

An in-vitro evaluation of the effect of two different herbal antioxidants on the microleakage of self-adhesive composite resin restoration after external tooth bleaching - A stereomicroscopic study

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Abstract

Background & Objectives: The aim of this study was to assess the efficacy of 5% grape seed extract solution and 5% guava seed extract solution on the decreasing the adverse effect caused by bleaching agents, by evaluating the marginal leakage of self- adhesive composite resin restoration. Further the study aimed to evaluate the effects of the following three different antioxidant application times, 10 minutes, 30 minutes, and 120 minutes.

Methods: Box-shaped class V cavities were made on the buccal surface on the cervical third of of one hundred and five human premolar teeth. The teeth were then randomly divided into three groups. GROUP I (Control) (n = 15): Teeth were bleached followed by restoration

with Dyad Flow, GROUP II (n = 45): Teeth were bleached followed by application of 5% grape seed extract solution as antioxidant. GROUP III (n = 45): Teeth were bleached followed by application of 5% guava seed extract solution as antioxidant. Groups II and III were further divided into 3 subgroups: Sub group A (10 min), Sub group B (30 min) and Sub group C (120 min), comprising 15 specimens each, based on the time of application of antioxidant. After restoration, all the teeth were age hardened by thermocycling. All the surfaces of the teeth, except for a 1-mm zone surrounding the restorations’ margins, were covered with two coats of nail polish. The specimens were then immersed in 2% methylene blue for 24 hours at room temperature. After staining, the teeth were washed and

cleaned. The teeth were then sectioned longitudinally, from buccal to lingual, through the center of the restorations. The microleakage was assessed by judging the depth of the stain.

Results: Kruskal Wallis test followed by Mann Whitney post hoc Analysis was used to compare the mean microleakage scores between the Group I and the Sub Groups A, B, and C of Group II and Group III at different time intervals. The results demonstrated a highly significant difference in the microleakage between the three study groups both occlusally and gingivally. Multiple comparison of mean difference in microleakage scores showed a very highly significant difference between Group I (Control) and the experimental groups, Group II and Group III. 5% guava seed extract application (Group III) showed lower microleakage with increase in time when compared with the 5% grape seed extract application (Group II) though it was not statistically significant. Application of 5% guava seed extract at 120 minutes (Group III C) showed the least microleakage.

Interpretation & conclusion: The present study showed considerable decrease in the microleakage following application of 5% grape seed and 5% guava seed extract containing antioxidants before bonding to the bleached tooth. Hence it could be considered to be a viable alternative to delaying restoration for 3 weeks after bleaching. Even though 120-minute application of 5% guava seeds extract had the least microleakage among all the groups, application time of 10 minutes of 5 % guava seed extract would be the most clinically feasible.

Keywords: microleakage, tooth bleaching, antioxidants, self-adhesive composites Introduction

Introduction

Demand for esthetic dental restorations has significantly increased. Patients pay more attention to smile esthetics and their appearance than before. Thus, treatment of tooth discolorations has become increasingly popular. 1 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.²

Vital tooth bleaching generally involves application of hydrogen peroxide on the tooth surface in an office technique or application of carbamide peroxide in home technique.³

Researchers have suggested postponement of composite restorations for four weeks following bleaching procedure in order to achieve bond strength values comparable to that obtained for unbleached tooth. When it comes to aesthetic treatment, time is an important factor in determining the success of procedure, as most patients that seek aesthetic treatment demand treatment without delay. However, the need for a waiting period prior to restorative procedure could result in unsatisfactory patient compliance. Various techniques have been proposed to resolve this clinical problem encountered after bleaching procedure like: conditioning the bleached enamel with alcohol before restoration, removing the surface layer of enamel, and employing adhesives containing organic solvents.

It is highly desirable to find a suitable antioxidant agent which could result in the reversal of the adverse reaction of bleaching facilitating the completion of restorative procedures in the same appointment as bleaching without causing any delay in treatment. Application of various antioxidant agents such as 10% sodium ascorbate, Vitamin E, and proanthocyanidin (grape seed extract) post bleaching have been proposed.² These

agents enhance the bond of composite to bleached tooth surface^{4,5}. Antioxidants absorb the free oxygen radicals and increase the redox potential of the enamel surface. Hence, the rationale of this in vitro study is to compare and evaluate the effects of 5% grape seed extract solution and 5% guava seed extract solution on the decreasing the adverse effect caused by bleaching agents, by evaluating the marginal leakage of self-adhesive composite resin restoration. Further the study aimed to evaluate the effects of the following three

different antioxidant application times, 10 minutes, 30 minutes, and 120 minutes.

Materials and methods

This in-vitro study was undertaken in the Department of Conservative Dentistry & Endodontics, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore. Box-shaped class V cavities were made on the buccal surface on the cervical third of one hundred and five human premolar teeth.

Table 1: Materials used for the study

Type	Material	Manufacturer	Application technique
Self-adhering flowable composite resin	Dyad flow	Kerr	Fix the applicator tip to the tube and build up cavity with moderate pressure. Light cure for 20 seconds
Bleaching agent	Opalescence Boost PF 40% hydrogen peroxide	Ultra-dent	Apply a 0.5–1.0 mm thick layer of gel to the labial surface of the teeth. Allow the gel to remain on the teeth for 20 minutes per application

Table 2: antioxidant preparation and application technique.

Antioxidant	Preparation of solution	Application technique
Grape seed extract (SINEW Nutrition Capsule)	5gm of grape seed extract in the form of powder collected from commercially available capsules and dissolved in 100ml of distilled water to make 5% proanthocyanidin solution	Solution applied using an applicator tip. Applied on to all the surfaces of the cavity walls and left as per the required time before rinsing
Guava seed extract	Dry guava seeds are to be ground and 5gm of powder should be dissolved in 20ml of distilled water. Homogenous mixture after shaking the solution was filtered with Whatman’s filter paper. Distilled water was added to reach volume of 100ml to obtain 5 % guava seed solution.	Solution applied using an applicator tip. Applied on to all the surfaces of the cavity walls and left as per the required time before rinsing.

The teeth were then randomly divided into three groups. GROUP I (Control) (n = 15): Teeth were bleached followed by restoration with Dyad Flow, GROUP II (n = 45): Teeth were bleached followed by application of 5% grape seed extract solution as antioxidant. GROUP III (n = 45): Teeth were bleached followed by application of 5% guava seed extract solution as antioxidant. Groups II and III were further divided into 3 subgroups: Sub group A (10 min), Sub group B (30 min) and Sub group C (120 min), comprising 15 specimens each, based on the time of application of antioxidant. After restoration, all the teeth were age hardened by thermocycling. All the surfaces of the teeth, except for a 1-mm zone surrounding the restorations' margins, were covered with two coats of nail polish. The specimens were then immersed in 2% methylene blue for 24 hours at room temperature. After staining, the teeth were washed and cleaned. The teeth were then sectioned longitudinally, from buccal to lingual, through the center of the restorations. The microleakage was assessed by judging the depth of the stain.

Statistical analysis

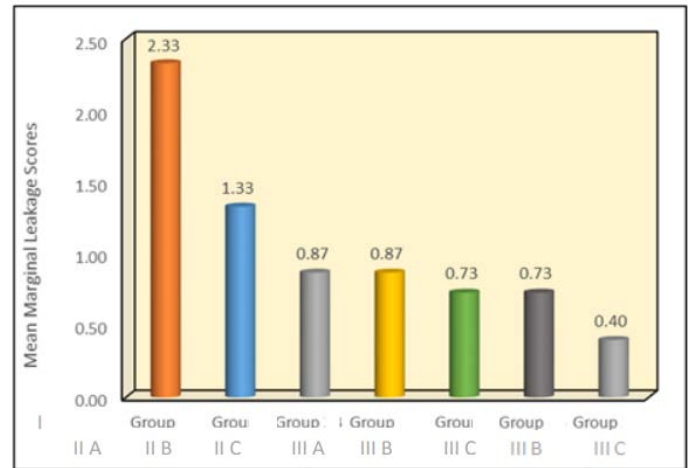
Kruskal Wallis test followed by Mann Whitney post hoc Analysis was used to compare the mean microleakage scores between the Group I and the Sub Groups A, B, and C of Group II and Group III at different time intervals.

Results

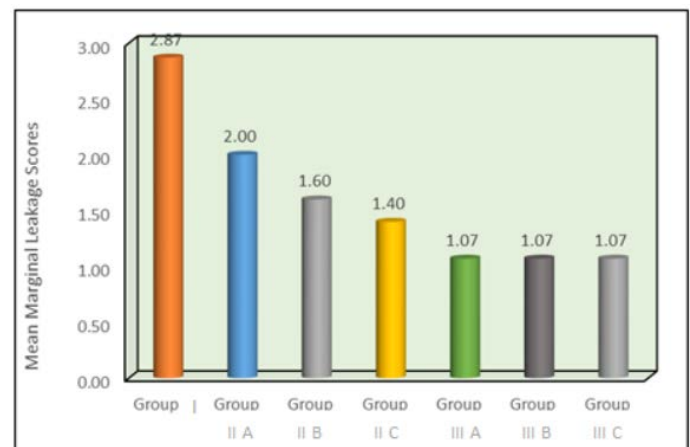
The results demonstrated a highly significant difference in the microleakage between the three study groups both occlusally and gingivally. Multiple comparison of mean difference in microleakage scores showed a very highly significant difference between Group I (Control) and the experimental groups, Group II and Group III. 5% guava seed extract application (Group III) showed lower microleakage with increase in time when compared with

the 5% grape seed extract application (Group II) though it was not statistically significant. Application of 5% guava seed extract at 120 minutes (Group III C) showed the least microleakage.

Graph 1: mean microleakage score at the occlusal level between the study groups



Graph 2: mean microleakage score at the gingival level between the study groups.



Discussion

At present, the gold standard to negate the adverse effects of bleaching on the restorative procedure of composite resins, is to delay any restorative procedure for 4 days to 4 weeks following bleaching.^{6,7,8} The structural changes caused by the incorporation of peroxide ions are eliminated after 24 hours - 3 weeks as the peroxide ions decomposes and the substituted hydroxyl radicals re-enter the hydroxyapatite lattice.⁹

However it is highly desirable to reduce the chair side time by treating the bleached teeth with agents that have free radical scavenging ability, that allows for the restoration of the tooth with composite resin in the same appointment without any deleterious effect to the restorative procedure. Hence the need for the present study.

In vitro studies have confirmed that the free radical scavenging ability and the antioxidant potential of Oligomeric Proanthocyanidin Complexes (OPCs) are 50 times greater than those of vitamin C and 20 times greater than those of vitamin E.^{10,11} Studies by Leong and Shui, Hernández-Acosta et al and others, suggest the high level of antioxidant activity of guava seed by various extraction methods.^{12, 13, 14} This high antioxidant activity of guava seed is due to the phenolic content present in the seeds.¹³ Phenols are very well known antioxidant which can reduce a variety of oxidative compounds and free radicals.

Previous studies that tested the efficacy of grape seed extract in reversing the effects of bleaching using concentrations of 2.5%, 5%, 6%, 10% and 15% and concluded with 5% being the most effective one. In the former studies the most common time period of application was 10 minutes.^{1-3, 9, 15 - 22} Whereas, in a study by Ying Xin XU, application time was 1 minute²² and by Hulas Gogia et al, application time of 120 minutes was found to increase the shear bond strength of composite to the bleached tooth.² Regarding the relationship between the application time and concentration of guava seed extract, only one study was done so far.² The former studies have demonstrated that increasing the period of antioxidant application increased the bond strength of composite to enamel. This occurred till 120 minutes.² It was also observed that not many studies had been done to correlate the relationship

between the concentration of extract and the time of application. Therefore, in this study, 5 % of both grape seed extract and guava seed extract was applied for 10 minutes, 30 minutes and 120 minutes to evaluate and propose the most time dependent clinically apt procedure.

The result of the present study showed that there was considerable increase in the microleakage in the composite resin restorations of Group I (Control) in which no antioxidant was applied prior to composite restoration, compared to that of Group II and Group III in which antioxidants was applied prior to composite restoration. This could be explained in conjunction with the study of Tor neck et al. (1990) who observed under SEM evaluation that the by-products of hydrogen peroxide may impair the intimate contact between resin restoration and tooth structure.²³ Demarco et al. (1998), under SEM evaluations observed that a precipitate was formed over the smear layer in contact with hydrogen peroxide. This precipitate formation could be due to a chemical reaction involving hydrogen peroxide, which is an oxidant, and the highly organic smear layer.²⁴ Another explanation for the increase in microleakage could be the free oxygen originated by breakdown of hydrogen peroxide, which acts adversely on the degree of curing. Oxygen has an inhibitory effect on curing (Ruyter 1981, Ulu Kapi H, Benderli Y and Ulu Kapi I (2003) and insufficient curing of the resin material may contribute to adhesive failures (Yanagawa & Finger 1994).^{25, 26, 27, 28, 29} The reasons given by Barkhordar, Daniel Kempler, Octavia Plesh (1997) was that, hydrogen peroxide alters the tubular permeability and interprismatic area, facilitating the persistence of peroxide which inhibits polymerization and leading to high rate of microleakage.³⁰

Another finding observed was that, the gingival microleakage in Group I was greater than that of the occlusal microleakage. Majority of authors agree with higher leakage values in gingival rather than occlusal aspects of cavities.^{31, 32, 33} In the gingival region it is more likely to have a dentin margin. Therefore, the microleakage can be attributed to the difficulty in establishing a strong bond to dentin rather than enamel.³⁴ Dentin adhesion is more complicated in that, dentin is 50% (by volume) organic material and water. Another consideration is the presence of dentin tubules that may enhance the rate of penetration of the bleaching agents and residual oxygen diffusion. The consequences may be a higher concentration of residual oxygen in the more porous dentin margins than on the enamel margins, thereby increasing micro leakage.³⁵

Among the groups in which the antioxidant was applied, the Group II, with the the grape seed extract had lower score of microleakage than Group I. The possible mechanism of the antioxidant activity of grape seed could be via the presence of donor sites on oligomeric proanthocyanidin complexes that enhance free radical scavenging and increase the antioxidative effect by esterification of polyphenols in proanthocyanidin complex.1 It is stated that the high molecular weight of proanthocyanidin is an important factor responsible for its less penetration into tooth structure and reducing oxygen free radicals. Therefore, it is capable of eliminating free radicals and can increase the bond strength of composite to bleached enamel. This finding was confirmed by Vidhya et al, 9 attributing this property to the following reasons

1. The specificity of Oligomeric Proanthocyanidin Complexes (OPCs) for hydroxyl free radicals.

2. The presence of multiple donor sites on Oligomeric Proanthocyanidin Complexes (OPCs) that trap superoxide radicals.

3. The esterification of epicatechin by gallic acid in Oligomeric Proanthocyanidin Complexes (OPCs), which enhances the free radical scavenging activity.

Microleakage values were considerably higher in all the three subgroups of Group II compared to that of Group III. This result was consistent with the study by Hulas Gogia et al, in which the improvement of bond strength by the application of antioxidant after bleaching was lower in 10% grape seed extract application than 10% guava seed extract.2

There was a decrease in the microleakage of grape seed extract samples over the time period of 10 minutes, 30 minutes and 120 minutes (Sub Group II A, Sub Group II B and Sub Group II C). Sub Group II C with an application time of 120 minute showed the least microleakage. This is in accordance with the previous studies, which stated that the bond strength increases with the increase in antioxidant application time till 120 min.^{36, 37, 38} These findings were relatable to the studies done previously that suggests an inverse relationship between the shear bond strength and microleakage.³⁹⁻⁴¹

Overall, the micro leakage was markedly lower in Group III than Group I (Control). This would be probably due the free radical scavenging ability of the antioxidant present in the guava seed extract in Group III and its absence in Group I.¹³ It was also observed that the microleakage scores were lesser than in Group II with the least microleakage seen in Sub group C at a time interval of 120 minutes. This could be verified by the studies of Leong and Shui, HernándezAcosta et al who suggested high potency of antioxidant activity of guava seed by various extraction methods.^{12, 13, 14} Earlier studies have indicated the potency of different herbal

products to neutralize the adverse effect of bleaching which depends on its composition and mechanism of action. Guava seed represented relatively high contents of total phenolics. These were predominantly composed of flavonoids (monomeric flavanols, proanthocyanidin, flavanols, flavanones, is flavonoids and neoflavanoids), hydrolysable tannins (ellagic acid derivatives involved), phenolic acid derivatives, benzophenones, lignan, phenylethanoid, stilbenoid and dihydrochalcones.

When comparing Sub Group II, A to Sub Group III A, Sub Group II B to Sub Group III B and Sub Group II C to Sub Group III C, the overall values of microleakage were lower in all the three Sub Group of Group III to that of Group II although it was not statistically significant. The better performance of guava seed could be due the potency of the antioxidants in the extracts. The antioxidant property of different products vary based on the composition, Changjiang Guo et al conducted a study on the antioxidant activities of peel, pulp and seed fractions of 28 common fruits as determined by FRAP assay and found out that the guava seeds was at the fourth highest place in terms of antioxidant property. According to this assay guava seeds have 4.71 \pm 0.24 times the potency of that of ascorbic acid.⁴² Further studies can be done in evaluate on the formulation of the antioxidant in gel form to improve their action.

Lai et al. used sodium ascorbate solution for 3 hours and suggested that sodium ascorbate should be used for at least one-third of the amount of time that the oxidizing bleaching agent was applied (e.g. bleaching gel was generally applied for 8 h a day). In the present study application time of 10 minutes, 30 minutes and 120 minutes were investigated. The result showed considerable decrease in microleakage within 10 minutes of application. Application for 10 minutes prior to

restoration seems to be a practical approach clinically. Although an increase in the application time to 30 minutes and 120 minutes showed a slight decrease in microleakage, it was not statistically significant and maybe impractical to apply the antioxidants for this proposed length of time prior to composite resin restoration.

Conclusion

The present study showed considerable decrease in the microleakage following application of 5% grape seed and 5% guava seed extract containing antioxidants before bonding to the bleached tooth. Hence it could be considered to be a viable alternative to delaying restoration for 3 weeks after bleaching. Even though 120-minute application of 5% guava seeds extract had the least microleakage among all the groups, application time of 10 minutes of 5 % guava seed extract would be the most clinically feasible.

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