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Original Research Article

EVALUATION OF EFFECT OF NATURAL ANTIOXIDANTS ON THE SHADE OF THE BLEACHED ENAMEL USING SPECTROPHOTOMETRY

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Abstract:

Background and Aim: The bleached enamel surface requires application of antioxidant as there will be continuous release of free oxygen radicals. It would be beneficial if remineralizing agent which is recommended after bleaching also provides an antioxidant therapy. The natural antioxidants are colored. Hence, the aim of the present study was to evaluate the effect of natural antioxidants on the shade of the bleached enamel.

Materials and Methods: Thirty single rooted maxillary anterior teeth were selected for the study. For all the samples middle third portion of the labial surface of the teeth was standardized using computerized cellophane stickers. Bleaching was carried out with 22% Carbamide peroxide gel (Philips Zoom Nite White). Based on the type of antioxidant solution application, samples were divided into three groups of ten samples each. Group I control (n=10) Group-II (n=10) 10% wheat grass solution (Sresta natural Bio products, India) Group-III (n=10) 5% grape seed extract (Zenith nutrition, Medizen Labs Pvt.Ltd, India). L, a, b values were recorded with spectrophotometry (Vita Zahnfabrik H. RauterGmbH & Co. KG,Bad Sackingen, Germany) thrice for each sample at the three respective time periods i.e at baseline, after bleaching and after antioxidant application.

Results: ΔE values were subjected to statistical analysis using one way ANOVA with p>0.05 and the results were tabulated. It was observed that there was statistical insignificant difference between the groups with a p value of 0.295(p>0.05). **Conclusion:** The antioxidants 5% grape seed extract and 10% wheat grass extract did not affect the shade of the bleached enamel.

Keywords: tooth bleaching, antioxidant, shade, spectrophotometry

Introduction:

Teeth discolorations are caused by extrinsic and intrinsic factors and restoring the aesthetics is a major challenge to dentists. [1] The compromised smile can affect social and psychological well-being of a person.

Aesthetic dentistry offers various treatment modalities to correct discoloration, like bleaching, laminates and crowns. Bleaching is considered most conservative, simple and easily affordable treatment option. [3]

Hydrogen peroxide and Carbamide peroxide have been used successfully as bleaching agents. [4] These reactive

molecules interact with chromophore molecules and oxidize the macromolecules and pigment stains. [5]

However bleaching can cause mild deleterious effects on enamel like morphological changes in surface, [6] reduction in wear resistance and reduction in shear bond strength to composite resin. The decreased bond strength is due to the presence of oxygen ions which interfere with resin polymerization. [7] It can be improved by delaying placement of composite resin for 1-3 weeks following the bleaching procedure. [8] Many patients would prefer immediate restorative treatment if indicated.

The use of antioxidants after bleaching procedure to overcome the compromised bond strength may be a viable alternative for immediate restorative procedure after bleaching. Previous studies suggested that reduced bond strength can be reversed by use of antioxidants such as sodium ascorbate because of its antioxidant property. However its remineralizing property is not reported in the literature.

However the interest in natural antioxidants of plant origin has greatly increased in recent years.

Proanthocyanidin, a grape seed extract, is a natural antioxidant having greater potential to scavenge oxygen free radicals. [8] Its antioxidant potency has been shown to be 20 times more than that of sodium ascorbate.

Wheatgrass is used as a health drink to cure many diseases in folk medicine. It has a potent antioxidant efficacy. Previous investigations have reported that 10% wheat grass solution and 5% grape seed extract solution are effective in reducing the surface morphological alterations caused by bleaching with 22% carbamide peroxide. The added advantage of remineralisation potential of Wheat grass powder and GSE can be utilized along with their antioxidant potential. [9]

According to previous studies, these extracts are colored in nature. Grape seed extract contains Anthocyanin pigment and Wheat grass extract contains Chlorophyll pigments which are responsible for their respective colors, purple and green. [9]

So there is a need to evaluate the effect color on the shade of the bleached enamel using advanced research equipment. Hence, the present study was designed to evaluate the same.

The research hypothesis tested was that, antioxidants 5% grape seed extract and 10% wheat grass extract may not affect the shade of the bleached enamel.

Materials and methods

Sample size calculation: It was performed for shade on bleached enamel as primary outcome using G Power 3.1.9.2 considering 95% confidence level with 80% power with effect size of 0.618 based on the results of pilot study. The estimated desired sample size was 30.

10g of wheat grass (Sresta natural Bio products, India), which is available in the form of powder was dissolved in 100 ml of distilled water to make 10% wheat grass solution.

GSE (Zenith nutrition, Medizen Labs Pvt.Ltd, India) which is available in the form of capsules was taken and powder was collected after removing the shell. 5 grams of GSE powder was dissolved in 100 ml of distilled water to make 5% GSE solution.

Thirty extracted human maxillary anterior teeth with no lesion or defects were collected. The middle third portion of the labial surface of the teeth was standardized using computerized cellophane stickers.

The baseline shade of the samples was evaluated using Vita easy shade advance spectrophotometer (Vita Zahnfabrik H. RauterGmbH & Co. KG,Bad Sackingen, Germany). L,a,b values depicted on the spectrophotometer were noted. 22% Carbamide peroxide gel was applied on the standardized area for 4 hours per day for a period of three days according to manufacturers' instructions. After 4 hours, the bleaching gel was completely rinsed off from the surface with distilled water. The samples were stored in artificial saliva during the time between the successive bleaching sessions. After the bleaching, the shade of the sample was evaluated using Vita easy shade advance spectrophotometer.

Based on the type of antioxidant solution applied, samples were divided into three groups of ten samples each.

Group 1 – (control) - Sample without any antioxidant application

Group-2 - Sample with application of antioxidant 10% wheat grass solution

Group-3 – Sample with application of antioxidant 5% grape seed extract solution.

Antioxidant solution was agitated with using micro applicators for a period of 10 minutes followed by thorough rinsing with distilled water (Fig 1 a-f). The shade of the sample was evaluated using Vita easy shade advance spectrophotometer. L,a,b values depicted on the spectrophotometer were noted (Fig 2 a-c).



Figure 1: (a) grape seed extract (b) commercial product of grape seed extract (c) application of grape seed extract (d) Wheat grass extract (e) commercial product of wheat grass extract (f) application of wheat grass extract







Figure 2: (a) Shade evaluation with vita easy shade advance spectrophotometer (b), (c) L,a.b Values depicted on Spectrophotometer

Interpretation of ΔE (Color difference): L,a,b values were recorded thrice for each sample at the three respective time periods i.e at baseline, after bleaching and after antioxidant application. The average of the L,a,b values obtained for each sample at different time periods was calculated. L1,a1,b1 mean values represented the baseline samples, L2,a2,b2 mean values represented the samples after the bleaching, L3,a3,b3 mean values represented the samples after wheat grass extract application and L4,a4,b4 mean values represented the samples after grape seed extract application. ΔE was calculated by subtracting the initial data from final data i.e L2-L1, L3-L2 and L4-L2 and similarly ΔE and E

➤ Constant difference in the color was calculated using the following formula

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]\frac{1}{2}$

- L Lightness (0 for black and 100 for white)
- a Amount of red color (positive values) or green color (negative values)
- b Amount of yellow color (positive values) or blue color (negative values)
- ➤ ΔE values were calculated between bleached and baseline samples, bleached and wheat grass extract samples and bleached and grape seed extract samples and subjected to statistical analysis using one way ANOVA with p>0.05 and the results were tabulated.

Results

The results were analyzed through statistical analysis done using One Way Anova test.

Table I: descriptive statistics of inter group comparison of mean values of ΔE , standard deviation and standard error.

Groups	No of	Mean of	Std	Std
	samples	ΔΕ	deviation	error
Group 1 -control	10	7.7030	2.50905	0.79343
Group 2 wheat	10	8.8820	3.91983	1.23956
grass extract				
Group 3 grape	10	9.7697	1.88307	0.59548
seed extract				

p>0.05

It was observed that there was statistical insignificant difference between the groups with a p value of 0.295(p>0.05) and statistical insignificant difference within the groups with a p value of 0(p>0.05). Table I shows descriptive statistics of inter group comparison of mean values of ΔE , standard deviation and standard error.

Although, there were differences in the mean values of color difference between the groups, these differences were not statistically significant.

Discussion

Discoloration of teeth negatively influences the esthetics of a person. Bleaching is the most conservative and preferred option for it. Carbamide peroxide (CP) upon dissolving in water or saliva, dissociates into hydrogen peroxide and urea. [14] Hydrogen peroxide is a strong oxidizing agent which degrades into water and oxygen free radicals. The free radicals oxidize the pigmented macromolecules into smaller and lighter molecules. The whitening effect is due to production of these shorter wavelength compounds which are colourless or lighter in shade. [15]

Studies reported increased roughness, porosities and etching like patterns on the dental hard tissues with the use of bleaching materials. Another most common adverse effect associated with vital tooth bleaching is tooth sensitivity occurring during and after the whitening treatment.

Moreover, bleaching is also known to have adverse effect on the bond strength of adhesive restorations to the bleached surfaces, if the bonding procedure is carried out immediately. This was attributed to residual reactive oxygen species that would interfere with the bonding. [15]

Application of antioxidants was reported to restore shear bond strength of composite resin to bleached enamel due to its protective role against free Radical reactions. Previous studies suggested that reduced bond strength can be reversed by use of antioxidants such as sodium ascorbate because of its antioxidant property. However its remineralizing property is not reported in the literature. [7] It will be definitely advantageous if the antioxidant is also having remineralization property.

Grape seed extract and wheat grass extract have efficiently decreased the morphological alterations on bleached enamel and increased the bond strength of composite resin to enamel. [13]

Wheat grass has scavenging ability on hydrogen peroxide, hydroxyl free radicals and nitric oxide radical. The significant improvement in the bond strength can be attributed to its scavenging ability on various free radicals and to the presence of free hydroxyl and alternate double bonds in wheat grass which might have donated the electrons to stabilize the free radicals. [17]

Grape seed extract was reported to have a free radical scavenging ability. It was reported that, Gallic acid, one of the major constituents of grape seed extract and galla chinensis, facilitates mineral deposition, predominately on the surface layer.^[10]

These extracts are colored in nature. Grape seed extract contains Anthocyanin pigment and Wheat grass extract contains Chlorophyll pigments which are responsible for their respective colors, purple and green. The improved color after bleaching should not get discolored after the application of these colored antioxidants.

Suspecting a discoloration tendency of these antioxidants, there is a need to evaluate their effect on the shade of the bleached enamel using advanced research equipment.

There are no studies regarding the change in the shade of the bleached enamel after the application of the antioxidants. Hence the current study was designed to evaluate changes that might occur in the shade of the bleached enamel after the application of antioxidants namely

- 1. Grape seed extract
- 2. Wheat grass extract

A total of thirty human maxillary anterior teeth were collected and the middle third portion of the labial surface of the teeth was standardized using computerized cellophane stickers to confine the shade evaluation at different time periods to that particular area for appropriate reproduction of evaluation.

Based on the type of antioxidant solution applied, these thirty samples were divided into three groups of ten samples each. Shade evaluation was done at three different time periods, baseline, post bleaching and after antioxidant application using Vita easy shade advance spectrophotometer which is the next milestone in digital shade determination. The innovative enhancement of this shade measurement device has significantly extended the applications for clinical practice and laboratory use.

The results are displayed in both international shade standards – VITA classical A1-D4 and VITA SYSTEM 3DMASTER.

This equipment quantifies the shade through a system named CIELAB- (L*a*b*) (Commission Internationale de L'Eclairage, Vienna, Austria). The L* value indicates lightness and varies between 0 for black and 100 for white; a* determines the amount of red color (positive values) or green color (negative values); and b* exhibits the amount of yellow color (positive values) or blue color(negative values). At each time period, three measurements of L*, a* and b*were recorded and Δ L, Δ a and Δ b were calculated by subtracting the initial data from the final data within each time period. The constant difference in color (Δ E) was then calculated using the following formula:

 $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]1/2$ (Friele-Max Adam-Chickering - Comission Internationale de L'Eclairage, 1976)

According to Douglas et al the advantage of this system is that the shade differences are expressed numerically, and these can be related to visual perception and clinical significance. According to Figueiredo et al values of L*, a* and b* were collected, as they were needed in the formula used to calculate the $\Delta E,$ which represents the constant difference in color. $^{[18]}$

The results of color change were analyzed through statistical analysis using One Way Anova test (Table I)

There was statistical insignificant difference between the groups with a p value of 0.295(p>0.05) and statistical insignificant difference within the groups with a p value of 0(p>0.05). Application of natural antioxidants did not produce undesirable effect on the bleached surfaces in the present study.

The probable reason could be that anthocyanin pigment present in grape seed extract has the minimum staining time of 120 minutes, $^{[19]}$ and chlorophyll pigment present in wheat grass extract has a minimum staining time of 105 minutes. $^{[20]}$ The application time required for the present protocol was far less than staining time of antioxidants. As reported by many studies, 10 minutes application time of antioxidants is adequate to reverse the surface irregularities on enamel thus enhancing bond strength of resin to enamel. $^{[7]}$. $^{[13]}$

- Since, the application of grape seed extract was confined to 10 min in the present study, the color difference observed between the groups was insignificant.
- Grape seed extract reduces the melanocytes proliferation thus reducing the melanin pigmentation in MELASMA/CHLOASMA which might be one of the reasons for insignificant color difference between the groups. [21]

The research hypothesis is accepted as the application of antioxidants 5% grape seed extract and 10% wheat grass extract did not affect the shade of the bleached enamel.

The limitations of this study are, results of in vitro testing of samples which is an important initial screening, cannot be extrapolated to clinical performance. Lab testing shows different degrees of clinical relevance. A more clinically relevant testing necessitates the simulation of the oral environmental factors, including temperature changes, masticatory forces, pH fluctuations, and others.

Conclusion

Application of natural antioxidant grape seed extract produced insignificant color change on bleached enamel

Application of natural antioxidant wheat grass extract produced insignificant color change on bleached enamel

Clinical significance of the study

- ➤ Bleaching is known to have adverse effect on the bond strength of adhesive restorations to the bleached surfaces, if the bonding procedure is carried out immediately.
- Thus, the application of 5% grape seed extract and 10% wheat grass extract can overcome the adverse effects of bleaching without compromising the results as well as help in planning for immediate bonding after bleaching.

Further ex vivo as well as in vivo studies are required to validate the results of the present study.

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