EFFECT OF MORINGA LEAF (MORINGA OLEIFERA LAM.) ETHANOL EXTRACT 10% AND ALPHA TOCOPHEROL 10% AS ANTIOXIDANT ON THE SHEAR BOND STRENGTH OF COMPOSITE RESIN TO ENAMEL FOLLOWING EXTRA CORONAL BLEACHING

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ABSTRACT

Introduction: After bleaching treatment, patients often require additional intervention for composite resin restorations. The initial composite restorations after bleaching with hydrogen peroxide (H_2O_2) 25-40% cannot be done immediately, due to the presence of free radical residues as a result of oxidation of the bleaching material it is recommended to delay bonding for 1-3 weeks. If the adhesive restoration are carried out immediately, the shear bond strength of the restoration would reduced. Free radicals can interfere with resin infiltration and inhibit complete polymerization. The use of antioxidants as an effective alternative treatment to overcome free radical residues and increase the shear strength of post-bleaching restorations. Alpha tocopherol is an antioxidant that has been known to bind free radicals, that adhesive action can be carried out immediately after bleaching. The potential natural resource being developed is moringa leaves (*moringa oleifera lam)* which is rich in antioxidants. The purpose of this study was to compare the differences in the use of antioxidants 10% alpha tocopherol and 10% ethanol extract of moringa leaves can increase the shear bond strength of the attachment in nanofilled composite restorations on teeth after bleaching with 40% H_2O_2.

Material and methods: Twenty seven insisivus were divided into three groups. Samples were treated with bleaching treatment, group I the application of 10% alpha tocopherol antioxidant, group II with ethanol extract of Moringa leaves for 10 minutes. Group III is the group without antioxidants. Then all samples were restored with nanofilled composites. The shear bond strength was measured by a universal testing machine. The data obtained were statistically tested with one-way analysis of variance

(ANOVA), while the LSD test was used to see the difference in the effect of the shear bond strength between groups.

Results: The highest shear bond strength of the composite resin was in the bleaching group of 40% hydrogen peroxide and using 10% alpha tocopherol as an antioxidant, while the lowest was in the control group bleaching using 40% hydrogen peroxide and immediate restoration without antioxidants

Conclusion: The application of antioxidant alpha tocopherol and ethanol extract of moringa leaves increased the shear bond strength of composite restoration to enamel following extra coronal bleaching.

Keyword : bleaching, antioxidant, ethanol extract of moringa leaves, shear bond strength

Introduction

Discolored teeth frequently affect people of various ages and are multifactorial in nature. With the evolution in esthetic dentistry, in-office and at-home bleaching procedures have become popular. Extra-coronal bleaching is considered a safe, popular, conservative and well-accepted treatment modality for discolored teeth Bleaching agents in varying concentration, namely carbamide peroxide (35% to 37%) or hydrogen peroxide (30% to 40%) have been used to achieved rapid esthetic results. (Abaraham S et al., 2013). Furthermore, hydrogen peroxide and carbamide peroxide have been used successfully for many years to achieve lighter and more desirable tooth color. (Vidhya S et al., 2010).

However, bleaching agents have certain adverse effects such as hypersensitivity, gingival irritation, micromorphological defects due to demineralization, and reduced enamel microhardness (Goldberg et al., 2010). In addition, bleaching agents affect the bonding of enamel and dentin tissues when restorations are applied immediately postbleaching. (Lai et al.,2002). This problem is more notable when the desired results after bleaching are not achieved, and the patient wishes for additional esthetic options such as direct and indirect veneers.(Gogia et al., 2018).

Some techniques have been suggested to solve the clinical problems related to post bleaching compromised bond strength, such as treated bleached enamel with alcohol before restoration, removal of the superficial layer of enamel, and the use of adhesives containing organic solvents (Whang et al., 2015). Another techniques have been proposed to resolve this clinical problem are as follows: conditioning the bleached enamel with alcohol before restoration, removing the surface layer of enamel, and employing adhesives containing organic solvents. Nonetheless, at present, the universal approach is to adjourn any bonding procedure for 4 days to 4 weeks. (Cavalli V et al., 2002). However, the general approach is to postpone any bonding procedure for a period after bleaching, because the reduction in bond strength has been shown to be temporary. The waiting period for bonding procedures after bleaching has been reported to vary from 24 hours to 4 weeks. Therefore, to overcome this delay in restorative treatment, several studies have proposed the use of antioxidant agents application of various antioxidant agents such as 10% sodium ascorbate, Vitamin E, and proanthocyanidin post bleaching have been proposed.(Kaya AD et al., 2008).

Alpha-tocopherol is the most active component of the vitamin E complex and is a powerful antioxidant in the human body in the lipid phase. The critical role of alpha-tocopherol protecting against free-radical reactions becomes apparent when considering the vast number of diseases and conditions, such as aging, many types of cancer, atherosclerosis and other circulatory diseases, arthritis, cataract formation, senile dementia, and respiratory diseases induced by pollution that are thought to be caused by these reactions (Whang et al., 2015). The utilization of natural antioxidants such as plant extracts as alternative treatments for synthetic and chemical antioxidants has been reported over the past few years (Himanshu et al., 2016). Themoringa extract (Moringa oleifera L) is made from moringa leaf, it contains potential source of antioxidants, phenols, flavonoids and also has antibacterial and antifungal activities (Lansky and Newman, 2007). Since moringa leaf grows abundance in Indonesia, then this material can be developed as an antioxidant after bleaching.

However, no studies have been conducted to compare the effects of alpha tocoferol to moringa extracts on the bond strength of bleached enamel. Hence, the aim of this in vitro study was to evaluate and compare the effects of 10% alpha-tocopherol and 10% moringa extracts on the bond strength of enamel following extra-coronal bleaching using 40% hydrogen peroxide.

Material and Method

This study was approved by Universitas Sumatera Utara Research Ethics Committee. Twenty seven incisorsfrom a dentist's practice with the following inclusion criteria: (1) maxillary incisors, (2) have no crown fracture, (3) have no caries and (4) the crowns remains was intact. The roots were sectioned approximately 2 mm apically of cemento-enamel junction (CEJ) using microtome. Each crown of teeth then was cut in mesial distal direction, and the buccal side of crowns were used in this study. Buccal side of crowns were embedded in an acrylic resin block, keeping only the buccal portion exposed, and were flattened with 600 grit silicon carbide paper to obtain flat and rough enamel surfaces.

All specimens were assigned randomly into 3 groups of 9 each. Group 1, specimens were bleached specimens were bleached using 40% hydrogen peroxide (Opalescence Xtra Boost 40%, Ultradent, South Jourdan, UT, USA) as manufacturer's direction. After bleaching, specimens were applied 10% alpha-tocopherol for 15 minutes,and specimens were acid etched with 37% phosphoric for 15 seconds, rinsed for 30 seconds and air dried for 10 seconds. A thin layer of adhesive material (Universal Bond, Dentsply) was applied on the etched enamel, gently spread with compressed air and light-cured for 10 seconds. Increments of a composite resin (G-Aenial, GC Japan) were inserted into tooth and increment was light cured for 20 seconds..(Fig 1)Group 2, specimens were bleached as in group 1, specimens were applied moringa leave extract and were applied composite resin as in group 1.Group 3 as control, specimens were bleached as group 1. After bleaching, composite resin restorations were performed immediately. The specimens were stored in an artificial saliva for 24 hours. Moringa leaf extracts were prepared by maceration method with ethanol solvent and were dilutes using distilled water to obtain 10% concentration

Each specimen was loaded in universal testing machine for shear bond strength. The long axis of the specimen was perpendicular to the direction of the applied forces. The knife edge was loaded at the interface between the composite and enamel surface (Fig 2).The shear bond strength was measured in shear mode at a crosshead speed of 1 mm/min until fracture occurred. The results were expressed in MPa, and were analyzed using a one-way ANOVA, followed by LSD test. Fracture analysis of the bonded enamel surface was performed using a scanning electron microscopy (SEM) at a magnification of X1000. The types of fractures were considered and classified as adhesive failure, cohesive failure or mixed failures.

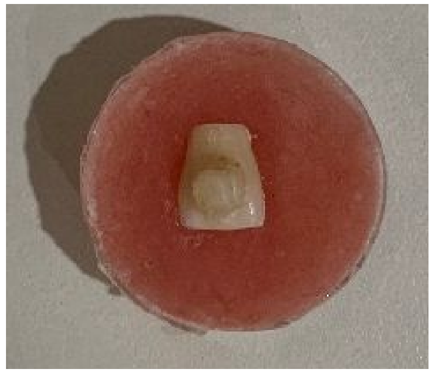

Fig 1. The specimen after applied resin composite

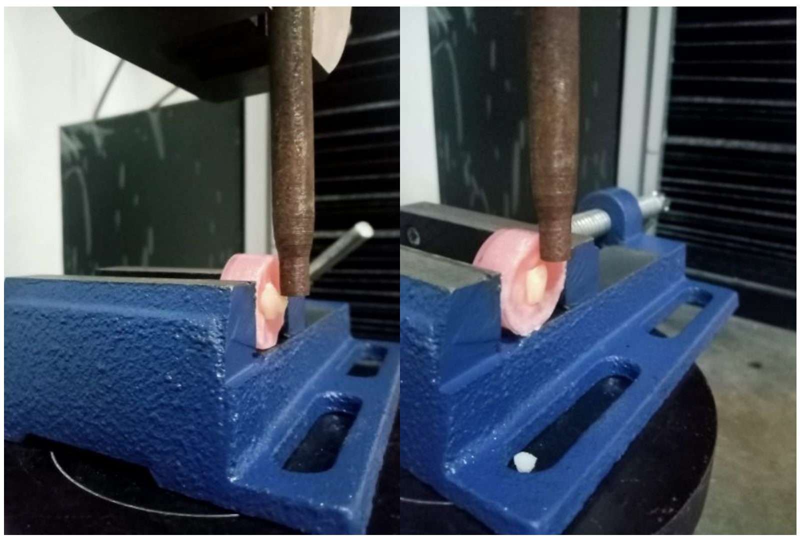

Fig 2. The knife edge was loaded at the interface between the composite and enamel surface.

Results

One-way ANOVA showed significant differences in shear bond strength among the groups ($p<0.05$). Results revealed (Table 1) that specimens were restored using composite resin immediately following bleaching, had the lowest shear bond strength compared to other groups ($p<0.05$).

No.	Shear Bond Strength	$\bar{x}$	SD	*p-value**
1.	Control	3,621	0,357	0,00001
2.	Alpha Tocoferol	10,538	0,224	
3.	Moringa Extract	6,478	0,330	

Table 1.showed a significant difference between each treatment groupwith p-value <0.05 using One-way ANOVAtest analysis

In table 2. shows that the difference in the mean value between the groups tested has a p-value <0.05, which means that there is a significant relationship between the test groups.

No.	Comparation Between Group	$\Delta \bar{x}$	*p-value**
1.	Alpha Tocoferol VS Control	6,917	0,0001
2.	Moringa Extract VS Control	2,857	0,0001
3.	Alpha Tocoferol VS Moringa Extract	4,060	0,0001

Table 2. showed result comparation between all group

Among antioxidants groups, 10% alpha-tocopherol revealed the highest shear bond strength compare to other antioxidants groups. Scanning electron microscopy observation of the fractured specimens demonstrated that the majority of failure of the specimens, which were restored immediately using composite resin following bleaching, were adhesive type (Fig. 3,4). In contrast the majority of specimens, which were applied antioxidants before restoration, produced mixed failures between adhesive and cohesive types (Fig. 5,6).

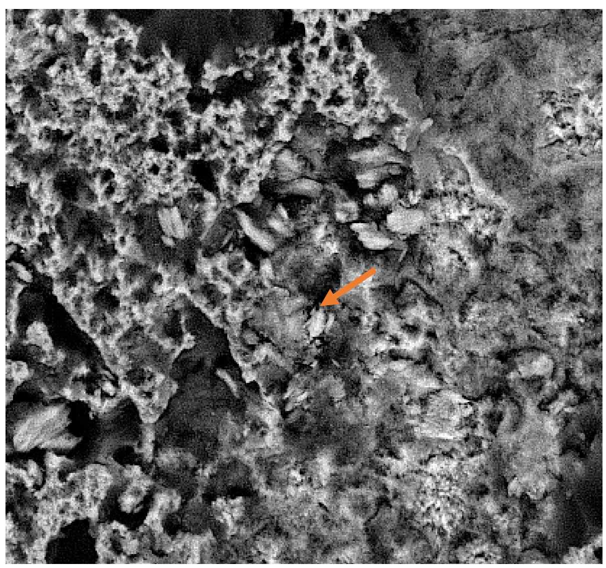

Fig 3. Showed enamel irregularity

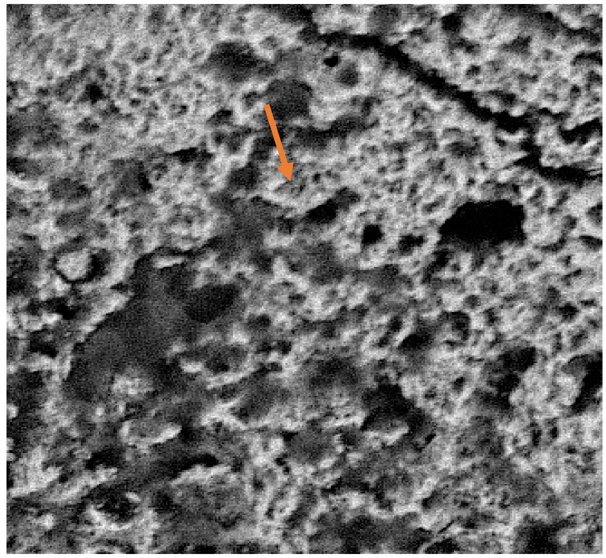

Fig 4. Showed adhesive type of failure

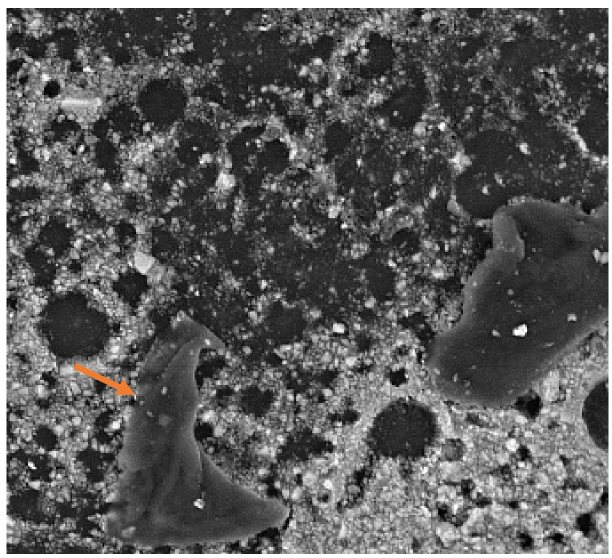

Fig 5. Showed mixed failure between cohesive and adhesive

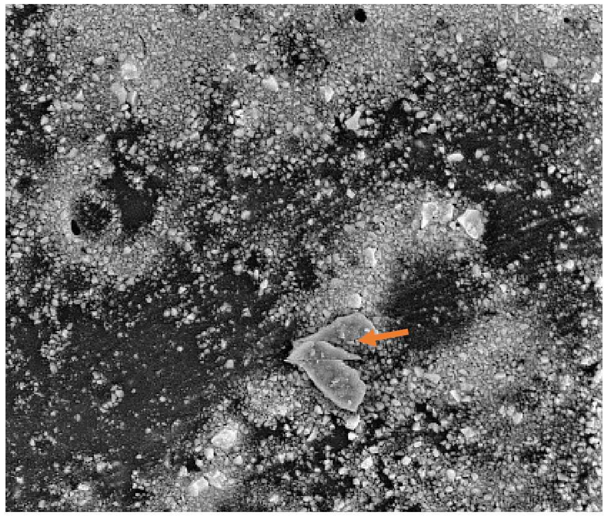

Fig 6. Showed mixed failure between cohesive and adhesive

Discussion

The bleaching process results in a color change through a redox reaction, namely the degradation of hydrogen peroxide which produces free radical ions and reactive oxygen then these ions penetrate into the enamel an diffuse into the dentin and the complex pigment molecules will transform into small molecules and produce a lighter color (Basting et al, 2004 Setzer, 2016). In the present study, it was observed that the extra-coronal bleaching procedure using 40% hydrogen peroxide resulted in a significant decrease of bond strength values compared to the unbleached group. The bleaching agents release free radicals as the nascent oxygen and hydroxyl or peri-hydroxyl ions when they are applied to the dental structure. (Nari-ratih et al., 2019). Free radical is any molecule that has one unpaired electron, providing it high reactivity. These molecules are able to react with the electron-rich regions of the pigments inside the dental structure, breaking down large pigmented molecules into smaller, less pigmented ones. On the other hand, this property could be deleterious to the bonding of resinous materials. One theory proposed to explain the influence of bleaching agents on bonding suggests that peroxides and their by-products present inside the dental structure are capable to interfere with the polymerization process of the adhesive material (Perchyonok et al., 2015).

The bleached specimens in the group 3, which were immediately restored using composite resin without any antioxidant treatment showed the lowest bond strength values with bond failure at the interface between the tooth substrate and the bonding agent. This was probably due to residual oxygen produced by bleaching agent on the tooth surface inhibiting polymerization of the bonding agent. As a result, the oxygen-rich tooth structure did not provide a good surface for bonding (Nari-ratih et al., 2019). In addition, as reported by (Moosavi et al., 2013) the effect of the bleaching agent reached the inside of the tooth structure. (Whang et al., 2015) observed using SEM, revealed that 40% hydrogen peroxide produces a much more irregular pattern on dentin, with shallow erosive areas covering the sample surface.

It has been shown that bleaching treatment with hydrogen peroxide, hydroxyl radicals in the apatite lattice are substituted by peroxide ions, resulting in the formation of peroxide-apatite. After a two-week waiting period, peroxide ions may decompose and substituted hydroxyl radicals re-enter the apatite lattice, resulting in elimination of the structural changes caused by the incorporation of peroxide ions (Kavitha et al., 2013).

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify thesereactive products. The degree of oxidative stress experienced by cells will be a function of the activity of reactive oxygen species (ROS) generating reactions and the activity of the ROS scavenging system (Sies, 1991). Therefore, antioxidants are needed to remove reactive oxygen species (ROS) that are produced excessively (Poljsak et al, 2013).

Furthermore, Sharafeddin*et al.* (8) reported that dentin and dentinal fluid can act as a peroxide and oxygen reservoir. The reservoirs of gaseous or dissolved oxygen products could persist until removed by pulpal micro-circulation and diffusion from the external surface. A greater surface diffusion would be expected based on a reduced pulpal microcirculation. Thus, levels of peroxide or oxygen higher than normal may be present in bonding interface, inhibiting the polymerization reaction and reducing bond strengths.

Hydrogen peroxide, as a bleaching agent, cancaused collagen denaturation so that there is noeffective hybrid layer that results in weak bondformation. The low mechanical properties of thecomposite resin and the greater denaturation ofcollagen will result in lower shear bond strength.
(Van Noort, 2007).

Antioxidant agents may facilitate free radical polymerization of the adhesive resin without the occurrence of early termination. Neutralizing process from antioxidant agents to free radicals is categorized into 3 types, i.e., the prevention of continuous (full-time prevention), active detoxification of oxidative stress, and passive detoxification. Alpha-tocopherol and Moringa leaf are included in passive detoxification that can neutralize free radicals and belong to non-enzyme antioxidants. However, none of the antioxidant agents was capable of completely neutralizing the deleterious effects of bleaching on bond strength. (Nari-ratih et al ., 2019).

Vitamin E is the term used for a group of tocopherols and tocotrienols, of which alpha-tocopherol has the highest biological activity. Vitamin E functions as a chain-breaking antioxidant that prevents propagation of free radical reactions. Some studies reported that alcohol application on bleached enamel increased bond strength, although the values did not return to the levels of the nonbleached group. The presence of alcohol in the composition of the 10% α-tocopherol solution formulated for this study may have contributed to the good response in re-versing the compromised bond strength of bleached enamel since 10% α-tocopherol was not miscible in water solutions. Thus, the phenomenon observed might be not only due to the antioxidant agent of α-tocopherol but also to the presence of alcohol. Furthermore, vitamin E is more oxidizing and stable than ascorbate because of its hydrophobicity. (Nari-ratih et al ., 2019).

The results also demonstrated that moringa extract reversed the reduction in shear bond strength although it has lower shear bond strength value compared to alpha-tocopherol. It can be explained that moringa extract used are the whole of moringa leaves so that there are other ingredients apart from antioxidants that play a role that lower the value than alpha tocopherol.

In this study group alpha tocoferol 10% and moringa extract 10% the porosity pattern was not visible because of the aprismatic enamel and adhesive layer covering the surface of the enamel. The surface looks uniform and not porous (porefree). Control group showed a granular and porous surface (bubble appearance). Examination using Scanning Electron Microscopy on the teeth after bleaching showed that the resin tag was short and irregular, and it was not present on some surfaces (Nugraheni, 2017). The presence of granular and porous features with bubbles on the surface between resin and enamel after bleaching treatment. These bubbles can block the infiltration of the bonding material into the tooth structure (Freire et al., 2009). The adhesion of adhesive material is largely determined by the resin tag which will form the micro-retension of the etched enamel (Hamdy, 2018).

(Whang et al., 2015) evaluated the scanning electron microscope (SEM) images of interfaces between resin and bleached enamel and observed fragmented and poorly refined resin tags that penetrated to a lesser depth when compared with unbleached controls. In addition, the entrapment of peroxide ions into the bleached enamel resulted in a resin-bleached enamel interface that was granular and porous with a bubbled appearance. The alteration in organic substance, loss of calcium, and a decrease in micro-hardness added to this effect.

The results of this present study showed that alpha-tocopherol showed greater shear bond strength than moringa extracts. This condition is probably caused by lower molecular weight in alpha-tocopherol, although in this study molecular weight was not measured of each antioxidant agent. Consequently, the lower molecular weight of alpha-tocopherol cause these antioxidants to penetrate deeply into enamel than moringa extracts.

Conclusions

Within the limitation of this study, it can be concluded that application of antioxidant alpha tocopherol and moringa extract of increased the shear bond strength of composite restoration to enamel following extra coronal bleaching. On examination using Scanning Electron Microscopy the tooth surface of the group using antioxidant alpha tocoferol 10% and moringa extract showed mixed failure, and control group showed adhesive failure.

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