsed. The surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond. This was followed by composite build-up in increment technique.

Group V (n=10): It was the control group; specimens were not subjected to bleaching. The labial enamel surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond. Followed by composite build-up with increment technique.

Shear Bond Strength testing: Before SBS testing was performed in an universal testing machine (Instron, UK), all specimens were stored in distilled water for 24 hours. The SBS was calculated at a crosshead speed of 0.5 mm/min in shear mode until fracture occurred.

Groups	Bleaching Agent	Antioxidants Used	Composite Build
Group I(n=10)	40% Hydrogen Peroxide	None	Done immediately
Group II(n=10)	40% Hydrogen Peroxide	5% Grape Seed Solution	Done immediately
Group III(n=10)	40% Hydrogen Peroxide	5% Pine Bark Solution	Done immediately
Group IV(n=10)	40% Hydrogen Peroxide	5% Pomegranate Solution	Done immediately
Group V(n=10) (Control group)	None	None	Done immediately

[Table/Fig-1]: The distribution of specimens and study groups.

STATISTICAL ANALYSIS

The values obtained were statistically analyzed using computer software Statistical Package for Social Sciences (SPSS) version 16.0. One-way analysis of variance (ANOVA) followed by Mann Tukey Post hoc test was used to analyze the data. Significance was established at $p< 0.05$ level.

RESULTS

The results of this study are shown in [Table/Fig-2]. Graphic representation of the comparison of mean SBS among groups is given in [Table/Fig-3].

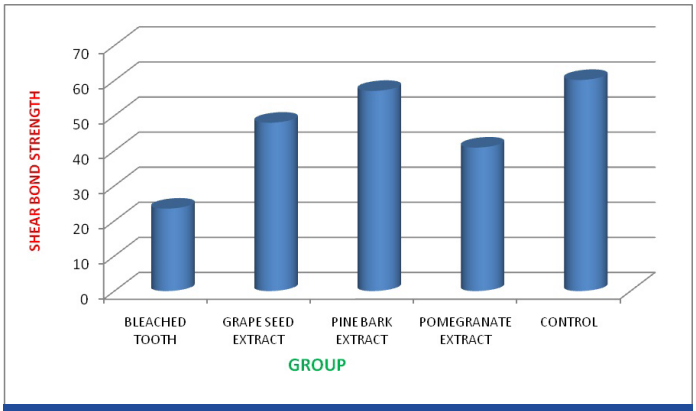
DISCUSSION

In present day practice tooth discoloration has always been a factor of utmost concern as more stress is being placed on aesthetics. With the developing awareness of aesthetic options, there is a great demand for various treatment modalities for discoloured teeth. While conserving the tooth structure it also permits a successful aesthetic outcome at minimal expense [11,12]. Vital tooth bleaching procedures are the most commonly used conservative and effective treatment options to treat discoloured teeth [5].

In spite of the constructive results obtained with the bleaching agents like carbamide peroxide and hydrogen peroxide, there

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		ANOVA	POST-HOC*
					Lower Bound	Upper Bound		
Bleached Tooth (1)	10	23.501	2.400	0.7591	21.783	25.218	F=793.47 p<0.05	1<2,3,4,5
Grape Seed Extract (2)	10	47.992	1.192	0.3771	47.138	48.845		3,5>2>1,4
Pine Bark Extract (3)	10	57.070	1.746	0.5521	55.820	58.319		5>3>1,2,4
Pomegranate Extract (4)	10	32.008	1.457	0.4609	30.965	33.050		1<4<2,3,5
Control (5)	10	60.181	1.202	0.3802	59.320	61.041		5>1,2,3,4

[Table/Fig-2]: Comparison of mean SBS values of different groups (MPa).
N denotes corresponding group number; bold numbers indicate statistically significant differences between groups.



[Table/Fig-3]: Graphic representation of the comparison of mean SBS among groups (MPa).

are many studies that have observed morphological alterations of teeth, increased surface irregularities, roughness and reduced microhardness of enamel. Another important complication is decreased bond strength of composite resin to enamel [5,7,13].

Following bleaching the compromised SBS is due to the fact that the bleaching agent leaves behind a free residual oxygen layer which inhibits the polymerization of composite resin and interferes with the resin infiltration into etched enamel [14,15]. The structural changes caused by the incorporation of peroxide ions are eliminated upon storage for 24 hours - 3 weeks as the peroxide ions decomposes and the substituted hydroxyl radicals re-enter the hydroxyapatite lattice [5,7,8].

Utilization of natural antioxidants like plant extracts as a viable alternative to chemical and synthetic antioxidants have been reported in recent years [16]. Hence, in this study, emphasis was placed on the use of pomegranate peel, grape seed and pine bark extract as antioxidants immediately following the bleaching procedure to reverse the compromised bond strength of composite resin to bleached enamel.

All these three antioxidants were capable of reversing the reduced SBS following bleaching [5,17,18]. The reduced SBS in Group I when compared to Group II, III, IV and V may be due to the residual oxygen layer left behind following the bleaching process which could have interfered with the resin infiltration into etched enamel and inhibited the polymerization of composite resin [8,14,18,19].

Group II specimens showed significantly higher SBS than that of Group I and IV but lesser than that of Group III and V. These findings were in accordance with other studies which states that this could be attributed to the specificity of OPCs for hydroxyl free radicals, the presence of multiple donor sites on OPCs that trap superoxide radicals and the esterification of (-) epicatechin by gallic acid in OPCs, which enhances the free radical scavenging activity [5,8].

OPCs are a class of polyphenolic bioflavonoids found most commonly in fruits and vegetables. They are present in grape seed extract, pine bark extract, cranberries, lemon tree bark, hazel nut tree leaves, etc. They have free radical scavenging and antioxidant activity. They also have antibacterial, antiviral, anti-inflammatory, antiallergic, anticarcinogenic, and vasodilatory actions [20].

The composition of grape seed extract consists of OPCs in the form of monomeric phenolic compounds such as catechin, epicatechin, and epicatechin-3-0-gallate (in dimeric, trimeric, and tetrameric procyanidin form) and free flavanol monomers [21].

Group III specimens showed a higher mean SBS value than that of Group I, II and IV specimens. The difference in the antioxidant activity of grape seed, pine bark and pomegranate peel extract and could likely be attributed to their different phenolic compositions [8,10].

The pine bark extract consists of phenolic compounds generally divided into monomers like catechin, epicatechin, taxifolin as well as compacted flavonoids like oligomeric to polymeric proanthocyanidins [22].

Group IV specimens showed significantly higher SBS than that of Group I but lesser than that of Group II and III. These findings were in accordance with other studies which state that this could be attributed to their different phenolic compositions [8,10].

Group V specimens showed significantly higher SBS than that of Group I, II, III and IV, this may be due to absence of the residual oxygen layer. These findings were in accordance with other studies done by Subramonian R and Arumugam MT et al., [8,12]. On the contrary, the study done by Vidhya S et al., reported that treatment with as 5% grape seed extract increases SBS significantly compared with other experimental groups and control group [5].

Studies have shown that the application of 5% grape seed extract as an antioxidant for 10 minutes reversed the compromised bond strength [5,7,12]. Application of 5% pine bark extract as an antioxidant for 10 minutes yielded almost the same bond strength as that of 10% sodium ascorbate [9]. Application of 5% pomegranate extract as an antioxidant for 10 minutes yielded almost the same bond strength as that of 5% grape seed extract and green tea extract [10]. In this study, 5% solution of grape seed extract, 5% pine bark extract and 5% pomegranate peel extract were applied for 10 minutes in order to evaluate and compare the effect of natural antioxidants on the reversal of compromised SBS of composite resins to bleached enamel.

In this study, solution of pomegranate peel extract (5%) was used to neutralize the negative effect of free radicals on the bonding agent polymerization and it was shown to be effective in reversal of reduced bond strength to bleached enamel [10].

Clinical Implication: Use of antioxidants immediately following the bleaching procedure to reverse the compromised bond strength of composite resin to bleached enamel without waiting for a period of 24 hours to 3 weeks.

- Comparisons of present study with previous similar studies

Previous study	Present study
1. Vidhya S et al., (2011) concluded that OPCs shows higher SBS than that sodium ascorbate [5].	The aim of this in vitro study was to evaluate and compare the effect of 5% grape seed extract, 5% pine bark extract, and 5% pomegranate extract on the SBS of composite resin to bleached enamel and concluded that of 5% pine bark extract significantly increases the SBS of composite resin to bleached enamel than that of 5% grape seed extract and 5% pomegranate peel extract.
2. Mageshwaran Thandalam et al., (2014) showed significantly higher bond strength compared to proanthocyanidin and lycopene [12].	
3. Subramonian R, et al., (2015) concluded that 10% pine bark extract application after bleaching showed better bond strength [8].	

LIMITATION

- Height (5mm) of composite resin build up does not simulate the clinical condition like veneer restorations.
- As it was an in-vitro study, direct estimation of these results to clinical application requires further in-vivo studies.

CONCLUSION

Under the limitations of this in-vitro study, it can be concluded that treatment of the bleached enamel surface with 5% grape seed extract or 5% pine bark extract or 5% pomegranate reverses the reduced SBS of composite resin. Among antioxidants, the use of 5% pine bark extract significantly increases the SBS of composite resin to bleached enamel than that of 5% grape seed extract and 5% pomegranate peel extract.

REFERENCES

[1] Watts A, Addy M. Tooth discolouration and staining: A review of the literature. *Br Dent J*. 2001;190(6):309-16.
[2] Wray A, Welbury R. Treatment of intrinsic discoloration in permanent anterior teeth in children and adolescents. *Int J Paediatr Dent*. 2001;11(4):309-15.

[3] McCloskey RJ. A technique for removal of fluorosis stains. *J Am Dent Assoc*. 1984; 109(1):63-64.
[4] Kimyai S, Oskoee SS, Rafighi A, Valizadeh H, Ajami AA, Helali ZN. Comparison of the effect of hydrogel and solution forms of sodium ascorbate on orthodontic bracket-enamel SBS immediately after bleaching: an in vitro study. *Indian J Dent Res*. 2010; 21(1):54-58.
[5] Vidhya S, Srinivasulu S, Sujatha M, Mahalaxmi S. Effect of grape seed extract on the bond strength of bleached enamel. Oper Dent. 2011; 36(4):433-38.
[6] Minoux M, Serfaty R.Vital tooth bleaching: biologic adverse effects-A review. *Quintessence Int* . 2008;39(8):645-59.
[7] Carlos RG, Kand FA, Alessandra BB. The effects of antioxidant agents as neutralizers of bleaching agents on enamel bond strength. *Braz J Oral Sci*. 2006; 5(16):971-76.
[8] Subramonian R, Mathai V, Christaine Angelo JB, Ravi J. Effect of three different antioxidants on the SBS of composite resin to bleached enamel. An in vitro study. *J Conserv Dent*. 2015; 18(2):144-48.
[9] Aksakalli S, Ileri Z, Karacam N. Effect of pine bark extract on bond strength of brackets bonded to bleached human tooth enamel. *Acta Odontol Scand.* 2013; 71(6):1555-59.
[10] Sharafeddin F, Motamedi M, Modiri Sh. Effect of immediate application of pomegranate peel, grape seed and green tea extracts on composite SBS of in-office bleached enamel. *Res J Biologic Scien*. 2013;8:83–87.
[11] Sharafeddin F, Jamalipour GR. Effects of 35% carbamide peroxide gel on surface roughness and hardness of composite resins. *J Dent.* 2010;7(1):6-12.
[12] Arumugam MT, Nesamani R, Kittappa K, Sanjeev K, Sekar M. Effect of various antioxidants on the SBS of composite resin to bleached enamel: An in vitro study. *J Conserv Dent*. 2015; 17 (1):22-26.
[13] Cavalli V, de Carvalho RM & Giannini M. Influence of carbamide peroxide based bleaching agents on the bond strength of resin-enamel/dentin interfaces. *Brazilian Oral Research*. 2005;19(1):23-29.
[14] Kimyai S, Valizadeh H. The effect of hydrogel and solution of sodium ascorbate on bond strength in bleached enamel. *Oper Dent*. 2006; 31(4):496-99.
[15] Khoroushi M, Feiz A, Khodamoradi R. Fracture resistance of endodontically-treated teeth: Effect of combination bleaching and an antioxidant. *Oper Dent*. 2010; 35(5):530-37.
[16] Wood JE, Senthilmohan ST, Peskin AV. Antioxidant activity of procyanidin containing plant extracts at different pHs. *Food Chem*. 2002;77:155-61.
[17] Ferreira R, Nunes TV, Luiz BK, Garcia RN. Effects of catalase, 2% chlorhexidine gel and 1% sodium hypochlorite on the microtensile bondstrength of teeth bleached with 35% hydrogen peroxide. *RSBO*. 2011;8(3):266-70.
[18] Nour El-din AK, Miller BH, Griggs JA, Wakefield C. Immediate bonding to bleached enamel. *Oper Dent*. 2006;31(1):106-14.
[19] Turkun M, Celik EU, Kaya AD, Arici M. Can the hydrogel form of sodium ascorbate be used to reverse compromised bond strength after bleaching? *J Adhes Dent*. 2009;11(1):35-40.
[20] Fine AM, Oligomeric proanthocyanidin complexes: History, structure, and phytopharmaceutical applications. *Altern Med Rev*. 2000; 5(2):144-51.
[21] Perumalla AVS, Hettiarachchy NS. Green tea and grape seed extracts potential applications in food safety and quality. *Food Research International*. 2011;44(4):827–39.
[22] Min Kim S, Kang SW, Jeon S, Hun Um B. Comparison of Pycnogenol and bark extracts from pinus thunbergii and pinus densiflora: Extractability, antioxidant activity and proanthocyanidin composition. *J Med Plants Res*. 2012;6(14):2839-49.

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