COMPARATIVE EVALUATION OF THE EFFECT OF HERBAL ANTIOXIDANT ON MICROLEAKAGE OF COMPOSITE RESTORATION TO BLEACHED TEETH: AN IN-VITRO STUDY

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Abstract

Introduction- Bleaching is done to manage discolored teeth. Free radicals released by bleaching agents break down the stains and lighten teeth. These free radicals interfere with the bonding of composite to tooth and may result in microleakage at the tooth-composite interface. Use of antioxidants have been recommended to remove free radicals and immediately bond composite to tooth. Aloe vera is rich in antioxidants. Hence, the study was undertaken to evaluate aloe vera as an antioxidant post bleaching.
Aim-The aim of this study was to evaluate the neutralizing effect of aloe vera on the microleakage of composite restorations in endodontically treated teeth after intracoronal bleaching.
Materials and methods- 50 single-rooted, single- canal premolars were endodontically treated and divided into 5 groups (n=10):
Group A- no bleaching was done and composite was immediately bonded.
Group B- bleaching was done and composite was immediately bonded.
Group C- bleaching was done and composite restoration was delayed for 2 weeks.
Group D- post bleaching, 10% sodium ascorbate solution was used for 30 mins followed by composite bonding
Group E- post bleaching 10% aloe vera solution was applied for 30 mins followed by composite bonding.
Specimens were subjected to microleakage test for 2 days and then longitudinally split. Microleakage was tested under a stereomicroscope.
Statistical Analysis- Data was analyzed using Chi-Square test, Kruskal-Walis test and Mann-Whitney U test. p value was set at<0.05.
Results: Comparison of dye penetration among all the groups was done using Chi-square test and a statistically significant difference was found between the groups. ($p<0.01$) Group A (mean= 0.40) and group C (mean= 0.45) showed similar microleakage values while the microleakage seen in group D (mean=0.70) and group E (mean= 0.75) was greater than group A and C, but it was not statistically significant. Group B shows the highest microleakage (mean=2.15). Thus, a statistically significant difference was seen. ($p<0.01$)
Conclusion-Delayed bonding and antioxidant application can significantly reduce microleakage after non-vital bleaching. 10% aloe vera is effective as an antioxidant for reducing the microleakage.

Keywords: Aloe vera, Bleaching, Microleakage, Root canal treatment, Sodium ascorbate

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1. Introduction

In today's world, people are more conscious of their looks and desire the best. For this reason, many are seeking dental treatment to enhance their smiles.[1]Discolouration of teeth is amongst the top reasons why a smile may be perceived as unattractive.[2]Bleaching is a clinical procedure that helps in lightening the shade of teeth with the use of certain chemicals and has gained popularity as it is considered to be a conservative treatment option.[3]

Bleaching of non-vital teeth was first proposed by Truman in 1864.[4] It is considered to be a simple, efficient and cost-effective treatment modality for endodontically treated teeth that are discoloured.[5]Hydrogen peroxide, carbamide peroxide, and sodium perborate are the commonly used bleaching agents.[2]

Even though bleaching has proven to be a simple and effective technique for whitening of teeth, certain demerits have been reported. Microscopic alterations of the enamel and dentin have been observed by various researchers which results in increase in the porosity and increase in the surface roughness. This in turn negatively affects the mechanical properties of enamel and dentin which results in reduction in strength, wear resistance and fracture toughness that ultimately leads to cracking of the tooth.[6]

Previous studies have shown that post bleaching, the composite bonding to the tooth is compromised. This could be attributed to the residual oxygen radicals present in the enamel and dentin from the bleaching agents. This leads to reduction in the bond strength and microleakage in the restoration.[7]

Literature reveals that the reduction in bond strength post bleaching is temporary and for this reason the delayed bonding approach is adopted, where a waiting period of 24 hours to 4 weeks is observed before the composite restoration is done. Studies have shown that this waiting period provides sufficient time for the residual oxygen to leach out of the tubules and the bonding of the composite to tooth is not hampered.[8]

However, clinically, in certain situations, bonding in the same session is required, for instance, when the patients have time constraints or live far away. It is also desirable as it is convenient for both the patient and dentist.[9] Usage of antioxidants has been recommended as a solution for this problem. Antioxidants act by removing the reactive oxygen species left in enamel and dentin pores by the bleaching gels.[10]

Sodium ascorbate is the most thoroughly investigated antioxidant to be used after bleaching and has shown promising clinical results in terms of reducing the microleakage and restoring the bond strength of composite resin to tooth, after bleaching.[11]

Aloe vera (*Aloe barbadensis miller*) is a short succulent herb with fleshy, green, and spiny leaves.[12] It is readily available and easy to grow and the leaves are rich in many bioactive compounds. The leaf extracts of aloe vera is said to have potent antioxidant activity.[13] Insufficient literature is available on the efficacy of aloe vera as an antioxidant on reducing the microleakage in composite restorations after bleaching.
Hence, this study was undertaken to evaluate the effect of aloe vera on microleakage of composite resins to bleached teeth.

2. Materials and Methods

50 single rooted, single canal human premolars that were extracted for orthodontic and periodontal reasons were selected for this study. After extraction, the teeth were cleaned of any residual tissue tags and calculus using periodontal scalers and were stored in normal saline till further use. The saline water was changed weekly to ensure that bacterial growth was avoided. Teeth included in the study wereintact and non-carious, single rooted, single canal human premolars. Teeth with hypoplastic defects, cracks and previous endodontic treatment were excluded from the study.

Grouping of specimens
Thesamples were divided into 5 groups, with 10 specimens in each group (**n=10**), depending upon the bleaching protocol, the time of composite bonding and the antioxidant treatment:

GROUPS	BLEACHING	TIME OF BONDING	ANTIOXIDANT TREATMENT
A (Positive control)	No	Immediate	No
B (Negative control)	Bleached with 35% Hydrogen peroxide	Immediate	No
C	Bleached with 35% Hydrogen peroxide	After 14 days (Delayed bonding)	No

D	Bleached with 35% Hydrogen peroxide	Immediate	10% Sodium ascorbate solution
E	Bleached with 35% Hydrogen peroxide	Immediate	10% Aloe Vera solution

Table 1: Grouping of specimens

Root canal treatment
Access opening was done with a round bur and Endo Z bur (Mani, Japan) in a high-speed contra-angled handpiece under water coolant till coronal orifice was reached. Following access opening, working length was determined by placing a #10 K file (Mani, Japan) in the root canal and taking a working length radiograph. After working length determination, the canal was prepared till F3 hand Protaper (Dentsply). Irrigation was done with sodium hypochlorite after every file used and a final rinse was done with normal saline. Canals were dried with absorbent paper points and obturated.

Intracoronal barrier placement
Excess coronal gutta percha was removed to create a space of 2mm beyond the coronal orifice (CEJ), for the placement of Glass Ionomer cement (GIC) over the root canal filling.

Bleaching procedure
Except for the samples in Group A, specimens of the other groups underwent intra-coronal (non-vital) bleaching. Pola Office tooth whitening system (SDI) was used for this purpose. Bleaching agent was mixed according to manufacturer's instructions.The gel was then applied in the pulp chamber with a microtip brush. A cotton pellet soaked in the gel was placed in theaccess cavity and the cavity was sealed with Avue Temp (Dental Avenue). The samples were stored in an incubator at 37^{o}C and 100% relative humidity for 5 days.

Delayed bonding
After removing the bleaching agent from the access cavity of the samples of Group C**,** they were thoroughly rinsed under a three-way syringe. The samples were again sealed with Avue Temp (Dental Avenue) and stored in saline water for 2 weeks.

Antioxidant preparation and application

- Preparation of antioxidant solution- 1g of antioxidant powder (sodium ascorbate and aloe vera) was measured on a weighing balance. This was mixed with 10 ml of distilled water to create a 10% antioxidant solution.
- Application of antioxidant solution- Bleaching agent was removed from samples of Group D and Group E and thoroughly washed.
  1 ml of the antioxidant solution (depending on the group) was delivered into the access cavities of the samples with a syringe. A cotton pellet soaked with the antioxidant solution was left in the cavity for 30 minutes. 1 ml of 10% sodium ascorbate solution was placed each sample of Group D. Similarly, 10% aloe verasolution was placed in samples of Group E. After 30 minutes, the solutions were rinsed out from the access cavities.

Restorative procedure
All the access cavities were bonded using Etch and Rinse technique using BestEtch (Waldent) for 30 seconds. The etchant was washed off and the cavity was blot dried. Then Tetric-N-Bond Universal bonding agent (Ivoclar, Vivadent) was applied and cured for 40 seconds. Following this Tetric-N-Ceram composite resin (Ivoclar, Vivadent) was placed in increments to restore the access cavities.Every increment was light cured for 40 seconds.

Thermocycling procedure
The specimens were subjected to 500 thermal cycles between water baths of 5^{o}C and 55^{o}C with a dwell time of 30 seconds.

Dye Penetration test
The root apices of the samples were covered with modelling wax. The surface of the samples was covered with 2 coats of nail varnish except for 1mm around the tooth-restoration interface. The teeth were then submerged for 24 hours in 1.5% methylene blue solution. After 24 hours, the samples were rinsed thoroughly under tap water to remove excess dye. The wax and nail varnish were removed from the surface.

Sectioning of samples
The samples were sectioned longitudinally from labial to lingual direction passing through the centre of the tooth with a low-speed diamond disc.

Evaluation under stereomicroscope
Samples were evaluated under 15X magnification of stereomicroscope (Stereo Zoom, Olympus) to visualise the complete access cavity and determine the depth of dye penetration. The software used

was MV Captor (Version 2.2.1) and the images were captured with a digital camera (NikonCoolpix950,Nikon,Japan).

Scoring criteria

Dye penetration was scored as [14]-

0: No dye penetration.

1: Dye penetration only within the enamel

2: Dye penetration up to half of the cavity depth

3: Dye penetration greater than score 2 without involvement of gutta-percha

4: Dye penetration with involvement of gutta-percha

Microleakage was evaluated. The depth of dye penetration wasassessed, and a score was assigned according to

the given scoring criteria.

Statistical Analysis

The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables etc**.**Chi square analysis was used to find the significance of study parameters on categorical scale.

Based on the results of normality test (Kolmogorov Smirnov & Shapiro Wilk test), it was concluded that part of the data is not following the normal distribution, hence non parametric tests were used. Mann Whitney U test was used to find the significance of study parameters on continuous scale between two groups. Kruskal Wallis test was used to find the significance of study parameters between three or more groups.

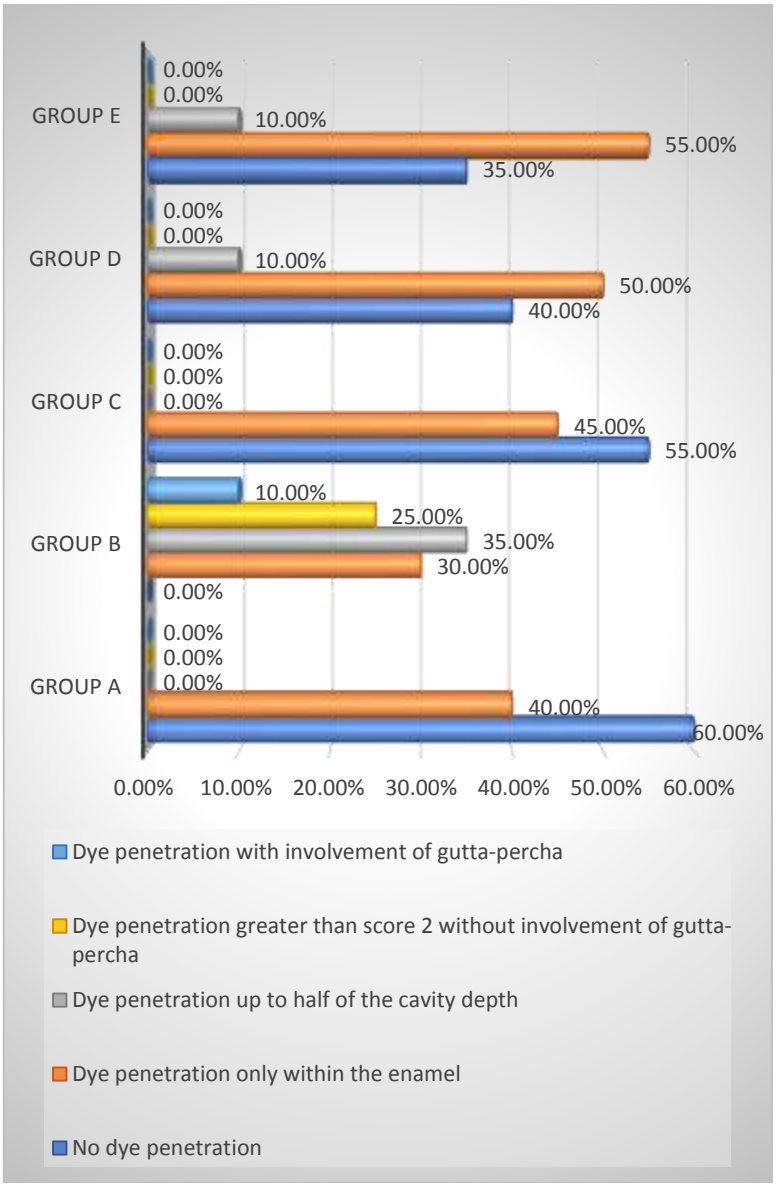
Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

3. Results

Comparison of dye penetration among all the groups was done using Chi-square test and a statistically significant difference was found between the groups. ($p<0.01$) [Table 2 and Graph 1]

Group		Depth					Total
		No dye penetration	Dye penetration only within the enamel	Dye penetration up to half of the cavity depth	Dye penetration greater than score 2 without involvement of gutta-percha	Dye penetration with involvement of gutta-percha	
Group A	**Count**	12	8	0	0	0	20
	% Within Group	60.0%	40.0%	0.0%	0.0%	0.0%	100.0%
Group B	**Count**	0	6	7	5	2	20
	% Within Group	0.0%	30.0%	35.0%	25.0%	10.0%	100.0%
Group C	**Count**	11	9	0	0	0	20
	% Within Group	55.0%	45.0%	0.0%	0.0%	0.0%	100.0%
Group D	**Count**	8	10	2	0	0	20
	% Within Group	40.0%	50.0%	10.0%	0.0%	0.0%	100.0%
Group E	**Count**	7	11	2	0	0	20
	% Within Group	35.0%	55.0%	10.0%	0.0%	0.0%	100.0%
Total	**Count**	38	44	11	5	2	100
	% Within Group	38.0%	44.0%	11.0%	5.0%	2.0%	100.0%
Chi square value: 56.328 p value: <0.001**							

Table 2: Comparison of depth penetration among all the groups using chi square test.



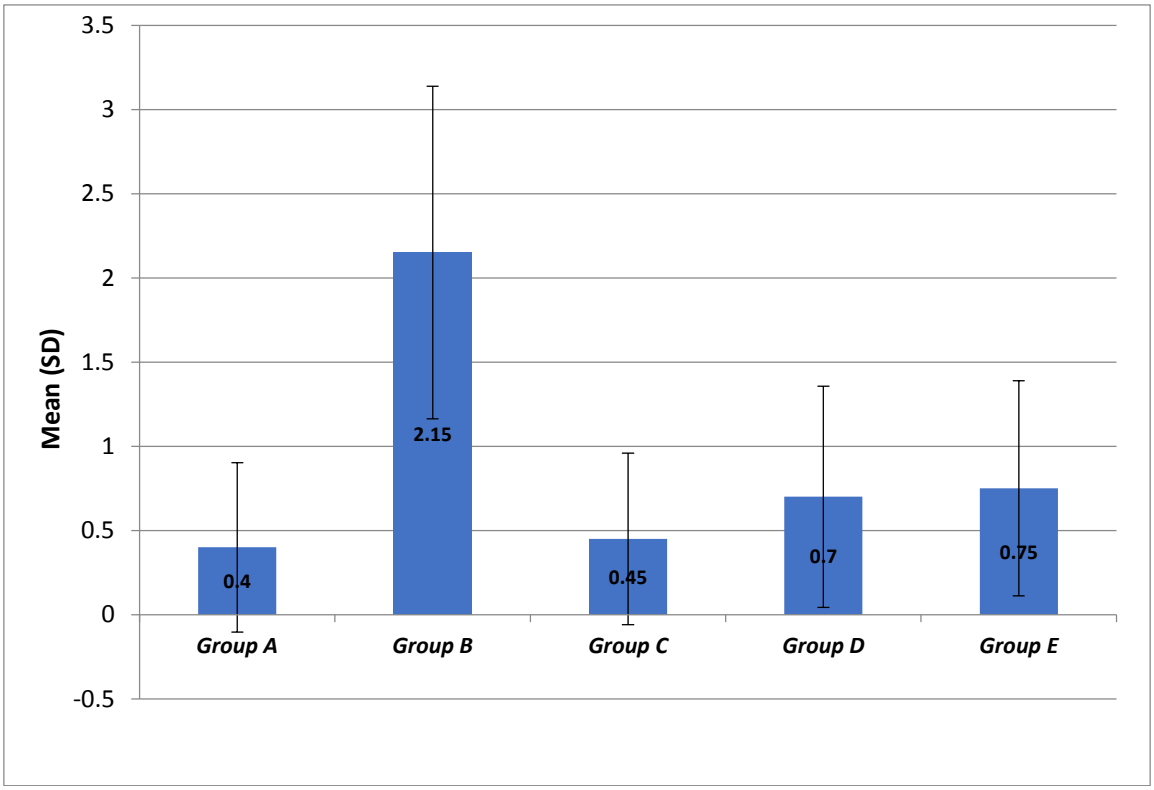
Graph 1: Comparison of depth penetration among all the groups using chi square test.

The comparison of depth penetration values in terms of {Mean (SD)} among all the groups using Kruskal Wallis test. It was seen that group A (mean= 0.40) and group C (mean= 0.45) showed similar microleakage values while the microleakage seen in group D (mean=0.70) and group E (mean= 0.75) was greater than group A and C, but it was not statistically significant. Group B shows the highest microleakage (mean=2.15). Thus, there was a statistically significant difference. ($p<0.01$) [Table 3, Graph 2]

Group	***N***	***Mean***	***Std. Deviation***	***Chi square value***	***P value***
Group A	20	0.40	0.503	**39.752**	**<0.001****
Group B	20	2.15	0.988		

Group C	20	0.45	0.510		
Group D	20	0.70	0.657		
Group E	20	0.75	0.639		
Total	100	0.89	0.931		

Table 3: Comparison of depth penetration values in terms of {Mean (SD)} among all the groups using Kruskal Wallis test



Graph 2: Comparison of depth penetration values in terms of {Mean (SD)} among all the groups using Kruskal Wallis test

Further, when individual groups were compared using Mann Whitney U test, a significant difference was found between group A and group B, group B and group C, group B and group D, group B and group E. ($p<0.01$) There was no statistically significant difference seen amongst groups A and group C, group A and group D, group A and group E. Similarly, no statistically significant difference was seen between group C and group D, group C and group E, group D and group E. ($p>0.05$) [Table 4]

Group	**Compared to**	**P value**
Group A	**Group B**	**<0.001****
	Group C	0.752

	Group D	0.143
	Group E	0.077
Group B	**Group C**	**<0.001****
	Group D	**<0.001****
	Group E	**<0.001****
Group C	**Group D**	0.234
	Group E	0.134
Group D	**Group E**	0.786

Table 4: Individual comparison using Mann Whitney U test.

Thus, from the results of the study we can conclude that immediate bonding after bleaching causes an increase in microleakage. Delayed bonding of 2 weeks showed results like immediate composite bonding without any bleaching procedure, which proves that delay in bonding after bleaching reverses the effects of the bleaching agents. Sodium ascorbate and aloe vera showed similar microleakage values. Hence, aloe vera is as effective as sodium ascorbate as an antioxidant and can be used in appropriate clinical scenarios.

4. Discussion

Bleaching is a procedure that helps in lightening the shade of teeth and is popular as a conservative treatment option.[3] Out of the various methods formulated for non-vital tooth bleaching, the walking bleach technique has gained the most popularity- where 35% of hydrogen peroxide is placed, sealed and left for 3-7 days in the pulp chamber.[15] The hydrogen peroxide acts over time and leads to whitening of the teeth.[16] Even though bleaching is a widely popular treatment technique, many problems have been reported that occur post-bleaching. Post operative sensitivity, irritation of the pulp, microstructural alterations of the enamel and dentin, reduction in the bond strength and increase in microleakage in restorations.[17] This happens because the free radicals of hydrogen peroxide combine with hydroxyapatite and produces a structure called peroxide apatite by replacing the hydroxyl ions.[18] Peroxide apatite significantly decreases the calcium and phosphate content in enamel and dentin, resulting in less substrate for bonding.[19]Another reason could be that the residual oxygen radicals present in the enamel and dentin pores, interfere with resin penetration and polymerization.[19] These free radicals are said to remain active in the dentinal tubules for a certain period after bleaching. This results in an oxygen-inhibited polymerization of the composite that is directly in contact with dental hard tissues and this layer is not capable of withstanding masticatory forces, leading to debonding and microleakage in the restoration.[7] Microleakage is the main cause ofdiscolouration of the restoration margins, regression of colour of non-vital teeth and failure of endodontic treatment.[20] This happens because of loss of seal at the tooth-restoration interface that allows the ingress of fluids in the oral environment into the restored cavity. [21] Even though advent of adhesive dental materials has enhanced the seal at the tooth-restoration interface, certain reports have shown that microleakage is increased after bleaching.[19] Evaluation of the effect of walking bleach technique on the microleakage of composite restorations is necessary because placement of adhesive restorations in the coronal access cavity is the next clinical step after bleaching.[22] In the present study, group Ashowed the least microleakage. This is in accordance with other studies.[14, 21, 22, 23, 24]It was also seen that microleakage increased drastically post bleaching when immediate bonding was done (Group B). A statistically significant difference was seen between Group A and Group B. These findings are in accordance with the findings in other studies that concluded immediate placement of composite restorations post bleaching increases the microleakage drastically. [21, 23, 24, 25] The present in-vitro study confirmed that delaying the bonding procedure (Group C) for 14 days restored

the sealing ability to a level like the unbleached control (Group A). There was no statistically significant difference seen between Group A and Group C. A 14-day waiting time was chosen as Texiera et al [22] and Shinohara et al [26] suggested a 14-day delay was adequate to reduce microleakage in the coronal access restorations. This proves that the effects of bleaching are temporary and reversible. This is possible because hydrogen peroxide, is an unstable compound and loses its potency with time,the residual oxygen present in the bleached surface leaches out with time, and the peroxide apatite break down allowing the hydroxyl ions to re-enter the apatite lattice. [19, 27] While delayed bonding is an effective method to reduce microleakage, immediate restoration after non-vital bleaching is preferred to prevent ingress of bacteria from the saliva, reduce waiting time and complete treatment in one appointment for clinician and patient convenience. [9,14]Thus, antioxidant treatment of bleached teeth has been advocated to reverse the deleterious effects of bleaching gels and reduce the waiting period between bleaching and composite restorations.[28] Antioxidants act by neutralizing free radicals.[29]Neutralizing process of antioxidants are classified as active detoxification and passive detoxification.[29] Sodium ascorbate and aloe vera, the antioxidants evaluated in this study, act via as passive detoxification. Action of antioxidants depends on the time, concentration and form of the antioxidant used. In this study, 10% concentration of the antioxidant, for 30 minutes, in solution form was used. Solution of the antioxidants was prepared from the powder form like Nair R et al. [30] 10% concentration of antioxidant was used as Turkun et al proved that 10% sodium ascorbate was more effective than 2.5% and 5% of sodium ascorbate in reversing the decreased shear bond strength of composites.[31] With regards to the duration of antioxidant application,using sodium ascorbate gel for a period of time equal to one third of the bleaching time was recommended by Lai et al. [32]Some researchers have recommended 10 minutes application.[33] In this study, since bleaching was done by walking bleach technique for 5 days, 30 minutes was selected as an appropriate time, similar to another study.[24] In the present study, a statistically significant difference was present between group B and group D (10% sodium ascorbate) and between group B and group E (10% aloe vera). Both the experimental antioxidants were able to significantly reduce the microleakage when compared to Group B. This is in accordance with many studies which have reported a decrease in microleakage after antioxidant application post bleaching.[14, 21,23,24,34] Sodium ascorbate is the sodium salt of ascorbic acid with a pH of 7.4. It contains a carbon-carbon double bond that has a high reducing potential. It readily donates hydrogen and electrons to the bleaching agents.[35]But some concerns about the mutagenic nature of sodium ascorbate have been raised.[24] In the past decade, a surge in research evaluating the effects of naturally occurring antioxidants derived from plant extracts have increased as they can be feasible alternatives to synthetic and chemical antioxidants. [36,37]Hence, this study evaluated the potential of aloe vera as antioxidant in reducing microleakage post bleaching. The antioxidant effect of aloe vera is attributed to the multitude of active agents contained in aloe vera that have a synergistic effect.[38]*Aloe barbadensis miller* is rich in polysaccharides, polyphenols, anthroquinones, indoles and alkaloids.[39] It also contains ascorbic acid, vitamin E (tocopherol) and vitamin A. [40,41] All of these compounds are known to exert antioxidant properties by neutralizing free radicals.[39] 10% Aloe vera solution (Group E) was prepared from spray dried aloe vera (*Aloe barbadensis*) powder. Spray dried aloe vera is prepared from the extracts of freshly harvested whole aloe leaves at a temperature of 60^{O}C to ensure that there is no loss of biological activity.[42] This type was selected because the whole leaf extract of aloe vera plant comprises the gel and latex. Both components are rich in the active ingredients responsible for the antioxidant properties.[43] The powder used did not have added alcohols or other solvents that could affect the findings of the study.[43] There was also no statistically significant difference present between group D and group E, proving that 10% aloe vera was as effective as 10% sodium ascorbate. This is in accordance with Kadiyala A [44] et al and Nair R et al[30]. In the present study, no statistically significant difference was present between unbleached samplesand the antioxidant groups (Sodium ascorbate and aloe vera). However, the mean values of microleakage noted for antioxidant groups was greater than the mean microleakage in the unbleached group (Table 2), even though it was not of statistical significance. This is accordance with Nari-Ratih et al [29], where the antioxidants did not reverse the effects of bleaching completely and Dabas et al [45], where 30-minute application of the antioxidant increased the bond strength but not to the levels of the unbleached group. Longer period of antioxidant application might be necessary in non-vital bleaching to achieve microleakage similar unbleached group. According to the results of this study, 10% aloe vera has similar efficacy as 10% sodium ascorbate in terms of antioxidant activity and can be used as an immediate solution to reduce the deleterious effects of bleaching agents.

5. Conclusion

Within the limitations of this in-vitro study, it was observed that-

1. The unbleached samples showed the least microleakage.
2. Immediate restoration of bleached samples with composite showed the highest microleakage.
3. Delaying the bonding of composite by 14 days helped reduce the microleakage and attain a mean valuelike the unbleached group.
4. Ten percent sodium ascorbate showed reduced microleakage after 30-minute application on bleached teeth. The mean value of microleakage for 10% sodium ascorbate was greater than the unbleached groupbut it was not of statistical significance.
5. 10% aloe vera showed reduced microleakage after their 30-minute application on bleached teeth. The mean value of microleakage for 10% aloe vera was greater than the unbleached group but it was not of statistical significance.
6. As an antioxidant, 10% aloe vera was as effective as 10% sodium ascorbate.

Hence, it can be concluded that delayed bonding and antioxidant application can significantly reduce microleakage after non-vital bleaching. 10% aloe vera is effective as an antioxidant for reducing the microleakage.

Limitations

The limitations of the current study are that it is an in-vitro study. Findings of an in-vitro study cannot be extrapolated to in-vivo situations where teeth and restorations are constantly subjected to cyclic loading. Thus, more in-vivo studies are required for affirmation of findings regarding concentrations, application time, and consequence of different types of antioxidants on microleakage of bleached tooth surface with different concentrations of bleaching agents.

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Conflict of Interest

The authors report no conflict of interest.

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