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# The Effect of Natural Antioxidants on Free Radicals Clearance after Tooth Bleaching

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Restorative Dentistry
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## *ABSTRACT*

**Purpose:** This in-vitro study aimed to assess the impact of natural antioxidants on free radical's removal at different time intervals. **Materials and methods:** A total of sixty intact upper central incisors were randomly split into four groups (n=15 each) according to the antioxidant used and all groups were bleached using gel containing 40% hydrogen peroxide (HP): G1 (control group): Bleaching with no antioxidant. G2: Bleaching followed by 5% grape seed extract solution for 10 mins G3: Bleaching followed by 5% green tea extract solution for 10 mins G4: Bleaching followed by 10% sodium ascorbate solution for 10 mins. The assessment of free radicals for each group was evaluated by colorimetric method at three-time intervals; baseline, after 24 and after 48 hrs. **Results:** Regarding effect of antioxidants on free radical clearance at different time intervals**,** results of this study showed that there was statistical substantial disparity between all groups at all time intervals. The greatest mean percentage change was recorded for sodium ascorbate, followed by green tea, then grape seed extract whereas the least value was recorded for the control. **Conclusion:** All the antioxidants employed in this study were capable of removal of free radicals after bleaching. It was concluded that, sodium ascorbate showed statistically significant decrease in free radicals after tooth bleaching when compared to natural antioxidants.

***KEYWORDS***

*Green tea, grape seed extract, sodium ascorbate, bleaching, colorimetric method.*

## INTRODUCTION

In restorative dentistry, discoloration of the anterior teeth is a cosmetic issue that requires efficient treatment. In recent years, the relevance of teeth whitening for patients and customers has led in

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- Radicals Clearance after Tooth Bleaching".

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a significant growth in the number of solutions available. The most frequent procedure for treating tooth discoloration is bleaching. The mechanism of tooth whitening using peroxides occurs by penetration of peroxide through the porosities of enamel prism to the dentin. Oxidation and formation of free radicals lead to lighten of colored species and achieve rapid aesthetic results [1].

The most potent free radicals are hydroxyl radical per–hydroxyl nascent oxygen and superoxide anions, which react with the electron rich zones of pigment inside the tooth, resulting in the dissociation of larger colored molecules into smaller less pigmented ones. Free radicals disrupt resin polymerization and limit resin tag production, both of which have a negative impact [2].

Presence of free radicals of oxygen after bleaching can increase microleakage of resin composite restorations. Research propose delaying composite application for one to four weeks following bleaching treatment to ensure appropriate bond strength comparable to pretreatment baseline values [3].

There are many methods that have been projected to decrease microleakage of resin composite which occurs due to bleaching, as alcohol treatment, utilization of organic solvent containing adhesives, peeling of the external surface of enamel, and the use of antioxidants [4].

Among all methods, the antioxidant application produced instant results that might reverse the increase in microleakage and might be viable alternative to delayed restorations after bleaching. Antioxidants bind to free radicals and boost the redox potential of the enamel surface [5]. Antioxidants found in herbs can repair the lower adhesion force of composite to bleached enamel; nevertheless, different research revealed varying effectiveness values for herbal products; in addition, a significant number of researches contrasted only one or two organic antioxidants with sodium ascorbate [6].

However, literature is spares in comparing the effect of natural versus chemical antioxidants at different time intervals, thus the goal of this study was to examine the impact of chemical and natural antioxidants on free radical clearance at different time intervals.

## MATERIAL AND METHODS

**Study design:** This study was based on measurement of the residual free radical after bleaching and antioxidant treatment of teeth at different time intervals. Ethical consideration**:** The research was carried out after the approval of local ethic committee of Faculty of Dentistry, Al-Azhar University in accordance with international guiding principles.

**Sample size calculation:** The sample size for this study depends on: there is a difference "false negative", i.e. Type II or β error=10%). Acceptable level of significance $p<0.05$ (Type I or α error=5%). This means that we are ready to accept that the probability that the observed difference "false positive" due to chance is 5% Power of the study =0.8. The "power" of the study then is equal to (1 –β). This means that we are ready to accept a 10% failure to detect a difference when actually

Standard deviation is the measure of dispersion or variability in the data [7].

Assessment of the residual free radicals after bleaching of teeth: Collection of teeth: A total of 60 intact permenant human upper central incisors were extracted from diabetic patients age range (18-25) according to the inclusion and exclusion criteria of previous studies [8]. These anonymous teeth were disposed in medical waste container after use. Inclusion criteria: sound and intact teeth were freshly extracted from diabetic patients. Exclusion criteria: The teeth with caries, extreme wear, dissolution, hypoplastic areas, degradation, splits, cracks, dehydrated teeth with developmental flaws and teeth that had history of treatment were excluded. Preparation of teeth: The teeth after

extraction were cleaned using ultrasonic scaler (Dentsply Cavitron Gen-131) to remove soft tissues, dental calculus and stains then immersed in 0.9% saline solution at 4°c until use within one month. Teeth grouping: A total of sixty teeth were randomly categorized into four groups (n=15 each) according to the antioxidant applied; G1: bleaching with HP gel with no antioxidant. G2: whitening with HP accompanied by 5% grape seed extract solution for 10 mins G3: bleaching with HP followed by 5% green tea extract solution for 10 mins G4: whitening with HP followed by 10% sodium ascorbate solution for 10 mins. Each group was measured at three time-intervals; baseline, after 24 and after 48 hrs. Preparation of antioxidant solutions: Natural: Each one of the antioxidants was prepared in 5% concentration for green tea extract and grape seed extract. The extracts were prepared in pharmacy college (M.U.S.T university) where 5gms of grape seed were ground and 5gms of green tea weighted by digital balance (Alfred becht GmbH, Offenburg, Germany). Hot distilled water was added to green tea and grape seed extract powder each of them in a separate flasks then they were filtered by 0.5mm filter paper. The filtrate was then transferred to a rotary evaporator to isolate the solvent from the extract. The extract was dried by lyophilizer to get pure extract powder free of water. The antioxidant solutions were prepared by adding 5gms of each antioxidant powder in 100ml of its distilled water to obtain 5% concentration [8]. Synthetic: For preparation of sodium ascorbate, 100ml distilled water were added to 10gms of sodium ascorbate powder to obtain 10% concentration. Specimens preparation: The roots were cut at 2mm below the cementoenamel junction using a diamond bur (Size 2 Brassler, Savannah, georgia, USA) and mounted on high-speed hand piece (NSK,Japan) under water and air spray. The labial surface of each tooth was polished with papers made of silicon carbide (3M 101Q) to get smooth and homogenous enamel in all samples.

Mounting of teeth in acrylic resin mold: A rectangular hollow plastic mold was used for embedment of the selected tooth in transparent auto polymerizing acrylic resin (Acrostone, Dental &Medical Supplies, Cairo, Egypt) to provide a method for holding the teeth, and resin was combined in accordance to the manufacturer's directions (Fig.1).

Figure (1): Teeth mounted on acrylic resin

Bleaching procedure: Bleaching of the specimens was performed using the cosmetic tooth whitening (white smile-Germany) with 40 %HP for 45 minutes (3x15mins) according to manufacturer instructions. Teeth were cleaned and dried, bleaching gel was applied on the enamel of the labial surface for 15 mins then rinsed and the process was conducted three times to reach 45 mins as a total time of bleaching. Teeth were rinsed carefully to make sure that no remnants of bleaching gel were left on the surface. Then teeth were dried with air spray and antioxidants were applied to the specimens in group 2, 3and 4 only.

Application of antioxidant: Each antioxidant was applied to the specimens according to the type used in each group A2, A3, A4 using a brush soaked in the antioxidant solutions and then applied to wet the labial surface of the bleached teeth. Then the antioxidants were left for 10 mins then rinsed with air water spray, dried and the residual free radicals resulted from bleaching were examined.

Assessment of free radical clearance in the specimens: Number of free radicals remained were measured after bleaching for control group (A1) and after antioxidant treatment for the groups (A2), (A3), (A4). The test was performed at three time-interval; baseline, 24 and 48 hrs using hydrogen peroxide colorimetric method according to manufacturer instructions that suggested the preparation of specimen solution, standard solution and blank solution as follows;

1)-Specimen solution: After application of the antioxidants according to the tested groups, each specimen was applied in a tube containing 500 microliters of distilled water. All the tubes containing the specimen solution were shaken for few minutes to release the free radicals from the teeth in the solution.0.05ml of each solution was applied in separate tubes until use.2)-Standard solution;_To provide a standardized reference for measuring the free radicals in the specimen's solution, a standard solution was prepared to provide a reference for comparing the released free radicals in each tested tooth. Diluted $H_2O_2$ (diluted 1000 times before use 10 microliter+10ml distilled water) was added into small tubes. This standard solution was discarded after finishing the test each time.3)-Blank solution: A solution was prepared where no hydrogen peroxide was added but only 0.05ml of sample solution and 0.5ml of distilled water.4)-Colorimetric method:_0.5ml of Chromogen was added to specimen solution, standard solution and blank solution. 0.5ml of enzyme included in the kit was added to specimen and standard solutions. The reaction resulted in changing the color of the solutions. The resulted color was reddish and its intensity was related to the amount of residual free radicals. All tubes were incubated 10 mins at 37C°.The specimen and standard were read against blank at UV wavelength 510 nm by spectrophotometer (Ser.No: S2505UV01057, Los Angeles, USA) Calculation: H2O2 concentration (mM/L) = sample/standard x 0.5[9]. Statistical analysis: The descriptive statistics version 18 was used for data processing and statistical analysis. The median, averages, standard deviations, minimum, maximum, and confidence intervals were used to sum up numerical data. The p-values are all two-sided. P-values of less than 0.05 were considered significant.

## RESULTS

Effect of antioxidants on free radical clearance at different time intervals [Interaction between groups regarding percentage change]: From Baseline (BL) to 24hrs: Results revealed that there was statistical substantial distinction in mean percent change between all the groups (p=0.00), the greatest mean percent decrease was recorded in sodium ascorbate group (-58±35.07), followed by green tea group (-47.73±.71), then grape seed group (-46.51±1.84) with the least value recorded in control group (-26.97±6.39). Tukey's post hoc test showed a substantial variation between control group and each of the other groups. From 24hr to 48hr: Test showed that the variation between groups was statistically substantial (p=0.042) the greatest mean percent decrease was recorded in sodium ascorbate group (-50.85±27.17), followed green tea group (-43.48±6.07), then grape seeds group (-40.61±22.69) with the least value recorded in control group (-36.69±.73). Tukey's post hoc test indicated a statistical substantial variation between green tea set and each of the other two sets. From baseline to 48hrs: Results indicated that the difference between groups was statistically relevant (p=0.00), the greatest mean percentage change was recorded in sodium ascorbate (-78.36±8.53) group, followed by green tea group (-73.77±2.92), then grape seeds group (-65.65±11.97) with the lowest value recorded in control group (-53.58±3.92). Tukey's post hoc test indicated a statistical substantial variation between control group and each of the other groups. Table (1) and figure (2,3).

**Table (1):** *The mean percentage change, standard deviation (SD) values, and results of ANOVA test for comparison between groups regarding the percent change in each interval:*

| | | Mean percentage change | Std. Dev | Std. Error | 95% Confidence Interval for Mean | | Min | Max | F value | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | | | |
| From. BL to 24hr | Control | -26.97[b] | 6.39 | 1.65 | -30.19 | -23.11 | -40.72 | -10.89 | 7.001 | .000* |
| | Grape seeds | -46.51[a] | 1.84 | 0.47 | -48.53 | -46.49 | -49.62 | -45.47 | | |
| | Green tea | -47.73[a] | 0.71 | 0.18 | -47.12 | -46.33 | -48.03 | -45.45 | | |
| | Sodium ascorbate | -58.20[a] | 35.07 | 9.06 | -74.62 | -35.78 | -84.85 | 52.54 | | |
| From 24hr to 48hr | Control | -36.69[b] | 0.73 | 0.19 | -37.10 | -36.29 | -37.50 | -35.65 | 2.911 | .042* |
| | Grape seeds | -40.61[b] | 22.69 | 5.86 | -47.17 | -22.04 | -47.62 | 9.09 | | |
| | Green tea | -43.48[a] | 6.07 | 1.57 | -54.05 | -47.33 | -57.69 | -43.51 | | |
| | Sodium ascorbate | -50.85[b] | 27.17 | 7.02 | -48.90 | -18.80 | -67.78 | 0.00 | | |
| from. BL. to 48hr | Control | -53.58[b] | 3.92 | 1.01 | -55.75 | -51.41 | -61.86 | -44.30 | 27.834 | .000* |
| | Grape seeds | -65.65[a] | 11.97 | 3.09 | -72.28 | -59.03 | -73.60 | -42.86 | | |
| | Green tea | -73.77[a] | 2.92 | 0.75 | -75.39 | -72.15 | -76.92 | -70.52 | | |
| | Sodium ascorbate | -78.36[a] | 8.53 | 2.20 | -82.09 | -72.64 | -84.85 | -50.85 | | |

*Substantial level p≤0.05, *significant*

*Tukey's post hoc test: the identical letter is not statistically different within each comparison (observation time).*

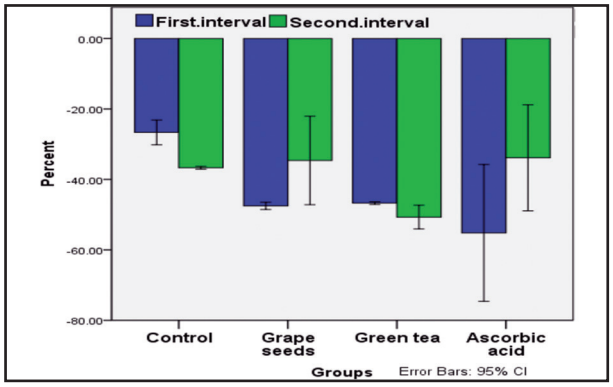


Figure. (2): Bar chart illustrating mean percent change in values of free radicals in different groups from baseline to 24 hours and from 24 to 48 hrs

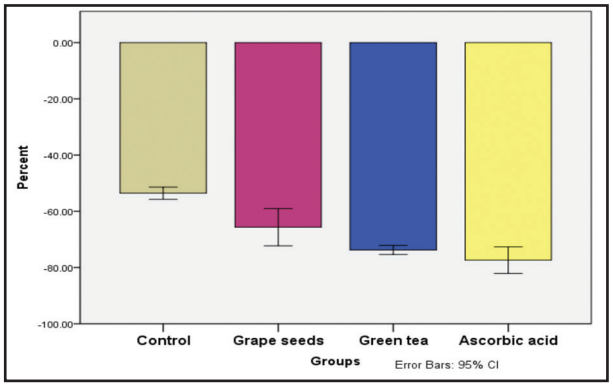


Figure (3): Bar char illustrating mean overall percentage change in values of free radicals in different groups from baseline to 48 hrs.

Comparison between different groups regarding each interval: Baseline: Test showed that the variation between groups was statistically relevant (p=0.00), the greatest mean value was determined in control group (0.545±0.03), followed by grape seeds group (0.261±0.014), then green tea group (0.202±0.011), with the least value recorded in ascorbic acid group (0.029±0.008). Tukey's post hoc test showed a substantial variation between each 2 groups. After 24hrs: Test showed that the difference between groups was statistically relevant (p=0.00), the highest mean value was recorded in

control group (0.399±0.021), followed by grape seeds group (0.137±0.009), then green tea group (0.108±0.006), with the least value recorded in sodium ascorbate group (0.013±0.008). Tukey's post hoc test showed a substantial variation between each 2 groups. After 48hrs: Test showed that the difference between groups was statistically relevant (p=0.00). The greatest mean value was calculated in control group (0.252±0.014), followed by grape seeds group (0.090±0.032), then green tea group (0.053±0.006), with the least value recorded in sodium ascorbate group (0.007±0.003). Tukey's post hoc test revealed a significant difference between each 2 groups. Table (2) and figure (4)

**Comparison within same group regarding each interval:**

Table (2) and figure (4) show the mean values, standard deviation within the same group at different time intervals and results of repeated measures ANOVA test.

For all groups: Results showed that, there was a statistically substantial difference between different time intervals (p=0.00). There was statistical substantial decrease in the mean value of free radicals after one day as compared to baseline. The highest statistically substantial reduction in mean value of remaining free radicals was recorded at 48 hrs followed by 24 hrs.

**Table (2)** *Mean, standard deviation and results of repeated measures ANOVA at different time intervals within the same group:*

| | Baseline | | After 24 hr | | After 48 hours | | P value |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| **Control** | 0.545[a] | 0.030 | 0.399[b] | 0.021 | 0.252[c] | 0.014 | **0.00*** |
| **Grape seeds** | 0.261[a] | 0.014 | 0.137[b] | 0.009 | 0.090[c] | 0.032 | **0.00*** |
| **Green tea** | 0.202[a] | 0.011 | 0.108[b] | 0.006 | 0.053[c] | 0.006 | **0.00*** |
| **Sodium ascorbate** | 0.029[a] | 0.008 | 0.013[b] | 0.008 | 0.007[c] | 0.003 | **0.00*** |

*Substantial level p≤0.05, *significant*

*Tukey's post hoc test: within each comparison (group-row), means the same superscript letter are not significantly different*

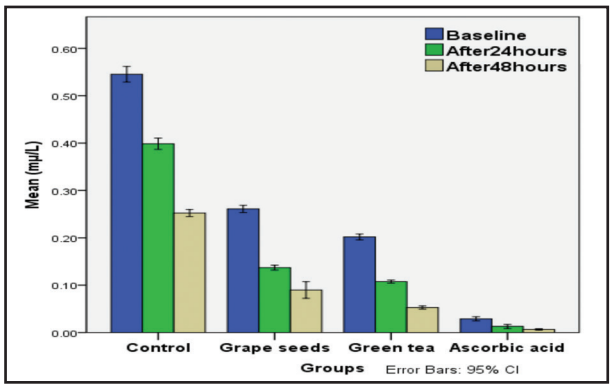


Figure (4): Bar chart illustrating mean value of free radicals in different groups

Comparison within the same group regarding the percent change in each interval: Control group: Results revealed that there was a statistical substantial variation in the same group between different time intervals, the highest mean percent change was recorded in the interval from baseline to 48 hours (p=0.00). Tukey's post hoc test showed that the difference between each two intervals was statistically relevant. Grape seeds group: Results revealed that there was a statistical substantial difference in the same group between different time intervals, the highest mean percent change was recorded the interval from baseline to 48 hours (p=0.00). Tukey's post hoc test revealed

that the difference between each two intervals was statistically significant. Green tea group: Results revealed that there was a statistically significant difference in the same group between various time intervals, the highest mean percent change was recorded in the interval from baseline to 48 hours (p=0.00). Tukey's post hoc test showed no statistical substantial variation between the first interval (baseline to 24 hours) and the second interval (24 to 48 hours). Sodium ascorbate group: Results demonstrated that there was a statistical significant difference in the same group between different time intervals, the highest mean percent change was recorded in the interval from baseline to 48 hours (p=0.00). Tukey's post hoc test revealed that the difference between each two intervals was statistically relevant. Table (3) and figure (5).

**Table (3)** *The mean percentage change, standard deviation (SD) values, and results of ANOVA test for comparison within the same group regarding the percent change in each interval:*

| | | Mean percentage change | Std. Dev | Std. Error | 95% Confidence Interval for Mean | | Min | Max | F value | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | | | |
| **Control** | **From BL to 24 hrs** | -26.65[a] | 6.39 | 1.65 | -30.19 | -23.11 | -40.72 | -10.89 | 146.9 | **0.00*** |
| | **-From 24hrs to 48 hrs** | -36.69[b] | 0.73 | 0.19 | -37.10 | -36.29 | -37.50 | -35.65 | | |
| | **From BL to 48 hrs** | -53.58[c] | 3.92 | 1.01 | -55.75 | -51.41 | -61.86 | -44.30 | | |
| **Grape seeds** | **-From BL to 24hr** | -47.51[d] | 1.84 | 0.47 | -48.53 | -46.49 | -49.62 | -45.47 | 16.57 | **0.00*** |
| | **-From 24hrs to 48 hrs** | -34.61[e] | 22.69 | 5.86 | -47.17 | -22.04 | -47.62 | 9.09 | | |
| | **-From BL to 48hr** | -65.65[f] | 11.97 | 3.09 | -72.28 | -59.03 | -73.60 | -42.86 | | |
| **Green tea** | **-From BL to 24hr** | -46.73[g] | 0.71 | 0.18 | -47.12 | -46.33 | -48.03 | -45.45 | 207.4 | **0.00*** |
| | **-From 24hrs to 48 hrs** | -50.69[g] | 6.07 | 1.57 | -54.05 | -47.33 | -57.69 | -43.51 | | |
| | **-From BL to 48hrs** | -73.77[h] | 2.92 | 0.75 | -75.39 | -72.15 | -76.92 | -70.52 | | |
| **Sodium ascorbate (S.A)** | **-From BL to 24hr** | -55.20[i] | 35.07 | 9.06 | -74.62 | -35.78 | -84.85 | 52.54 | 10.39 | **0.00*** |
| | **-From 24hrs to 48 hrs** | -33.85[j] | 27.17 | 7.02 | -48.90 | -18.80 | -67.78 | 0.00 | | |
| | **-From BL to 48hr** | -77.36[k] | 8.53 | 2.20 | -82.09 | -72.64 | -84.85 | -50.85 | | |

*Substantial level p≤0.05, *significant. Tukey's post hoc test: within each comparison (group), means the same superscript letter are not significantly different*

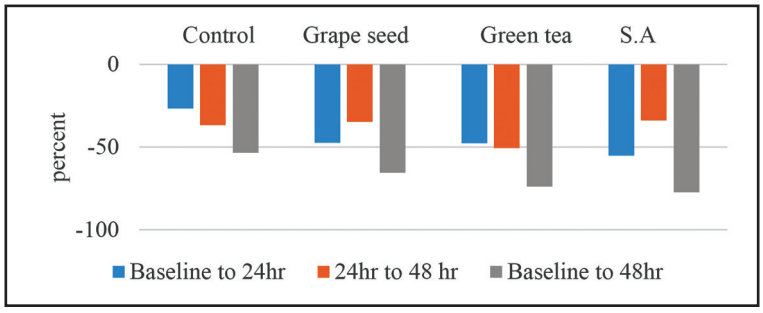


Figure (5): Bar chart illustrating mean percentage change in values of free radicals in each group from baseline to 24 hours and from 24 to 48 hrs.

## DISCUSSION

Hydrogen peroxide undergoes ionic dissociation resulting in the creation of free radicals such as nascent oxygen, hydroxyl radical, per-hydroxyl, and superoxide anions. These free radicals have the potential to produce issues ranging from postoperative discomfort to pulpal inflammation, tooth structural changes, or microleakage of fillings[10].

Another significant problem after bleaching process is the reduced bond strength of composite resin to enamel when bonding is carried out spontaneously after the bleaching process; this is related to the existing of remaining peroxide that counteract with resin adhesion and limits resin polymerization. As a result, several research papers advocated the use of various antioxidant compounds following the bleaching treatment. These researches investigated the influence of various antioxidants on the removal of residual free radicals and the bond strength of resin composite restorations immediately following antioxidant treatment [11]. As there is increasing public interest in the use of natural products, it was found worthy to study the effect of natural antioxidants versus synthetic products on free radical clearance after bleaching at different time intervals; immediate, 24 and 48 hrs.

For this in vitro study, freshly extracted human incisors were used as substrate. Human teeth are recommended in vitro dental research because they enable for testing of study assumption in more clinically relevant substrate. Nevertheless, some variations with use of human teeth exist, such as the differences in patient's age and environmental factors that lead to difference in enamel characteristics. Those effects were minimized in this study by including teeth from patient's age range (18-25)[12]. As the incisors are mostly included for esthetic intervention so they were enrolled in this study. Moreover, enamel tissue was used in this study as a substrate because enamel is considered the first pathway for discoloration and caries [12].

The residual free radicals were evaluated using the spectrometric method based on the CIE-Lab system has been regarded as a trustworthy and objective technique for quantifying tooth color change which was related to the amount of residual free radicals as described by previous study [13].

Results of the current study revealed that there was a statistical substantial difference between all groups at all time intervals (table 1, fig 2,3). The greatest mean percentage change was recorded for sodium ascorbate, followed by green tea, then grape seed extract whereas the lowest value was recorded for the control group.

The current study showed that lowest mean percent decrease was recorded in control group in all time intervals. This was due to missing of any means of antioxidant agents to buffer or neutralize the effect of HP free radicals. These results were supported by previous studies which recorded that the free radicals liberated by bleaching and the accumulated oxygen affected the structural and physical properties of tooth structure. The free radicals resulted in dramatical reduction in shear bond strengths of direct and indirect adhesive restoration [14,15].

In group (A4) treated with sodium ascorbate, results showed the greatest mean percent decrease in all time intervals among all other groups. There was a statistically significant difference in HP free radical clearance between sodium ascorbate group and the control group after bleaching. These results were probably related to the mechanism of action of sodium ascorbate that has antioxidizing ability to neutralize and reverse the oxidizing effects of the bleaching agent. This explanation could be supported by a recent study that evaluated sodium ascorbate is a neutral, nontoxic, and biocompatible antioxidant that when administered as a 10% solution with a 10-minute application period, may restore the lower bond strength of bleached enamel[10]. Furthermore, other study demonstrated that the quantity of sodium ascorbate necessary for reduction of hydrogen peroxide is directly linked to the concentration of the latte, and 10% was sufficient to make significant difference [16]. The present study's findings were also consistent with prior studies which revealed

that employing this agent at a 10% concentration for correcting the weakened bond strength had a significant effect [17].

Moreover, group (A3) treated with green tea showed higher statistically significant decrease in the mean percentage change of residual free radicals than control group and grape seed groups. This might be related to the chemical composition of green tea and stability of the material itself. This was supported by a previous study that evaluated that green tea contains herbal antioxidants - epigallocatechin gallate - that equally compensate the reduced bond strength of composite to bleached enamel more than grape seeds group [18]. Also results showed that, there was no statistical remarkable difference between green tea group and sodium ascorbate group in free radical clearance in different time intervals. The results of this study were supported by previous researchers which compared sodium ascorbate and green tea, they reported that there was no statistical significant difference in bond strength between bleached samples treated with sodium ascorbate and green tea, and that both materials could neutralize the unfavorable effect of bleaching on composite restoration [12]. This was in agreement with a previous study revealed that green tea extract, like ascorbic acid, may be important for increasing bonding strength instant after bleaching[19]. But these results disagrees with a previous study which evaluated that green tea extract showed relevant higher bond strength compared to Proanthocyanidin, tocopherol, and sodium ascorbate[20].

Also, in this study, the grape seed extract group recorded greater statistically significant decrease in the mean percentage change of residual free radicals than control group. Results also revealed no statistically significant difference when compared to sodium ascorbate as well as green tea at different time interval. This may be due to due to Oligomeric Proanthocyanidin Complexes present in grape seed, which are rapidly metabolized and show antioxidants activity. This was in accordance with a study which showed that grape seed extract contains Oligomeric Proanthocyanidin Complexes (OPCs) that have free radical scavenging ability [21]. This also was in agreements with another study that showed the use of grape seed extract as an antioxidant resulted in removal of free radicals after bleaching and significantly enhanced the bond strength of resin composite to bleached enamel. However, the result of this study agreed with a study which investigated that grape seed extract and green tea had the same impact on the shear bond strength of home-bleached enamel, and neither created a statistical significant increase in its value [22].

Concerning the effect of different time intervals on the residual free radicals after bleaching, there was a statistical remarkable decrease in the mean value of residual free radicals from baseline to 24 hrs, from 24 hrs to 48 hrs and from baseline to 48 hrs. All groups after 48hrs results showed favorable results in which free radicals were neutralized and removed. There was a statistical substantial difference between control group and all other groups after 48 hrs which could be related to the absence of antioxidant treatment in control group. This was in agreement with a study that concluded that after 48 hrs. the quantity of peroxide emitted decreased considerably, especially when sodium ascorbate was used [23].

## CONCLUSION

Both synthetic and natural antioxidants were effective in clearance of free radicals after bleaching. There was time dependent improvement in clearance of free radicals

## RECOMMENDATIONS

More research papers are demanded to determine the impact of greater antioxidant concentrations with shorter treatment times. Other studies are needed to evaluate the effects of other natural antioxidants at different time intervals in comparison to sodium ascorbate.

**CONFLICT OF INTEREST:** There is no conflict of interest

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