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# INFLUENCE OF NATURAL ANTIOXIDANTS ON MICROSHEAR BOND STRENGTH TO BLEACHED ENAMEL: CHEMICAL VERSUS LASER ASSISTED BLEACHING

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## *ABSTRACT*

**Objective:** To investigate the effect of some natural antioxidants on microshear bond strength to enamel bleached using chemical and laser assisted bleaching.

**Methods:** Sound human maxillary central incisors were used in the study. The labial surfaces were ground to obtain flat enamel surfaces. Bleached specimens were divided into 2 main groups according to the bleaching protocol; chemical bleaching and laser assisted bleaching. Each group was subdivided into 4 subgroups according to the antioxidant used; 10% Ascorbic acid, 10% Alpha-tocopherol, 10% Hesperidin and No antioxidant. Non-bleached specimens were prepared to be used as a control group. Bonding procedures were done 1h after bleaching. Microshear bond strength ($\mu$SBS) was tested using a universal testing machine. Data were tabulated and statistically analyzed using Two-way ANOVA followed by Bonferroni's post-hoc test.

**Results:** The control group showed the highest $\mu$SBS value. Using hesperidin and ascorbic acid with both bleaching protocols recorded no significant difference compared to the control group. Also, laser bleaching without antioxidant was statistically comparable to the control group. Regarding type of antioxidant, hesperidin showed the highest $\mu$SBS results followed by ascorbic acid while $\alpha$-tocopherol showed the lowest values.

**Conclusion:** Hesperidin and ascorbic acid have the ability to regain microshear bond strength to bleached enamel with superior performance of hesperidin. Alpha tocopherol is not able to reverse compromised bond strength after tooth bleaching. Laser assisted bleaching could allow immediate bonding procedures without the need for antioxidant application.

**KEY WORDS:** Natural antioxidants, hesperidin, chemical bleaching, laser bleaching, microshear bond strength.

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## INTRODUCTION

Nowadays, the increasing demand for a brighter smile is the rationale behind visiting dental offices seeking for teeth bleaching. Dental bleaching is a conservative and non-invasive line of treatment [1] based on a complex oxidation reaction [2]. Hydrogen peroxide is the most commonly used bleaching material [3]. It has a strong oxidizing property which is attributed to its instability that makes it easily decomposed to form free radicals. It is able to penetrate through enamel prisms and reach dentin tissue to break down pigmented molecules and achieve a lighter appearance[4,5] due to its low molecular weight[5]. Power bleaching was introduced in an attempt to achieve quick and efficient teeth whitening[6] using high intensity light[7] as conventional halogen units, plasma arc lamps, LED lights and lasers [1,6].

Free radicals are molecules containing unpaired electrons which tend to capture electrons from other molecules to get stabilized and at the same time creating other free radicals. This continuous process leads to production of thousands of free radical reactions within seconds[8]. Oxygen, hydroxyl and perhydroxyl ions become trapped in tooth structure and inhibit polymerization of resin materials [9]. It was demonstrated that peroxide ions replace the hydroxyl ions in the apatite lattice during bleaching procedures. This lattice structure is unstable and subsequently reversed within 2 weeks of storage as the peroxide ions decomposed and the hydroxyl ions regain its position in the apatite lattice [10].

Since, bleaching is frequently associated with replacement of esthetic restoratives to obtain more acceptable shades [11], some clinicians prefer to delay any bonding procedures for a period of time ranging from 24 hours up to few weeks [12-14]. Another approach was advocated, is the use of antioxidants in order to save time and allow immediate performance of any bonding procedures [2,5,15].

Ascorbic acid and its salt are powerful antioxidants that are characterized by their capability of reducing many oxidative compounds[10,16]. The most active component of vitamin E is the alpha tocopherol which is characterized by its antioxidizing potential in the human body [17]. Vitamin E was used for enhancing the reversal of the bond strength to tooth tissue by scavenging the free radicals and the molecular oxygen in a mode of action similar to that of the ascorbic acid [18].

Hesperidin is a type of flavonoids which is abundant in citrus fruits. The flavonoid hesperidin is compromised of the hesperitin flavanone and the rutinose disaccharide. It is known to have a wide range of pharmacological effects as antioxidant, anti-inflammatory, anti-allergic, hypolipidemic, vasoprotective, and anticarcinogenic action [8,19].

Numerous studies have compared the anti-oxidizing potential of ascorbic acid and α-tocopherol and demonstrated conflicting results [2,15,18,20]. However, no researches were found regarding the antioxidant effect of hesperidin on bleached enamel. Hence, this study was conducted to evaluate the effect of some natural antioxidants on the microshear bond strength to enamel after bleaching with either chemical or laser assisted bleaching.

## MATERIALS AND METHODS

### Preparation of the solutions

Three natural antioxidants were prepared in a solution form for the current study; 10% ascorbic acid, 10% α-tocopherol and 10% hesperidin. The solutions were prepared by adding 10gm of each antioxidant powder in 100ml of its dissolving solution to obtain 10% concentration. Ascorbic acid powder ≥99% (Sigma–Aldrich; St. Louis, MO, USA) was dissolved in distilled water. Alpha-tocopherol powder ≥96% (Sigma–Aldrich; St. Louis, MO, USA) was dissolved in absolute ethanol (ELNasr Pharmaceutical Chemicals Co, Cairo, Egypt) and hesperidin powder ≥80% (Sigma–Aldrich; St. Louis, MO, USA) was dissolved in

dimethyl sulfoxide (Fisons Scientific Equipment; Bishop Meadow Road, *Loughborough*, LE11 ORG, UK).

**Specimens' preparation and grouping**

Sound human maxillary central incisors were used in the current study. The teeth were washed under running water, scaled from adhering soft tissue, plaque and calculus and then stored in distilled water at 4°C for not more than one month. Roots of the central incisors were cut 2 mm below the cemento-enamel-junction. The crowns were mounted in self-cured acrylic resin blocks using metal molds (2 cm x 3 cm) with the labial surface facing upward. Enamel was wet ground using 80 grit sandpaper discs to achieve flat enamel surfaces. A uniform smear layer was then achieved by utilizing wet 600 grit sandpaper discs.

The specimens were divided into 9 groups. Bleached specimens were divided into 2 main groups according to the bleaching protocol; chemical bleaching and laser assisted bleaching. Each group was subdivided into 4 subgroups according to the used antioxidant; 10% ascorbic acid, 10% α-tocopherol, 10% hesperidin and No antioxidant. Non-bleached specimens were prepared to be used as a control group.

**Bleaching procedures**

After flattening of labial enamel, two bleaching protocols were applied. Chemical bleaching was carried out using 40% hydrogen peroxide gel (WhiteSmile, POWER WHITENING YF, GmbH, Germany). The bleaching material was applied in a uniform thickness, left for 20min according to the manufacturer's instructions and rinsed using copious amount of water. Laser activated bleaching material was 35% hydrogen peroxide gel with activator (JW Power Bleaching Next, Heydent, GmbH, Germany). The bleaching material was applied in a uniform thickness for 15 min according to the manufacturer's instructions, activated for 30 sec using diode laser (Biolase, EPIC™ 10 Diode Laser, USA) with a wave length 940 nm and power 2W (Figure 1).

**Surface treatment of the specimens**

After 1 hour of the bleaching procedures, the specimens recommended for treatment with antioxidants were treated using 10% ascorbic acid, 10% α tocopherol and 10% hesperidin solutions using microbrushes. Each solution was applied for 10min, washed under running water and gently dried.


Fig (1) Specimens subjected to diode laser assisted bleaching using Biolase device

**Bonding procedures**

All the specimens were etched for 15 sec using 37% phosphoric acid etchant gel (UltraEtch®, ULTRADENT,Inc, USA), rinsed under running water and gently dried. Two coats of universal bonding agent (Single Bond Universal adhesive, 3M ESPE, St.Paul, MN, USA) were applied on the bleached enamel surfaces using disposable microbrushs. Rubber microtubes of 1 mm diameter and 1 mm height (Harvard tubing, USA) were placed on the treated surfaces of the specimens. The adhesive resin was light cured using LED light curing unit (Elipar S10 free light 3M ESPE) with light intensity 1200 mW/cm$^2$ for 10sec according to the manufacturer's instructions.

The microtubes were filled with flowable composite (Filtek™ Z350 XT Flowable Restorative, 3M ESPE, St.Paul, MN, USA). The microtubes were covered with celluloid strips (Stripmat, POLYDENTIA, CH-6805 Mezzovico, Switzerland) and cured for 20 sec with a total number of 10 resin composite cylinders for each group. After curing of resin composite, the rubber microtubes were sectioned longitudinally using sharp scalpel and removed and the excess adhesive resin was scraped. Samples which were debonded during removal of rubber microtubes, were excluded. The specimens were then stored in distilled water for 24 h before testing.

**Microshear bond strength testing (μSBS)**

The microshear bond strength was tested using a universal testing machine (Lloyd LR 5K, Lloyd Instruments Ltd, Hampshire, UK) with a cross head speed of 0.5mm/min. A thin metal wire (0.2 mm diameter) was looped around each resin composite cylinder and gently held flushed with the resin-dentin interface. The metal wire was secured in the upper compartment of the universal testing machine. Each resin composite cylinder was stressed to failure, and the force required for debonding was divided by the bonded area of the specimens to express the bond strength values in MPa.

**Statistical Analysis**

Numerical data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Microshear bond strength data showed parametric distribution. Two-way ANOVA test was used to study the effect of bleaching protocol, antioxidant and their interactions on microshear bond strength. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

**RESULTS**

Two-way ANOVA results showed that bleaching protocol, antioxidant and the interaction between the two variables had a statistically significant effect on mean microshear bond strength (table 1). Descriptive statistics of microshear bond strength values were represented by mean, standard deviation (SD), minimum, maximum and 95% Confidence Interval (95% CI) values in table 2.

TABLE (1) Two-way ANOVA results for the effect of different variables on mean microshear bond strength

| Source of variation | Sum of Squares | df | Mean Square | *F*-value | *P-value* |
|---|---|---|---|---|---|
| Bleaching | 134.719 | 1 | 134.719 | 35.954 | <0.001* |
| Antioxidant | 184.197 | 3 | 61.399 | 16.386 | <0.001* |
| Bleaching x Antioxidant interaction | 96.240 | 3 | 32.080 | 8.561 | <0.001* |

***df: degrees of freedom = (n-1), *: Significant at P ≤ 0.05***

Table 3 shows that without antioxidant application, there was no statistically significant difference between control group (No bleaching) and laser activated bleaching. Chemical bleaching without antioxidant showed the significantly lowest mean $\mu$-SBS. Using hesperidin as well as ascorbic acid revealed no significant difference in comparison to the non-bleached group with both chemical and laser activated bleaching. Alpha tocopherol showed the lowest mean $\mu$-SBS with both bleaching protocols.

The effect of antioxidant on $\mu$SBS is demonstrated in table 4. With chemical bleaching, hesperidin showed the significantly highest mean $\mu$SBS followed by ascorbic acid then α-tocopherol. No significant difference was recorded between No antioxidant group and α-tocopherol group. However, Laser bleaching revealed no statistically significant difference between no antioxidant group and hesperidin group; both showed the highest mean $\mu$SBS values among other antioxidants.

TABLE (2) Descriptive statistics of microshear bond strength values

| Bleaching | Antioxidant | Mean | SD | Minimum | Maximum | 95% CI | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower bound | Upper bound |
| Chemical Bleaching | Ascorbic acid | 15.3 | 2.5 | 11.9 | 18.7 | 12.7 | 17.9 |
| | α-tocopherol | 12.1 | 1.7 | 10.3 | 14.5 | 10.4 | 13.8 |
| | Hesperidin | 18.3 | 1.3 | 16.1 | 19.7 | 17.0 | 19.7 |
| | No antioxidant | 11.0 | 1.6 | 9.0 | 13.5 | 9.3 | 12.7 |
| Laser Bleaching | Ascorbic acid | 17.1 | 1.6 | 14.8 | 19.5 | 15.5 | 18.8 |
| | α-tocopherol | 14.7 | 2.1 | 12.0 | 18.0 | 12.4 | 16.9 |
| | Hesperidin | 19.2 | 1.7 | 17.5 | 21.8 | 17.4 | 20.9 |
| | No antioxidant | 19.1 | 2.9 | 16.0 | 23.7 | 16.1 | 22.2 |
| No Bleaching | Control | 18.7 | 1.5 | 16.8 | 21.3 | 17.1 | 20.3 |

TABLE (3) Mean, standard deviation (SD) values and results of comparison between bleaching protocols with different interactions of variables

| | ChemicalBleaching | | Laser Bleaching | | No bleaching | | P-value |
|---|---|---|---|---|---|---|---|
| Antioxidant | Mean | SD | Mean | SD | Mean | SD | |
| Ascorbic acid | 15.3 | 2.5 | 17.1 | 1.6 | 18.7 | 1.5 | 0.101 |
| α- tocopherol | 12.1 $^{B}$ | 1.7 | 14.7 $^{B}$ | 2.1 | 18.7 $^{A}$ | 1.5 | 0.028* |
| Hesperidin | 18.3 | 1.3 | 19.2 | 1.7 | 18.7 | 1.5 | 0.452 |
| No antioxidant | 11.0 $^{B}$ | 1.6 | 19.1 $^{A}$ | 2.9 | 18.7 $^{A}$ | 1.5 | <0.001* |

***: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different***

TABLE (4) Mean, standard deviation (SD) values and results of comparison between antioxidants with different interactions of variables

| Bleaching | Ascorbic acid | | Alpha tocopherol | | Hesperidin | | No antioxidant | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | |
| Chemical | 15.3 $^{B}$ | 2.5 | 12.1 $^{C}$ | 1.7 | 18.3 $^{A}$ | 1.3 | 11.0 $^{C}$ | 1.6 | <0.001* |
| Laser | 17.1 $^{B}$ | 1.6 | 14.7 $^{C}$ | 2.1 | 19.2 $^{A}$ | 1.7 | 19.1 $^{A}$ | 2.9 | <0.001* |

***: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different***

## DISCUSSION

The current study was carried out to investigate the effect of natural antioxidants on microshear bond strength to enamel bleached using chemical and laser activated bleaching materials.

Antioxidants are compounds that provide their protective effect via one of two mechanisms. The first mechanism is that it donates a hydrogen ion to the free radical (ArOH) and subsequently, itself becomes a radical: $R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet}$. The second mechanism via giving an electron to the free radical becoming itself a radical cation: $R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{\bullet+}$ [21].

In the present study, chemical bleaching without application of antioxidant resulted in massive reduction in $\mu$SBS in comparison to all tested groups. Numerous researches supported this finding [2,22-24]. Bleaching agents are usually blamed to reduce the bond strength due to the presence of residual oxygen on the enamel surface which became trapped within the adhesive [3,20,23]. Residual oxygen interferes with resin infiltration into bleached enamel and inhibits resin polymerization [3,10,16] forming spherical bubble-like structures observed along the resin-enamel junction [10,20,24]. In addition, changes in enamel surface as loss of prismatic structure, decreased mineral content especially calcium, alterations in organic substance, decreased microhardness, and increased porosity leading to an over-etched appearance are also considered responsible for the reduction in bond strength [1,3,16].

Laser assisted bleaching revealed an unaltered bond strength values as compared to the No bleaching group. This might be due to the accelerated elimination of residual free oxygen

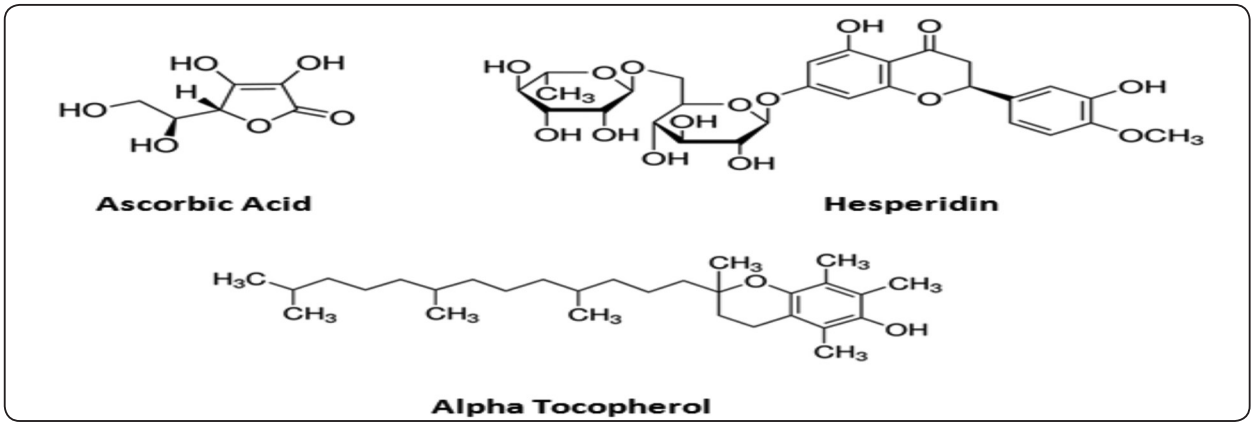


Fig. (2) Molecular structure of ascorbic acid, α-tocopherol and hesperidin

radicals as a result of the temperature rise caused by laser application [25]. *Dostalova et al*,[7] *and De Moor et al*,[26] reported that diode laser assisted bleaching do not induce surface morphological changes in dental enamel, which was previously reported to be a major cause of reduction of bond strength after teeth bleaching. This was in accordance with *Mirhashemi et al*,[3] who assessed the effect of laser assisted bleaching on shear bond strength at different time intervals and found that 1 hour is sufficient for the reversal of bond strength.

According to the results of the current study, Hesperidin application with both chemically bleached and laser bleached specimens was comparable to the control group. It has also recorded the highest bond strength values significantly among all tested antioxidants. This might be attributed to its molecular structure. Hesperidin is a member of flavanone group of flavonoids which are natural compounds characterized by their variable phenolic structures [19].

Phenolic compounds exhibit extensive free radical scavenging activities through their reactivity as hydrogen or electron-donating agents and metal ion chelating properties [27]. Several studies reported a direct relationship between total phenolic content and the antioxidant activity [27-29]. The antioxidant activity of the phenolic content seems to be related to their molecular structure, more precisely to the presence and number of hydroxyl groups [21]. According to the molecular structure as shown in figure 2, hesperidin possesses the highest number of hydroxyl groups among the tested antioxidants followed by ascorbic acid and the least is the α-tocopherol. *Kalpana et al*,[8] found that hesperidin has a higher radical scavenging activity than that of standards as ascorbic acid and tolox which is a water soluble analogue of vitamin E. They attributed this finding to the presence of hydroxyl group on B-ring in the hesperidin structure, which donates hydrogen and an electron to the hydroxyl radicals.

Use of ascorbic acid in the current study, showed high bond strength values of both chemical and laser bleached specimens with insignificant difference in comparison to the control group. This result is consistent with previous researches [5,10,22,23,25]. It has been suggested that ascorbic acid restores the compromised bonding as it alters the redox potential of the oxidized bonding substrate which allows polymerization of adhesive resin via avoidance of its premature termination [16,20]. Molecular structure of ascorbic acid contains 4 OH groups that can donate hydrogen to an oxidizing system [17]. On the other hand, it showed lower antioxidant effect than hesperidin. This may be explained by the lesser number of hydroxyl groups present in the molecular structure of ascorbic acid in comparison to hesperidin (figure 2) although, it seems that this number of hydroxyl groups is quiet sufficient to reverse the compromised bond strength after teeth bleaching.

Alpha tocopherol failed to improve bond strength in all tested groups. Again the molecular structure and number of hydroxyl groups might be the reason behind this finding. *Kavitha et al*,[18] found the same finding and attribute it to the non-aqueous nature of alpha tocopherol.

To summarize, chemical bleaching has a deleterious effect on microshear bond strength. In order to overcome this deleterious effect and to avoid time delay, the use of 10% hesperidin and 10% ascorbic acid is recommended as well as the use of laser assisted bleaching without the need for antioxidant application.

## CONCLUSIONS

Despite the limitations of the current study, the following could be concluded:

1- Hesperidin and ascorbic acid have the ability to regain microshear bond strength to bleached enamel with superior performance of hesperidin.

2- Alpha tocopherol is not able to reverse compromised bond strength after tooth bleaching.

3- Use of laser assisted bleaching could allow immediate bonding procedures without the need for an additional step as antioxidant application.

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