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Comparative Evaluation OD the Effects of Antioxidants on Oxidative Stress, Postoperative Tooth Sensitivity and Bleaching Efficacy in Smokers and Non-Smokers

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Abstract

Background: Antioxidants offer a large protection against oxidative damage in the process of bleaching. However, existing data is limited concerning the efficacy of antioxidants as grape seed extract in reversal of oxidative stress and tooth sensitivity after the process of bleaching.

Aim: The present study was aimed to comparatively evaluate the effects of antioxidants on oxidative stress, postoperative tooth sensitivity and bleaching efficacy in smokers and non-smokers.

Methods: The study assessed 120 subjects with 60 smokers and 60 non-smokers that presented to the Institute in the defined study period with shade as C2 or darker in six maxillary anterior teeth. In all subjects, in-office vital bleaching was done using 35% hydrogen peroxide in two sessions with 7 days difference between the sessions. A split-mouth method was used for grape seed extract application. Sensitivity was assessed postoperatively on VAS scale and shade was assessed using objective and subjective methods and alteration in oxidative stress biomarker level was assessed using ELISA.

Results: The study results showed that the level of oxidative stress biomarker (8-OHdG) showed a significant reduction in smokers at two different

intervals, whereas, in non-smokers, reduction was seen only at one interval after grape seed extract application. For postoperative sensitivity and bleaching efficacy, there was a reduction in postoperative sensitivity after grape seed extract application and was no effect on the color change. Decrease in sensitivity was significant smokers as well as non-smokers.

Conclusion: The present study concludes that application of grape seed extract poses no effect on the efficiency of bleaching for both smokers as well as non-smokers. The level for the oxidative stress biomarker was higher in smokers compared to non-smoker subjects which showed a reduction after the application of the grape seed extract.

Keywords: Grape Seed Extract, Hydrogen Peroxide, Tooth Sensitivity, VAS, 8-Hydroxy-Deoxyguanosine.

Introduction

Tooth color is a vital factor that play a major role in esthetics, especially during smiling. An unacceptable tooth color is a concern in many subjects. Tooth discoloration, intrinsic or extrinsic is affected by various factors as environmental and lifestyle factors. To attain cosmetic outcomes, tooth discoloration treatment needs different approaches for patients and clinicians. Various treatment alternatives must be considered till required outcome is gained. While treating tooth discoloration, systemic approach must be followed including minimally invasive methods that aim at restore and preserve dental structures. Invasive methods must only be considered if all conservative methods fail. One such conservative, affordable, and easy method for treating discolored teeth is dental bleaching.[1]

One of the most commonly used bleaching agents is hydrogen peroxide used at various concentrations of 20%, 35%, 38%, and 40%. On ionic dissociation, it releases free radicals as superoxide anions, per-hydroxyl, nascent oxygen, and hydroxyl radicals. These free oxygen radicals formed during bleaching are highly reactive and can penetrate areas having high electron density of the enamel and enamel-dentin junction causing oxidative stress in the cells of the pulp resulting in imbalance between exogenous and endogenous antioxidants and reactive oxygen species. Oxidative stress leads to instant cell death, oxidative damage to DNA, proteins, and lipids, stimulus in the pulp tissue, and cell differentiation.[2]

The harmful effects of using high concentration of hydrogen peroxide are impaired immediate bond strength of the composite, irritation in the gingival soft tissue, slight demineralization of the enamel surface, and temporary increase in tooth sensitivity. To mitigate the oxidative stress developed with hydrogen peroxide bleaching, exogenous antioxidants like grape seed extract, pine bark extract, aloe vera extract, green tea extract, lycopene, and sodium ascorbate has been advocated.[3]

Grape seed extract is a natural and richest source for OPCs (oligomeric proanthocyanidin complexes) is the most commonly used antioxidant that has ability to scavenge the free radicals which is 20-50 times stronger compared to vitamin E and vitamin C and can be efficacious to neutralize the remaining free radicals. Also, grape seed extract has inflammatory mediators that work in synergism with other antioxidants and give protection against oxidative damage while bleaching. However, existing literature data is scarce concerning the efficacy of grape seed extract in reversal of tooth sensitivity and oxidative stress after bleaching.[4] Hence, the present study was aimed to comparatively evaluate the effects of antioxidants on oxidative stress,

postoperative tooth sensitivity and bleaching efficacy in smokers and non-smokers.

Materials and methods

The present prospective split-mouth clinical study was aimed to comparatively evaluate the effects of antioxidants on oxidative stress, postoperative tooth sensitivity and bleaching efficacy in smokers and non-smokers. The study subjects were from Department of Dentistry of the Institute. Verbal and written informed consent were taken from all the subjects before study participation.

The study assessed 120 subjects with 60 smokers and 60 non-smokers that presented to the Institute in the defined study period with shade as C2 or darker in six maxillary anterior teeth. In all subjects, in-office vital bleaching was done using 35% hydrogen peroxide in two sessions with 7 days difference between the sessions. A split-mouth method was used for grape seed extract application. Sensitivity was assessed postoperatively on VAS scale and shade was assessed using objective and subjective methods and alteration in oxidative stress biomarker level was assessed using ELISA.

In smokers, they were further divided into 2 groups as bleaching with distilled water as placebo and bleaching with antioxidant, grape seed extract. Non-smokers were also divided into two groups as bleaching with distilled water as placebo and bleaching with antioxidant, grape seed extract. The inclusion criteria for the study were smokers with a habit of smoking a minimum of 10 cigarettes/day for a period ≥ 5 years and were referred to us from the Tobacco Cessation Center (TCC), with extrinsic or mild to moderate intrinsic discoloration having 6 maxillary anterior teeth that are at least shade C2 or darker following oral prophylaxis, vital teeth which was free of restorations or caries, and without periodontal disease, healthy patient having good general and oral health (ASA I), and subjects in age range of 18-40 years. The exclusion criteria for the study were subjects with no previous history of smoking in case of non-smokers, receiving drugs with analgesic or anti-inflammatory properties, with fixed orthodontic appliances, teeth with bruxism, pulp less teeth, spontaneous tooth sensitivity, gingival recession, visible cracks, and carious cervical lesions, maxillary anterior teeth with restoration, root canal therapy, or dental prosthesis, teeth having extreme discoloration, such as tetracycline staining and fluorosis, lactating or pregnant females, previously bleached teeth, known peroxide allergy, pre-operative tooth sensitivity, and poor oral hygiene.

1 week before bleaching, anterior teeth scaling was done and shade was assessed before and after bleaching using Vita classical shade guide and photographs. Guide was formed using condensation silicon impression with a window on labial surface of middle third of vestibule using metal device where the tip of the spectrophotometer was inserted.

After isolating the area, baseline GCF samples were collected randomly from maxillary anterior teeth with a micro capillary pipette tube considered as T0, it was transferred to Eppendorf tube and stored at a temperature of −20°C. The bleaching agent (Florence - In office Whitening System) was provided in a powder [potassium nitrate (KNO_3), potassium hydroxide, fumed silica] and liquid (30% hydrogen peroxide, glycerin) form. Potassium nitrate was advised to reduced postoperative sensitivity. The application of bleaching gel was done according to the manufacturer's recommendations (1 mm thick layer of 35% H_2O_2 was applied in two 10-minute applications per session)

After completing the first bleaching session, 5 grams of grape seed extract in powder form was collected from capsule and was dissolved in 100ml distilled water to form a solution of 5% which was applied on labial surface topically for 10 minutes using micro brush. In each half of the arch, antioxidant and distilled water was applied in split-mouth manner. ELISA was done to assess the biomarker level after collecting GCF to assess localized effect of oxidative stress. After 1 week of 1st bleaching session, second bleaching and antioxidant application was done in a similar manner.

The postoperative instructions included avoid brushing the area where gums were sensitive, avoid meals and drinks that leave stains, and avoid smoking for 48 hours. Tooth sensitivity was assessed on VAS scale of 10 at 10 minutes, 1 hours, 24 hours, and 48 hours of bleaching. Shade was assessed using objective and subjective methods at 1 week after session-1, 1 week after session-2, and 1 month after session-2. Biomarker 8-Hydroxy-deoxyguanosine (8-OHdG) was evaluated 10 min after the 2nd Session, 1 week after session-2, and 2 weeks after session-2 considered as T1, T2, and T3 respectively.

Postoperatively, tooth sensitivity was assessed on VAS on scale of 10 where 0 represented no sensitivity; 1-3 mild sensitivity; 4-6 moderate sensitivity; 7-9 severe sensitivity; and 10 represented the worst sensitivity ever. Change in level of 8-Hydroxy-deoxyguanosine (8-OHdG) in GCF was quantitively assessed using ELISA following manufacturer's recommendation.

While assessing the shade, the tooth was kept wet to avoid dehydration. The number of shade guide units (ΔSGU) that became lighter end of the value-oriented list of shade tabs was used to assess change in shade from activity phase beginning to individual recall timings. The variation in colour between the two assessment periods (ΔE) was calculated using the formula: $(\Delta E^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]1/2$

Statistical analysis of the collected data was done with Fisher's exact test, Chi-square test, Mann Whitney U test, and SPSS (Statistical Package for the Social Sciences) software version 24.0 (IBM Corp., Armonk. NY, USA) was used along with the ANOVA and student's t-test. The significance level was considered at a p-value of <0.05.

Results

The present prospective split-mouth clinical study was aimed to comparatively evaluate the effects of antioxidants on oxidative stress, postoperative tooth sensitivity and bleaching efficacy in smokers and non-smokers. The study assessed 120 subjects with 60 smokers and 60 non-smokers that presented to the Institute in the defined study period with shade as C2 or darker in six maxillary anterior teeth. In all subjects, in-office vital bleaching was done using 35% hydrogen peroxide in two sessions with 7 days difference between the sessions. For comparison in smokers and non-smokers with and without antioxidants after bleaching, in non-smokers where antioxidants were applied, mean scores were significantly lesser at all the time intervals with p=0.001, <0.001, and 0.001 at 10 minutes, 1 hour, and 24 hours respectively. At 48 hours, values were 0 in both the groups with p=1.000. In smokers, values were comparable with and without antioxidant application with p>0.05. on intergroup comparison, mean sensitivity scores were significantly lesser with antioxidants at all time intervals compared to without antioxidants with p>0.05 (Table 1).

It was seen that for VITA shade comparison in smokers and non-smokers with and without antioxidants after

bleaching, intergroup comparison showed that for smokers and non-smokers Without antioxidants, results were significantly lesser in subjects that were non-smokers compared to smokers with p=0.006, 0.01, 0.02, and 0.01 at 10 minutes, 1 hour, 24 hours, and 48 hours after bleaching. The values were significantly lower in smokers with antioxidants compared to no antioxidants with p=0.003, 0.01, 0.004, and <0.001 respectively. However, on intra group assessment, in smokers with and without antioxidants and non-smokers with and without antioxidants, the values were non-significant at all the time-intervals with p>0.05 (Table 2).

The study results showed that for ΔE comparison in smokers and non-smokers with and without antioxidants after bleaching, in smokers and non-smokers without antioxidants, the values were significantly lower in smokers without antioxidants compared to with antioxidants at 10 minutes, 1 hour, and 48 hours with p=0.02, 0.03, and 0.05 and were non-significant at 24 hours with p=0.899. In smokers and non-smokers with antioxidants, values were significantly lower in smokers with antioxidants compared to non-smokers with at 10 minutes, 1 hour, and 48 hours with p=0.02, 0.05, and 0.02 respectively. At 24 hours, the values were non-significant with p=0.876. On intra group comparison, in smokers with and without antioxidants, the values were comparable at 10 mins, 1 hour, 24 hours, and 48 hours with p=0.911, 0.951, 0.703, and 0.714 respectively. In non-smokers with and without antioxidants, the values were comparable at 10 mins, 1 hour, 24 hours, and 48 hours with p=0.967, 0.905, 0.625, and 0.820 respectively (Table 3).

Concerning biomarker value comparison in smokers and non-smokers with and without antioxidants after bleaching, on intergroup comparison, the values were significantly lower in non-smokers compared to smokers with p<0.001 at 10 mins, 1 hour, 24 hours, and 48 hours. In smokers and non-smokers, the values were significantly lower in non-smokers with antioxidants compared to non-smokers with p<0.001 at 10 mins, 1 hour, 24 hours, and 48 hours. On intra group comparison, in smokers with and without antioxidants, the values were lower in smokers with antioxidants compared to without antioxidants at 1 hour and 24 hours with p=0.02 and 0.03 and were comparable at 10 minutes and 48 hours with p=0.544 and 0.09. in non-smokers, the values were comparable with and without antioxidants at 10 mins, 24 hours, and 48 hours with p=0.318, 0.227, and 0.08 and were significantly higher in non-smokers with antioxidants than without antioxidants with p=0.227 (Table 4).

Discussion

The present study assessed 120 subjects with 60 smokers and 60 non-smokers that presented to the Institute in the defined study period with shade as C2 or darker in six maxillary anterior teeth. In all subjects, in-office vital bleaching was done using 35% hydrogen peroxide in two sessions with 7 days difference between the sessions. For comparison in smokers and non-smokers with and without antioxidants after bleaching, in non-smokers where antioxidants were applied, mean scores were significantly lesser at all the time intervals with p=0.001, <0.001, and 0.001 at 10 minutes, 1 hour, and 24 hours respectively. At 48 hours, values were 0 in both the groups with p=1.000. In smokers, values were comparable with and without antioxidant application with p>0.05. on intergroup comparison, mean sensitivity scores were significantly lesser with antioxidants at all time intervals compared to without antioxidants with p>0.05. These data were comparable to the previous

studies of Nagpal R et al[5] in 2026 and Seo YS et al[6] in 2023 where study design similar to the present study was also adopted by the authors in their studies on subjects undergoing bleaching.

The study results showed that for VITA shade comparison in smokers and non-smokers with and without antioxidants after bleaching, intergroup comparison showed that for smokers and non-smokers Without antioxidants, results were significantly lesser in subjects that were non-smokers compared to smokers with p=0.006, 0.01, 0.02, and 0.01 at 10 minutes, 1 hour, 24 hours, and 48 hours after bleaching. The values were significantly lower in smokers with antioxidants compared to no antioxidants with p=0.003, 0.01, 0.004, and <0.001 respectively. However, on intragroup assessment, in smokers with and without antioxidants and non-smokers with and without antioxidants, the values were non-significant at all the time-intervals with p>0.05. These results were consistent with the findings of Dalavai P[7] in 2020 and Degirmenci A et al[8] in 2020 where results reported by the authors for VITA shade comparison in smokers and non-smokers with and without antioxidants after bleaching were comparable to the results of the present study.

It was seen that for ΔE comparison in smokers and non-smokers with and without antioxidants after bleaching, in smokers and non-smokers without antioxidants, the values were significantly lower in smokers without antioxidants compared to with antioxidants at 10 minutes, 1 hour, and 48 hours with p=0.02, 0.03, and 0.05 and were non-significant at 24 hours with p=0.899. In smokers and non-smokers with antioxidants, values were significantly lower in smokers with antioxidants compared to non-smokers with at 10 minutes, 1 hour, and 48 hours with p=0.02, 0.05, and 0.02 respectively. At 24 hours, the values were non-significant with p=0.876. On intra group comparison, in smokers with and without antioxidants, the values were comparable at 10 mins, 1 hour, 24 hours, and 48 hours with p=0.911, 0.951, 0.703, and 0.714 respectively. In non-smokers with and without antioxidants, the values were comparable at 10 mins, 1 hour, 24 hours, and 48 hours with p=0.967, 0.905, 0.625, and 0.820 respectively. These findings were in agreement with the results of Maran BM et al[9] in 2020 and Torres CR et al[10] in 2014 where results for ΔE comparison in smokers and non-smokers with and without antioxidants after bleaching comparable to the present study were also reported by the authors in their respective studies.

For biomarker value comparison in smokers and non-smokers with and without antioxidants after bleaching, on intergroup comparison, the values were significantly lower in non-smokers compared to smokers with p<0.001 at 10 mins, 1 hour, 24 hours, and 48 hours. In smokers and non-smokers, the values were significantly lower in non-smokers with antioxidants compared to non-smokers with p<0.001 at 10 mins, 1 hour, 24 hours, and 48 hours. On intra group comparison, in smokers with and without antioxidants, the values were lower in smokers with antioxidants compared to without antioxidants at 1 hour and 24 hours with p=0.02 and 0.03 and were comparable at 10 minutes and 48 hours with p=0.544 and 0.09. in non-smokers, the values were comparable with and without antioxidants at 10 mins, 24 hours, and 48 hours with p=0.318, 0.227, and 0.08 and were significantly higher in non-smokers with antioxidants than without antioxidants with p=0.227. These results correlated with the findings of Silva RR et al[11] in 2023 and Esteves LMB et al[12] in 2022 where results reported by the authors for biomarker value

comparison in smokers and non-smokers with and without antioxidants after bleaching were similar to the results of the present study.

Conclusion

The present study, within its limitations, concludes that application of grape seed extract poses no effect on the efficiency of bleaching for both smokers as well as non-smokers. The level for the oxidative stress biomarker was higher in smokers compared to non-smoker subjects which showed a reduction after the application of the grape seed extract.

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Legend Tables

Table 1: Sensitivity comparison in smokers and non-smokers with and without antioxidants after bleaching

Sn.	Groups	10 mins	1 hour	24 hours	48 hours
1.	Non-smokers				
a)	With antioxidants	1.21±1.12	0.75±0.95	0.11±0.33	0.00±0.00
b)	Without antioxidants	1.75±1.23	1.00±0.95	0.28±0.45	0.00±0.00
2.	Smokers				
a)	With antioxidants	1.95±1.17	1.31±0.86	0.55±0.61	0.00±0.00
b)	Without antioxidants	4.11±2.41	2.48±1.74	1.21±1.12	0.00±0.00
	p-value				
3.	Intergroup				
a)	Smokers and non-smokers Without antioxidants	0.001	<0.001	0.001	1.000
b)	Smokers and non-smokers with antioxidants	0.01	0.006	0.001	1.000
4.	Intra group				
a)	Smokers with and without antioxidants	<0.001	0.004	0.01	1.000
b)	Non- Smokers with and without antioxidants	0.08	0.013	0.118	1.000

Table 2: VITA shade comparison in smokers and non-smokers with and without antioxidants after bleaching.

Sn.	Groups	10 mins	1 hour	24 hours	48 hours
1	Non-smokers				
a)	With antioxidants	9.35±1.53	5.68±1.82	3.24±1.64	2.65±1.39
b)	Without antioxidants	9.76±1.51	6.35±1.80	3.46±1.68	3.05±1.42
2.	Smokers				
a)	With antioxidants	12.23±3.54	8.87±4.41	6.68±4.38	7.05±4.68
b)	Without antioxidants	12.44±3.35	9.44±4.28	7.02±4.49	7.16±4.70
	p-value				
3.	Intergroup				
a)	Smokers and non-smokers Without antioxidants	0.006	0.01	0.02	0.01
b)	Smokers and non-smokers with antioxidants	0.003	0.01	0.004	<0.001
4.	Intra group				
a)	Smokers with and without antioxidants	8.14	0.363	0.833	0.08
b)	Non- Smokers with and without antioxidants	0.272	0.08	0.334	1.000

Table 3: ΔE comparison in smokers and non-smokers with and without antioxidants after bleaching.

Sn.	Groups	10 mins	1 hour	24 hours	48 hours
1.	Non-smokers				
a)	With antioxidants	10.29±2.79	6.47±2.75	0.25±0.55	13.29±2.62
b)	Without antioxidants	10.31±2.68	6.56±2.74	0.32±0.58	13.43±2.71
2.	Smokers				
a)	With antioxidants	8.52±3.23	5.32±1.37	0.23±0.55	11.51±3.16
b)	Without antioxidants	8.60±3.26	5.33±1.36	0.29±0.69	11.82±3.40
	p-value				
3.	Intergroup				
a)	Smokers and non-smokers Without antioxidants	0.02	0.03	0.899	0.05
b)	Smokers and non-smokers with antioxidants	0.02	0.05	0.876	0.02
4	Intra group				
a)	Smokers with and without antioxidants	0.911	0.951	0.703	0.714
b)	Non- Smokers with and without antioxidants	0.967	0.905	0.625	0.820

Table 4: Biomarker value comparison in smokers and non-smokers with and without antioxidants after bleaching.

Sn.	Groups	10 mins	1 hour	24 hours	48 hours
1.	Non-smokers				
a)	With antioxidants	423.36±28.47	480.08±24.76	464.23±24.11	446.67±29.21
b)	Without antioxidants	425.03±28.74	523.13±69.70	479.16±36.41	459.42±24.56
2.	Smokers				
a)	With antioxidants	496.33±38.92	540.03±42.38	517.24±39.28	505.06±36.54
b)	Without antioxidants	500.47±39.65	569.31±62.62	542.34±49.23	514.33±35.81
	p-value				
3.	Intergroup				
a)	Smokers and non-smokers Without antioxidants	<0.001	<0.001	<0.001	<0.001
b)	Smokers and non-smokers with antioxidants	<0.001	<0.001	<0.001	<0.001
4.	Intra group				
a)	Smokers with and without antioxidants	0.544	0.02	0.03	0.09
b)	Non- Smokers with and without antioxidants	0.318	0.007	0.227	0.08